

Non-covalent Inflammasome-Caspase-1 Complex Inhibitors: New Molecules Targeting Immune and Inflammatory Disorders Including Covid-19

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INTRODUCTION

The release in the cytosol of potent pro-inflammatory mediators interleukin-1 β and interleukin 18, triggered by activation of inflammasome-caspase 1 complex in response to danger signals, culminates in beneficial immune responses.^{1,2} However, there is a growing evidence of the relation between innate immunity, excessive release of pro-inflammatory IL-1 β and various immune and inflammatory disorders, including CNS diseases such as Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) diseases, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS).³ Moreover there is also a growing evidence⁴ that the pathway inflammasome NLRP3/Caspase-1 is overactivated by the SARS-Cov-2 and may be responsible for the high mortality observed in the COVID-19 patients due to the inflammatory internal organs collapse driven by the cytokine storm induced by the virus. It is now quite clear that an effective pharmacological approach to Covid-19 must comprise an antiviral drug in combination with an inflammation modulator able to regulate the innate immunity system response preserving its regular activity but quenching its overactivation. For all of these reasons a new caspase-1 inhibitor drug could be a useful tool to treat inflammation driven diseases including Covid-19.

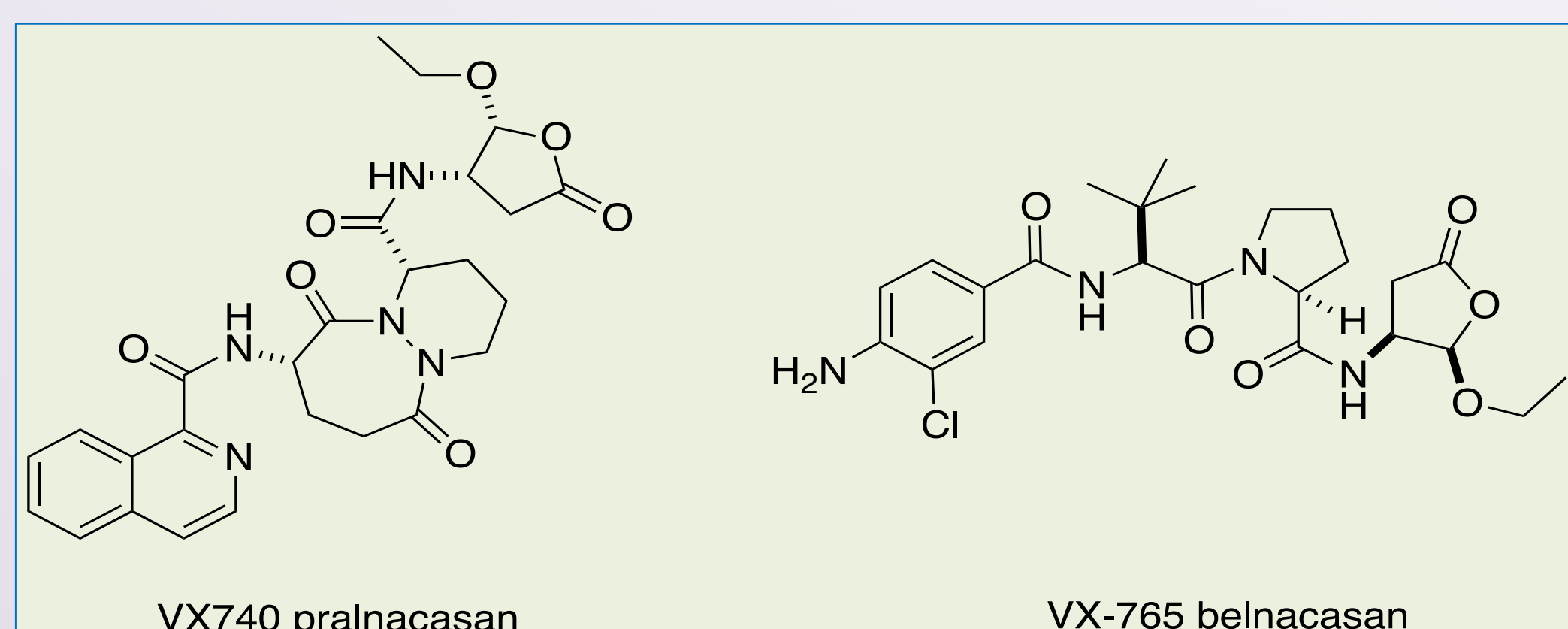


Figure 1. Caspase 1 inhibitors progressed in clinical trials

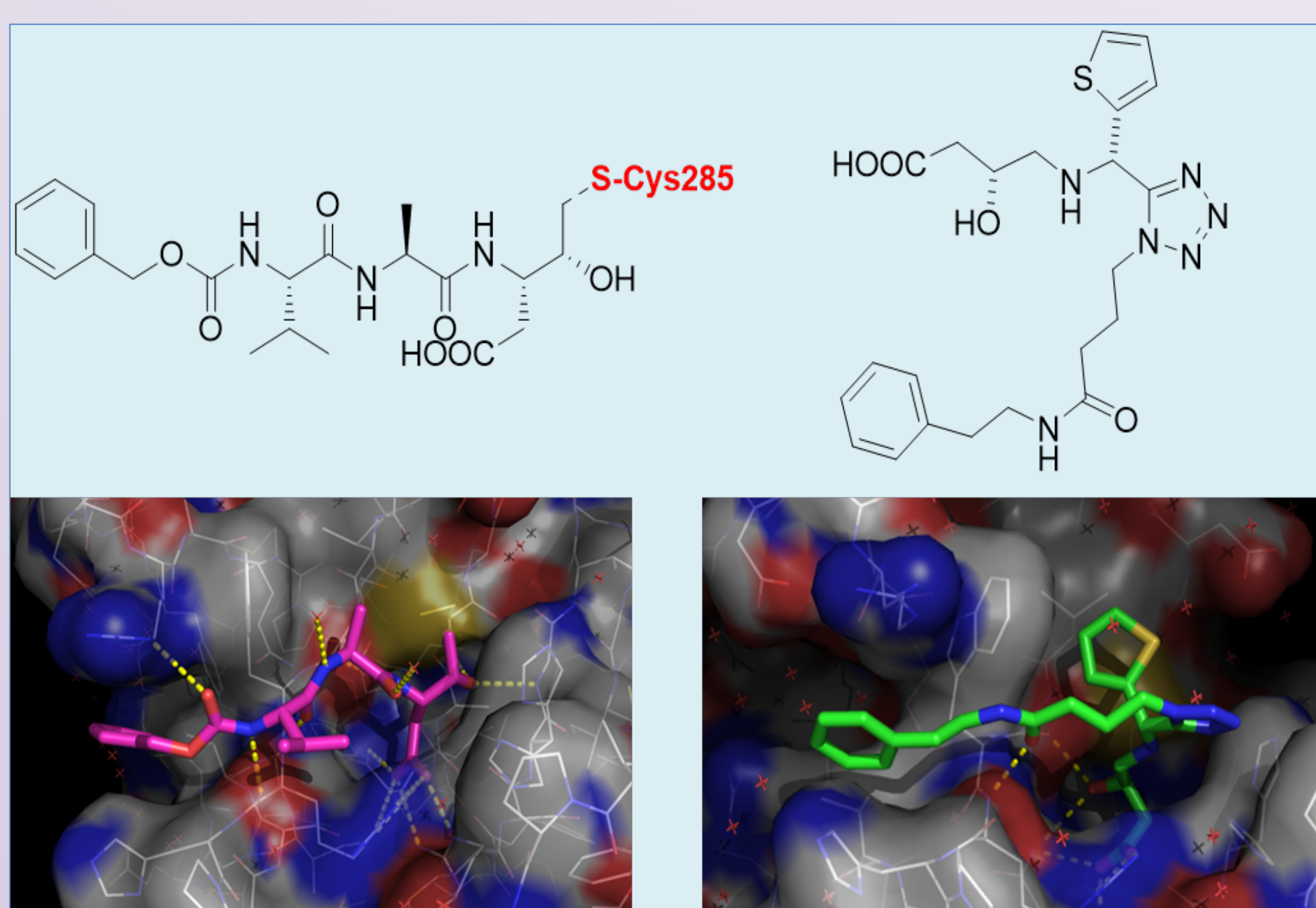


Figure 3

Compounds inhibiting the caspase-1 activity with covalent mode of action (MoA) have been proposed as promising new therapeutics by modulating immune and inflammatory response, but until now there are no caspase-1 inhibitor drugs approved for clinical use on the market (Figure 1). Novel, improved, compounds characterized by a different MoA are needed. We have designed a new class of non-covalent, non-peptidic, small molecule caspase-1 inhibitors by structure-based drug design in order to obtain a new class of stable and bioavailable inhibitors.

