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Manidipine is not a potential inhibitor against SARS-CoV-2 main protease

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The rapid spread of the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection highlights an urgent need for developing antivirals against coronavirus. An attractive drug target for COVID-19 is the main protease (Mpro), as this conserved enzyme plays a key role in virus replication and host immune evasion (1, 2). Given the urgency of identifying antivirals, repurposing approved drugs as effective Mpro inhibitors represent a promising strategy for COVID-19 treatment (3, 4). Recently, manidipine was identified as a potent Mpro inhibitor with the half-maximal inhibitory concentration (IC_{50}) of 10.4 ± 1.6 µM using fluorescence resonance energy transfer (FRET) assay (5). Nonetheless, a recent study suggested that manidipine is likely to be a promiscuous Mpro inhibitor because it is prone to colloidal aggregation in the enzymatic assay, resulting in the inactivation of Mpro enzyme (6). Considering the potential of manidipine in COVID-19 treatment, a rigorous validation for its Mpro inhibition is necessary.

For this purpose, we evaluated the *in vitro* inhibition of Mpro by manidipine using a robust high-throughput screening (HTS) platform, including FRET, fluorescence polarization (FP), and dimerization-dependent red fluorescent protein (ddRFP) assays (7–9). When we repeated the FRET assay developed by the authors (5), the IC_{50} value of manidipine was 24.14 ± 3.41 µM (Fig. 1A and B). In clear contrast, manidipine showed no inhibition in the FRET assay when Tween-20 was present in the reaction buffer (IC_{50} > 200 µM), suggesting that its Mpro inhibition is false positive (Fig. 1C). To further test this possibility, we separately evaluated Mpro inhibition by manidipine using FP and ddRFP assays in the reaction buffer containing 0.05% Tween-20. As expected, manidipine did not inhibit Mpro in both assays (IC_{50} > 200 µM) (Fig. 1D and E). Consistently, manidipine exhibited no detectable inhibition of Mpro using SDS-PAGE because the ddRFP biosensor could be cleaved into RFP-A_1 and RFP-B_1 fragments in the presence of manidipine (Fig. 1F). Thus, our results invalidated manidipine as a potential Mpro inhibitor *in vitro*. Intriguingly, a recent study independently confirmed our results using cell-based assays (10). In the presence of detergent, the aggregation caused by compounds is minimized in the enzymatic assay (11). Therefore, our results demonstrated the necessity for the addition of detergent such as Tween-20 or Triton X-100 into the assay buffer when assessing Mpro inhibitors, which could disrupt colloids and keep Mpro enzyme stable. Moreover, manidipine might have other targets to achieve the anti-SARS-CoV-2 effect, which can be further studied.

In summary, our data indicate that manidipine is not a potential inhibitor against Mpro based on the results from a set of *in vitro* assays. These results suggest that the colloidal aggregation caused by compounds should be considered when evaluating Mpro inhibitors. It is necessary to verify the inhibitory activity of candidate Mpro inhibitors using diverse biochemical assays due to the limitations of the FRET approach.

