CM554, A NEW POTENT AND SELECTIVE INOS INHIBITOR: SYNTHESIS, DOCKING STUDY, AND EX VIVO BIOLOGICAL EVALUATION.

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Endogenous nitric oxide (NO) is produced by NO synthases (NOS) by the conversion of L-arginine to L-citrulline. In the vascular system, the inducible NOS (iNOS) isoform generates high amounts of NO and other reactive oxygen species associated with decreased function of eNOS and subsequent impairment of both vasoconstriction and endothelium-dependent vasorelaxation. Several iNOS inhibitors have been published to date, most of them targeting the arginine binding site of the enzyme oxygenase domain, which is highly conserved among the NOSs isoforms. This makes the design of selective iNOS inhibitors quite challenging, being an excellent isoform selectivity, particularly over eNOS, an essential requisite for a given iNOS inhibitor (Macallini, 2015).

Here we report the synthesis of a new series of compounds structurally related to the leading scaffold of N-[(3-aminomethyl)benzyl] acetamidine (1400W), along with the results of a docking study and biological activity performed on the most promising compound of the series.

The substitution of the 1400W aminomethyl tail with the L-Ala, L-Ile and L-Leu residues gave potent iNOS inhibitors (IC50 =0.276 μM, 0.170 μM, and 0.253 μM, respectively) but substantially similar to the reference compound 1400. A significant decrease of the overall biological activity (vs. 1400W) was observed for other 3 molecules of this series. Other 10 compounds resulted quite inactive against iNOS.

Promising results were obtained from compound CM554 bearing the L-Pro residue: it gave a potent iNOS inhibition (IC50: 0.058 μM), and a selectivity of 4569 folds over eNOS significantly higher than 1400W ($p<0.01$). Moreover, CM554 did not inhibit nNOS (IC50>10 μM), demonstrating a i/n isoform selectivity higher than 1400W (172 folds vs. 77 folds). Docking results show for CM554 the same interactions observed for 1400W, i.e. between the acetamidine moiety and a GLU and a TRP residues of the iNOS binding site, and between the ethylamino group and one of the heme propionate arm, plus a further hydrogen bond between the proline amino group and the remaining propionate arm.

Chemical and enzymatic stability of CM554 were evaluated in phosphate buffer (pH = 7.4), HCl solution (pH = 2.0), NaOH solution (pH = 9.0), and human plasma. Solutions were kept at 37 °C, and monitored for 24 h. Compound CM554 half-life in plasma was 11 hours, and no loss of product was observed in each considered aqueous medium after 24 hours.

The iNOS selective inhibitory ability of CM554 was therefore evaluated in vessels isolated from rats treated in vivo with bacterial lipopolysaccharide (E. coli LPS, 30 mg kg-1, i.p.). The LPS-induced

hyporeactivity to noradrenaline (NA) largely depends on dysregulated production of NO generated by iNOS. In mesenteric vessels from LPS-treated rats, dose-dependent increase in perfusion pressure by NA (10 nM – 10 μM) under basal conditions (CTRL) were repeated under incubation with CM554 (0.06 μM/ 30 min), and subsequently with Nω-Nitro-L-arginine methyl ester (L-NAME, 100 μM/30 min), a non selective NOS inhibitor. With respect to NA CTRL curve, the maximal NA vasoconstriction (peak effect) was significantly increased under CM554 incubation ($p<0.05$ vs. each individual NA dose). As expected, and consistent with L-NAME ability to inhibit NO biosynthesis from all NOS isoforms, NA-mediated vasoconstriction was further enhanced under L-NAME incubation ($p<0.001$ vs. CTRL; $p<0.01$ vs. CM554). Similarly, the total extent of vasoconstriction induced by NA (AUC) in CTRL was increased in the presence of CM554 and L-NAME ($p<0.01$ vs. CTRL; $p<0.05$ vs. CM554). Altogether, results from docking study and ex vivo biological evaluation support the selective inhibitory activity of CM554 on iNOS.

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