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Design, Synthesis and Evaluation of an NLRP3 Inhibitor Diazirine Photoaffinity Probe

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The NLRP3 inhibitor MCC950/CRID3 ameliorates a remarkable number of inflammatory disorders in animal models. Herein we describe a trifluoromethyl phenyl diazirine (TPD) photoaffinity probe, called TPD-950-Br, to probe the molecular interactions of MCC950. We show that TPD-950-Br covalently captures proximal species upon photo-activation and inhibits IL-1β production in an NLRP3 inhibitor assay.

A fundamental aspect of drug discovery is the use of chemical probes to interrogate biological systems.1 Photoaffinity probes are masked reactive compounds that can preferentially interact with a molecular target. Ultraviolet (UV) photo-activation unveils a reactive intermediate that irreversibly binds proximal species.2 This is useful for probing transient molecular interactions, for example, target identification studies.3 This requires incorporation of a photo-cross-linking unit, such as a benzophenone, diazirine or aryl azide.

The molecular target and mechanism of action of MCC950 (also called CP-456,773 and CRID3) has been in question since the compound was reported in 2001, as a potent inhibitor of interleukin (IL)-1β activity.4 The molecular target of MCC950 was originally thought to be glutathione transferase omega 1; although, this was subsequently disproven.5,6 In 2015, MCC950 was demonstrated to selectively inhibit IL-1β production derived from NLRP3 (NOD [nucleotide oligomerisation domain], LRR [leucine-rich repeat] and pyrin domain-containing protein 3), with a half-maximal inhibitory concentration (IC50) of 8 nM.7 MCC950 was suggested to target NLRP3 or a closely associated protein.7 MCC950 then became a widely used tool compound for determining the role of NLRP3 in various inflammatory diseases and fuelled a flurry of interest in NLRP3 inhibitor development.8,9 However, at this stage the precise mechanism of action was unknown and there was no evidence of a physical interaction between MCC950 and NLRP3. In 2019, the understanding of MCC950’s molecular target and mechanism of action was further elucidated. Using photoaffinity labelling, a drug affinity responsive target stability assay and surface plasmon resonance, we demonstrated that MCC950 directly and reversibly interacts with the ATP-hydrolysis motif of NLRP3.10 Additionally, Tapia-Abellán and co-workers reported that MCC950 binding closes the ‘open’ conformation of NLRP3.11 Later work by Vande Walle and co-workers similarly used photoaffinity labelling to conclude that MCC950 interferes with NLRP3-ATP binding.12

The two MCC950 photoaffinity probes reported to date, BP-950 and PAL-CRID3, both utilise the benzophenone photo-cross-linking unit (Fig. 1).10,12 Benzophenones are the largest of the popular photo-cross-linkers. This size affects the NLRP3-inhibitor potency of benzophenone-MCC950 analogues, which are of approximately micromolar potency, compared to the 8 nM potency of the parent compound.10,12 Diazirines on the other hand, have a small steric footprint, allowing them to be nested within a compounds’ biologically active motif with less perturbation.13,14 Moreover, using various photoaffinity probes reduces the chance of target misidentification, improves the likelihood that all potential interactions are probed and provides insights into the overall structure of the inhibitor binding pocket.2,15 Herein we describe the design, synthesis and evaluation of an NLRP3-inhibitory photoaffinity probe, whereby the trifluoromethyl phenyl diazirine (TPD) photo-cross-linker is nested within the biologically active motif.

Figure 1. Benzophenone-MCC950 photoaffinity probes.
The design of a MCC950 photoaffinity probe was guided by prior studies of NLRP3 sulfonylurea inhibitors (Fig. 2). These studies indicated that: i) the sulfonylurea and (tricyclic) s-hexahydroindacene moiety are largely indispensable for nanomolar NLRP3 inhibitory activity; ii) halogens are tolerated at the 4-position of the s-hexahydroindacene; iii) the furan can be replaced with numerous aromatic groups; and iv) this aromatic group tolerates a broad range of substituents.

Figure 2. Design of TPD950-Br, an NLRP3-inhibitory photoaffinity probe.

Considering these structure activity relationships (SAR), TPD-950-Br was designed by incorporating two new features (Fig. 2). The furan of MCC950 was replaced with a trifluoromethyl phenyl diazirine (TPD) moiety and an aromatic proton was replaced by bromine. These changes were envisaged to cause minimal perturbation to NLRP3-inhibitory activity. A TPD was selected over other photo-cross-linking units due to its small topological footprint, aromatic proton was replaced by bromine. These changes were envisaged to cause minimal perturbation to NLRP3-inhibitory activity. However, when photo-activated, the resulting reactive carbene was intended to cross-link irreversibly to spatially proximal species.

TPD-950-Br, like many TPD-containing photoaffinity probes, required a lengthy synthesis. This synthesis has four phases (Scheme 1): i) substituting the trifluoromethyl acetophenone starting material with a synthetic handle, ii) installing the TPD moiety, and iii) functionalising the TPD with a sulfonamide, and iv) incorporating the TPD into the final bioactive compound. The instability of diazirine and sulfonylurea functional groups limited the feasible reaction conditions.

To provide a synthetic handle for subsequent modifications and to create the desired substitution pattern, commercially available trifluoromethyl acetophenone (1) was nitrated, using nitric and sulfuric acid, to afford meta-nitro trifluoromethyl acetophenone 2 (Scheme 2, A). The nitro group would eventually be reduced to the aniline.

