A natural microbicide (BP36) against HIV-1

4th Biennial Conference

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Microbicide as a potential solution to HIV transmission

- Sub-Saharan Africa burdened by HIV infection, Sexually active women contribute to > 50% infections
- A “microbicide” is a product that will prevent sexual transmission of HIV and potentially other STIs, and is likely to be applied topically to the vagina as a gel, cream, film, suppository, or vaginal ring
    - Alternative means for women to protect and control sexual transmission of HIV
    - Protects at main portal of transmission

Microbicides: Failures and successes

Candidate name	Mode of action	Comment
Nonoxynol-9 (N9)	Surfactant	Risk of acquiring HIV was increased with frequent use of product. Product shown to be harmful.
SAVVY®	Surfactant	Product did not produce a meaningful result owing to lower than expected HIV incidence in the study population.
Carraguard®	Non specific blockers (Electrostatic interference with virus)	Unsuccessful in demonstrating efficacy. Risk of acquiring HIV not reduced.
cellulose sulphate	Non specific blockers (Electrostatic interference with virus)	Unsuccessful in demonstrating efficacy and product may be harmful.
BufferGel™	Acidifying agent (Maintaining natural flora of vagina)	Unsuccessful in demonstrating efficacy.
0.5% PRO 2000	Non specific blockers (Electrostatic interference with virus)	Demonstrated efficacy but short of statistical significant levels.
Tenofovir	RT inhibitor	**Caprisa 004 study: reduced women's risk of infection by 39%** **Voice MTN 003: Gel was ineffective in reducing risk of infection**

