Article

Atazanavir Is a Competitive Inhibitor of SARS-CoV-2 M^{pro}, Impairing Variants Replication In Vitro and In Vivo

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Abstract: Atazanavir (ATV) has already been considered as a potential repurposing drug to 2019 coronavirus disease (COVID-19); however, there are controversial reports on its mechanism of action and effectiveness as anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Through the pre-clinical chain of experiments: enzymatic, molecular docking, cell-based and in vivo assays, it is demonstrated here that both SARS-CoV-2 B.1 lineage and variant of concern gamma are susceptible to this antiretroviral. Enzymatic assays and molecular docking calculations showed that SARS-CoV-2 main protease (M^{pro}) was inhibited by ATV, with Morrison's inhibitory constant (K_i) 1.5-fold higher than GC376 (a positive control) dependent of the catalytic water (H_2O_{cat}) content. ATV was a competitive inhibitor, increasing the M^{pro}'s Michaelis–Menten (K_m) more than sixfold. Cell-based assays indicated that different lineages of SARS-CoV-2 is susceptible to ATV. Using oral administration of ATV in mice to reach plasmatic exposure similar to humans, transgenic mice expression in human angiotensin converting enzyme 2 (K18-hACE2) were partially protected against lethal challenge with SARS-CoV-2 gamma. Moreover, less cell death and inflammation were observed in the lung from infected and treated mice. Our studies may contribute to a better comprehension of the M^{pro}/ATV interaction, which could pave the way to the development of specific inhibitors of this viral protease.

Keywords: SARS-CoV-2; COVID-19; repurposing drugs; atazanavir; protease inhibitor; pharmacokinetics; molecular docking

SUPPLEMENTARY MATERIAL

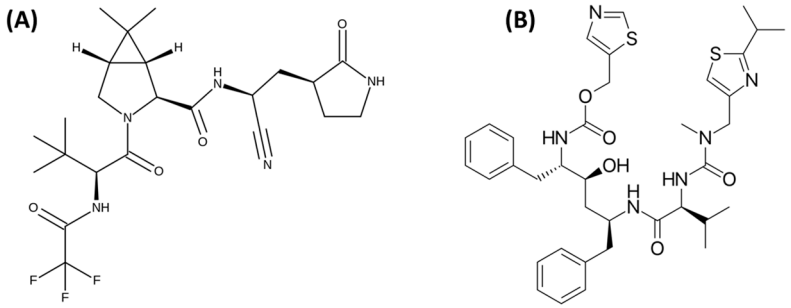

Figure S1. Chemical structure for (A) PF-07321332 and (B) ritonavir, the active principles of PAXLOVID™ from Pfizer.

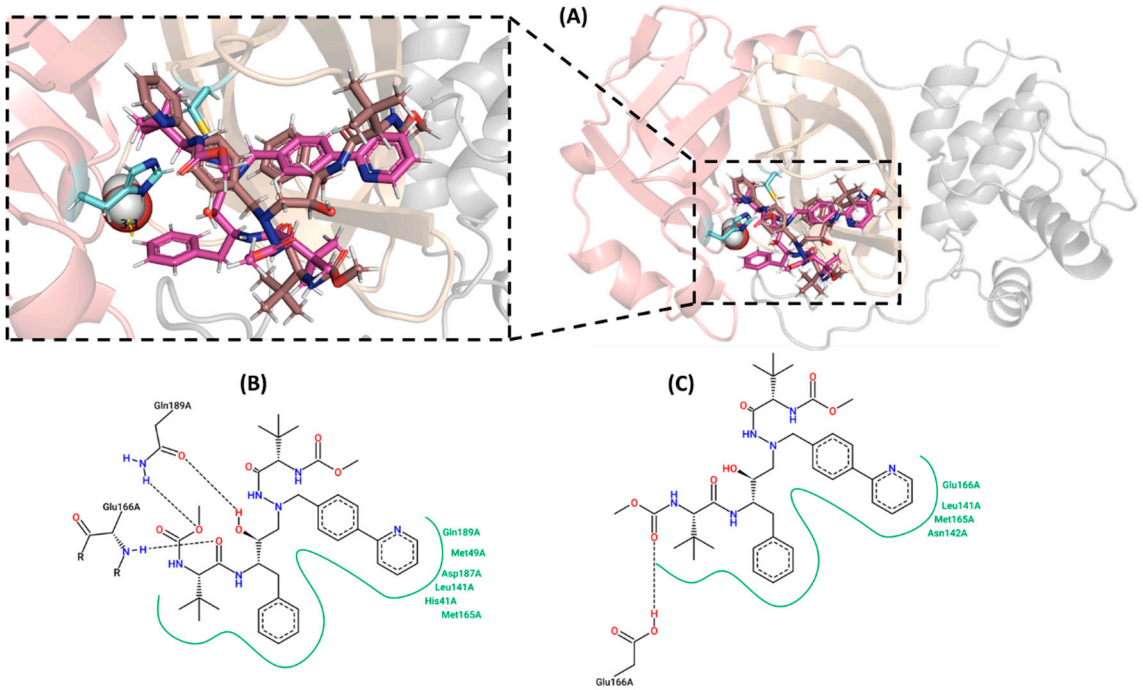

Figure S2. (A) Superposition of the best docking pose for the interaction between M^{pro} and ATV in the presence and without the catalytic water H_2O_{cat} (ATV in pink and brown, respectively). The 2D-plot image for the interaction among the amino acid residues from the catalytic pocket of M^{pro} with (B) ATV in the presence of H_2O_{cat} and (C) ATV without H_2O_{cat}. For better interpretation the M^{pro} structure was represented only in the monomeric form with the domains I, II, and III in light red, orange, and gray, respectively. The catalytic dyad His-41 and Cys-145 are represented as sticks in cyan, while the amino acid residues which interact hydrophobically with ATV are in green in the 2D-plot image.