DESIGN

Our design of non-covalent caspase-1 inhibitors is based on a substrate mimicry approach (Figure 2). The WEAD (trp-glu-ala-asp) sequence is generally recognized by caspase-1 and target proteins are always aspartyl-cleaved (P1 = Asp). Thus, we aimed to design non cleavable aspartyl mimicking scaffolds which would allow for easy modification in further positions to address P2-P4 sites. Multicomponent reaction (MCR) chemistry was used to address a large drug-like scaffold space and in particular the Ugi tetrazole variation (UT-4CR). Our design includes a 4-amino-3-hydroxy butanoic acid, as amine component, mimicking the aspartyl P1 side chain with significative interaction to protein bonding sites. (Figure 3)

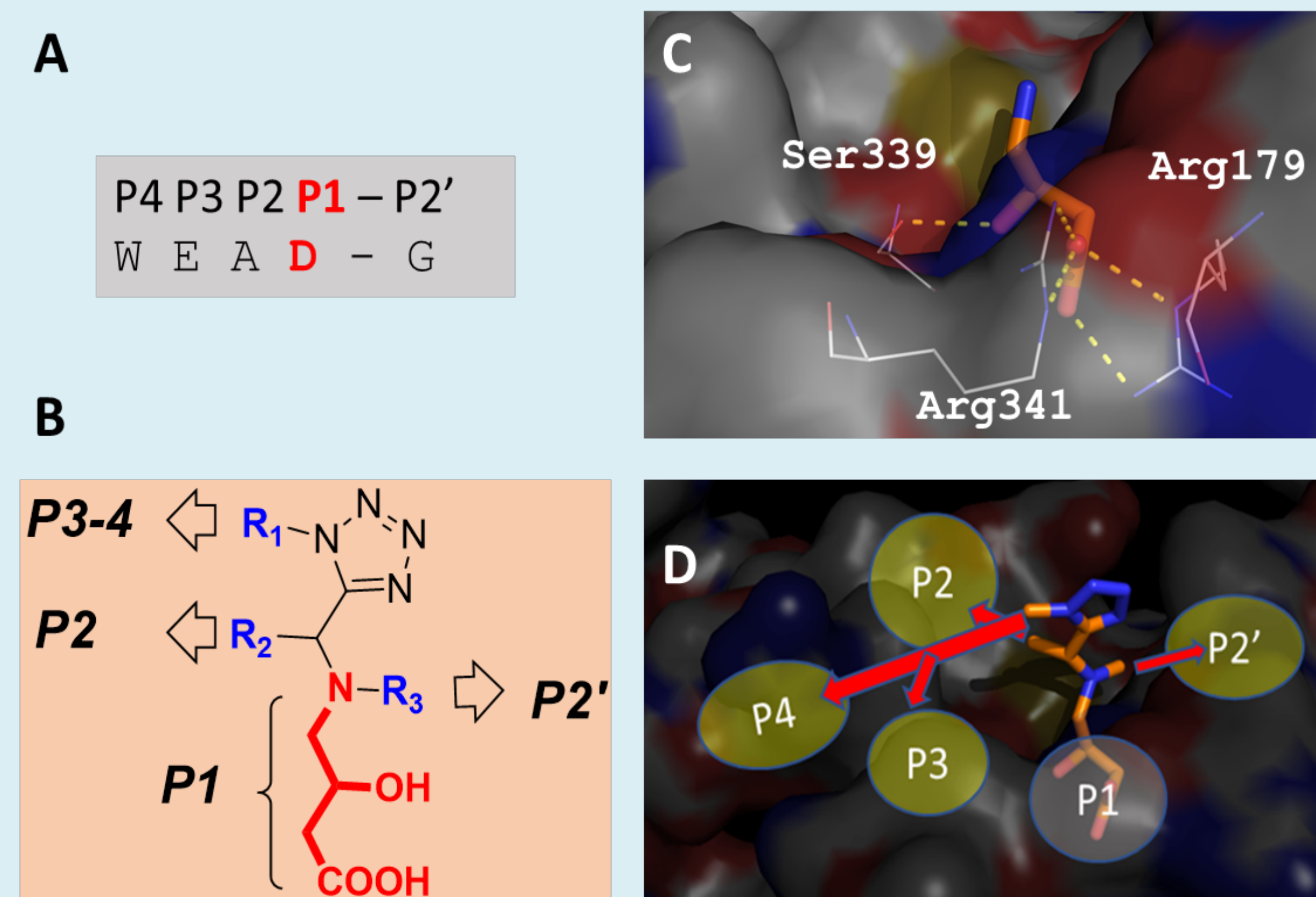
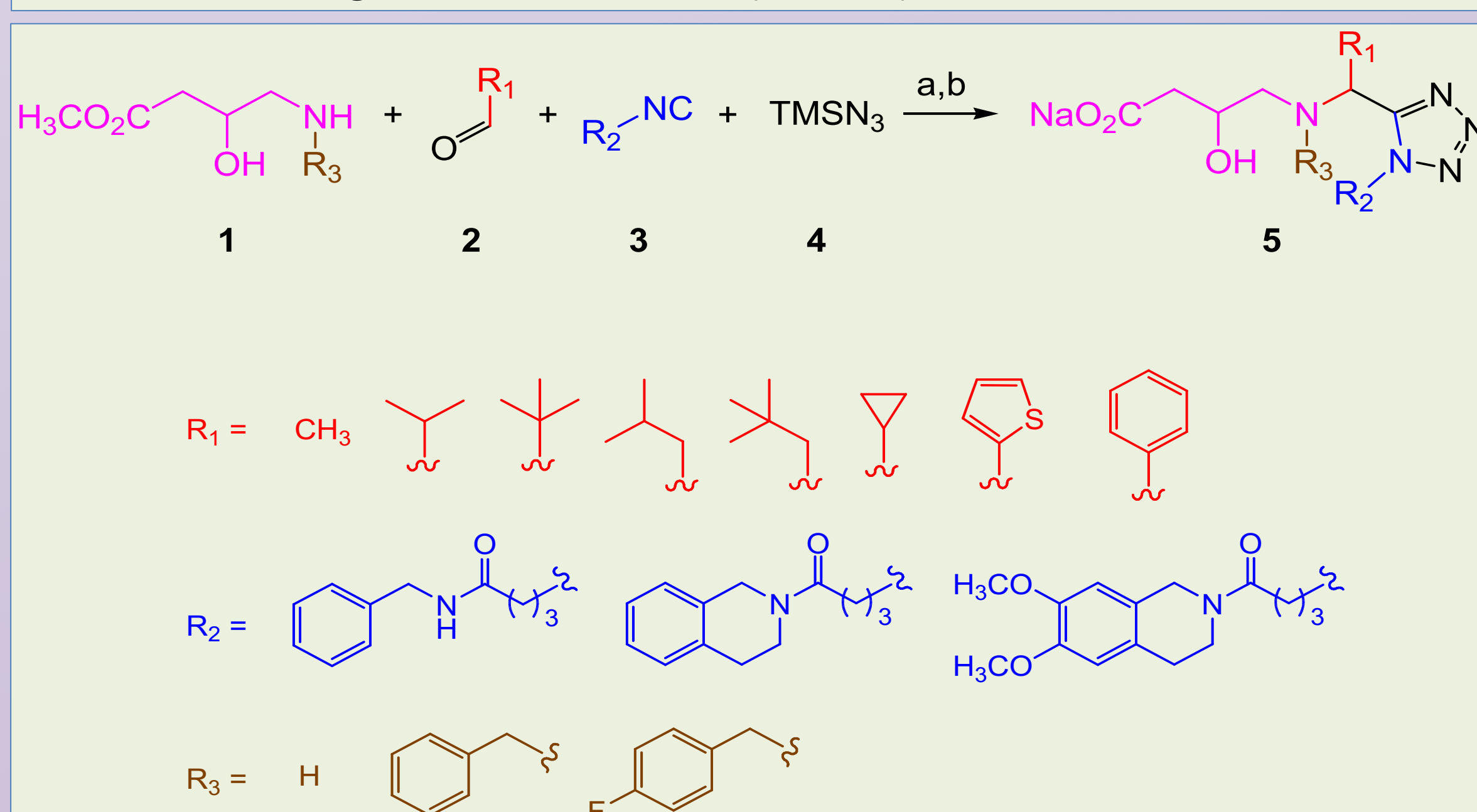


Figure 2. Caspase-1 inhibitor design.

SYNTHESIS OF TARGET COMPOUNDS 5

The multicomponent reaction (MCR) approach was used for the synthesis of desired compounds **5**, because it offers a significant advantage over conventional linear step synthesis permitting the one pot assembly of very complex structures (Scheme 1). In order to meet the requirements established by the computational approach four component (**1-4**) were needed for the Ugi tetrazole variation (UT-4CR).



Scheme 1. Reagents and conditions: (a) CH₃OH, Na₂SO₄, Et₃N, rt; (b) NaOH, CH₃OH

IC₅₀ ENZYMATIC TEST RESULTS

R	>100 μ M	>100 μ M		>100 μ M	>100 μ M	>100 μ M	>100 μ M
S						78 μ M	>100 μ M
R	>100 μ M	>100 μ M	>100 μ M	79 μ M	15 μ M	>100 μ M	>100 μ M
S					12 μ M	33 μ M	53 μ M
					51 μ M	68 μ M	
R							
S							
R		>100 μ M	>100 μ M	15 μ M	>100 μ M	>100 μ M	
S							

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