HIV-1 Pseudovirus Neutralisation by a Natural Compound: A Potential Microbicide

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BACKGROUND

A natural compound isolated from extracts of an indigenous plant in the Eastern Cape, South Africa, showed neutralisation activity against HIV-1 pseudoviruses. The compound can potentially be used in a topical microbicide as an alternative means for women to control the sexual transmission of HIV. The medicinal plant was used by a Traditional Health Practitioner and after a collaboration was set up with CSIR Biosciences, the anti-HIV active compound was isolated. A target based assay (time of addition assay) indicated that the compound acts as an entry inhibitor with the potential use as a topical microbicide. The efficacy of entry inhibitors can be measured with the Luciferase Reporter Gene Assay which detects the inhibition of HIV-1 envelope (env) pseudovirus infection in TZM-bl cells in-vitro. The pseudoviral particles are generated in 293T cells through co-transfection of env-expressing plasmids with backbone plasmid DNA. These pseudovirions can infect cells but are unable to reproduce due to an incomplete genome, termed single-round infection. The infections are detected in genetically engineered TZM-bl cells (HeLa cell clones) that express CD4, CCR5 and endogenous CXCR4 and contain a Tat-responsive firefly luciferase gene under the control of an HIV-LTR. The luciferase activity is detected and quantified by luminescence and is proportional to the infectious viral particles that have entered the cell. The specificity of the compound was also determined with this assay against a vasicular stomatitis virus glycoprotein (VSV-G) env pseudovirus. This measured the binding specificity of the compound to HIV while the cytotoxicity of the compound was determined with a MTS assay.

METHOD

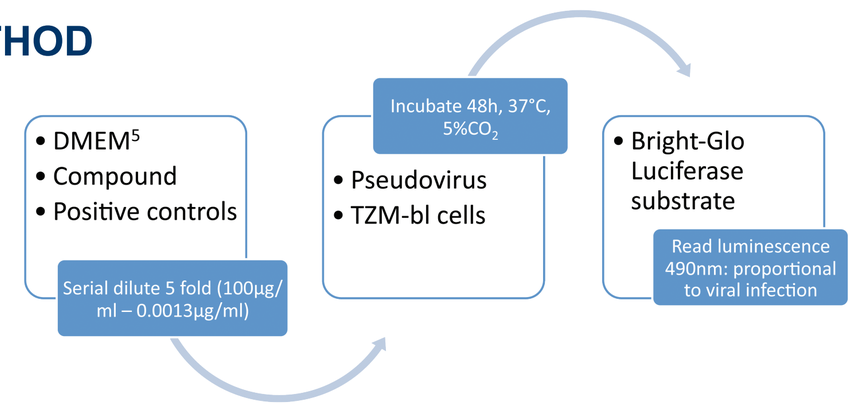


Figure 1: Luciferase Reporter Gene Assay protocol.

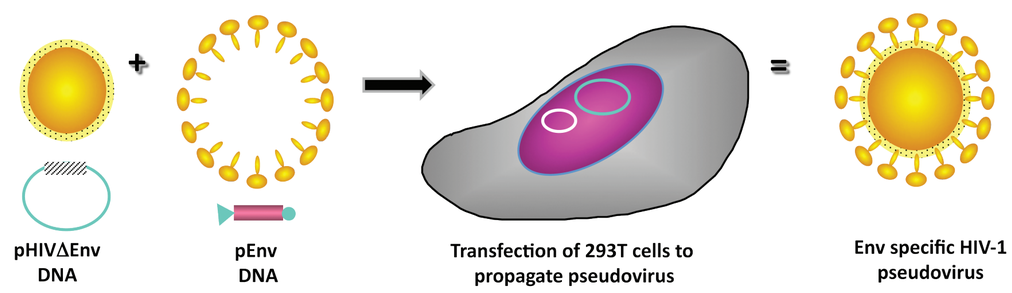


Figure 2: Molecular cloning of env pseudovirus.

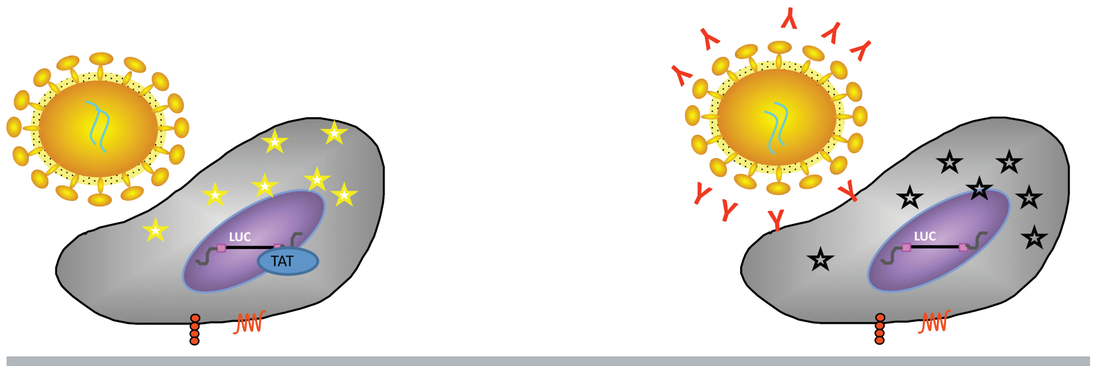


Figure 3: Principle of Luciferase Reporter Gene Assay in TZM-bl cells.

