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## Accepted Article

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# Sulfonylureas as concomitant insulin secretagogues and NLRP3 inflammasome inhibitors

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**Abstract:** Insulin-secretory sulfonylureas are widely used, cost-effective treatments for type 2 diabetes (T2D). However pancreatic  $\beta$ -cells are continually depleted as T2D progresses thereby rendering the sulfonylurea drug class ineffective in controlling glycaemia. Dysregulation of the innate immune system *via* activation of the NLRP3 inflammasome, and the consequent production of interleukin-1 $\beta$ , has been linked to pancreatic  $\beta$ -cell death and multiple inflammatory complications of T2D disease. One proposed strategy for treating T2D is the use of sulfonylurea insulin secretagogues which are also NLRP3 inhibitors. We report the synthesis and biological evaluation of nine sulfonylureas which inhibit NLRP3 activation in murine bone marrow-derived macrophages in a potent, dose-dependent manner. Six of these compounds inhibited NLRP3 at nanomolar concentrations and can also stimulate insulin secretion from a murine pancreatic cell line (MIN6). These novel compounds possess unprecedented dual modes of action, paving the way for a new generation of sulfonylureas that may be useful as therapeutic candidates and/or tool compounds in T2D and its associated inflammatory complications.

## Introduction

The NOD-like receptor, pyrin domain containing protein 3 (NLRP3) is a pattern recognition receptor (PRR) activated by wide-ranging stimuli to mediate the release of pro-inflammatory cytokines interleukin (IL)-1 $\beta$  and IL-18. Chronic activation of NLRP3 is implicated in a surprisingly wide variety of non-communicable diseases,<sup>[1]</sup> including metabolic dysregulation, T-cell mediated or organ specific autoimmune diseases, systemic autoimmune diseases and inflammatory reactions in skin, joints, muscle, heart and brain.<sup>[2]</sup> NLRP3 acts as an intracellular signaling molecule sensing pathogen, environmental and host-derived stress.<sup>[1]</sup> This contrasts with other PRRs which are primarily responsible for microbial recognition.<sup>[3]</sup> Several

proposed mechanisms of NLRP3 activation have been thoroughly reviewed elsewhere, all of which involve a two-signal process.<sup>[4]</sup> The first signal, known as priming, up-regulates the expression of inactive cytokines and inflammasome components. Priming is followed by an activation step, this involves oligomerisation of NLRP3 and recruitment of adapter proteins and pro-caspase-1 to form the NLRP3 inflammasome. The multi-protein complex serves as a scaffold for the activation of caspase-1, a proteolytic enzyme which cleaves inactive pro-inflammatory cytokines, pro-IL-1 $\beta$  and pro-IL-18, into their active secreted forms IL-1 $\beta$  and IL-18. These cytokines promote the release of pro-inflammatory mediators and amplify the inflammatory response.<sup>[5]</sup> Additionally, mature caspase-1 mediates a form of inflammatory cell death known as pyroptosis.<sup>[2b]</sup>

Investigation into the role of inflammation in type 2 diabetes (T2D) has attracted significant attention in recent years, illuminating the complexity of the metabolic disorder.<sup>[6]</sup> High-fat, high-calorie diets cause excessive glucose and free fatty acids (FFA) to stress pancreatic islets and adipose tissue, leading to immune cell recruitment and inflammation.<sup>[7]</sup> Obesity instigates T2D pathogenesis by up-regulating the expression of pro-inflammatory "M1" macrophages in adipose tissue which infiltrate the pancreas, promote insulin resistance and increase NLRP3 expression.<sup>[8]</sup> NLRP3 is primed, within both pancreatic  $\beta$ -cells and macrophages, by FFA or lipopolysaccharide (LPS) and subsequently activated by a range of danger signals prevalent amongst obese individuals.<sup>[9]</sup> These signals include glucose, FFA, ceramide, uric acid, cholesterol crystals and pancreatic amyloid deposits formed by islet amyloid polypeptide.<sup>[4]</sup> NLRP3 inflammasome activation and the consequent production of caspase-1, IL-18 and IL-1 $\beta$  promotes pancreatic islet inflammation, impairs insulin secretion and initiates pyroptotic and apoptotic pancreatic  $\beta$ -cell death.<sup>[10]</sup> Moreover, for diabetics the implications of aberrant NLRP3

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activation extends beyond pancreatic damage and impaired glycaemic control, with complications such as nephropathy, coronary atherosclerosis, neuroinflammation and wound healing all closely associated with NLRP3 activation.<sup>[4b, 11]</sup>

The validity of targeting IL-1 $\beta$  in T2D is supported by clinical evidence and animal models.<sup>[12]</sup> NLRP3 knockout mice fed a high-fat diet showed improved glucose tolerance and insulin sensitivity.<sup>[8c, 13]</sup> In the clinic, several biotherapeutics have successfully targeted IL-1 pathways. For example a clinical study of subcutaneously injected anakinra, a recombinant IL-1 receptor antagonist improved glycaemia,  $\beta$ -cell secretory function and reduced markers of systemic inflammation.<sup>[14]</sup> However this approach indiscriminately blocks IL-1 signalling, rendering subjects immunocompromised.<sup>[4b]</sup> In contrast NLRP3 inhibition is much more specific, leaving other IL-1 pathways fully responsive. Potent and selective NLRP3 inhibitors are required to fully understand the therapeutic potential of blocking only NLRP3 mediated IL-1 $\beta$  release in T2D.

A 2015 review by Baldwin *et al.* details many of the known NLRP3 inhibitors.<sup>[15]</sup> Thus far most NLRP3 inhibitors have potency in the micromolar range, are often unselective and/or contain reactive functional groups including Michael acceptors, thiol traps and/or epoxides. One exception is the sulfonylurea MCC950 (Figure 1), also known as CRID3, the most potent ( $IC_{50}$  = 8 nM), selective and well-characterised NLRP3 inhibitor to date.<sup>[16]</sup> Another previously identified NLRP3 inhibitor of the sulfonylurea class, albeit with activity only at micromolar concentrations, is Glyburide (Figure 1) and its sulfonamide precursor 16673-24-0.<sup>[17]</sup>

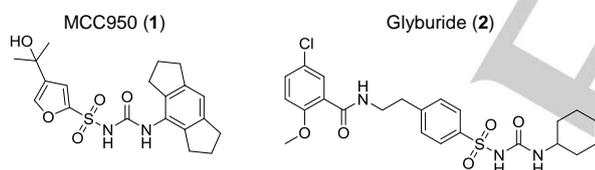


Figure 1. NLRP3-inhibitory sulfonylureas.

Sulfonylurea drugs, such as Glyburide (**2**), are used therapeutically in the treatment of T2D, triggering insulin secretion from pancreatic  $\beta$ -cells. The insulinotropic properties of sulfonylureas are due to binding with sulfonylurea receptors (SUR) in the pancreatic  $\beta$ -cell membrane. This interaction closes ATP-dependent potassium channels ( $K_{ATP}$ ) causing membrane depolarisation and  $Ca^{2+}$  influx through voltage-gated calcium channels.<sup>[18]</sup> High intracellular  $Ca^{2+}$  concentrations then stimulate the exocytosis of insulin-containing secretory granules ultimately reducing glycaemia.<sup>[19]</sup> Unfortunately as T2D progresses, sulfonylureas become decreasingly effective, due to progressive  $\beta$ -cell death.<sup>[10, 20]</sup> A means of stimulating insulin secretion while curtailing  $\beta$ -cell death could be a promising approach to long-term T2D treatment.

