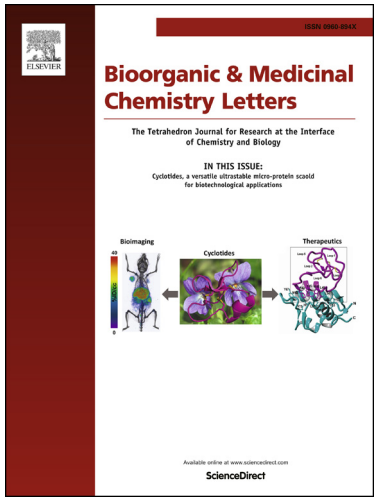
Accepted Manuscript



Synthesis of deuterium-labelled analogues of NLRP3 inflammasome inhibitor MCC950

Manohar Salla, Mark S. Butler, Nicholas L. Massey, Janet C. Reid, Matthew A. Cooper, Avril A.B. Robertson

PII: S0960-894X(17)31228-3
DOI: https://doi.org/10.1016/j.bmcl.2017.12.054
Reference: BMCL 25510

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 9 November 2017
Revised Date: 22 December 2017
Accepted Date: 23 December 2017

Please cite this article as: Salla, M., Butler, M.S., Massey, N.L., Reid, J.C., Cooper, M.A., Robertson, A.A.B., Synthesis of deuterium-labelled analogues of NLRP3 inflammasome inhibitor MCC950, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: https://doi.org/10.1016/j.bmcl.2017.12.054

Synthesis of deuterium-labelled analogues of NLRP3 inflammasome inhibitor MCC950

Manohar Salla [a], Mark S. Butler [a], Nicholas L. Massey [a], Janet C. Reid [a], Matthew A. Cooper [a, b*] and Avril A. B. Robertson [a*]

[a] *Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia*

[b] *Inflazome Ltd., The Tower, Trinity TEC, Pearse Street, Dublin, Ireland*

ABSTRACT

This study describes the syntheses of di, tetra and hexa deuterated analogues of the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome inhibitor MCC950. In di and tetra deuterated analogues, deuteriums were incorporated into the 1,2,3,5,6,7-hexahydro-*s*-indacene moiety, whereas in the hexa deuterated MCC950 deuteriums were incorporated into the 2-(furan-3-yl)propan-2-ol moiety. The di deuterated MCC950 analogue was synthesised from 4-amino-3,5,6,7-tetrahydro-*s*-indacen-1(2*H*)-one **5**. Tetra deuterated analogues were synthesised in 10 chemical steps starting with 5-bromo-2,3-dihydro-1*H*-inden-1-one **9**, whereas the hexa deuterated analogue was synthesised in four chemical steps starting with ethyl-3-furoate **24**. All of the compounds exhibited similar activity to MCC950 (IC_{50} = 8 nM). These deuterated analogues are useful as internal standards in LC-MS analyses of biological samples from *in vivo* studies.

The NLRP3 inflammasome is a key mediator of inflammatory processes. Its abnormal activation is involved in the pathogenesis of inherited disorders such as cryopyrin-associated periodic syndrome (CAPS) and complex inflammatory diseases, including metabolic disorders such as type 2 diabetes, atherosclerosis, and gouty arthritis.[1] Increasing evidence indicates that NLRP3 is also implicated in several central nervous system (CNS) diseases including multiple sclerosis, Alzheimer's and Parkinson's diseases.[2,3] A small molecule inhibitor of NLRP3 inflammasome, MCC950 (CRID3),[4,5] has been reported with IC_{50} 8 nM in human cell based assays, and impressive *in vivo* activity in multiple proof of concept models of inflammatory disease.[6-8] This molecule is also widely used as a tool to understand the role of NLRP3 in biological processes.[9-11]

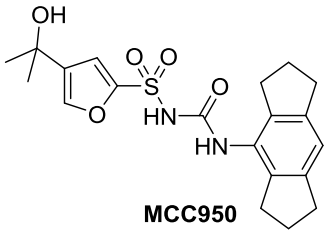


Figure 1. Structure of MCC950

Isotopically labelled compounds can be used as internal standards in quantification of drugs and associated metabolites in biological samples by using liquid chromatography-mass spectrometry (LC-MS).[12,13] Indeed, we recently identified and reported the major metabolite of MCC950.[14]

In this study, deuterium-labelled analogues of MCC950 have been synthesised for use as isotopic standards in pharmacokinetic analysis. Deuterium has been included in multiple positions of MCC950 such that the synthetic precursors can be used towards additional standards in the future.

