Supplementary Figure Legends

Supplementary Figure 1

^{1}H NMR spectrum of MCC950

Representative ^{1}H NMR spectrum of MCC950 in DMSO-*d6* (600 Mhz)

Supplementary Figure 2

^{13}C NMR spectrum of MCC950

Representative ^{13}C NMR spectrum of MCC950 in DMSO-*d6* (150 Mhz)

Supplementary Figure 3

COSY NMR spectrum of MCC950

Representative COSY NMR spectrum of MCC950 in DMSO-*d6*

Supplementary Figure 4

HSQC NMR spectrum of MCC950

Representative HSQC NMR spectrum of MCC950 in DMSO-*d6*

Supplementary Figure 5

HMBC NMR spectrum of MCC950

Representative HMBC NMR spectrum of MCC950 in DMSO-*d6*

Supplementary Figure 6

MCC950 inhibits NLRP3 activation in HMDM and PBMC and in BMDM in response to MSU

(**a**) Production of IL-1β and TNF-α from HMDM stimulated with LPS and ATP and treated with MCC950 (1–1,000 nM) measured by ELISA. Cytokine level is normalized to DMSO control treated cells. Data are expressed as mean ± S.E.M of twelve independent experiments carried out in triplicate. Non-linear regression analysis was performed and Log [M] MCC950 vs. normalized response (variable slope) curve is presented. (**b**) Production of IL-1β and TNF-α from PBMC stimulated with LPS and ATP and treated with MCC950 and glyburide measured by ELISA. Cytokine level is normalized to DMSO control treated cells. Data are expressed as mean ± S.E.M of three independent experiments carried out in triplicate. (**c**) Western blots of cell lysates and supernatants from PBMC stimulated with LPS and ATP and treated with MCC950 or glyburide. (**d–g**) Production of IL-1β, LDH, IL-1α and TNF-α from BMDM stimulated with LPS and MSU and treated with MCC950 as measured by ELISA (**d,f,g**) and LDH assay (**e**). Data are expressed as mean ± S.E.M of three independent experiments carried out in triplicate. (**h,i**) Production of IL-1β (**h**) and TNF-α (**i**) from PBMC treated with MCC950 and

stimulated with LPS for 24 h in full medium. Data are expressed as mean ± S.D and are representative of two independent experiments.

Supplementary Figure 7

MCC950 blocks NLRP3-dependent ASC speck formation

Representative images of ASC-cerulean cells treated with MCC950 and stimulated with nigericin, LeuLeu-Ome and lethal toxin. DRAQ5 nuclear stain (red), ASC-cerulean (green).

Supplementary Figure 8

MCC950 inhibits NLRP3 activation in PBMC from individuals with MWS *ex vivo*

Western blots of cell lysates and supernatants from PBMC isolated from individuals with MWS (with the indicated mutations) stimulated with LPS and treated with MCC950.

Supplementary Figure 9

Pharmacokinetics of MCC950

Mean plasma concentration of MCC950 after single dose i.v. and p.o. delivery as determined by LC-MS/MS *n*=3.

Supplementary Table 1

MCC950 structural assignments

Structural assignments from NMR Data of MCC950 recorded in DMSO-*d6*

Supplementary Table 2

Microsomal stability of MCC950

The half-life (T1/2) and % compound remaining after 60 min of MCC950, Testosterone, Diclofenac and Propafenone after incubation with human and mouse liver microsomes**.**

Supplementary Table 3

CYP substrate preparation

Details of preparation of substrates for 5 major CYP isozymes.