Sulfonylurea T2D drugs are loosely classified as first (Gen1) or second generation (Gen2) therapeutics, the primary difference being the increased affinity of Gen2 sulfonylurea for the SUR, attributed to the additional *p*- $\beta$ -arylcarboxamidoethyl group (see

Figure 2).<sup>[21]</sup> The Gen2 sulfonylurea Glyburide is particularly interesting, as it has a weak dual mode of action. Glyburide can act as an insulin secretagogue, *via* closure of  $\beta$ -cell  $K_{ATP}$  channels, and as a weak anti-inflammatory *via* inhibition of NLRP3 ( $IC_{50}$  = 20  $\mu$ M). The independence of these two pathways was demonstrated by Lamkanfi *et al.* (2009), where it was concluded NLRP3-mediated IL-1 $\beta$  production was not dependent on  $K_{ATP}$  channels.<sup>[17c]</sup> However improvements to the NLRP3 inhibitory-potency of sulfonylurea drugs are necessary to improve the likelihood of *in vivo* efficacy and avoid potential for high dose-associated toxicity.<sup>[22]</sup> We report herein the first *potent* dual action NLRP3 inhibitors, leveraging known structure-activity relationships (SAR) of insulin-secretory sulfonylurea drugs and NLRP3-inhibitory potency of MCC950.

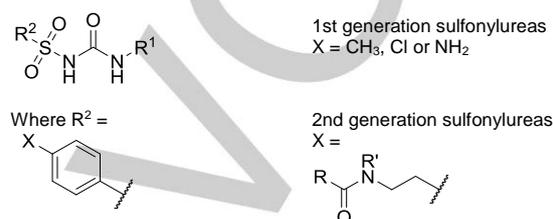


Figure 2. General structures of T2D sulfonylurea drugs.

## Results and Discussion

Known sulfonylurea drugs were tested for NLRP3-inhibition to establish initial SAR. We then synthesised nine molecular hybrids of these sulfonylureas incorporating the *s*-hexahydroindacene of MCC950 and found all exhibited potent NLRP3-inhibitory activity. The hybrids were tested for their insulin secretory properties and compared to Glyburide (**2**), from this work six novel dual action anti-inflammatory sulfonylureas were identified.

### NLRP3-inhibitory activity of known sulfonylureas

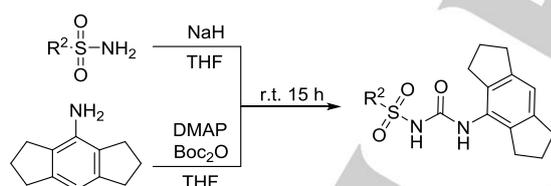
Eleven known T2D sulfonylurea drugs, their related sulfonamide precursors and the experimental antineoplastic agent sulofenur (**3**) were screened as NLRP3 inhibitors (Table 1).<sup>[23]</sup> The NLRP3-inhibition of each compound was tested by measuring IL-1 $\beta$  secretion from murine bone marrow-derived macrophages (BMDM). BMDM were primed with lipopolysaccharide (LPS), treated with the test compound (to a maximum concentration of 200  $\mu$ M) and finally stimulated with ATP. The half-maximal inhibitory concentration ( $IC_{50}$ ) was determined for each compound and compared to the previously reported NLRP3 inhibitor MCC950 (Table 1, left column).<sup>[16a]</sup> As expected MCC950 ( $IC_{50}$  = 8 nM) was a significantly more potent NLRP3 inhibitor than tested sulfonylurea drugs. Glyburide ( $IC_{50}$  = 20  $\mu$ M) was the most potent NLRP3 inhibitor of the sulfonylurea drugs tested and comparable to the experimental compound **3** ( $IC_{50}$  = 30  $\mu$ M). Glimepiride ( $IC_{50}$  = 52  $\mu$ M), gliquidone ( $IC_{50}$  = 100  $\mu$ M) and glisoxepide ( $IC_{50}$  = 156  $\mu$ M) had modest potency while remaining sulfonylureas failed to inhibit 50% of NLRP3 activity at 200  $\mu$ M. The data generated for Glyburide (**2**) glipizide (**10**) and sulofenur (**3**) reconciles with prior reports, with the remaining sulfonylureas having not been previously tested for NLRP3 inhibition.<sup>[16b, 17c]</sup>

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The sulfonamides, corresponding to the screened sulfonylureas, were likewise screened for NLRP3-inhibitory activity (Table 1, right column). The NLRP3 IC<sub>50</sub>'s of tested sulfonamides were above 200 μM, with the exception of **7a** (IC<sub>50</sub> = 159 μM). Even sulfonamide 16673-34-0 (**2a**), the Glyburide precursor, previously reported as an inhibitor of NLRP3 was not active at 200 μM in our hands, all controls were in line with expected values.<sup>[17a]</sup> In all but one instance, the sulfonamide was a less potent NLRP3 inhibitor than the corresponding sulfonylurea. This implies the sulfonylurea motif and/or the *s*-hexahydroindacene R<sup>1</sup> group are contributing to NLRP3 inhibition. It was also noted that compound **3**, the only compound, other than MCC950, to have an aromatic R<sup>1</sup> group, was more potent than all marketed sulfonylureas.

### Synthesis and NLRP3-inhibitory activity of MCC950-sulfonylurea hybrids

A medicinal chemistry strategy was devised to improve the NLRP3-inhibitory activity of known sulfonylurea compounds whilst maintaining insulinotropic properties. We focused on replacing the R<sup>1</sup> moiety of the aforementioned sulfonylurea set with the *s*-hexahydroindacene R<sup>1</sup> moiety of MCC950. The MCC950-sulfonylurea hybrids were prepared from the corresponding sulfonamide and hexahydro-*s*-indacen-4-amine *via* a two-step reaction (Scheme 1). Sulfonamides were treated with sodium hydride to form the corresponding sodium salts. Meanwhile the aniline was treated with di-*tert*-butyl dicarbonate (Boc<sub>2</sub>O) and *N,N*-dimethylpyridin-4-amine (DMAP) in tetrahydrofuran (THF) to form the isocyanate *in situ*.<sup>[24]</sup> The isocyanate and sodium salt mixtures were combined and stirred at room temperature until judged complete by LC-MS. All but three of the sulfonamides were commercially obtained. Sulfonamides **5a** and **10a** were synthesised *via* the cleavage of **5** and **10** respectively with phthalic anhydride and DMAP.<sup>[25]</sup> Sulfonamide **6a** was prepared from an acid chloride and primary amine (detailed in experimental section).