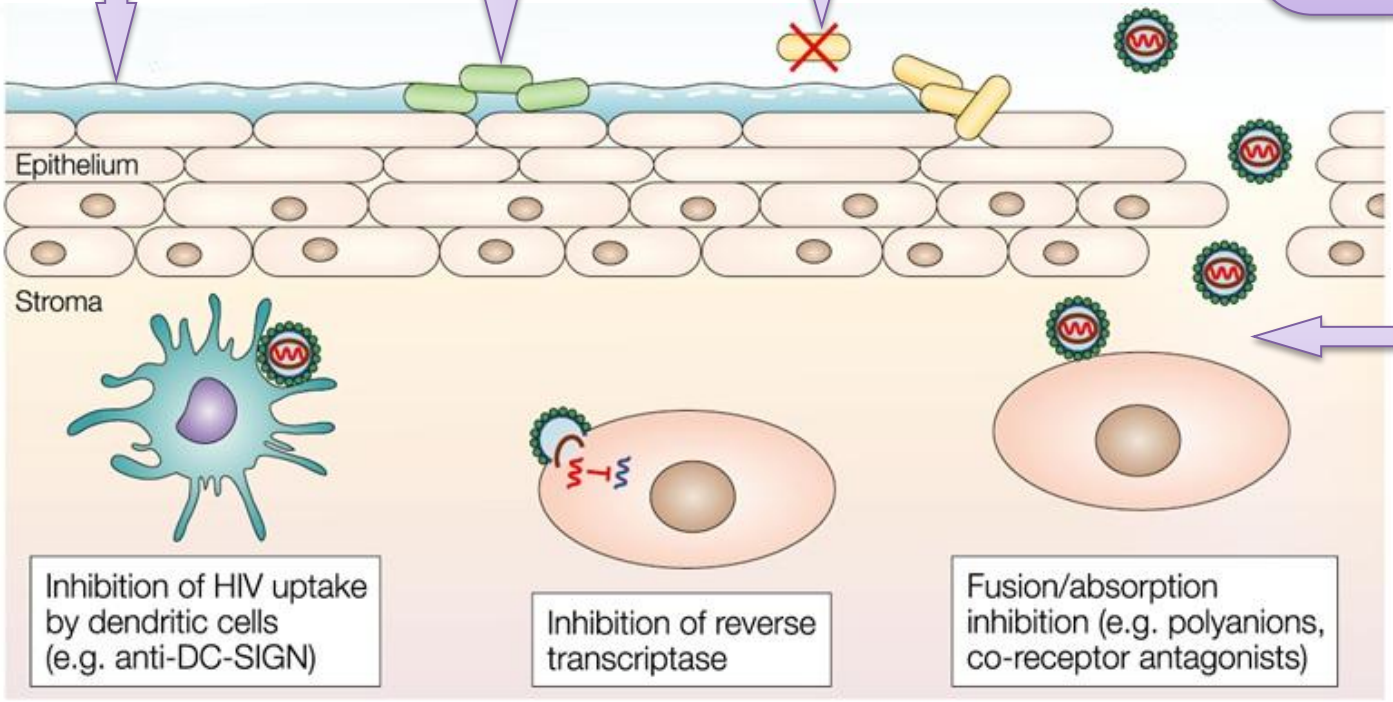
An ideal microbicide

Physical barrier, lubrication

Maintenance of normal microflora

Effective against Other STIs

- Non inflammatory
- Affordable
- Ease of use, acceptability - compliance



Multiple mode of action e.g. entry, fusion, RT inhibitors

Nature Reviews | Microbiology

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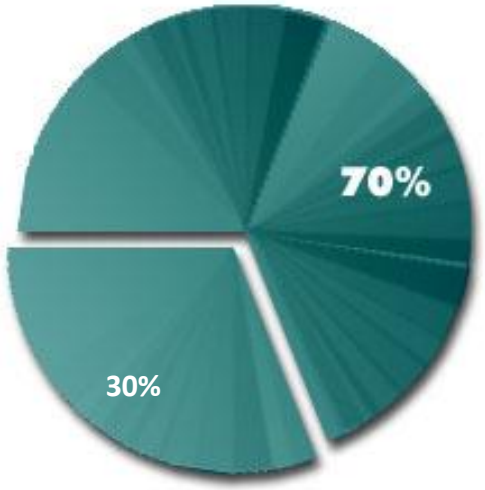
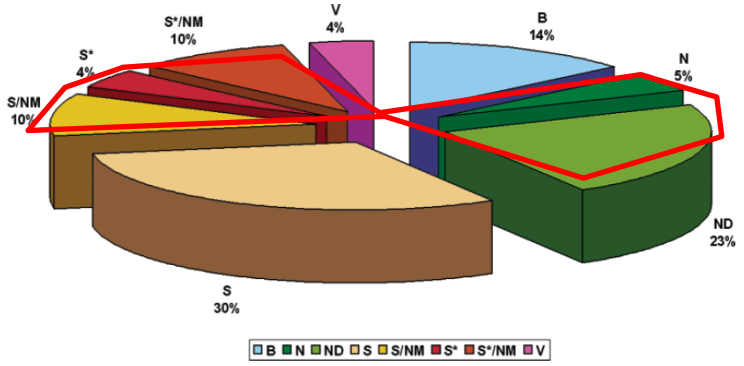
Traditional Medicines and relevance to HIV

- South Africa has a long tradition of medicinal use of indigenous plants
- >200 000 Traditional Health Practitioners (THP) active throughout country, ~ 20 000 university trained MD
- 70% of population visits a THP
- 350 commonly used medical plants
- Recently significant increase in HIV infected patients visiting THPs
- Scientific research based on TMs has significant potential to lead to new treatments for HIV
  - 25% of prescription medicines are plant derived