Supplementary Table 4

Effect of MCC950 on CYP

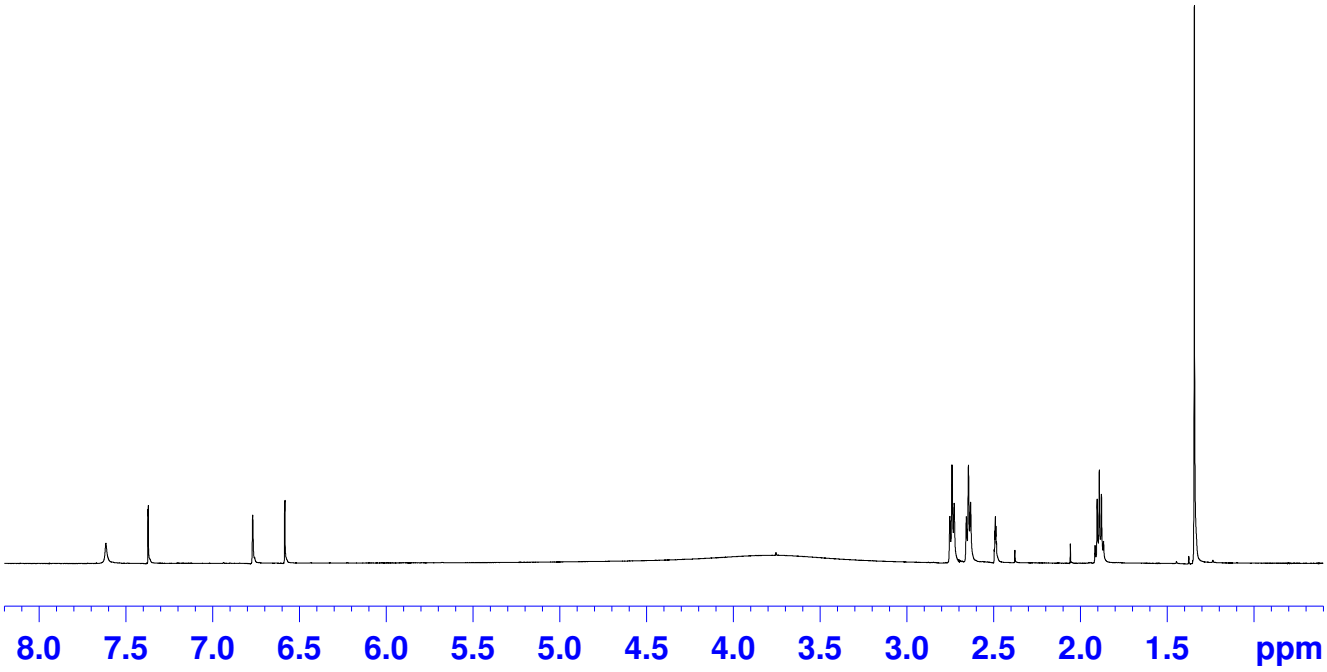
The % inhibition of 5 major CYP isozymes by MCC950 and known substrates after incubation with human liver microsomes.

