# Antiviral activity against SARS-CoV-2

## Molecule tested: Raloxifene

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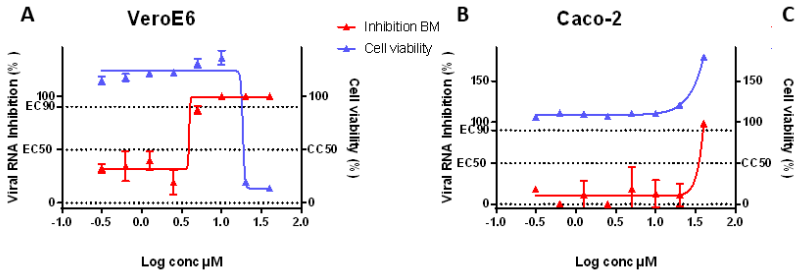
### Introduction

In response to the recent emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, the Unité des Virus Émergents performed preclinical evaluation of drug candidates against SARS-COV-2. **This interim report has not been peer-reviewed.** The data of this report are available upon request.

The molecule tested here is the Raloxifene: a Selective Estrogen Receptor Modulator (SERM) which is used to prevent and treat osteoporosis in postmenopausal women.

### *In vitro* evaluation of efficacy of Raloxifene

Raloxifene antiviral activity was, first, evaluated in vitro from 40 µM to 0.31 µM in Vero-E6 cells using a viral RNA yield reduction assay as previously described (Touret et al., 2019, Touret et al., 2020). Dose-response curves are presented in figure 1A. Raloxifene showed half maximal effective concentration (EC50) and 90% effective concentration (EC90) of 3.85 µM and 4.02 µM respectively, with a 50% cytotoxicity concentration of 18.8 (Figure 1C) resulting in a SI of 4.9. We then tested the raloxifene in Caco-2 cells (Fig 1B). Raloxifene showed EC50 and EC90 of 34.7 µM and 39.2 µM respectively with no cytotoxicity in this cell line. Of note: at 40 µM it seems that raloxifene stimulated the caco-2 cells growth resulting in an enhanced viability (Fig1B). As raloxifene was found to be effective against SARS-CoV-2 its antiviral activity was evaluated *in vivo*.




| µM | VeroE6 | Caco-2 |
|---|---|---|
| EC50 | 3,85 | 34,73 |
| EC90 | 4,02 | 39,25 |
| CC50 | 18,79 | >40 |
| SI | 4,88 | >1,15 |

**Figure 1**: *In vitro* evaluation of Raloxifene. A: Dose response and cytotoxicity evaluation in VeroE6 cells. B: Dose response and cytotoxicity evaluation in Caco-2 cells. C Raloxifene interpolated antiviral activity values. All assay were performed as previously described (Touret et al., 2020).

## *In vivo* evaluation of efficacy of *Raloxifene*

Experiments were approved by the local ethical committee (C2EA—14) and French authorities (APAFIS#23975). Four-week-old female Syrian hamsters were intranasally infected with $10^4$ TCID50 of the BavPat1 SARS-CoV2 strain as described previously (Driouich et al., 2020). To assess the efficacy of Raloxifene, a group of 6 hamsters received the drug, orally, two times a day (BID). We used one dose at 93 mg/kg/day. Treatment was initiated at day of infection, ended at 2 day post infection (dpi) and animals were sacrificed at 3 dpi. A control group of 6 infected/untreated animals received, orally, 0.9% Sodium Chloride solution. Viral replication was measured in lungs (viral RNA yields) and in plasma (viral RNA yields).

Results showed no significant reduction of viral replication in lungs and plasma compared to control group (**Figure 2**).

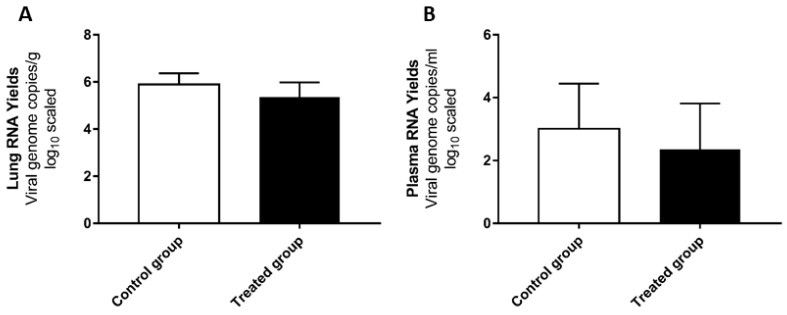



**Figure 2:** Viral replication in lungs (A) and in plasma (B). Lung and plasma viral load were measured using a viral RNA yields RT-qPCR assay as previously described (Driouich et al., 2020).

## Conclusion

Raloxifene was found to inhibit SARS-CoV-2 replication *in vitro* using Vero-E6 and Caco-2 cells. However, results obtained using a hamster model suggest that this molecule is a poor inhibitor of SARS-CoV-2 replication *in vivo.*

## References

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