70% of South Africans consult a THP

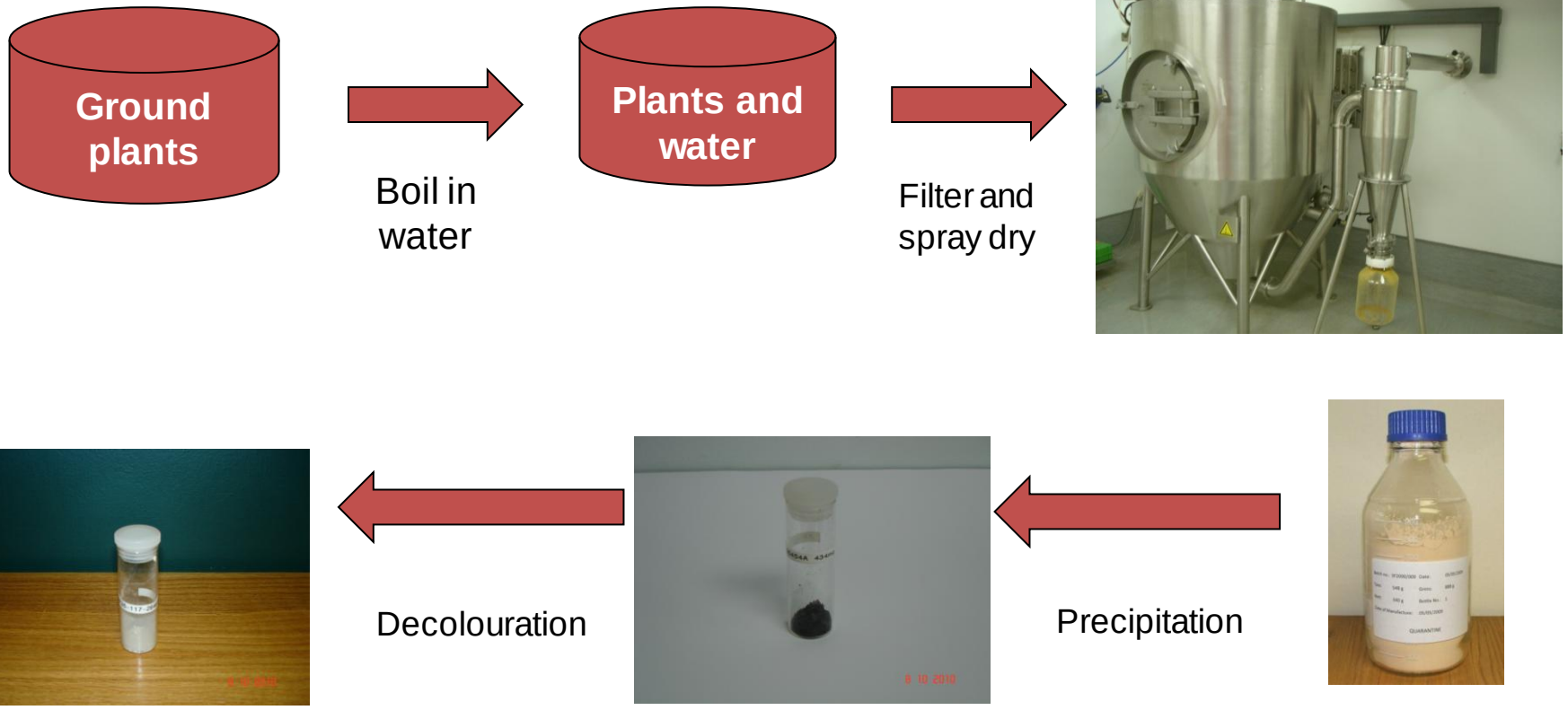
52% of all drugs has some relation to natural compounds



Background: Traditional use to BP36

- Information on traditional use of Eastern Cape indigenous plant species received during 2003
- Benefit Sharing Agreement signed in 2006 between knowledge holder and CSIR.
- Plant originally used to treat “skin diseases or ailments, womb problems and blood related diseases, arthritis, diabetes, high blood pressure, TB, cancers, eye and ear infection”.
- More recently used to treat HIV infected patients
- Method of Preparation
  - Leaves, stems dried and ground
  - Boiling water added to powder
  - Drink as strong tea