Supplementary Table 5

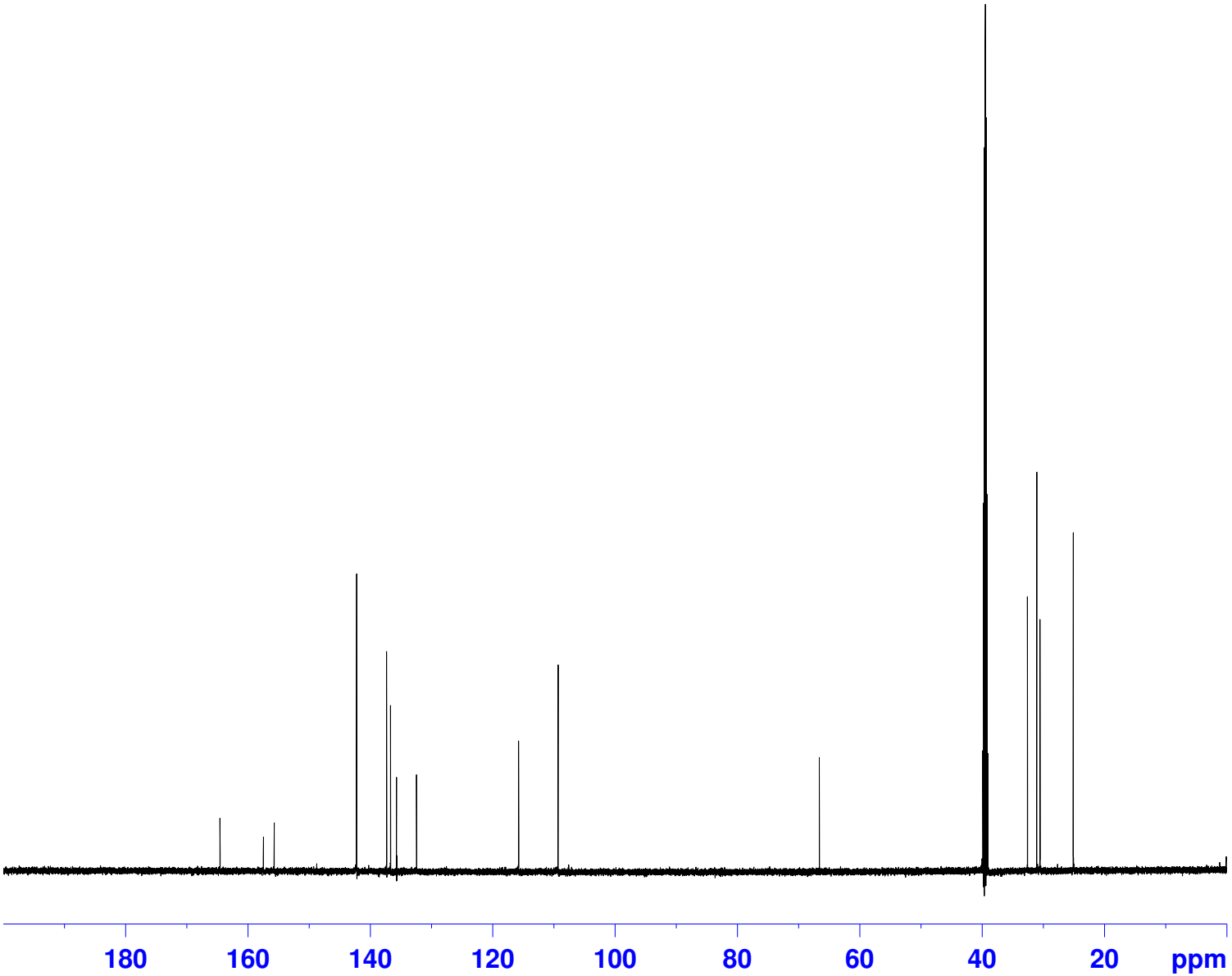
Effect of MCC950 on the hERG channel

The IC_{50} of MCC950 (*n*=3) and amitriptyline (*n*=2) on whole cell hERG currents determined by automated patch clamp method ($QPatch^{HTX}$).

