Inhibition of HIV-1 by a natural compound

NARINE VAN DEN BERG, PHINDIWE MBOBELA, PAMISHA PILLAY, VINESH MAHARAJ, MAKOBETSA KHATI
CSIR Biosciences, PO Box 395, Pretoria 0001, South Africa
Email: nvdberg@csir.co.za

INTRODUCTION

CSIR Biosciences investigated the anti-HIV properties of a plant indigenous to the Eastern Cape, commonly used by traditional healers. A natural compound isolated from the plant, coded BP36, inhibited infectivity of HIV-1 pseudoviruses. The data suggest that BP36 is an entry inhibitor and may therefore be suitable to be developed as a microbicide to control the sexual transmission of HIV.

METHODS

The efficacy of BP36 was measured with the Luciferase Reporter Gene Assay which detects the inhibition of HIV-1 envelope (env) pseudovirus infection in TZM-bl cells. The range of HIV-1 pseudoviral particles was generated in 293T cells through co-transfection of env-expressing plasmids with backbone plasmid DNA (Figure 1). The luciferase activity was detected and quantified by luminescence, which is proportional to the infectious viral particles that have entered the cells (Figures 2-3). The specificity of BP36 to HIV-1 was also determined with this assay against a vasicular stomatitis virus glycoprotein (VSV-G) env pseudovirus. The MTS assay was used to determine the cytotoxicity of the compound.

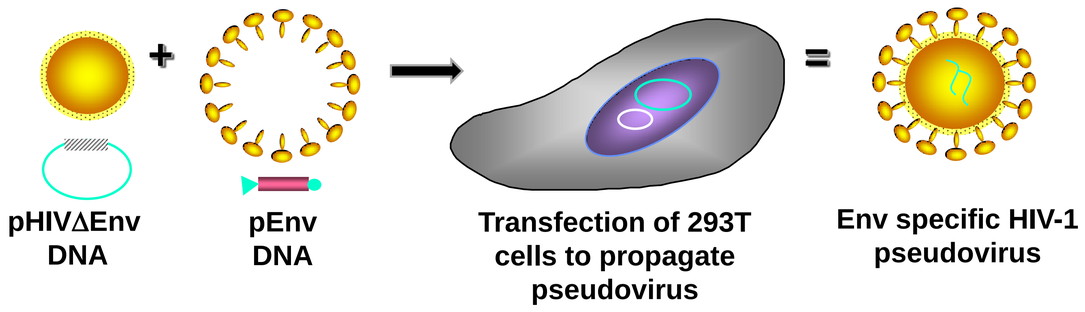

Figure 1: Molecular cloning of an env pseudovirus in 293T cells.

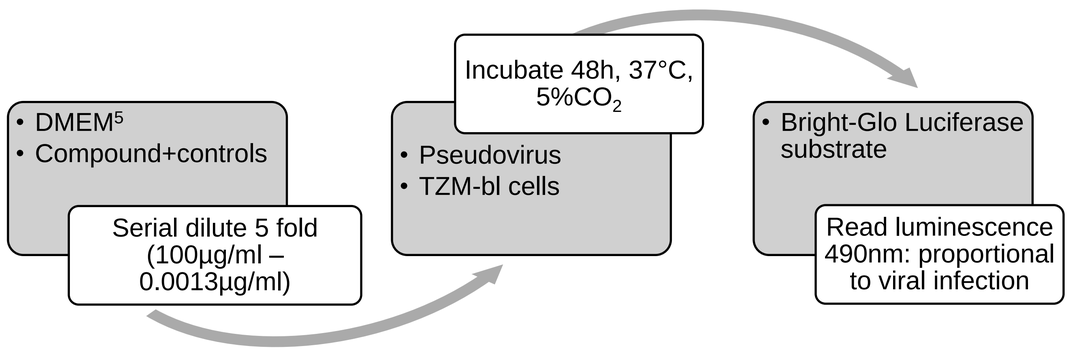

Figure 2: The Luciferase Reporter Gene Assay protocol.