Production of active ingredient

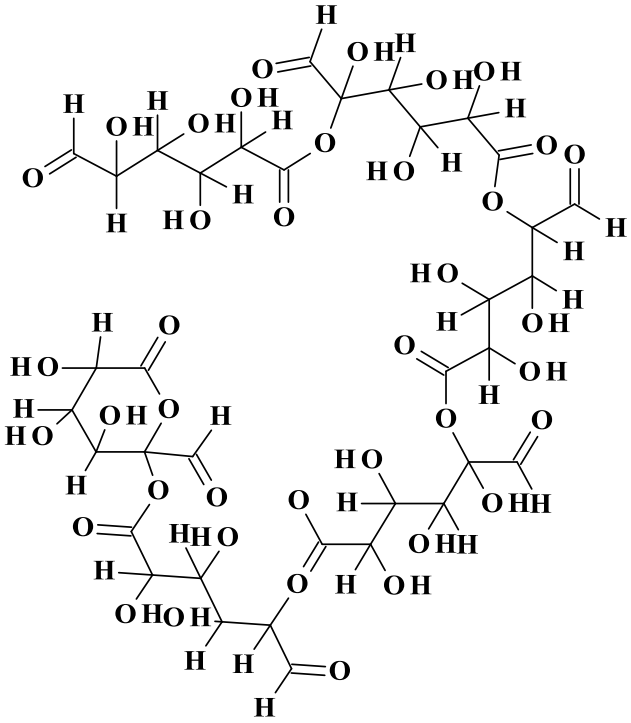


- Production in Clinical and Botanical Supplies Unit

Chemical characterization

- Hydrolysis, derivatization process followed by GC analysis

Monosaccharide	% of total carbohydrate present
Arabinose	11,5
Rhamnose	5,5
Xylose	1,9
Mannose	4
Galactose	10,5
Glucose	26
Glucuronic acid	4,3
Galacturonic acid	36,3



Pectin like molecule, high molecular weight

Anti HIV efficacy

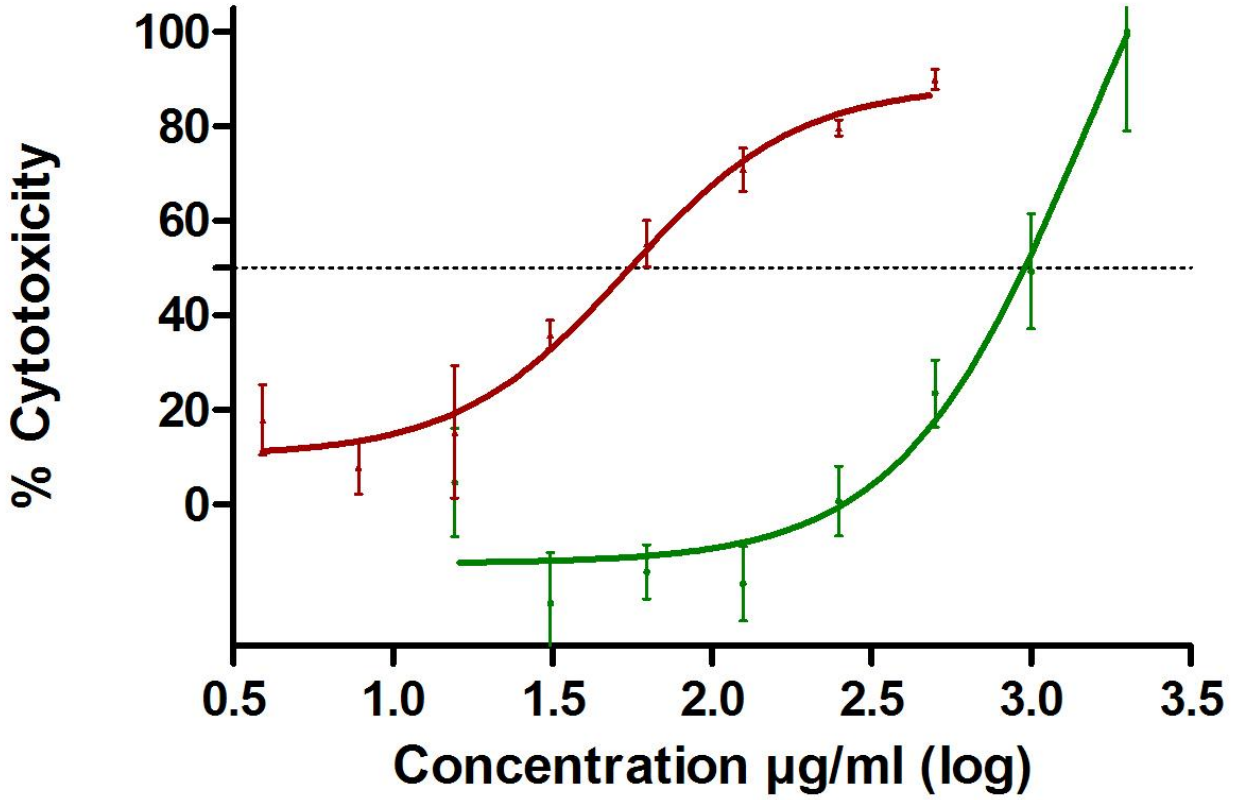
- **Testing of Active: HIV-1 pseudovirus inhibition assay**
- **Completed assaying against 11 subtype C, 3 subtype A and 3 subtype B strains**