Supplementary Figure 1
^{1}H NMR spectrum of MCC950



Supplementary Figure 2
^{13}C NMR spectrum of MCC950



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PROCNO	1
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SOLVENT	DMSO
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FIDRES	0.596046 Hz
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RG	3649.1
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DE	21.96 usec
TE	298.0 K
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D11	0.03000000 sec
TD0	4
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PL1	-2.40 dB
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SFO1	150.9194078 MHz
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NUC2	1H
PCPD2	70.00 usec
PL2	2.00 dB
PL12	21.06 dB
PL13	120.00 dB
PL2W	9.65199947 W
PL12W	0.11984429 W
PL13W	0.00000000 W
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SI	65536
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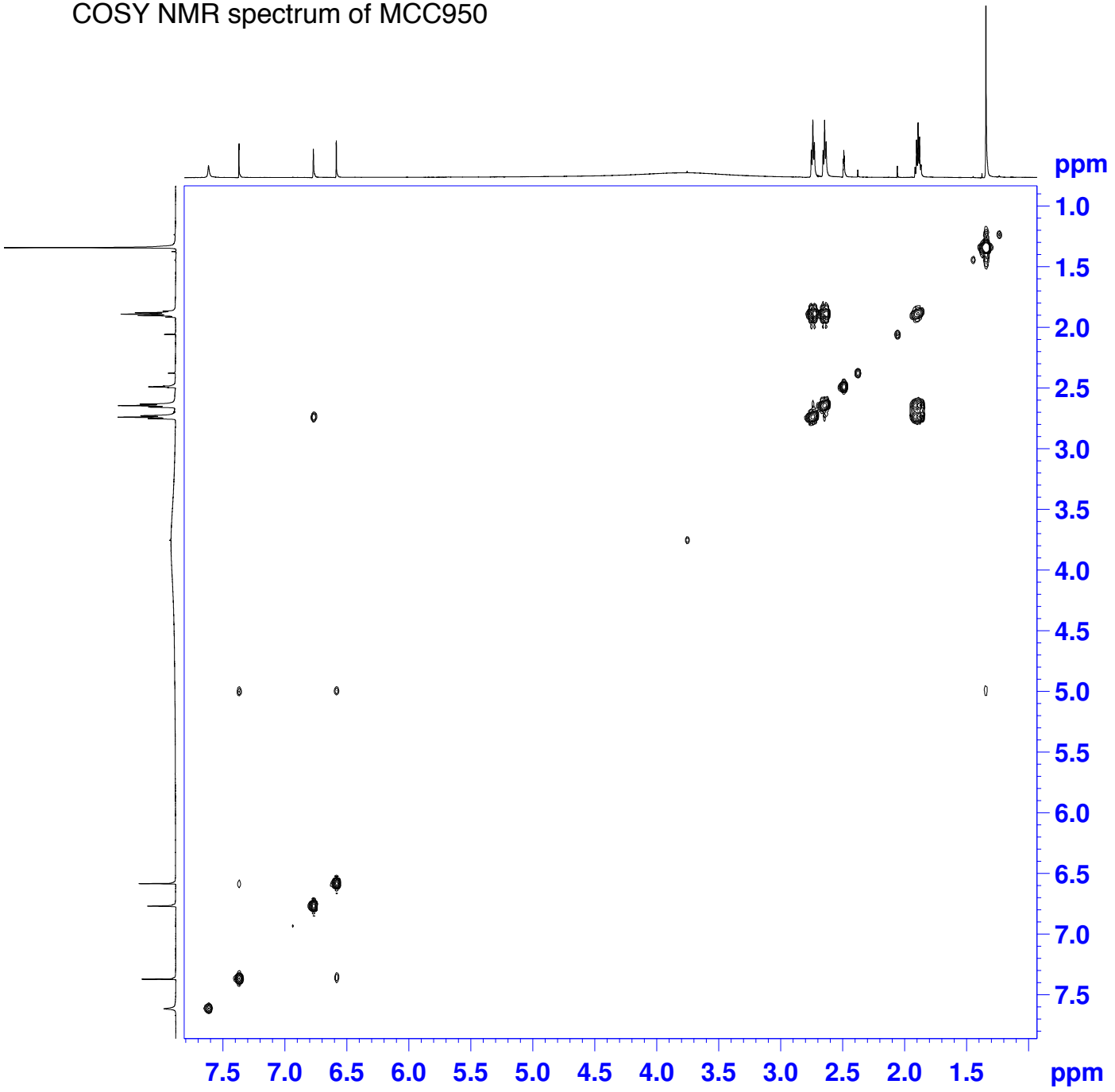
Supplementary Figure 3
COSY NMR spectrum of MCC950