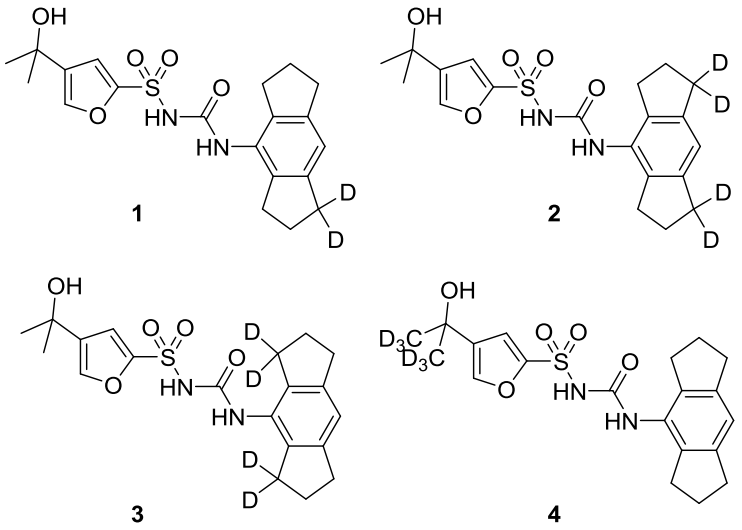
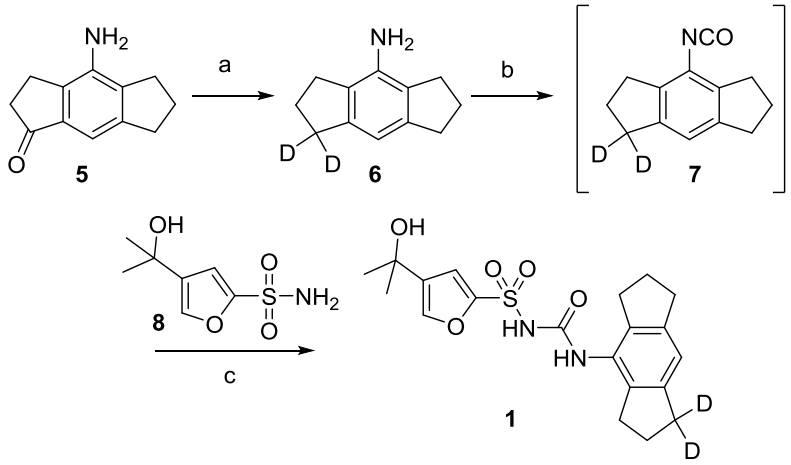