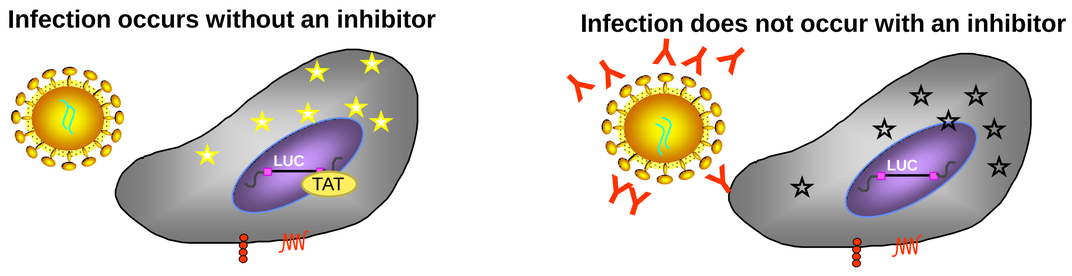

Figure 3: The principle of the Luciferase Reporter Gene Assay in TZM-bl cells.

RESULTS AND DISCUSSION

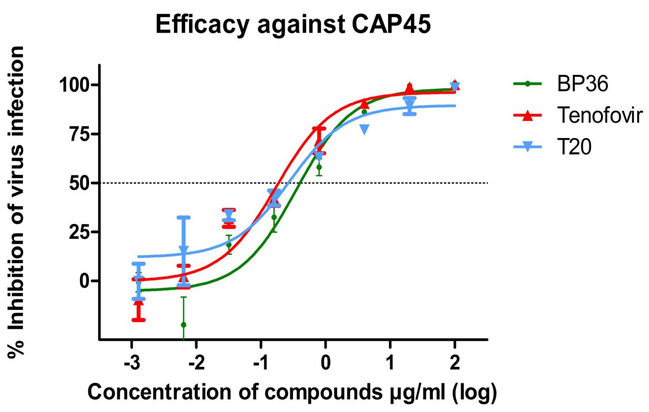

Figure 4: The inhibition efficacy (IC_{50}) curve of BP36 and positive controls (T20: fusion inhibitor, Tenofovir: reverse transcriptase inhibitor) against HIV-1 subtype C env pseudovirus CAP45 (CAPRISA clone). Pseudoviral infections are displayed against a log concentration range (µg/ml) of the compounds.

Table 1: Summary of the origin and IC_{50} values of the HIV-1 pseudovirions screened.

	Subtype	Mode of transmission	Country of origin	BP36 IC_{50} (µg/mL)	T20 Enfuviritide IC_{50} (µg/mL)	Tenofovir IC_{50} (µg/mL)
Cytotoxicity	To test for cytotoxicity			>500	>500	>1000
VSV-G	To test for HIV specificity			± 100	>100	0.5476
HXB2	B	Male to Male	France	0.1198	0.0551	0.4738
CAP45	C	Female sex worker	SA	0.3527	0.2773	0.1735
ZM53	C	Female to Male	Zambia	0.8461	0.1517	0.2512
ZM109	C	Male to Female	Zambia	0.2957	0.0100	0.0110
ZM135	C	Female to Male	Zambia	1.861	2.945	0.7787
ZM233	C	Female to Male	Zambia	1.1140	0.7699	0.2570
ZM249	C	Female to Male	Zambia	7.2050	2.0060	0.9125
Q168.a2	A	Male to Female	Kenya	0.2544	0.3509	0.3160

The activity of BP36 against several HIV-1 subtype C pseudovirions compared favourably with that of T-20 and Tenofovir (Table 1 and Figure 4). We have shown that the crude extracts of the plant (CC_{50}>100µg/ml) and the BP36 compound (CC_{50}>500 µg/ml) are not cytotoxic towards TZM-bl cells when tested in a MTS assay.

