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Identification, Synthesis, and Biological Evaluation of the Major Human Metabolite of NLRP3 Inflammasome Inhibitor MCC950

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Abstract : MCC950 is a potent and selective inhibitor of the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome that shows early promise for treatment of inflammatory diseases. The identification of major metabolites of lead molecule is an important step during drug development process. It provides an information about the metabolically labile sites in the molecule and thereby helping medicinal chemists to design metabolically stable molecules. To identify major metabolites of MCC950, the compound was incubated with human liver microsomes and subsequent analysis by (+)- and (−)-QTOF-ESI-MS/MS revealed a major metabolite formed due to hydroxylation on 1,2,3,5,6,7-hexahydro-s-indacene moiety of MCC950. This major metabolite can lose two water molecules and three possible regioisomers were synthesized. Co-elution of major metabolite with each of the synthesized compounds using HPLC-ESI-SRM-MS/MS revealed the structure of the metabolite (±) N-((1-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide. Subsequent synthesis of individual enantiomers and coelution in HPLC-ESI-SRM-MS/MS using a chiral column revealed the metabolite was R-(+)- N-((1-hydroxy-1,2,3,5,6,7-hexahydro-s-indacen-4-yl)carbamoyl)-4-(2-hydroxypropan-2-yl)furan-2-sulfonamide. To study the possible cytochrome P450 enzyme(s) responsible for the formation of major metabolite, MCC950 was incubated with a panel of cytochrome P450 enzymes. The result indicated that CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C18, CYP2C19, CYP2J2 and CYP3A4 are most likely responsible for the formation of the major metabolite. The biological activity of the major metabolite and the other synthesized regioisomers was also investigated by screening for for NLRP3 inflammasome inhibitory activity and cytotoxicity. The major metabolite had 170-fold less inhibitory activity (IC50-1238 nM) than MCC950 (IC50-7.5 nM). Interestingly, one regioisomer had shown nanomolar inhibitory activity (IC50-232 nM). However, no evidence of cytotoxicity was observed with any of these synthesized compounds when tested in human embryonic kidney 293 cells (HEK293) and human liver hepatocellular carcinoma G2 cells (HepG2). These key findings give an insight into the SAR of the hexahydroindacene moiety of MCC950 and reveal a metabolic soft spot which could be blocked by chemical modification.

Keywords : Cytochrome P450, inflammasome, MCC950, metabolite, microsome, NLRP3
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