Figure 2. Structures of deuterium-labelled MCC950 analogues

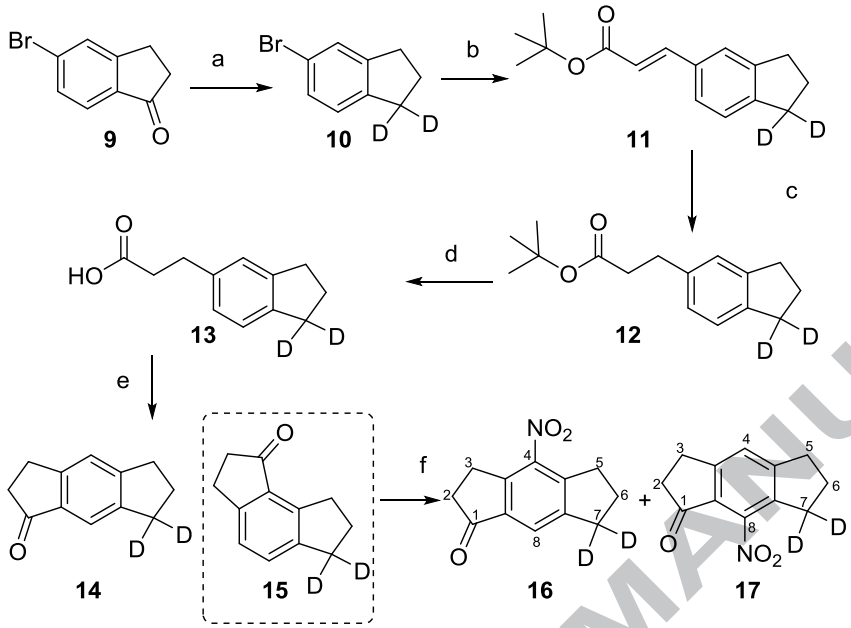
The synthesis of compound **1** (Figure 2) required key intermediate **5** (Scheme 1), which was synthesised by procedures reported in our previous publication.[14] Intermediate **5** was converted into deuterium labelled amine intermediate **6** by using $LiAlD_4/AlCl_3$ in anhydrous Et_2O at room temperature.[15] The structure of the amine **6** was confirmed by LC-MS and NMR analyses. Detection of the desired mass, *m/z* 176 $[M+H]^+$ on (+)-ESI-LC-MS, disappearance of the carbonyl carbon signal, appearance of a new carbon signal with reduced intensity in the aliphatic region (^{13}C NMR), and no additional signals in ^1H NMR spectra confirmed the structure of amine **6**. Amine **6** was converted into the corresponding isocyanate intermediate **7** using Boc_2O and DMAP in acetonitrile at room temperature.[16] Subsequent reaction with 4-(2-hydroxypropan-2-yl)furan-2-sulfonamide **8** in presence of NaH gave the desired product **1** in 98% isotopic purity (LC-MS).[17] In the (–)-ESI-LC-MS analysis, the desired *m/z* 405 $[M–H]^–$ (406 Da) was detected, which was 2 Dalton (Da) higher mass than MCC950 (404 Da, *m/z* 403 $[M–H]^–$). The ^1H NMR spectra showed two protons less than MCC950 in the hexahydroindacene moiety at the site of deuterium incorporation confirming the structure of compound **1**.



Scheme 1. Reagents and conditions: (a) $LiAlD_4$, $AlCl_3$, Et_2O, rt, 4 h, 32% (b) Boc_2O, DMAP, CH_3CN, 30 min (c) NaH, THF, rt, 16 h, 63% (2 steps).

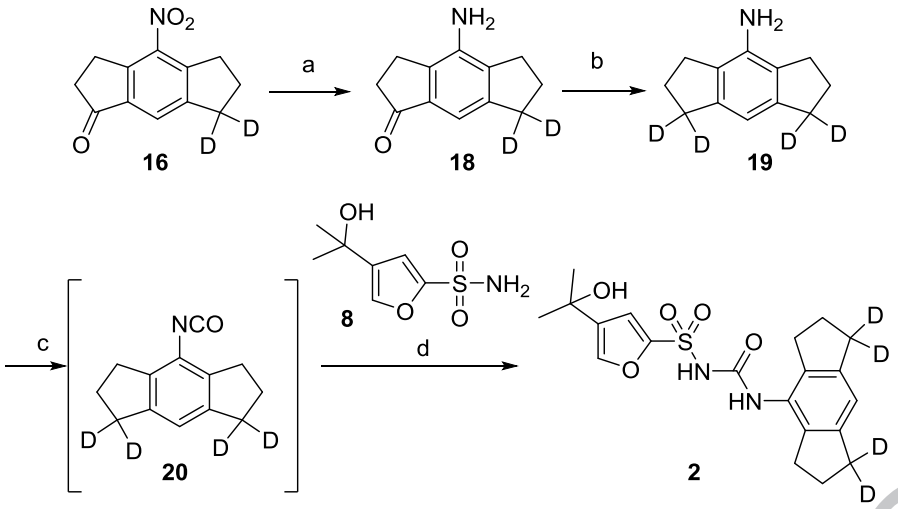
The synthesis of compounds **2** and **3** (Figure 2) required two key intermediates **16** and **17** (Scheme 2); these intermediates were synthesised using commercially available 5-bromo-2,3-dihydro-1*H*-inden-1-one (Scheme 2) as starting material. Reaction with $LiAlD_4/AlCl_3$ gave deuterated intermediate **10** in excellent yield. Heck coupling with *t*-butyl acrylate gave alkene intermediate **11**,[18] which was reduced to intermediate **12** by hydrogenation using Pd/C as catalyst over 1 h. The quantity of catalyst (10% wt/wt) was key to the success of this conversion. In initial efforts to convert intermediate **11** to **12**, with excess loading of catalyst (20% wt/wt) and prolonged reaction duration (typically overnight), >50% of deuterium to hydrogen exchange was observed. The optimal reaction conditions were found to be 10% catalyst loading with 1 hour reaction time under a hydrogen atmosphere. Deprotection of the *t*-butyl group was achieved using trifluoroacetic acid to obtain **13** in quantitative yield. The acid intermediate **13** was first chlorinated using oxalyl chloride and catalytic DMF at room temperature for 1 h, then subjected to cyclization *via* Friedel–Crafts acylation using $AlCl_3$ catalyst in 1,2 dichloroethane (DCE). During this cyclisation, we observed ca. 15% (by LC-MS) of regioisomer **15**; this was separated by column chromatography. ^{1}H NMR spectra of intermediate **14** showed two singlets as expected in the aromatic region, and two doublets for regioisomer **15**, confirming the structure of the regioisomers. Nitration of the intermediate **14** using concentrated H_2SO_4/HNO_3 (1:1) at 0 °C gave a mixture of regiosiomers **16** and **17**. The regioisomers were separated by column chromatography and the minor isomer was identified as **16** and the major as **17**.[14] The structures of intermediates **16** and **17** were confirmed by comparing the NMR spectra with their hydrogen