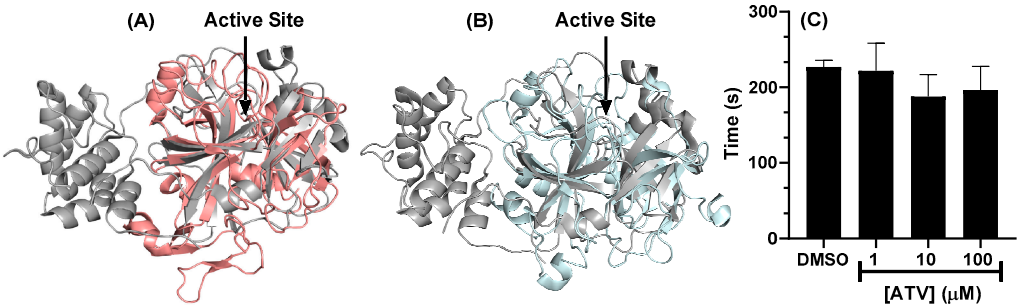

Figure S3. Superposition of the monomeric unit of M^{pro} (in gray, PDB code 7K40) with (A) FXa (in salmon, PDB code 2P16) and (B) thrombin (in cyan, PDB code 1KTS). For better interpretation the catalytic water (H_2O_{cat}) of M^{pro} is not shown. (C) Fibrin formation trial without and in the presence of three concentrations of ATV.

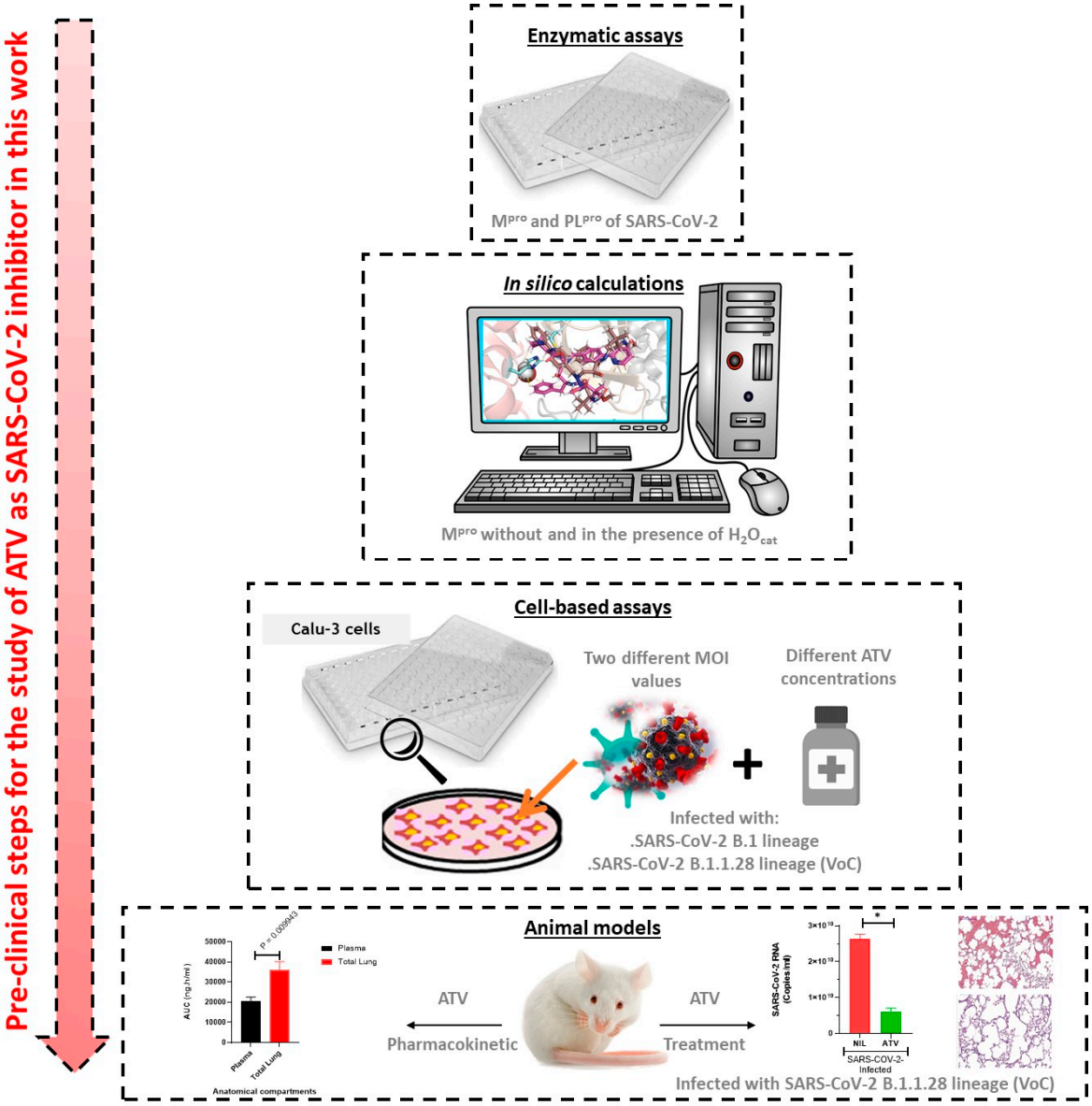

Figure S4. The flow charge indicating the main steps of the present work.