# Antiviral activity of 5-Hydroxytyrosol, a microbicidal candidate against HIV-1 transmission

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## Introduction

5-Hydroxytyrosol (5-HT. Figure 1) is a natural compound that has previously shown biochemical activity against HIV integrase and gp41 (1,2). In this work we show that 5-HT is able to diminish viral replication without toxic effects *in vitro*.

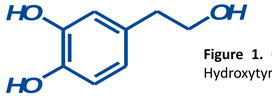


**Figure 1.** Chemical structure of 5-Hydroxytyrosol (5-HT).

## Materials and methods

Recombinant viruses carrying luciferase-renilla reporters (Figure 2) with different properties (Wild type R5 and X4 tropic HIV, VSV pseudotyped HIV or resistant HIV clones) were used to infect a cell line (MT-2) or primary lymphocytes (PBMCs). Different concentrations of 5-HT were used in each assay to determine its potency and toxicity (3).

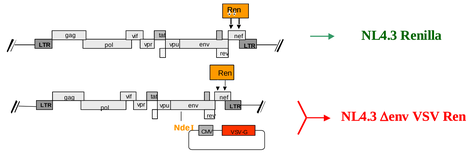



**Figure 2**. Scheme of the recombinant virus NL4.3-Renilla and NL4.3-Δenv-VSV-Ren. NL4.3 backbone (X4 tropic) was used as template and luciferase or renilla genes was inserted instead of *nef* gene. Recombinant HIV-1 with different properties (R5 tropic or VSV pseudotyped) were obtained inserting the R5 *env* gene instead of NL4.3 *env* gene or co-transfecting pNL4.3-delta-env-Luc with VSV g protein.

## Results

### 5-HT IS ACTIVE AGAINST WILD-TYPE HIV, SEVERAL HIV SUBTYPES AND FOUNDER VIRUS *IN VITRO*

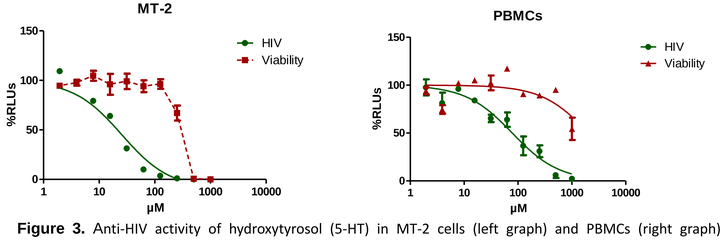



**Figure 3.** Anti-HIV activity of hydroxytyrosol (5-HT) in MT-2 cells (left graph) and PBMCs (right graph) with wild type X4 tropic recombinant virus NL4.3-Renilla, with VSV-pseudotyped HIV NL4.3-VSV-Luc or with R5 tropic recombinant virus JR-Renilla. Cell viability was measured in the same conditions as infections, but without virus infection. $IC_{50}$ MT-2: 35,65 µM; $IC_{50}$ PBMCs: 55,58 µM; $CC_{50}$ MT-2: >250 <1000. $IC_{50}$: Inhibitory Concentration 50; $CC_{50}$: Cytotoxic Concentration 50.

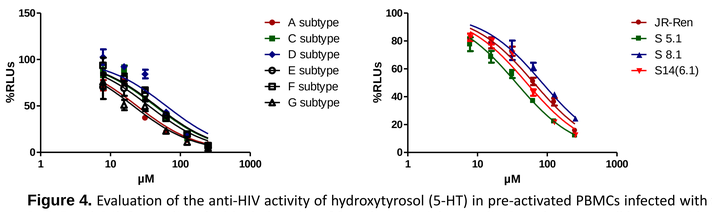



**Figure 4.** Evaluation of the anti-HIV activity of hydroxytyrosol (5-HT) in pre-activated PBMCs infected with viral strains of subtypes A, C, D, E, F and G (left panel) and with viral strains of founder HIV (right panel). $IC_{50}$ values were all between 30 and 60 µM with no significant difference among them. $IC_{50}$: Inhibitory Concentration 50

### 5-HT IS ACTIVE AGAINST HIV TRANSMISSION IN THE IMMUNE SYNAPSE

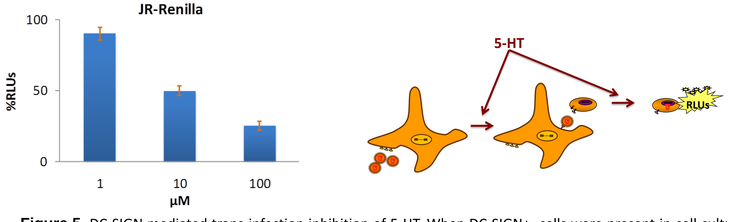



**Figure 5.** DC-SIGN mediated trans-infection inhibition of 5-HT. When DC-SIGN+ cells were present in cell culture, 5-HT anti-HIV potency was >10 fold higher, which would be essential for its microbicide activity, since mucosae infection is highly enhanced by Antigen Presenting Cells (APCs).