equivalents already confirmed by X-ray crystallography.[14] The ^{1}H NMR of the deuterated compounds **16** and **17** (Scheme 2) showed a triplet instead of multiplet for protons on C-6 carbon and no signal was observed for deuteriums on C-7 carbon.



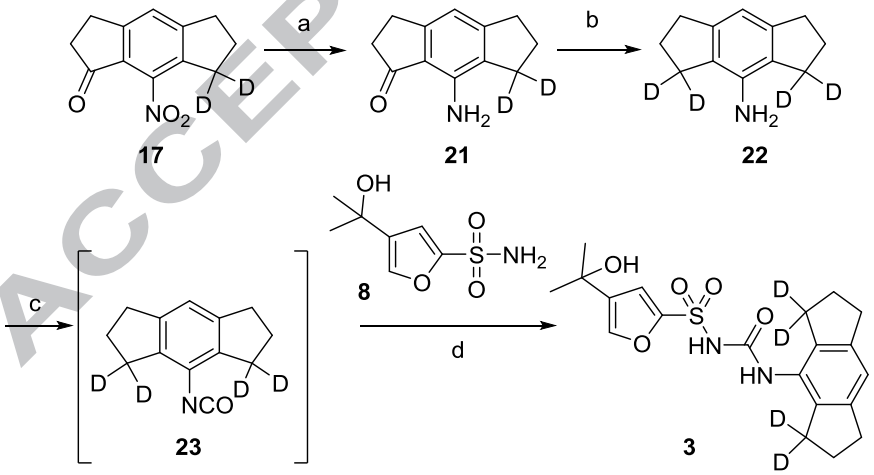
Scheme 2. Reagents and conditions: (a) $LiAlD_4$, $AlCl_3$, Et_2O, rt, 4 h, 94% (b) *t*-Butyl acrylate, $Pd(OAc)_2$, Tri(*p*-tolyl)phosphine, Et_3N, DMF, 100 °C, 47% (c) 10% Pd/C, H_2 (1 atm), MeOH, rt, 1 h, 97% (d) CF_3COOH, CH_2Cl_2, rt, 98% (e) i) $(CO)_2Cl_2$, DMF (cat.) ii) $AlCl_3$, DCE, rt, 1 h, 72% (f) con. HNO_3/H_2SO_4, 0 °C, 1 h, 16%, 62% of **16** and **17** respectively.

After confirming the structures (using unlabelled **16**) for the synthesis of compound **2** (Scheme 3), the nitro intermediate **16** was reduced to the corresponding amine **18** by hydrogenation using Pd/C as catalyst, followed by reaction with $LiAlD_4/AlCl_3$ to give the tetra deuterium-labelled amine **19**. This key amine intermediate **19** was converted into the corresponding isocyanate **20** as before, followed by reaction with sulfonamide **8** in presence of NaH to give the desired product **2** in 98% isotopic purity (LC-MS).