Compound 2 then underwent a four step reaction sequence, common in TPD synthesis (Scheme 2, B-E). Treating 2 with hydroxylamine hydrochloride and pyridine, converted the carbonyl to the E- and Z-oxime isomers (3). We initially performed column chromatography after this reaction; however, later replicates found that the crude oxime could be used without purification. Oxime 3 was then treated with tosyl chloride, 4-dimethylaminopyridine (DMAP) and triethylamine, affording N-tosylate 4. Reaction with liquid ammonia caused nucleophilic attack and intramolecular cyclisation to form diaziridine 5. Subsequent oxidation using iodine and trimethylamine in dichloromethane afforded diazirine 6. Each reaction in this TPD sequence afforded good to excellent yields (77-99%).

With the diazirine ring now formed, we turned our attention to functionalising the TPD moiety with a sulfonamide (Scheme 2, F-H); being mindful of TPD sensitivity towards high temperatures, harsh reducing agents and strong acids. Nitro TPD 6 was reduced to TPD aniline 7 using sodium dithionite. Wixe and co-workers have demonstrated TPD aniline 7 can also be prepared via an N-Boc protection/deprotection strategy. Next, we utilised...
Scheme 2. TPD-950-Br Synthesis. Reagents and conditions: A) H$_2$SO$_4$, HNO$_3$, -5 °C to r.t., 2 h, 95%. B) NH$_2$OH.HCl, pyridine, reflux, 4 h, 99%. C) TsCl, DMAP, Et$_2$N, CH$_3$Cl, r.t., 16 h, 77%. D) NH$_4$·SO$_4$, CuCl, <5 °C, 2 h, 98%. E) Na$_2$S$_2$O$_8$, THF/water, r.t., 16 h, 56%. F) I$_2$, NaNO$_2$, HCl, water, <5 °C, 15 min; g) SO$_2$, CuCl, <5 °C, 2 h, 68%. h) NH$_3$, THF, -78 °C to r.t., 3 h, 92%. i) NBS, NH$_3$·OAc, CH$_3$CN, r.t., 10 min, 98%. j) Triphosgene, Et$_3$N, THF, r.t., 4 h, 96%. k) NaH, THF, r.t., 16 h, 55%.

To confirm that TPD-950-Br could undergo photo-cross-linking, we irradiated it in deuterated methanol and observed the formation of the methanol adduct 13 by $^{19}$F NMR and tandem MS. TPD photolysis proceeds via the formation of a carbene, directly or via the linear diazo. Carbenes then undergo carbon-hydrogen or heteroatom-hydrogen insertion with proximal species. This is exemplified in Scheme 3, whereby TPD-950-Br exposed to UV light forms carbene 14, directly or via linear diazo 15. Carbene 14 then undergoes deuterium-oxygen insertion to form methanol adduct 13. Both the $^{79}$Br and $^{81}$Br methanol-adducts (13) were observed by tandem MS. However, the fragmentation of 13 in MS/MS may weaken the isotopic signature when analysing tryptic peptides.

The trifluoromethyl group associated with the TPD, diazo and methanol adduct each exhibit characteristic $^{19}$F NMR shifts of 67, 59 and 78 ppm, respectively. During UV exposure of TPD-950-Br, $^{19}$F NMR spectra were recorded at various intervals over 14 minutes. Plotting the relative $^{19}$F NMR integrals, the photolysis of TPD-950-Br can be observed, simultaneously with the formation of diazo 15 and methanol adduct 13 (Fig. 3). This photolysis provides guidance for future cell-based photo-cross-linking experiments.
To confirm biological activity, TPD-950-Br was screened in a cell-based NLRP3 inhibitor assay. IL-1β release from murine bone-marrow derived macrophages was measured, as per previously reported methods,7,16 The IL-1β IC_{50} of TPD-950-Br was 164 nM (pIC_{50} = 6.8 ± 0.1, n = 4). In comparison, the IL-1β IC_{50} of MCC950 = 8 nM (pIC_{50} = 8.1).7 From this we infer that TPD-950-Br has retained its NLRP3 inhibitor properties, consistent with the literature NLRP3 sulfonylurea SAR. Specifically, replacing the furan with a TPD group and introducing a bromine on the s-hexahydroindacene only resulted in a minor reduction in potency.

In summary, we have synthesised TPD-950-Br, a diazirine-containing analogue of the widely studied NLRP3 inhibitor MCC950. In doing so, we have expanded the scope of TPD-compatible nucleophilic aromatic substitutions. The photo-cross-linking properties of TPD-950-Br were confirmed using ^{19}F NMR and tandem MS. Our NLRP3 inhibitor assay demonstrated that TPD-950-Br is a potent inhibitor of IL-1β production (IC_{50} = 164 nM), in line with the literature SAR. TPD-950-Br may be useful for studying MCC950-protein interactions.

Experimental

Please see supplementary for experimental details.

Conflicts of interest

RC, AR and KS are co-inventors on granted patents (US 10,538,487, EP 3259253) and patent applications (WO2018215818, WO2017140778, WO2016131098) for NLRP3 inhibitors, which are licensed to Inflazome Ltd, a company headquartered in Dublin, Ireland. Inflazome is developing drugs that target the NLRP3 inflammasome to address unmet clinical needs in inflammatory disease.

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References