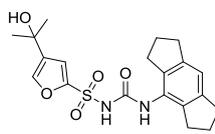
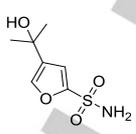
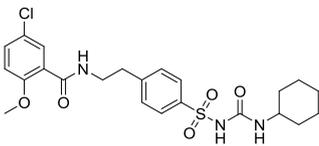
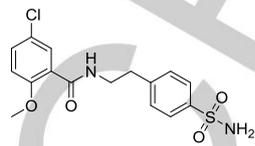
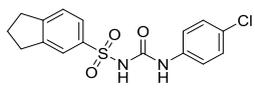
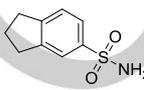
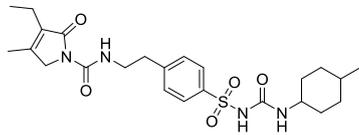
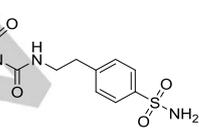
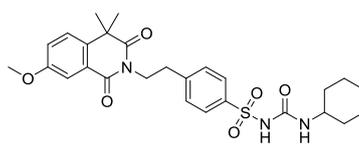
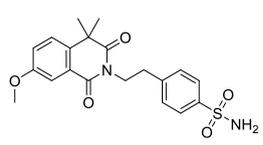
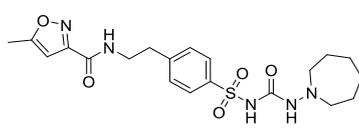
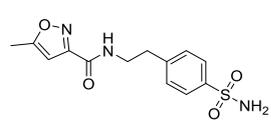
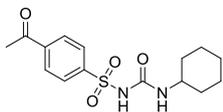
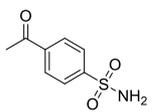
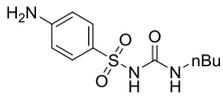
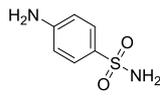
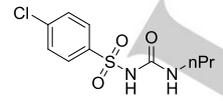
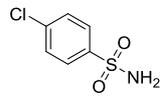
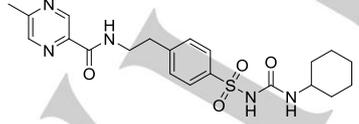
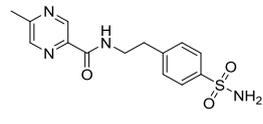
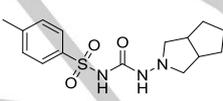
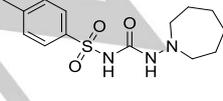
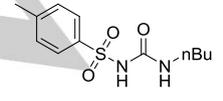


**Scheme 1.** Sulfonylurea synthesis.

Comparing the MCC950-sulfonylurea hybrids (Table 2) to the parent sulfonylurea (Table 1) a striking increase in potency was observed. This series of NLRP3 inhibitors ranks amongst the most potent published to date, superseded by only MCC950 and the sesquiterpene lactone arglabin.<sup>[16, 26]</sup> Interestingly the hybrids based on Gen1 sulfonylureas are generally more potent NLRP3 inhibitors than the higher molecular weight Gen2 sulfonylurea hybrids. The notable exception being the glimepiride hybrid (**4b**). These results represent a significant improvement of NLRP3-inhibitory potency in comparison to Glyburide. The scope of variation in R<sup>2</sup> suggests it does not contribute significantly to NLRP3 potency, and can be altered to interact with a second target, leading to an additional mode of action, in this case insulin secretion.

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Table 1. NLRP3-inhibitory activity of sulfonyleureas and precursor sulfonamides.

Compound	Structure	IC <sub>50</sub> (pIC <sub>50</sub> ±SD) <sup>[#]</sup>	Compound	Structure	IC <sub>50</sub> (pIC <sub>50</sub> ±SD) <sup>[#]</sup>
MCC950 (1)		7.5 nM <sup>[a]</sup>	1a		>200 μM <sup>[e]</sup>
Glyburide (2)		20 μM <sup>[b]</sup> (4.7±0.1)	16673-24-0 (2a)		>200 μM <sup>[d]</sup>
Sulofenur (3)		30 μM <sup>[c]</sup> (4.5±0.1)	3a		>200 μM <sup>[e]</sup>
Glimepiride (4)		52 μM <sup>[d]</sup> (4.3±0.1)	4a		>200 μM <sup>[e]</sup>
Gliclidone (5)		100 μM <sup>[d]</sup> (4.0±0.1)	5a		>200 μM <sup>[e]</sup>
Glisoxepide (6)		156 μM <sup>[d]</sup> (3.8±0.2)	6a		>200 μM <sup>[e]</sup>
Acetohexamide (7)		>200 μM <sup>[d]</sup>	7a		169 μM (3.8±0.1) <sup>[e]</sup>
Carbutamide (8)		>200 μM <sup>[d]</sup>	8a		>200 μM <sup>[e]</sup>
Chlorpropamide (9)		>200 μM <sup>[e]</sup>	9a		>200 μM <sup>[e]</sup>
Glipizide (10)		>200 μM <sup>[b]</sup>	10a		>200 μM <sup>[e]</sup>
Gliclazide (11)		>200 μM <sup>[e]</sup>			
Tolazamide (12)		>200 μM <sup>[d]</sup>			
Tolbutamide (13)		>200 μM <sup>[e]</sup>			

[#] The NLRP3 assay was performed using LPS primed, ATP stimulated BMDM. IC<sub>50</sub> values were determined by vehicle-normalised ELISA quantification of IL-1β. Data expressed as the mean of *n* biological replicates performed in triplicate. [a] *n* = 6, previously reported in R. Coll *et al. Nat. Med.* (2015).<sup>[16d]</sup> [b] *n* = 4, [c] *n* = 3, [d] *n* = 2, [e] *n* = 1.

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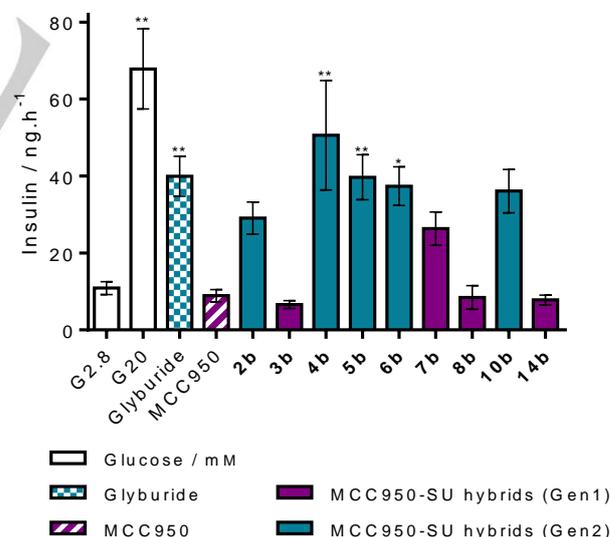
Table 2. NLRP3-inhibitory activity of MCC950-sulfonylurea hybrids.

Compound	Structure	IC <sub>50</sub> (pIC <sub>50</sub> ±SD) <sup>[#]</sup>	Compound	Structure	IC <sub>50</sub> (pIC <sub>50</sub> ±SD) <sup>[#]</sup>
MCC950 (1)		7.5 nM <sup>[a]</sup>	6b		167 nM <sup>[d]</sup> (6.8±0.1)
2b		536 nM <sup>[b]</sup> (6.3±0.1)	7b		59 nM <sup>[d]</sup> (7.2±0.2)
3b		52 nM <sup>[e]</sup> (7.3±0.1)	8b		15 nM <sup>[e]</sup> (7.8±0.4)
4b		42 nM <sup>[c]</sup> (7.4±0.2)	10b		318 nM <sup>[c]</sup> (6.5±0.1)
5b		230 nM <sup>[d]</sup> (6.6±0.1)	14b		57 nM <sup>[f]</sup> (7.2±0.3)

[#] The NLRP3 assay was performed using LPS primed, ATP stimulated BMDM. IC<sub>50</sub> values were determined by vehicle-normalised ELISA quantification of IL-1β. Data expressed as the mean of *n* biological replicates performed in triplicate. [a] *n* = 6, previously reported in R. Coll *et al. Nat. Med.* (2015).<sup>[16d]</sup> [b] *n* = 5, [c] *n* = 4, [d] *n* = 3, [e] *n* = 2, [f] *n* = 1.