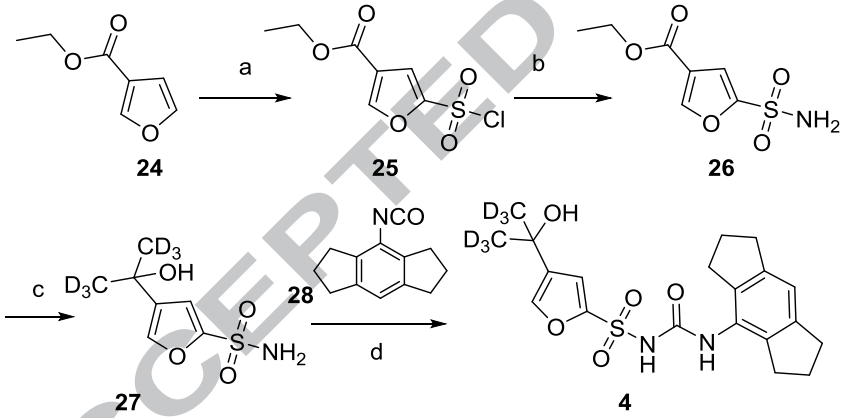
Scheme 3. Reagents and conditions: (a) 10% Pd/C, H_2 (1 atm), MeOH, rt, 1 h, 94% (b) $LiAlD_4$, $AlCl_3$, Et_2O, rt, 4 h, 31% (c) Boc_2O, DMAP, CH_3CN, 30 min (d) NaH, THF, rt, 16 h, 68% (2 steps).

Compound **3** (Figure 2) was synthesised from 8-nitro-3,5,6,7-tetrahydro-*s*-indacen-1(2*H*)-one-7,7-d_2 **17** (Scheme 4) *via* the same procedures used for compound **2** (Scheme 3) in 97.3% isotopic purity (LC-MS). In the (–)-ESI-LC-MS analysis for compounds **2** and **3** (408 Da), the desired *m/z* 407 $[M–H]^-$ was detected, 4 Da higher than MCC950 (404 Da, *m/z* 403 $[M–H]^-$). ^1H NMR spectra showed four protons less than MCC950 in the hexahydroindacene moiety where deuteriums were incorporated, confirming the structures of compounds **2** and **3**.



Scheme 4. Reagents and conditions: (a) 10% Pd/C, H_2 (1 atm), MeOH, rt, 1 h, 89% (b) $LiAlD_4$, $AlCl_3$, Et_2O, rt, 4 h, 40% (c) Boc_2O, DMAP, CH_3CN, 30 min (d) NaH, THF, rt, 16 h, 56% (2 steps).

Compound **4** (Scheme 5) was synthesised from ethyl-3-furoate as starting material. This was reacted with chlorosulfonic acid in anhydrous CH_2Cl_2 for 72 hours at room temperature. Pyridine was then added at –10 °C followed by phosphorous pentachloride (PCl_5) and the resulting solution stirred for 16 h at room temperature to give sulfonyl chloride **25**. This sulfonylchloride was treated with liquid NH_3 at –78 °C to obtain sulfonamide **26** in excellent yield without any purification. Intermediate **26** was reacted with *in situ* generated CD_3MgI in Et_2O at room temperature to give the desired deuterium-labelled sulfonamide intermediate **27** in high yield. The sulfonamide **27** was coupled with previously synthesised isocyanate **28** as before to give the desired sulfonylurea **4** in 100% isotopic purity (LC-MS). In the (–)-ESI-LC-MS analysis for compound **4** (410 Da), the desired *m/z* 409 $[M–H]^-$ was detected, 6 Da higher than MCC950 (404 Da, *m/z* 403 $[M–H]^-$). In the ^1H NMR spectrum, no signal was detected for deuterium-labelled methyl groups, confirming the structure of compound **4**.



Scheme 5. Reagents and conditions: (a) i) $ClSO_3H$, CH_2Cl_2, rt, 72 h ii) Pyridine, PCl_5, rt, 12 h, 79% (b) NH_3, rt, 3 h, 94% (c) CD_3MgI, Et_2O, rt, 16 h, 89% (d) NaH, THF, rt, 16 h, 47%.