RESULTS

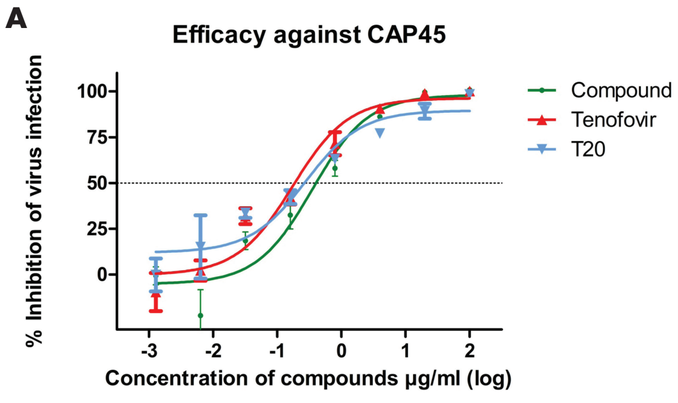
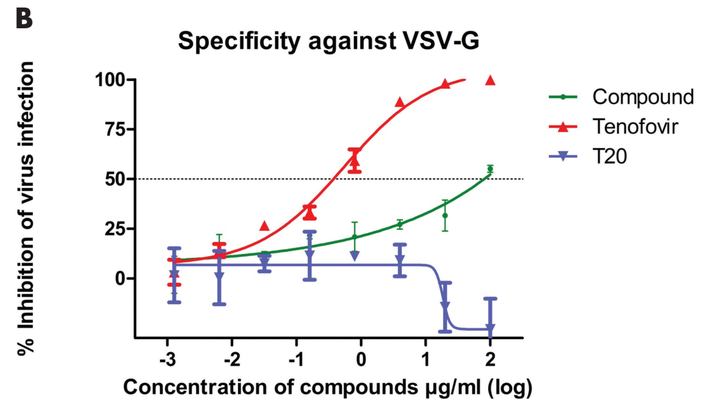


Figure 4: The inhibition efficacy (IC_{50}) of the compound and positive controls (T20: fusion inhibitor, Tenofovir: reverse transcriptase inhibitor) against HIV-1 env pseudoviral infections displayed against a log concentration range (µg/ml) of the compounds. a) The efficacy curve of the compounds against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

Figure 4 *(cont)*: b) The HIV-1 gp120 specificity curve of the compounds against VSV-G env pseudovirus. Tenofovir shows neutralisation activity against VSV-G because it acts downstream of viral entry (eg. reverse transcription).



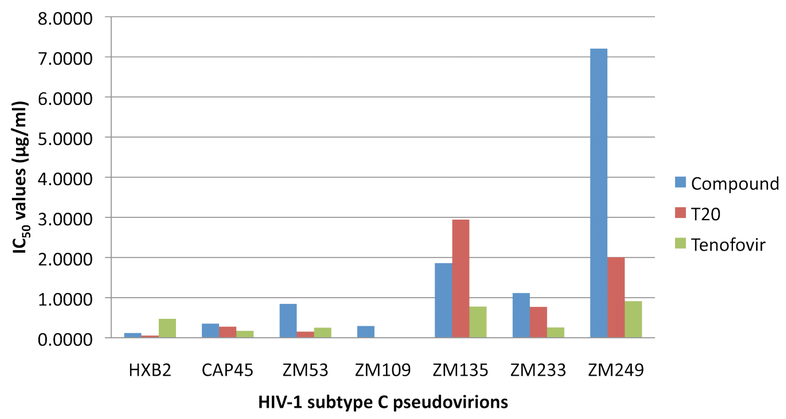


Figure 5: IC_{50} values against HIV-1 subtype C env pseudovirions.

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

	Subtype	Mode of transmission	Country of origin	Compound IC_{50} (µg/mL)	T20 Enfuviritde IC_{50} (µg/mL)	Tenofovir IC_{50} (µg/mL)
Cytotoxicity	To test for cytotoxicity			>100	>100	
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

DISCUSSION AND FUTURE WORK

Safety and efficacy evaluations in-vitro and in animal models are essential before a potential microbicide can be screened in human clinical trials. The pseudovirus inhibition assay used in this study serves as an early identification step to determine the susceptibility or resistance of the isolates to the potential compound. The compound's neutralisation activity (IC_{50} 0.1198-7.2µg/mL) against the screened pseudovirions in a Luciferase Reporter Gene Assay seems to be comparable with that of the entry inhibitor T-20 (IC_{50} 0.01-2.94µg/mL) and the reverse transcriptase inhibitor Tenofovir (IC_{50} 0.01-0.9125µg/mL). T20 is a more relevant drug for comparative purposes as it is an entry inhibitor and indications on our compound of interest point to it also acting in a similar manner. The mode of action of the compound is, however, yet to be determined. We have shown that the extracts of the plant and the compound are not cytotoxic towards TZM-bl cells when tested in a MTS assay at concentrations up to 100 µg/mL. In addition these did not show any neutralisation activity against VSV-G which has a similar glycoprotein to HIV-1 indicating specificity to HIV-1. The compound will be screened against more HIV-1 subtype C molecular clones to determine the percentage efficacy of the compound to the strains commonly found in sub-Saharan Africa.

ACKNOWLEDGEMENTS

CSIR Thematic Funding programme
CSIR Biosciences Aptamer Technology Group

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