	Env clone	type	Patient	ge of ction	CoR	Compound	T20 (Enfuviritde)	Tenofovir IC_{50} µg/mL)
Durban, Sout Africa								.2 ± 0.5
								.6 ± 0.1
CAPRISA004 isolates, Durban, Sout Africa								2 ± 0.01
								.0 ± 0.4
								5 ± 0.02
								.1 ± 0.5
								.8 ± 0.2
South Africa								.4 ± 0.5
								.7 ± 0.4
Lusaka, Zambia								3 ± 0.04
								.0 ± 0.1
France	HXB2	B	Adult	Acute	X4	0.1 ± 0.1	0.06 ± 0.004	0.5 ± 0.1
	VSV-G					± 100	> 100	

IC_{50} range against HIV-1 subtype C pseudoviruses

- BP36 active: 0.1 – 7.9µg/ml
- T20: 0.1 – 7.5µg/ml
- Tenofovir: 0.2 – 1.2µg/ml

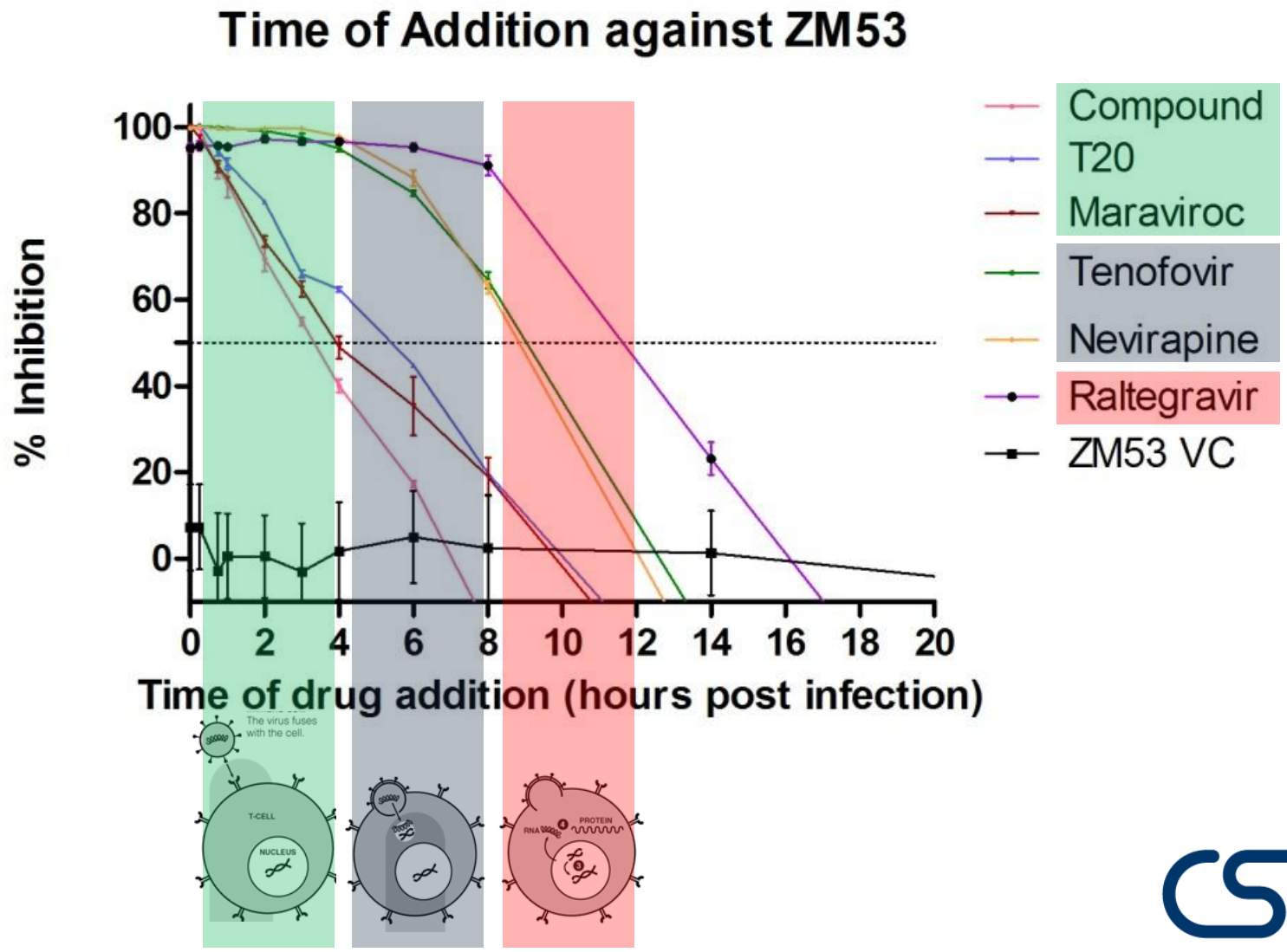
Cytotoxicity towards non infected cells



Compound CC_{50}: 1477µg/ml