A time of addition assay indicated that the compound acts during the early stages of HIV infection, similar to the T20 and Maraviroc entry inhibitors (Figure 5). These data suggest that BP36 is an entry inhibitor. This mode of action supports its potential use as a microbicide.

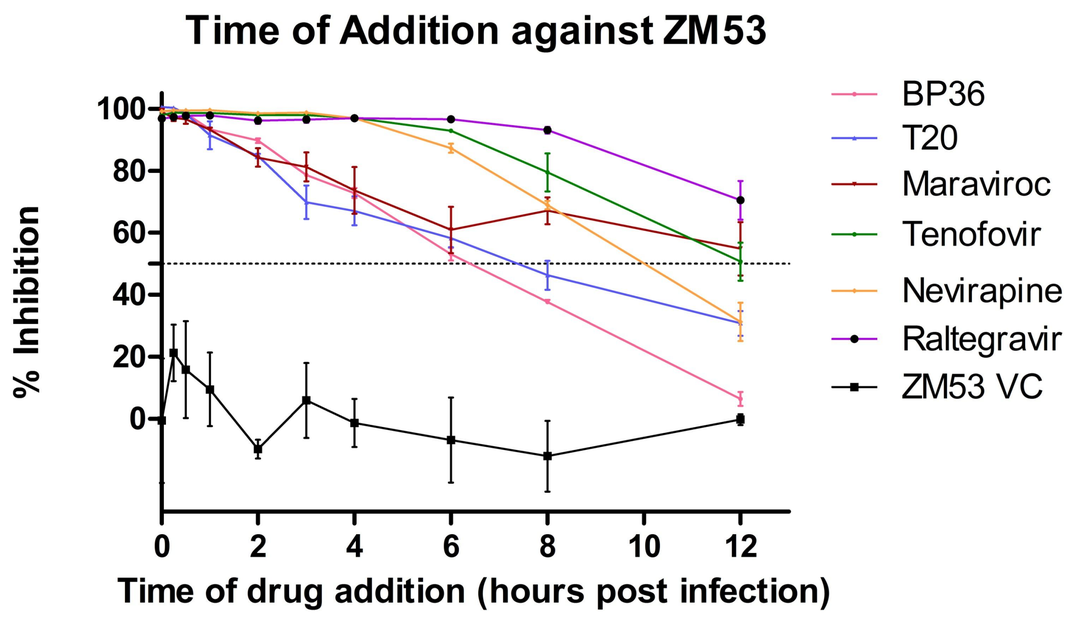

Figure 5: The stage of viral inhibition was determined with the time of addition assay by adding the compound and positive controls (antiretrovirals acting at different stages of the viral lifecycle) at specific time points post viral (HIV-1 subtype C env pseudovirus ZM53) infection of TZM-bl cells. BP36 acts at a similar stage as entry inhibitors, T20 and Maraviroc.

FUTURE WORK

The BP36 compound will be screened against more HIV-1 subtype C molecular clones to determine its efficacy. An intensive programme is currently underway to develop suitable formulations of the compound as a topical microbicide.

ACKNOWLEDGEMENTS

➢Lynn Morris, HIV/AIDS Unit, NICD, South Africa for providing the HIV-1 pseudovirion constructs and target cells.
➢CSIR for funding.

REFERENCES

➢Montefiori D.C. (2005) Current Protocols in Immunol, Chapter 12:Unit 12.11
➢Gray E.S., Meyers T., Gray G., Monterfiori D.C. and Morris L. (2006) PLoSmed 3:7, e225
➢Seaman M.S., Janes H., Hawkins N., Grandpre L.E., Devoy C., Giri A., Coffey R.T., Harris L. Wood B., Daniels M.G., Bhattacharya t., Lapedes A., polonis V.R., McCutchan F.E., Gilbert P.B., Self G.S., Korber B.T., Montefiori D.C., Mascola J.R. (2010) Journal of Virology 84:3, 1439-1452