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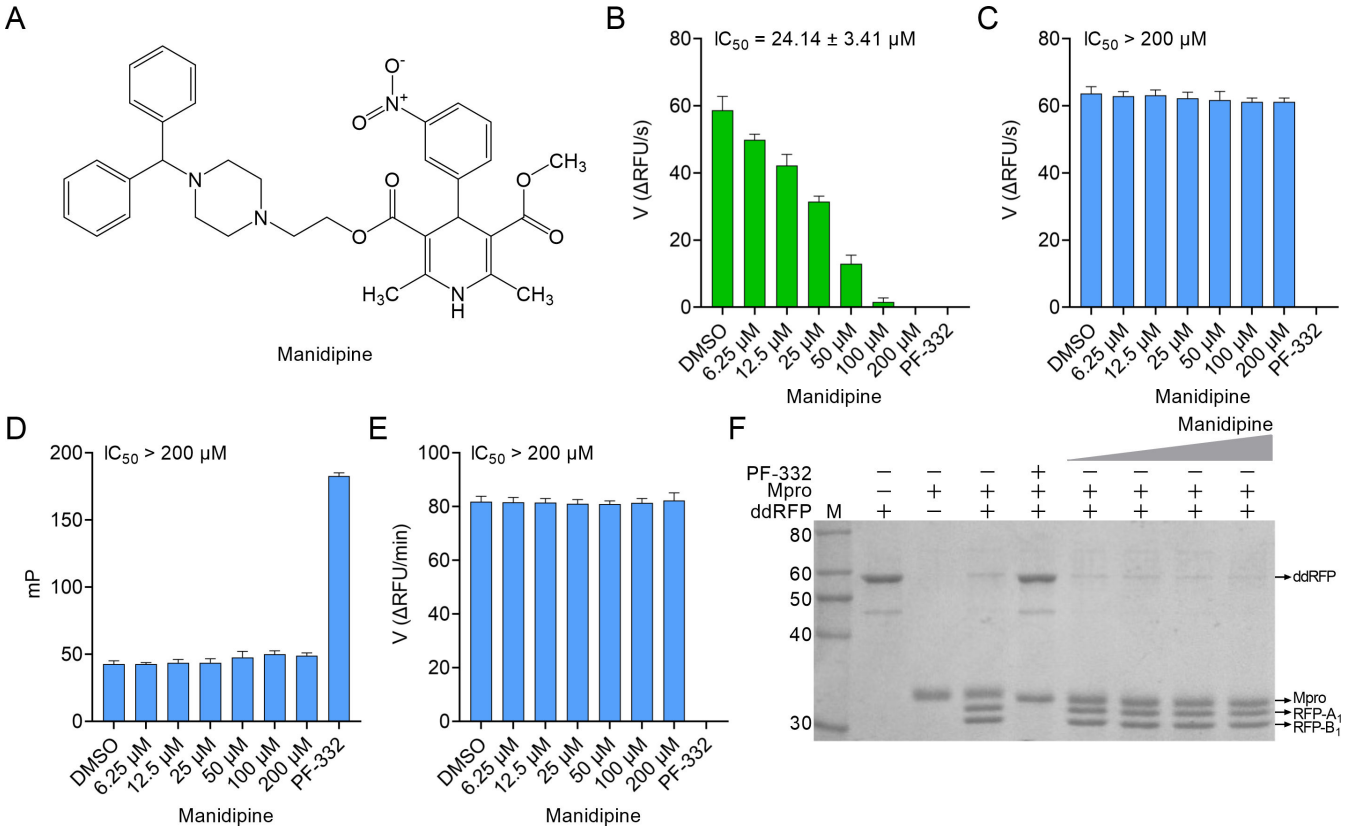

FIG 1 Inhibition of SARS-CoV-2 main protease (Mpro) by manidipine *in vitro*. (A) The chemical structure of manidipine. (B) Evaluation of the activity of manidipine against Mpro using FRET assay developed by authors (5). (C and D) Evaluation of the activity of manidipine against Mpro using FRET and FP assays in the reaction buffer containing 0.05% Tween-20. In these biochemical assays, nirmatrelvir (PF-332, 1 μM) and DMSO served as the positive and negative controls, respectively. The IC_{50} value of manidipine was shown. (E) Inhibition of Mpro by manidipine through monitoring the initial velocity of Mpro enzyme reaction initiated by ddRFP biosensor. The IC_{50} value of manidipine was shown. (F) Gel-based assay of ddRFP biosensor cleavage by manidipine *in vitro*. In the absence of Mpro inhibitors, the ddRFP biosensor (55 kDa) can be cleaved by Mpro (34 kDa) to generate RFP-A_1 (top band, 29 kDa) and RFP-B_1 fragments (bottom band, 26 kDa) in the gels. Nirmatrelvir (PF-332) and manidipine were purchased from TargetMol (Shanghai, China). The concentrations of manidipine used in this experiment were 25, 50, 100, or 200 μM. In the ddRFP assay, nirmatrelvir (PF-332, 10 μM) and DMSO served as the positive and negative controls, respectively. All assays were conducted as previously described (7–9, 12). The reaction buffer was 10 mM HEPES, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.05% Tween-20 (vol/vol), pH7.0.

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