Compounds **1**-**4** were tested for their NLRP3 inflammasome inhibitory activity in a human cell based assay. Assays were conducted in two or three biological repeats performed in triplicate and the results are listed in Table 1. NLRP3-induced production and release of the pro-inflammatory cytokine interleukine-1β (IL-1β) from lipopolysaccharide (LPS) primed human

monocyte-derived macrophages (HMDMs), stimulated with nigericin, was tested in the presence and absence of increasing concentrations of test compounds. The determined IC_{50} values were then compared to that for MCC950 used in this assay as positive control. As anticipated, all of the compounds exhibited similar activity to MCC950 (IC_{50} 8 nM).

Table 1. NLRP3 inhibitory activity results for compounds **1-4**

Compound	**IL1-β IC_{50}[a]±SD[b] (nM) (HMDM)**
MCC950	5±4[c]
1	6±2
2	7±3
3	6±1[d]
4	5±1

[a]IC_{50} data reported as the mean of three biological replicates performed in triplicate; [b]Standard deviation
[c]Previously reported 8 nM [4]; [d]IC_{50} data reported as the mean of two biological replicates performed in triplicate.

In summary, we have successfully synthesised di, tetra and hexa deuterium labelled analogues of NLRP3 inflammasome inhibitor MCC950. These derivatives are useful as internal standards for LC-MS analyses of biological samples from *in vivo* studies. Furthermore, the synthetic routes and intermediates developed for these deuterated tricyclic systems (compounds **1-3**, Figure 2) can be used more widely for other lead molecules now progressing towards the clinic.

Acknowledgments

The authors are thankful to National Health and Medical Research Council (NHMRC) for the financial support in terms of funding (NHRMRC grant APP1086786). Manohar Salla is supported by a UQ International Scholarship (UQI) Ph.D. scholarship. We would like to acknowledge The Australian Red Cross Blood service for their supply of buffy coat tested blood for biological assays. MAC currently holds a fractional Professorial Research Fellow appointment at the University of Queensland with his remaining time as CEO of Inflazome Ltd. a company headquartered in Dublin, Ireland that is developing drugs to address clinical unmet needs in inflammatory disease by targeting the inflammasome.

Supplementary data

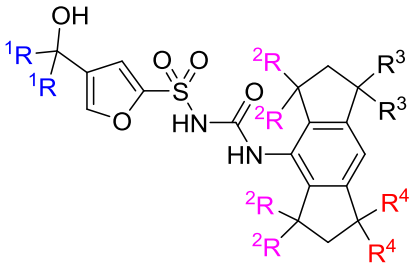
Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/xxx/j.bmcl.2017.xx. xx.