## Insulin secretion of NLRP3 inhibitors

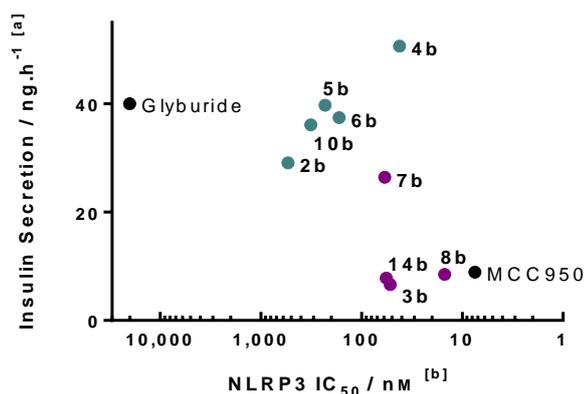
To determine if the MCC950-sulfonylurea hybrids had retained their insulinotropic properties we tested insulin secretion from MIN6 cells in the presence of a single concentration (10 μM) of test compound (Figure 3). Six hybrids (**2b**, **4b**, **5b**, **6b**, **7b** and **10b**) demonstrated insulin secretion comparable to Glyburide. The insulin-secretory hybrids, with the exception of **7b**, contained the *p*-β-arylcarboxamidoethyl motif common to Gen2 sulfonylureas, which is reported to increase K<sub>ATP</sub> affinity 100-fold.<sup>[21, 27]</sup> While **7b** lacks a *p*-β-arylcarboxamidoethyl motif, the carbonyl facilitates insulin secretion, perhaps acting as a hydrogen bond acceptor in SUR binding. The hybrids based on Gen1 sulfonylureas were significantly less potent, failing to stimulate insulin secretion beyond basal glucose levels. Prior to our study, Glyburide was the only reported compound to concomitantly stimulate insulin secretion and inhibit NLRP3 (albeit weakly), however our results suggest improvements to this dual activity are certainly achievable.



**Figure 3.** Insulin-secretory activity of MCC950-sulfonylurea (SU) hybrids. Insulin secretion from MIN6 cell line in response to basal 2.8 mM glucose (G2.8), elevated 20 mM glucose (G20) or test compounds at 10 μM concentration. Insulin secretion was quantified by radioimmunoassay. Data are presented as mean ± SEM of three biological replicates in triplicate. \*\* *p*<0.01 vs. G2.8, \* *p*<0.05 vs. G2.8.

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Plotting insulin secretion versus NLRP3 inhibition (Figure 4), it can be seen these MCC950-sulfonylurea hybrids fill the chemical space between the purely NLRP3-inhibitory MCC950 and the primarily insulin-secretory Glyburide. While small-molecule inhibition of NLRP3 is yet to be fully explored in models of T2D pathogenesis, a multi-targeted approach may prove useful on combating this complex disease. Multi-targeted molecules may have pharmacokinetic and regulatory advantages in comparison to combination treatments.<sup>[28]</sup> For these reasons, compounds possessing significant NLRP3-inhibitory and insulin-secretory properties should undoubtedly be further explored.



**Figure 4.** Dual action sulfonylureas.

[a] Insulin secretion, quantified by radioimmunoassay, from a MIN6 cell line was measured in response to 10  $\mu\text{M}$  compound concentration. Data are presented as mean of three independent experiments performed in triplicate, normalised to the mean secretion measured in response to 20 mM glucose concentration. [b] The NLRP3 assay was performed using LPS primed, ATP stimulated BMDM. IC<sub>50</sub> values were determined by vehicle-normalised ELISA quantification of IL-1 $\beta$ . Data expressed as the mean of  $n$  biological replicates performed in triplicate as outline in Table 1 and 2.

### Cytotoxicity

The MCC950-sulfonylurea hybrids and their parent sulfonylureas were not cytotoxic up to a top concentration of 80  $\mu\text{M}$ . Cytotoxicity was determined against a human embryonic kidney (HEK293) cell line using the cell viability indicator resazurin. Tamoxifen was used as a positive control, showing a 50% cytotoxicity concentration (CC<sub>50</sub>) = 58  $\mu\text{M}$ . All data are detailed in supplementary and represents the mean of two independent experiments performed in triplicate.

### Conclusions

Screening marketed sulfonylurea drugs for NLRP3 inhibition showed Glyburide (IC<sub>50</sub> = 20  $\mu\text{M}$ ) to be the most potent NLRP3 inhibitor, while glimepiride and gliquidone also showed modest NLRP3 inhibition (IC<sub>50</sub> <100  $\mu\text{M}$ ). The corresponding sulfonamides were similarly screened, and found to be largely inactive in our BMDM NLRP3 assay. Interestingly the precursor sulfonamide of Glyburide (16673-24-0) failed to inhibit NLRP3, despite prior reports.<sup>[17]</sup> A series of MCC950-sulfonylurea hybrids were then synthesised and screened in cell based assay against NLRP3, identifying some of the most potent NLRP3 inhibitors published (IC<sub>50</sub> = 15 – 536 nM) to date. Remarkably six NLRP3-inhibitory sulfonylureas appear to have retained their insulin-secretory properties when tested at 10  $\mu\text{M}$ . This highlights the

ability to functionalise MCC950 with other bioactive motifs to create small molecule chimeras. Our dual action hybrids fill a useful chemical space between the purely insulin secretory anti-diabetic sulfonylureas and highly potent NLRP3 inhibitors such as MCC950. These promising compounds are valuable tools to further understand NLRP3 activation in T2D and may be of significant therapeutic value.

## Experimental Section

### Mouse primary macrophage cell culture

Macrophages from C57BL/6 mice were differentiated from bone marrow as previously described (Schroder *et al.* 2012).<sup>[29]</sup> The University of Queensland Animal Ethics Committee approved all experimental protocols involving mice. BMDM were cultured in RPMI 1640 medium (Life Technologies) supplemented with 10% heat inactivated fetal calf serum, 2 mM GlutaMAX (Life Technologies) and 50 U/mL penicillin-streptomycin (Life Technologies) and 100 ng/mL recombinant human M-CSF (endotoxin free, expressed and purified by the University of Queensland Protein Expression Facility).

### NLRP3 inflammasome assay

BMDM were seeded at 1 x 10<sup>6</sup> cells/mL in 96 well plates. The following day the overnight medium was replaced with Opti-MEM® reduced serum medium (Life Technologies) and cells were stimulated with 100 ng/mL ultrapure *Escherichia coli* K12 LPS (Invivogen) for 3 h. Compounds were prepared as 10 mM stock solutions in 80% DMSO / 20% 10 mM ammonium bicarbonate and serially diluted with Opti-MEM® to allow final well concentrations of 0.001-200  $\mu\text{M}$ . Compounds or vehicle control were added to the LPS primed cells for 30 mins before stimulation with 2.5 mM adenosine 5'-triphosphate disodium salt hydrate (ATP) (Sigma Aldrich) for 1 h. IL-1 $\beta$  levels in cell-free supernatants were analysed by ELISA (ReadySetGo!® eBioscience). IC<sub>50</sub> values were determined from ELISA quantification of IL-1 $\beta$  by performing non-linear regression analysis of inhibitor vs. normalised response (variable slope) using Prism Software (GraphPad).

