CONCEPTION OF COVALENTLY REVERSIBLE semi-PEPTIDIC INHIBITORS OF TMPRSS2 FOR SARS-COV-2 TREATMENT

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COVID-19, an infectious respiratory disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has led to a global pandemic with profound public health implications and significant economic impacts worldwide. The Type-II transmembrane serine protease (TMPRSS2) has been identified as the proteolytic driver of SARS-CoV-2 activation and replication, making the dysregulation of TMPRSS2 activity a highly effective host-directed therapeutic strategy.

The previously reported TMPRSS2 inhibitor **N-0385** exhibits unfavorable pharmacokinetic properties, including excessively high bioavailability (99%). To address the issue of significant systemic exposure, we designed a small library of peptidomimetic compounds with P3 site modifications by substituting proteinogenic amino acids and further evaluated their potency, *in vitro* efficacy, and pharmacokinetic profiles.

Exceptionally, compound **9**, with Asp at the P3 position, resulted in a 2-fold increase in TMPRSS2 sub-nanomolar inhibitory potency (Ki of 13 ± 0.03 nM), while achieving >700-fold selectivity over Factor Xa and a superior selectivity profile against other proteases (matriptase, TMPRSS6, thrombin, and furin). An *in vitro* air-liquid interface (ALI) model of pulmonary epithelium revealed that compound **9** demonstrated a 1.5-fold decrease in permeability compared to **N-0385**, with sustained lung (11 h) and plasma (13 h) stability, suggesting its potential for daily prophylactic or therapeutic intranasal administration.