New non-sulfonylurea NLRP3 inhibitors: discovery and selection of INF200, a 1,3,4-oxadiazol-2-one-based NLRP3 inhibitor

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The cytosolic multiprotein complex NLRP3, nucleotide-binding oligomerization domain leucine rich repeat and pyrin domain containing protein 3 inflammasome, plays an important role in the initiation and maintenance of the inflammation. Once activated and assembled, the NLRP3 inflammasome triggers the auto-proteolytic cleavage of pro-caspase-1 into the active caspase-1, converting the pro-inflammatory cytokines pro-interleukin (IL)-1β and pro-IL-18 into their active forms and causing pyroptotic cell death. An aberrant activation of NLRP3 inflammasome has been detected in chronic inflammatory diseases such as neurodegenerative, autoimmune, and cardiovascular diseases. The inhibition of the NLRP3 inflammasome activation represents an interesting new approach for the development of a new class of anti-inflammatory drugs. To date, the most studied NLRP3 inhibitor is MCC950, a disubstituted sulfonylurea derivative (figure 1). However, its *in vivo* use in humans has been limited due to hepatic toxicity.[1,2] Recent research has highlighted the key interactions of sulfonylurea inhibitors with the NACHT domain of the NLRP3 protein.[3] Inspired by this discovery, and through the use of computational techniques, we designed and synthesized a new series of compounds by replacing the sulfonylurea moiety with different heterocycles. The compounds were evaluated for *in vitro* ability to reduce IL-1β release and prevent NLRP3-dependent pyroptosis in human macrophages. The 1,3,4-oxadiazol-2-one derivative, **INF200**, showed the most promising results being able to prevent NLRP3-dependent pyroptosis triggered by LPS/ATP and LPS/MSU by 66.3 ± 6.6% and 61.6 ± 11.5% at 10 μM and to reduce IL-1β release with an IC_{50} of 16.6 ± 2.6 μM. **INF200** was tested in an *in vivo* model of high-fat-diet (HFD)-induced metaflammation in rats to evaluate its cardiometabolic effects. **INF200** proved able to reverse the unfavorable cardiometabolic dysfunction associated with obesity and to reduce systemic inflammation and anthropometric changes in HFD rats.[4]

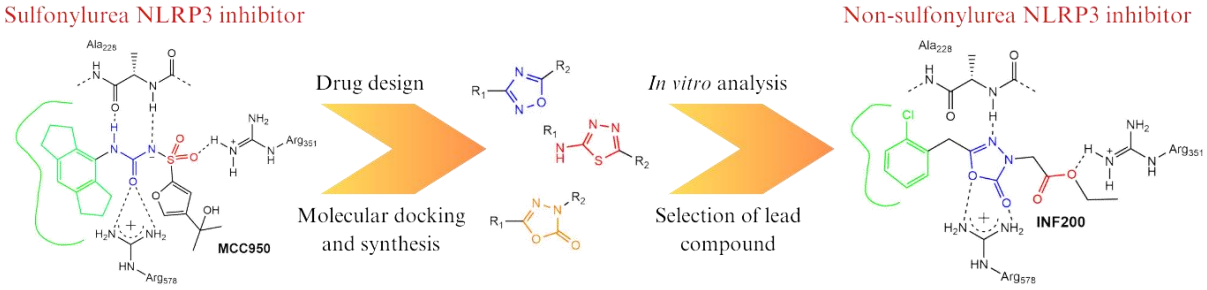

***Figure 1**. Structures of the investigated scaffolds and of the selected new NLRP3 inhibitor, **INF200**.*

[1] Swanson, K.V. et al. *Nat Rev Immunol*. 2019, **19**, 477–489.
[2] Blevins, H.M. et al. *Front Aging Neurosci*. 2022, **14**, 879021.
[3] Dekker, C. et al. *J. Mol. Biol*. 2021, **433**, 167309.
[4] Gastaldi, S. et al. *Eur. J. Med. Chem*. 2023, **257**, 115542.

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