### MIN6 insulin assay

MIN6 cells (passage 29-37) were incubated for 1 h in 2.8 mM glucose containing Krebs-Ringer buffer (KRB). The cells were then stimulated for 1 h in either 2.8 mM KRB, 20 mM glucose KRB, or 2.8 mM glucose KRB plus test compound. Supernatants were collected and then assayed using rat insulin radioimmunoassay kit (RI-13K, Merck Millipore). One-way ANOVA with Tukey's honest significance test was performed using Prism Software.

### Cytotoxicity Assay

Cytotoxicity of the MCC950-sulfonylurea hybrids (2b, 3b, 4b, 5b, 6b, 7b, 8b, 10b, 14b) and their parent sulfonylureas (Glyburide, MCC950, 3, 4, 5, 6, 7, 8, 10, 14) was assessed against a Tamoxifen (Sigma Aldrich) positive control using a HEK293 (ATCC®CRL-1573) cell line and the cell viability indicator resazurin. HEK293 cells were seeded at 5,000 cells per well, in black, clear bottomed 384-well tissue culture treated plates, in a volume of 20  $\mu\text{L}$  DMEM medium (GIBCO™) supplemented with 10% foetal bovine serum (Scientifix) and 50 units/mL of penicillin and 50  $\mu\text{g}/\text{mL}$  of streptomycin (GIBCO™). Compounds, prepared in triplicate, at 80  $\mu\text{M}$  to 0.625  $\mu\text{M}$  in two-fold dilutions were added to each well to a final volume of 40  $\mu\text{L}$  per well and incubated for 20 h at 37 °C, 5% CO<sub>2</sub>. After the incubation, 5  $\mu\text{L}$  of 100  $\mu\text{M}$  resazurin (Sigma Aldrich) in PBS was added to each well. The plates were then incubated for 3 h. The fluorescence (FI) was read at 560 nm excitation 590 nm emission using a TECAN M1000

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Pro monochromator plate reader. The data was then analysed using PrismSoftware. Cell viability was calculated as an average percentage of control  $\pm$ SD for each set of duplicate wells using the following equation: Cell viability (%) =  $(F_{\text{Sample}} - F_{\text{Negative}} / F_{\text{Untreated}} - F_{\text{Negative}}) * 100$ . Using nonlinear regression analysis of log(concentration) vs. normalized cytotoxicity using variable fitting,  $CC_{50}$  (concentration at 50% cytotoxicity) were calculated.

## Chemistry

## General

All solvents, reagents and compounds were purchased and used without further purification unless stated otherwise. Sulfonamides were purchased from Sigma Aldrich or Tokyo Chemical Industry.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded using a Bruker Avance 600 MHz spectrometer (operating at 600 MHz for  $^1\text{H}$  NMR and 151 MHz for  $^{13}\text{C}$  NMR)  $^{13}\text{C}$  and  $^1\text{H}$  chemical shifts ( $\delta$ ), reported in ppm, were internally referenced to tetramethylsilane. LC-MS analysis was performed using a 0.05% (v/v) formic acid<sub>(aq)</sub> / 0.05% (v/v) formic acid in  $\text{CH}_3\text{CN}$  solvent system on a Shimadzu Prominence instrument equipped with an Agilent Eclipse XDB-Phenyl column (3.5 x 100 mm, 3  $\mu\text{m}$ ) maintained at 40  $^\circ\text{C}$ , SPD-M20A diode array UV-Vis detector, ELSD-LT II evaporative light scattering detector (ELSD) and LC-MS-2020 mass spectrometer. High resolution mass spectrometry (HRMS) was performed on a Bruker MicroTOF mass spectrometer with electrospray ionisation (ESI). Medium pressure liquid chromatography (MPLC) purification was conducted on a Grace Reveleris® X1 using two serial 12 g Grace C18 columns with a 10 mM  $\text{NH}_4\text{HCO}_3$ (aq) /  $\text{CH}_3\text{CN}$  solvent system flowing at 30 mL/min. HPLC purification was performed on a Gilson PLC 2020 instrument using an Agilent Eclipse XDB-Phenyl column (21.2 mm x 100 mm, 5  $\mu\text{m}$ ) with 10 mM  $\text{NH}_4\text{HCO}_3$ (aq) /  $\text{CH}_3\text{CN}$  solvent system flowing at 20 mL/min.

## Sulfonylurea synthesis - General method A

An isocyanate was prepared *in situ* from the corresponding amine by Boc<sub>2</sub>O (1.1 eq.) in THF (3 mL / mmol amine), treating with DMAP (1.1 eq.) and stirring for 5 min at room temperature before adding the amine (1 eq.) and stirring for a further 20 min. Meanwhile a sulfonamide sodium salt was prepared *in situ* by dissolving the sulfonamide (1 eq.) in THF (3 mL / mmol), treating with NaH (1.0 eq., 60% oil dispersion) and stirring under reduced pressure until effervescence ceased (approximately 10 min). The sulfonamide salt and isocyanate solutions were combined and stirred at room temperature for 15 h under  $\text{N}_2$  atmosphere, monitored by LC-MS. Reaction mixtures were concentrated *in vacuo*, dissolved in the minimum volume 1:1  $\text{CH}_3\text{CN}$  / *N,N*-dimethylformamide (DMF) and purified *via* reverse phase MPLC. Typically a four minute aqueous wash followed by a 15 min 10 mM  $\text{NH}_4\text{HCO}_3$ (aq) /  $\text{CH}_3\text{CN}$  gradient. HPLC purification was performed where necessary using a 15 min 0.1% formic acid<sub>(aq)</sub> /  $\text{CH}_3\text{CN}$  gradient, loaded in 10% DMF /  $\text{CH}_3\text{CN}$ , whereby fractions were neutralised with the equivalent volume of 10 mM  $\text{NH}_4\text{HCO}_3$ .

5-Chloro-*N*-(4-(*N*-(1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)phenethyl)-2-methoxybenzamide (2b)

5-Chloro-2-methoxy-*N*-(4-(sulfamoylphenethyl) benzamide (2a) (300 mg, 0.81 mmol) was treated as per *general method A* to afford the titled compound as a white solid (325 mg, 70%):  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.74 (br s, 1H), 8.27 (t,  $J$  = 5.7 Hz, 1H), 8.01 (s, 1H), 7.86 (d,  $J$  = 8.3 Hz, 2H), 7.65 (d,  $J$  = 2.8 Hz, 1H), 7.50 (dd,  $J$  = 8.9, 2.8 Hz, 1H), 7.47 (d,  $J$  = 8.3 Hz, 2H), 7.13 (d,  $J$  = 8.9 Hz, 1H), 6.89 (s, 1H), 3.78 (s, 3H), 3.59 – 3.49 (m, 2H), 2.92 (t,  $J$  = 7.1 Hz, 2H), 2.75 (t,  $J$  = 7.4 Hz, 4H), 2.53 (t,  $J$  = 7.4 Hz, 4H), 1.89 (p,  $J$  = 7.5 Hz, 4H);  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO}-d_6$ )  $\delta$  163.5, 155.6, 153.8, 144.4, 142.8, 136.9, 136.9, 131.4, 129.4, 129.4, 128.9, 127.1, 124.6, 124.2, 117.4, 114.0, 56.1, 40.2, 34.6, 32.3, 30.0, 24.9; HRMS (ESI-TOF)  $m/z$  calculated for  $\text{C}_{29}\text{H}_{29}\text{ClN}_3\text{O}_5\text{S}$  [M-H]<sup>-</sup>: 566.1522, found 566.1543; LC-MS  $m/z$  566.2 [M-H]<sup>-</sup>, purity >95% (ELSD).