### 5-HT IS ACTIVE AGAINST SIV AND SHIV *IN VITRO*

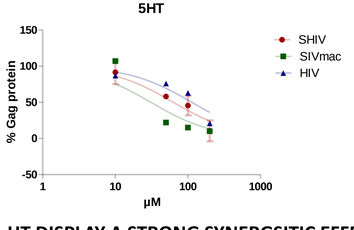
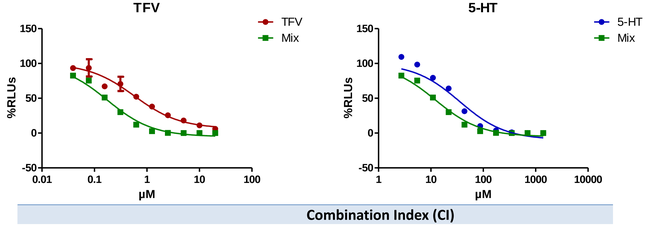



**Figure 6.** Evaluation of SIV, SHIV and wild type HIV inhibition by 5-HT by gag protein quantification. $IC_{50}$ were 9,25 µM for SIVmac and 28 µM for SHIV. $IC_{50}$: Inhibitory Concentration 50.

### 5-HT DISPLAY A STRONG SYNERGSITIC EFFECT WITH TENOFOVIR *IN VITRO*




| Combination Index (CI) | | | |
|---|---|---|---|
| | IC50 | IC75 | IC90 |
| PBMCs IL-2 | 0,307 | 0,252 | 0,390 |
| PBMCs PHA/IL-2 | 0,237 | 0,223 | 0,380 |
| MT-2 | 0,558 | 0,490 | 0,469 |

**Figure 7.** Evaluation of the anti-HIV activity of 5-Hydroxytyrosol (5-HT) combined with TFV (ratio 70/2) in MT-2 cells and PBMCs pre-activated with IL-2 (ratio 1/30) or with PHA/IL-2 (ratio 1/40). Data obtained were analysed using CalcuSyn software. Figure shows MT-2 combinations experiments. $IC_{50}$: Inhibitory Concentration 50; CI: Combination Index, **CI >1.30: antagonism, 1.10–1.30: weak antagonism, 0.90–1.10: additive, 0.70–0.90: weak synergy and <0.70: strong synergy.**

### 5-HT TARGETS VIRAL TRANSCRIPTION AND DO NOT BLOCK VIRAL ENTRY

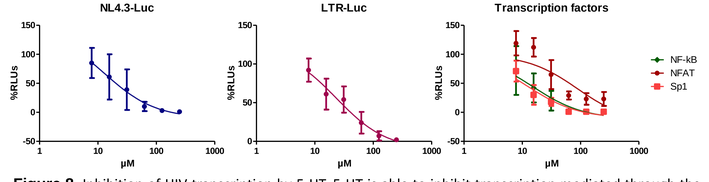



**Figure 8.** Inhibition of HIV transcription by 5-HT. 5-HT is able to inhibit transcription mediated through the whole HIV provirus (NL4.3-Luc) and the HIV promoter (LTR-Luc). This effect is mainly due to NF-kB and Sp1 inhibition.

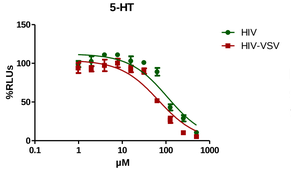



**Figure 9.** 5-HT inhibits HIV and psedotyped HIV replication, suggesting that its effect is viral envelope independent and thus do not interfere with viral entry.

### VAGINAL TOLERANCE OF HYDROXYTYROSOL GEL IN RABBITS

**Figure 10.** Topical administration of 5-HT GEL at three different concentrations (30, 100 and 200 mM) caused no morphological alterations in the rabbit vagina.

## Conclusions

**Although 5-HT potency is not in the highest range, it is active against HIV in a wide range of situations including resistant and founder viruses and is devoid of toxicity at doses 100 times higher than $IC_{50}$. Moreover, 5-HT formulation as vaginal gel is easy and cheap and TFV/5-HT combinations shows a synergic effect in vitro. These characteristics make 5-HT a good microbicide candidate either alone or in combination with other antiretroviral drugs.**

## Acknowledgments and Disclaimer

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Responsibility for the information and views set out in this poster lies entirely with the authors.

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