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TD	2048
SOLVENT	DMSO
NS	1
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FIDRES	3.507768 Hz
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RG	512
DW	69.600 usec
DE	12.00 usec
TE	298.0 K
D0	0.00000300 sec
D1	1.00000000 sec
D13	0.00000400 sec
D16	0.00020000 sec
IN0	0.00013920 sec
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P1	7.80 usec
PL1	2.00 dB
PL1W	9.65199947 W
SFO1	600.1330006 MHz
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GPZ1	10.00 %
P16	1000.00 usec
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TD	256
SFO1	600.133 MHz
FIDRES	28.062141 Hz
SW	11.971 ppm
FnMODE	QF
SI	1024
SF	600.1299933 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.00
SI	1024
MC2	QF
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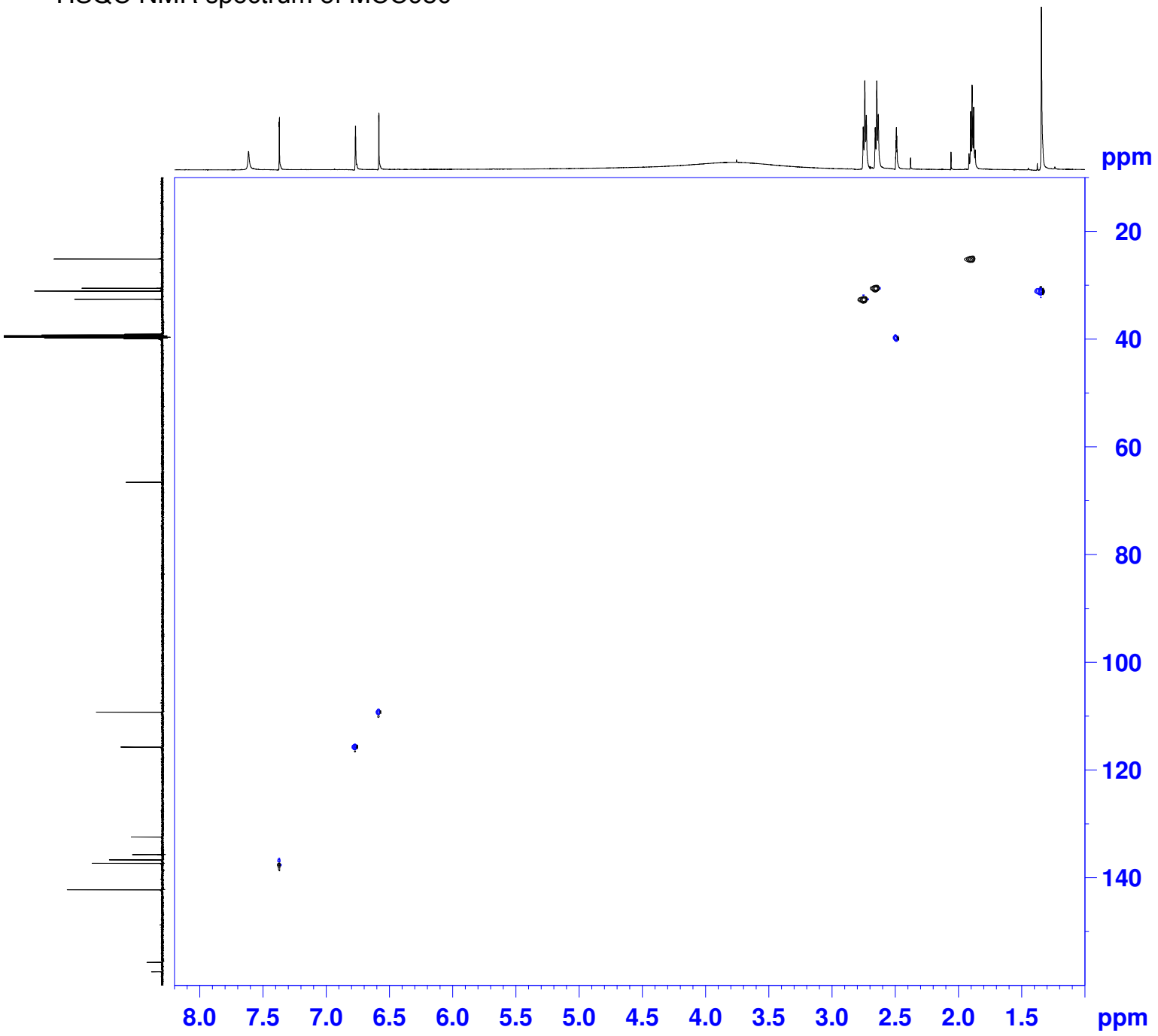
GB	0