*N*-(4-(4-Chlorophenyl)carbamoyl)-2,3-dihydro-1*H*-indene-5-sulfonamide (3)

4-Chlorobenzoic acid (1.0 g, 6.30 mmol) dissolved in toluene (10 mL) was treated with DMF (1 drop) and thionyl chloride (9.68 mmol, 0.7 mL) then heated to reflux for 18 h. The solvent was removed *in vacuo* to afford crude 4-chlorobenzoyl chloride (1.11 mg) as a brown oil. A solution of sodium azide (550 mg, 8.46 mmol) in water (8 mL) and acetone (6 mL) was cooled to 0  $^\circ\text{C}$ , and the crude acid chloride dissolved in acetone (5 mL) was added dropwise. The toluene (2 x 16 mL) extract was concentrated *in vacuo* (without heat) to 8 mL volume and this azide solution added dropwise into refluxing toluene under argon atmosphere. After addition the solution was heated, under argon atmosphere, until Curtius rearrangement was complete (2 h). Removal of solvent *in vacuo* afforded the 1-chloro-4-isocyanatobenzene crude as a brown oil (400 mg, 41%). 2,3-Dihydro-1*H*-indene-5-sulfonamide (107 mg, 0.54 mmol) dissolved in THF (1 mL) was treated with NaH in 60% oil dispersion (24 mg, 0.63 mmol) and agitated for 10 min. Once effervescence had ceased the crude 1-chloro-4-isocyanatobenzene dissolved in THF (0.5 mL) was added, and allowed to stir at room temperature for 16 h. The reaction was concentrated *in vacuo* and purified as per *general method A* to afford the titled compound as a white solid (60 mg, 32%):  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.90 (s, 1H), 8.90 (s, 1H), 7.74 (d,  $J$  = 1.4 Hz, 1H), 7.68 (dd,  $J$  = 7.9, 2.0 Hz, 1H), 7.41 – 7.35 (m, 3H), 7.26 (d,  $J$  = 9.0 Hz, 2H), 2.91 (q,  $J$  = 7.7 Hz, 4H), 2.05 (p,  $J$  = 7.6 Hz, 2H);  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO}$ )  $\delta$  151.2, 148.9, 144.2, 139.3, 138.0, 128.4, 125.7, 125.5, 124.1, 122.9, 119.9, 32.2, 32.0, 24.9; HRMS (ESI-TOF)  $m/z$  calculated for  $\text{C}_{16}\text{H}_{14}\text{ClN}_2\text{O}_3\text{S}$  [M-H]<sup>-</sup>: 349.0419, found, 349.0418; LC-MS  $m/z$  351.0 [M+H]<sup>+</sup>, purity >95% (ELSD).

*N*-(1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)-2,3-dihydro-1*H*-indene-5-sulfonamide (3b)

2,3-Dihydro-1*H*-indene-5-sulfonamide (3a) (200 mg, 1.01 mmol) was treated as per *general method A* to afford the titled compound as a white solid (48 mg, 12%):  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.68 (br s, 1H), 8.02 (s, 1H), 7.75 (d,  $J$  = 1.6 Hz, 1H), 7.69 (dd,  $J$  = 7.9, 1.6 Hz, 1H), 7.41 (d,  $J$  = 7.9 Hz, 1H), 6.91 (s, 1H), 2.92 (m, 4H), 2.77 (t,  $J$  = 7.4 Hz, 4H), 2.53 (t,  $J$  = 7.4 Hz, 4H), 2.05 (p,  $J$  = 7.5 Hz, 2H), 1.91 (p,  $J$  = 7.4 Hz, 4H);  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO}-d_6$ )  $\delta$  149.5, 149.4, 144.5, 142.9, 138.2, 137.0, 128.7, 125.3, 124.4, 122.8, 117.7, 32.3, 32.2, 32.0, 30.0, 25.0, 24.9; HRMS (ESI-TOF)  $m/z$  calculated for  $\text{C}_{22}\text{H}_{23}\text{N}_2\text{O}_3\text{S}$  [M-H]<sup>-</sup>: 395.1435, found 395.1430; LC-MS  $m/z$  397.1 [M+H]<sup>+</sup>, purity >95% (ELSD).

3-Ethyl-*N*-(4-(*N*-(1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)phenethyl)-4-methyl-2-oxo-2,5-dihydro-1*H*-pyrrole-1-carboxamide (4b)

3-Ethyl-4-methyl-2-oxo-*N*-(4-(sulfamoylphenethyl)-2,5-dihydro-1*H*-pyrrole-1-carboxamide (4a) (100 mg, 0.29 mmol) was treated as per *general method A* to afford the titled compound as a white solid (78 mg, 50%):  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta$  10.73 (br s, 1H), 8.38 (t,  $J$  = 5.8 Hz, 1H), 8.04 (s, 1H), 7.84 (d,  $J$  = 8.4 Hz, 2H), 7.45 (d,  $J$  = 8.4 Hz, 2H), 6.90 (s, 1H), 4.16 (s, 2H), 3.52 – 3.45 (m, 2H), 2.89 (t,  $J$  = 7.2 Hz, 2H), 2.75 (t,  $J$  = 7.5 Hz, 4H), 2.52 (d,  $J$  = 7.4 Hz, 4H), 2.18 (q,  $J$  = 7.5 Hz, 2H), 2.00 (s, 3H), 1.90 (p,  $J$  = 7.5 Hz, 4H), 0.97 (t,  $J$  = 7.5 Hz, 3H);  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO}-d_6$ )  $\delta$  171.8, 152.1, 151.7, 149.8, 144.7, 143.0, 138.6, 137.2, 131.9, 129.1, 129.0, 127.4, 117.8, 51.9, 40.2, 35.3, 32.5, 30.1, 25.0, 16.0, 12.9, 12.8; HRMS (ESI-TOF)  $m/z$  calculated for  $\text{C}_{29}\text{H}_{33}\text{N}_4\text{O}_5\text{S}$  [M-H]<sup>-</sup>: 549.2177; found 549.2169. LCMS:  $m/z$  573.47 [M+Na]<sup>+</sup>, purity >95% (ELSD).