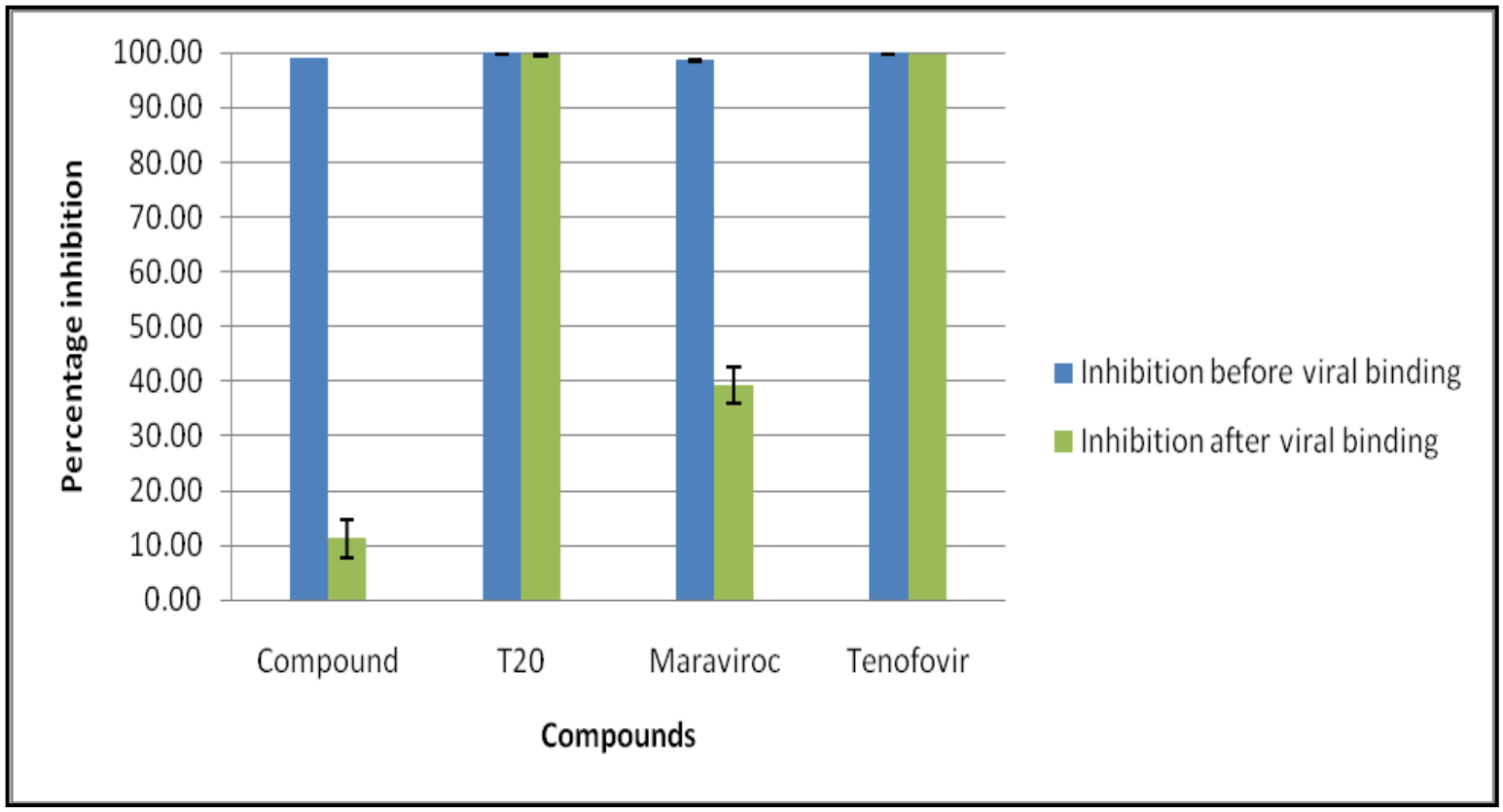
Etoposide CC_{50}: 54.37µg/ml

Mode of action – stage of viral cycle compound inhibits



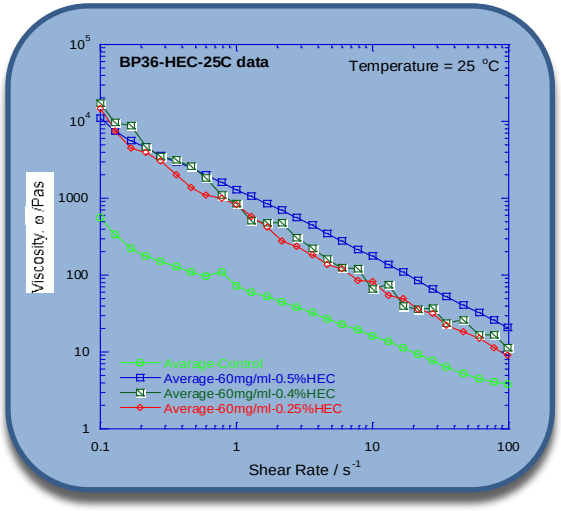
Fusion arrest experiments

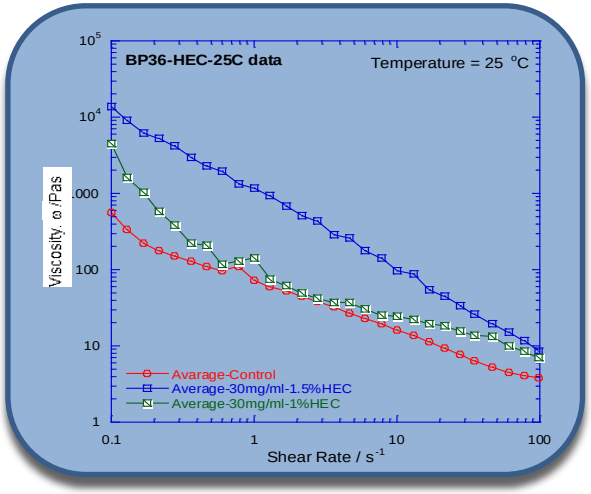
Active ingredient loses its ability to inhibit infection after viral attachment suggesting that it acts as an attachment inhibitor