Supplementary Figure 4
HSQC NMR spectrum of MCC950

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PROCNO                 1
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TD                  2048
SOLVENT              DMSO
NS                      6
DS                     32
SWH            7183.908 Hz
FIDRES         3.507768 Hz
AQ             0.1426604 sec
RG               14596.5
DW                69.600 usec
DE                12.00 usec
TE                298.0 K
CNST2         135.0000000
CNST17         -0.5000000
D0             0.00000300 sec
D1             1.50000000 sec
D4             0.00185185 sec
D11            0.03000000 sec
D16            0.00020000 sec
D21            0.00370370 sec
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IN0            0.00001950 sec
L31                     1

======== CHANNEL f1 ========
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P1                   7.80 usec
P2                  15.60 usec
P28                  0.00 usec
PL1                  2.00 dB
PL1W          9.65199947 W
SFO1         600.1327006 MHz

======== CHANNEL f2 ========
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P3                  12.00 usec
P14                500.00 usec
P24               2000.00 usec
P63               1500.00 usec
PL0                120.00 dB
PL2                 -2.40 dB
PL12               11.58 dB
PL0W          0.00000000 W
PL2W        120.04185486 W
PL12W         4.80101109 W
SFO2         150.9148808 MHz
SP3                 4.18 dB
SP7                 4.18 dB
SP14                5.66 dB
SP31               11.68 dB
SPNAM3   Crp60,0.5,20.1
SPNAM7      Crp60comp.4
SPNAM14  Crp32,1.5,20.2
SPNAM31  Crp32,1.5,20.2
SPOAL3              0.500
SPOAL7              0.500
SPOAL14             0.500
SPOAL31             0.500
SPOFFS3             0.00 Hz
SPOFFS7             0.00 Hz
SPOFFS14            0.00 Hz
SPOFFS31            0.00 Hz