4-(2-(7-Methoxy-4,4-dimethyl-1,3-dioxo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)benzenesulfonamide (5a)

*N*-(Cyclohexylcarbamoyl)-4-(2-(7-methoxy-4,4-dimethyl-1,3-dioxo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)benzenesulfonamide (5) (504 mg, 0.96 mmol) dissolved in pyridine (8 mL) was treated with phthalic anhydride (143 mg, 0.97 mmol) and DMAP (11.8 mg, 0.097 mmol), then heated at reflux for 5 h under  $\text{N}_2$  atmosphere. The reaction mixture was purified by MPLC, affording the titled compound as an amorphous white solid (291 mg, 77%):  $^1\text{H}$  NMR (600 MHz,  $\text{DMSO}-d_6$ )  $\delta$  7.72 (d,  $J$  = 8.5 Hz, 2H), 7.61 (d,  $J$  = 8.8 Hz, 1H), 7.53 (d,  $J$  = 2.9 Hz, 1H), 7.40 (d,  $J$  = 8.5 Hz, 2H), 7.33 – 7.26 (m, 3H), 4.13 (t,  $J$  = 7.4 Hz, 2H), 3.84 (s, 3H), 2.93 (t,  $J$  = 7.4 Hz, 2H), 1.45 (s, 6H);  $^{13}\text{C}$  NMR (151 MHz,  $\text{DMSO}-d_6$ )  $\delta$  176.3, 163.1, 158.0, 142.6, 142.2, 137.2, 129.1, 127.4, 125.6, 124.1, 121.5, 110.5, 55.3, 42.3,

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40.4, 32.9, 28.8; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{20}H_{21}N_2O_5S$  [M-H]<sup>-</sup> 401.1177, found 401.1174; LC-MS  $m/z$  403.1 [M+H]<sup>+</sup>, purity >95% (ELSD).

***N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)-4-(2-(7-methoxy-4,4-dimethyl-1,3-dioxo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)benzenesulfonamide (5b)**

4-(2-(7-Methoxy-4,4-dimethyl-1,3-dioxo-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)benzene sulfonamide (**5a**) (111 mg, 0.276 mmol) was treated as per *general method A* to afford the titled compound as a white solid (85 mg, 52%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 10.72 (br s, 1H), 7.91 (s, 1H), 7.80 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.7 Hz, 1H), 7.53 (d, *J* = 2.9 Hz, 1H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.29 (dd, *J* = 8.7, 2.9 Hz, 1H), 6.88 (s, 1H), 4.13 (t, *J* = 7.5 Hz, 2H), 3.83 (s, 3H), 2.93 (t, *J* = 7.4 Hz, 2H), 2.76 (t, *J* = 7.4 Hz, 4H), 2.55 (t, *J* = 7.4 Hz, 4H), 1.90 (p, *J* = 7.4 Hz, 4H), 1.42 (s, 6H); <sup>13</sup>C NMR (151 MHz, DMSO) δ 176.3, 163.1, 158.0, 150.1, 143.5, 142.8, 137.1, 136.9, 129.1, 129.0, 127.4, 127.1, 125.6, 124.1, 121.4, 117.4, 110.4, 55.3, 42.3, 40.3, 33.0, 32.3, 30.0, 28.8, 24.9; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{33}H_{34}N_3O_6S$  [M-H]<sup>-</sup> 600.2174, found 600.2183; LC-MS  $m/z$  602.4 [M+H]<sup>+</sup>, purity >95% (ELSD).

**5-Methyl-*N*-(4-sulfamoylphenethyl)isoxazole-3-carboxamide (6a)**

5-Methylisoxazole-3-carboxylic acid (150 mg, 1.18 mmol) dissolved in toluene (2 mL) was treated with DMF (1 drop) and thionyl chloride (1.42 mmol, 103 μL) then heated to reflux for 5 h. The solvent was removed *in vacuo* to afford crude 5-methylisoxazole-3-carbonyl chloride (155 mg, 1.06 mmol) as a brown oil. The crude acid chloride was dissolved in THF (4 mL), treated with Et<sub>3</sub>N (155 μL, 1.06 mmol) and stirred for 5 min, before adding 4-(2-aminoethyl)benzenesulfonamide (220 mg, 1.10 mmol). The reaction was stirred at room temperature for 15 h under N<sub>2</sub> atmosphere. The reaction was concentrated *in vacuo* and purified by MPLC, affording the titled compound as an amorphous white solid (205 mg, 62%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 8.79 (t, *J* = 5.8 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.30 (s, 2H), 6.50 (q, *J* = 0.8 Hz, 1H), 3.49 (m, 2H), 2.91 (t, *J* = 7.2 Hz, 2H), 2.45 (d, *J* = 0.8 Hz, 3H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 171.0, 158.8, 158.5, 143.3, 142.0, 129.0, 125.6, 101.1, 39.8, 34.3, 11.7; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{13}H_{14}N_3O_4S$  [M-H]<sup>-</sup> 308.0711, found 308.0708; LC-MS  $m/z$  308.0 [M+H]<sup>+</sup>, purity >99% (ELSD).

***N*-(4-(*N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)phenethyl)-5-methylisoxazole-3-carboxamide (6b)**

5-Methyl-*N*-(4-sulfamoylphenethyl) isoxazole-3-carboxamide (**6a**) (14 mg, 0.044 mmol) was treated as per *general method A*. Further MPLC purification used 0.05% formic acid(aq) / 0.05% formic acid in CH<sub>3</sub>CN gradient elution. Lyophilisation of the appropriate fractions afforded the titled compound as a white solid (14 mg, 62%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 10.73 (br s, 1H), 8.81 (t, *J* = 5.8 Hz, 1H) 8.09 (s, 1H), 7.86 (d, *J* = 8.2 Hz, 2H), 7.47 (d, *J* = 8.2 Hz, 2H), 6.92 (s, 1H), 6.49 (s, 1H), 3.49 (m, 2H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.76 (t, *J* = 7.2 Hz, 4H), 2.52 (m, 4H), 2.44 (s, 3H), 1.90 (p, *J* = 7.2 Hz, 4H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 171.0, 158.7, 158.5, 148.9, 145.0, 142.9, 137.8, 137.1, 129.1, 128.5, 127.2, 117.9, 101.1, 39.9, 34.4, 32.3, 29.9, 24.9, 11.70; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{26}H_{27}N_4O_5S$  [M-H]<sup>-</sup> 507.1708, found 507.1709; LC-MS  $m/z$  509.4 [M+H]<sup>+</sup>, purity >95% (ELSD).

**4-Acetyl-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)benzenesulfonamide (7b)**

4-Acetylbenzenesulfonamide (**7a**) (100 mg, 0.50 mmol) was treated as per *general method A* to afford the titled compound as a white solid (31 mg, 16%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 11.03 (br s, 1H), 8.08 (d, *J* = 8.5 Hz, 2H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.07 (br s, 1H), 6.87 (s, 1H), 2.75 (t, *J* = 7.4 Hz, 4H), 2.62 (s, 3H), 2.56 (t, *J* = 7.4 Hz, 4H), 1.90 (p, *J* = 7.4 Hz, 4H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 197.3, 151.8, 142.6, 138.9, 136.9, 129.7, 128.2, 127.2, 117.1, 32.4, 30.1, 26.9, 24.9; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{21}H_{21}N_2O_4S$  [M-H]<sup>-</sup> 397.1228, found 397.1225; LC-MS  $m/z$  399.1 [M+H]<sup>+</sup>, purity >95% (ELSD).