References

1. Guo, H.; Callaway, J. B.; Ting, J. P. *Nat. Med.* **2015**, *21*, 677-687.
2. Strowig, T.; Henao-Mejia, J.; Elinav, E.; Flavell, R. *Nature* **2012**, *481*, 278- 286.
3. Walsh, J. G.; Muruve, D. A.; Power, C. *Nature. Rev. Neurosci.* **2014**, *15*, 84- 97.
4. Coll, R. C.; Robertson, A. A.; Chae, J. J.; Higgins, S. C.; Munoz-Planillo, R.; Inserra, M. C.; Vetter, I.; Dungan, L. S.; Monks, B. G.;Stutz, A.; Croker, D. E.; Butler, M. S.; Haneklaus, M.; Sutton, C. E.;Nunez, G.; Latz, E.; Kastner, D. L.; Mills, K. H.; Masters, S. L.;Schroder, K.; Cooper, M. A.; O'Neill, L. A. *Nat. Med.* 2015, 21, 248-255.
5. Primiano, M. J.; Lefker, B. A.; Bowman, M. R.; Bree, A. G.; Hubeau, C.; Bonin, P. D.; Mangan, M.; Dower, K.; Monks, B. G.; Cushing, L.; Wang, S.; Guzova, J.; Jiao, A.; Lin,L.L.; Latz, E.; Hepworth, D.; Hall, J. P., *J. Immunol.* **2016**, 197, 2421-2433.
6. Mridha, A. R.; Wree, A.; Robertson, A. A.; Yeh, M. M.; Johnson, C. D.; Van Rooyen, D. M.; Haczeyni, F.; Teoh, N. C.; Savard, C.; Ioannou, G. N.; Masters, S. L.; Schroder, K.; Cooper, . . M. A.; Feldstein, A. E.; Farrell, G. C. *J. Hepatol.* **2017**, 66, 1037-1046
7. Dempsey, C.; Rubio Araiz, A.; Bryson, K. J.; Finucane, O.; Larkin, C.; Mills, E. L.; Robertson, A. A.; Cooper, M. A.; O'Neill, L. A.; Lynch, M. A. *Brain Behav. Immun.* **2017**, *61*, 306-316.
8. Van Hout, G. P.; Bosch, L.; Ellenbroek, G. H.; de Haan, J. J.; van Solinge, W. W.; Cooper, M. A.; Arslan, F.; de Jager, S. C.; Robertson, A. A.; Pasterkamp, G.; Hoefer, I. E. *Eur. Heart J.* **2016**, 38, 828-836
9. Fuster, J. J.; MacLauchlan, S.; Zuriaga, M. A.; Polackal, M. N.; Ostriker, A. C.; Chakraborty, R.; Wu, C. L.; Sano, S.; Muralidharan, S.; Rius, C.; Vuong, J.; Jacob, S.; Muralidhar, V.; Robertson, A. A.; Cooper, M. A.; Andres, V.; Hirschi, K. K.; Martin, K. A.; Walsh, K. *Science* **2017**, 355, 842-847
10. Pinar, A.; Dowling, J. K.; Bitto, N. J.; Robertson, A. A.; Latz, E.; Stewart, C. R.; Drummond, G. R.; Cooper, M. A.; McAuley, J. L.; Tate, M. D.; Mansell, A. *J. Biol. Chem.* **2017**, *292*, 826-836.

11. Gaidt, M. M.; Ebert, T. S.; Chauhan, D.; Schmidt, T.; Schmid-Burgk, J. L.; Rapino, F.; Robertson, A. A.; Cooper, M. A.; Graf, T.; Hornung, V. *Immunity* **2016**, *44*, 833-46.
12. Liu, R. H.; Lin, D.-L.; Chang, W.-T.; Liu, C.; Tsay, W.-I.; Li, J.-H.; Kuo, T.-L., *Anal. Chem.* **2002**, 74, 618–626
13. Mutlib, A. E., *Chem. Res. Toxicol.* **2008**, *21*, 1672-1689.
14. Salla, M.; Butler, M. S.; Pelingon, R.; Kaeslin, G.; Croker, D. E.; Reid, J. C.; Baek, J. M.; Bernhardt, P. V.; Gillam, E. M.; Cooper, M. A.; Robertson, A. A. *ACS Med. Chem. Lett.* **2016**, *7*, 1034-1038.
15. Michaelides, M.; Hansen, T.; Dai, Y.; Zhu, G.; Frey, R.; Gong, J.; Penning, T.; Curtin, M.; Mcclellan, W.; Clark, R.; Torrent, M.; Mastracchio, A.; Kesicki, E. A.; Kluge, A. F.; Patane, M. A.; Ji, Z.; Wang, C. WO Patent 2016044770, 2015.
16. Knölker, H. J.; Braxmeier, T.; Schlechtingen, G. *Angew. Chem.* **1995**, *34*, 2497-2500.
17. Urban, F. J.; John Jasys, V.; Raggon, J. W.; Buzon, R. A.; Hill, P. D.; Eggler, J. F.; Weaver, J. D. *Synth. Commun.* **2003,** *33*, 2029-2043.
18. Whitcombe, N. J.; Hii, K. K. M.; Gibson, S. E. *Tetrahedron* **2001**, *57*, 7449-7476.

Graphical abstract



Compound-**1**: $R^1 = CH_3$, $R^2 = H$, $R^3 = H$, $R^4 = D$
Compound-**2**: $R^1 = CH_3$, $R^2 = H$, $R_3 = D$, $R^4 = D$
Compound-**3**: $R^1 = CH_3$, $R^2 = D$, $R_3 = H$, $R^4 = H$
Compound-**4**: $R^1 = CD_3$, $R^2 = H$, $R_3 = H$, $R^4 = H$