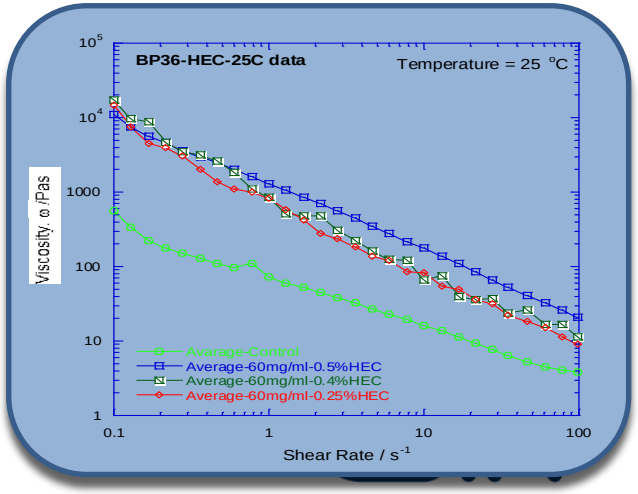


Formulation studies – gels

- Work done by CSIR materials scientists
- Formulation of active into hydroxyethyl cellulose (HEC) gel and maintaining rheological properties
- Investigated rheometry of 3 different concentrations of active, 5, 30, 60mg/mL gel
- 60mg/mL active combined with 0.1% or less HEC - gel much higher viscosity profile
- HEC concentration cannot be dropped further as gel-like properties will not be retained in the vagina without leakage occurring.

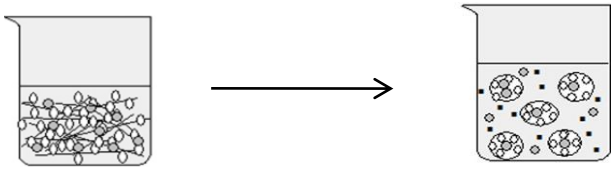


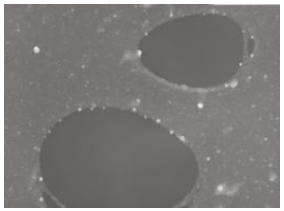


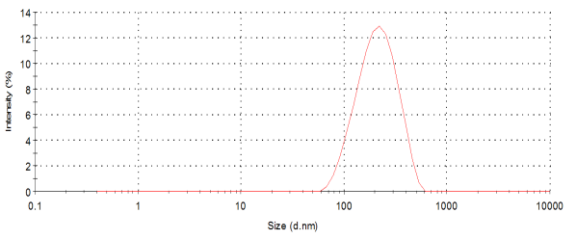


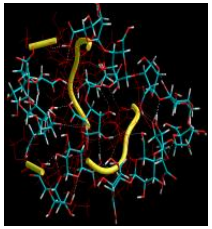
Formulation studies – Caplet

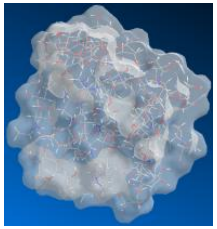
- Work done by Wits University, Drug Delivery Platform
- Intra vaginal composite polymeric drug delivery system
- Target – slow release of actives over 30-60 days through insertion of a caplet in posterior fornix of vagina
- BP36 and AZT microsphere encapsulation prepared with extended release

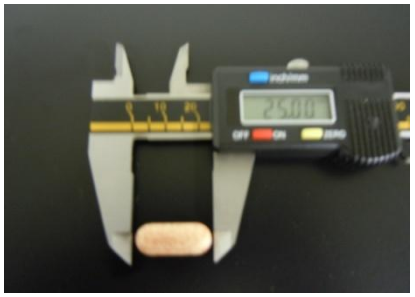


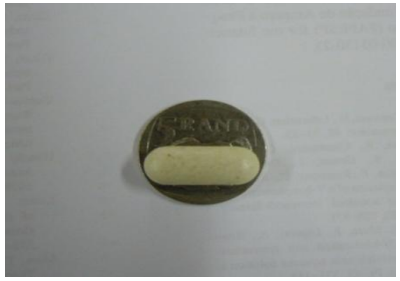












BP36 & AZT loaded caplet

Production of BP36: Cultivation trials

- Work done by CSIR Enterprise Creation for Development
- Obtained regulatory approval for the collection of seeds of the indigenous plant species (BP36)
- Developed methods for germination of the seed of BP36
- Seedlings have been transplanted to establish a mother plantation
  - Ensures commercial scale cultivation can be undertaken without disturbance of wild populations

Way ahead

- Preclinical Screening
  - Efficacy against other viral infections e.g. herpes simplex virus type 2 (HSV-2), human papillomavirus (HPV)
  - Additional Efficacy in PBMC assay
- Toxicity profiles
  - *in vitro*
  - *Lactobacilli*
  - Inflammatory
- Drug release profiles and irritancy
  - pH stability
  - *In vivo* (pig vagina study)

Thank you

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