**4-Amino-*N*-((1,2,3,5,6,7-hexahydro-*s*-indacen-4-yl)carbamoyl)benzenesulfonamide (8b)**

*N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)-4-nitrobenzenesulfonamide (**14b**) (40.0 mg, 0.100 mmol) was dissolved in EtOAc:DMF (4:1, 2.5 mL) and stirred at room temperature for 1 h under hydrogen atmosphere with 10% palladium on carbon (5 mol%) catalyst. Filtration through celite and purification by MPLC afforded the titled compound as a white solid (16 mg, 43%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 10.08 (br s, 1H), 7.95 (s, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 6.91 (s, 1H), 6.59 (d, *J* = 8.8 Hz, 2H), 6.05 (s, 2H), 2.77 (t, *J* = 7.5 Hz, 4H), 2.55 (t, *J* = 7.5 Hz, 4H), 1.92 (p, *J* = 7.5 Hz, 4H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 153.0, 149.4, 142.9, 136.8, 129.2, 128.9, 124.9, 117.6, 112.1, 32.3, 30.0, 24.9; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{19}H_{20}N_3O_5S$  [M-H]<sup>-</sup> 370.1231, found 370.1225; LC-MS  $m/z$  372.1 [M+H]<sup>+</sup>, purity >95% (ELSD).

**5-Methyl-*N*-(4-sulfamoylphenethyl)pyrazine-2-carboxamide (10a)**

*N*-(4-(*N*-(Cyclohexylcarbamoyl)sulfamoyl)phenethyl)-5-methylpyrazine-2-carboxamide (**11**) (250 mg, 0.56 mmol) dissolved in pyridine (8 mL) was treated with phthalic anhydride (83 mg, 0.56 mmol) and DMAP (10 mg, 0.082 mmol), then heated at reflux for 5 h under N<sub>2</sub> atmosphere. The reaction mixture was purified by MPLC affording the titled compound as an amorphous white solid (113 mg, 63%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 9.03 (d, *J* = 1.4 Hz, 1H), 8.96 (t, *J* = 6.0 Hz, 1H), 8.61 (m, 1H), 7.73 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 8.4 Hz, 2H), 7.30 (s, 2H), 3.57 (dt, *J* = 7.3, 6.0 Hz, 2H), 2.95 (t, *J* = 7.3 Hz, 2H), 2.58 (s, 3H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 162.8, 156.7, 143.4, 142.7, 142.3, 141.9, 141.9, 129.0, 125.6, 39.9, 34.6, 21.2; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{14}H_{17}N_4O_3S$  [M+H]<sup>+</sup> 321.1016, found 321.1029; LC-MS  $m/z$  321.0 [M+H]<sup>+</sup>, purity >95% (ELSD).

***N*-(4-(*N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)sulfamoyl)phenethyl)-5-methylpyrazine-2-carboxamide (10b)**

5-Methyl-*N*-(4-sulfamoylphenethyl)pyrazine-2-carboxamide (**10a**) (100 mg 0.312 mmol) was treated as per *general method A* to afford a crude white solid (72 mg). Further HPLC purification using a 0.1% formic acid(aq) / CH<sub>3</sub>CN mobile phase and 10% DMSO / CH<sub>3</sub>CN loading solvent, whereby fractions were neutralised with 10 mM NH<sub>4</sub>HCO<sub>3</sub>(aq), yielded a white solid (14 mg, 9%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 9.03 (d, *J* = 1.0 Hz, 1H), 8.93 (t, *J* = 5.9 Hz, 1H), 8.59 (d, *J* = 1.0 Hz, 1H), 7.77 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 6.84 (s, 1H), 6.53 (br s, 1H), 3.58 – 3.51 (m, 2H), 2.93 (t, *J* = 7.3 Hz, 2H), 2.74 (t, *J* = 7.3 Hz, 4H), 2.58 (s, 3H), 2.55 (t, *J* = 7.3 Hz, 4H), 1.88 (p, *J* = 7.4 Hz, 4H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 162.8, 156.7, 147.7, 143.0, 142.7, 142.5, 142.3, 141.9, 141.6, 136.7, 128.4, 126.8, 125.6, 116.7, 39.9, 34.6, 32.4, 30.1, 24.9, 21.2; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{27}H_{30}N_5O_4S$  [M+H]<sup>+</sup> 520.2020, found 520.2030; LC-MS  $m/z$  520.1 [M+H]<sup>+</sup>, purity >95% (ELSD).

***N*-((1,2,3,5,6,7-Hexahydro-*s*-indacen-4-yl)carbamoyl)-4-nitrobenzenesulfonamide (14b)**

4-Nitrobenzenesulfonyl chloride (300 mg, 1.35 mmol) dissolved in acetone (1 mL) was added drop-wise to NH<sub>4</sub>HCO<sub>3</sub>(aq) (450 mg, 5.42 mmol, 4 mL) and stirred at room temperature for 2 h. The reaction mixture was then acidified with 1 M HCl (to pH ~2) and extracted with EtOAc (3 x 10 mL). The organic layers were combined, washed with brine (20 mL), water (20 mL) and dried (MgSO<sub>4</sub>). Solvent was removed *in vacuo* to afford 4-nitrobenzenesulfonamide as a pale orange solid (157 mg, 57%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 8.42 (d, *J* = 9.0 Hz, 2H), 8.06 (d, *J* = 9.0 Hz, 2H), 7.74 (s, 2H); HRMS (ESI-TOF)  $m/z$  calculated for  $C_8H_5N_2O_4S$  [M-H]<sup>-</sup> 200.9976, found 200.9984; LC-MS  $m/z$  200.9 [M-H]<sup>-</sup>, purity >99% (ELSD). 4-Nitrobenzenesulfonamide (124 mg, 0.613 mmol) was treated as per *general method A* to afford the titled compound as a pale yellow solid (148 mg, 60%): <sup>1</sup>H NMR (600 MHz, DMSO-*d*<sub>6</sub>) δ 10.00 (br s, 1H), 8.21 (d, *J* = 9.0 Hz, 2H), 7.97 (d, *J* = 9.0 Hz, 2H), 7.45 (s, 1H), 6.75 (s, 1H), 2.73 (t, *J* = 7.4 Hz, 4H), 2.61 (t, *J* = 7.4 Hz, 4H), 1.87 (p, *J* = 7.4 Hz, 4H); <sup>13</sup>C NMR (151 MHz, DMSO-*d*<sub>6</sub>) δ 158.0, 153.8, 147.4, 141.9, 136.4, 132.5, 127.8, 122.8, 115.4, 32.5, 30.4, 25.0; HRMS (ESI-TOF)  $m/z$  calculated for  $C_{19}H_{18}N_3O_5S$  [M-H]<sup>-</sup> 400.0973, found 400.0979; LC-MS  $m/z$  402.1 [M+H]<sup>+</sup>, purity >95% (ELSD).

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**Keywords:** NLRP3 • inflammasome • diabetes • inflammation • multi-targeted

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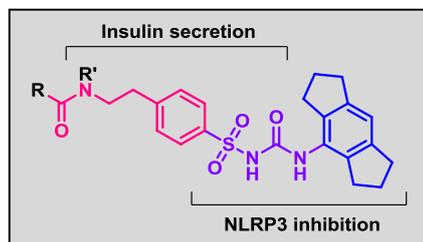
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The hybridisation of the potent NLRP3 inhibitor *MCC950* with anti-diabetic sulfonylureas has created dual mode of action compounds which concomitantly inhibit NLRP3 and stimulate insulin secretion. These hybrids are interesting tool compounds for studying the role of inflammation in type 2 diabetes, promising therapeutics and highlight the ability to functionalise *MCC950* with other bioactive motifs to create small-molecule chimeras.