====== GRADIENT CHANNEL =====
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GPNAM2          sine.100
GPNAM3          sine.100
GPNAM4          sine.100
GPZ1               80.00 %
GPZ2               20.10 %
GPZ3               11.00 %
GPZ4               -5.00 %
P16              1000.00 usec
P19               600.00 usec
ND0                     2
TD                    256
SFO1           150.9149 MHz
FIDRES        100.216911 Hz
SW               170.000 ppm
FnMODE     Echo-Antiecho
SI                   2048
SF          600.1299964 MHz
WDW                 QSINE
SSB                     2
LB                   0.00 Hz
GB                      0
PC                   1.00
SI                   1024
MC2         echo-antiecho
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SSB                     2
LB                   0.00 Hz
GB                      0
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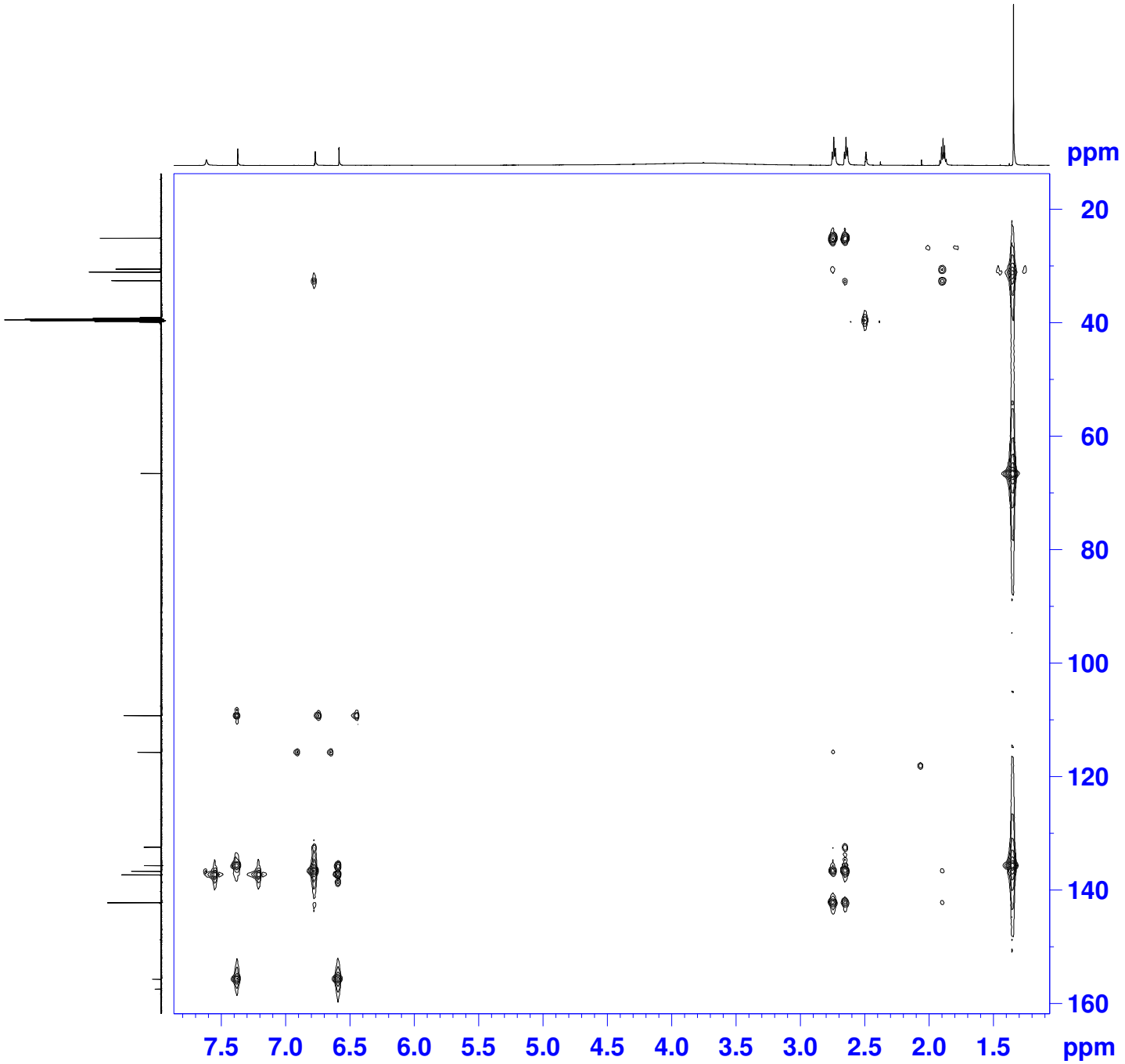
Supplementary Figure 5
HMBC NMR spectrum of MCC950



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TD	4096
SOLVENT	DMSO
NS	4
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SWH	7183.908 Hz
FIDRES	1.753884 Hz
AQ	0.2852012 sec
RG	29193
DW	69.600 usec
DE	12.00 usec
TE	298.0 K
CNST2	135.0000000
CNST13	8.0000000
D0	0.00000300 sec
D1	2.00000000 sec
D2	0.00370370 sec
D6	0.06250000 sec
D16	0.00020000 sec
IN0	0.00001380 sec
======== CHANNEL f1 ========	
NUC1	1H
P1	7.80 usec
P2	15.60 usec
PL1	2.00 dB
PL1W	9.65199947 W
SFO1	600.1330006 MHz
======== CHANNEL f2 ========	
NUC2	13C
P3	12.00 usec
PL2	-2.40 dB
PL2W	120.04185486 W
SFO2	150.9178988 MHz
====== GRADIENT CHANNEL =====	
GPNAM1	sine.100
GPNAM2	sine.100
GPNAM3	sine.100
GPZ1	50.00 %
GPZ2	30.00 %
GPZ3	40.10 %
P16	1000.00 usec
ND0	2
TD	256
SFO1	150.9179 MHz
FIDRES	141.484116 Hz
SW	239.998 ppm
FnMODE	QF
SI	1024
SF	600.1299890 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.00
SI	1024
MC2	QF
SF	150.9028566 MHz
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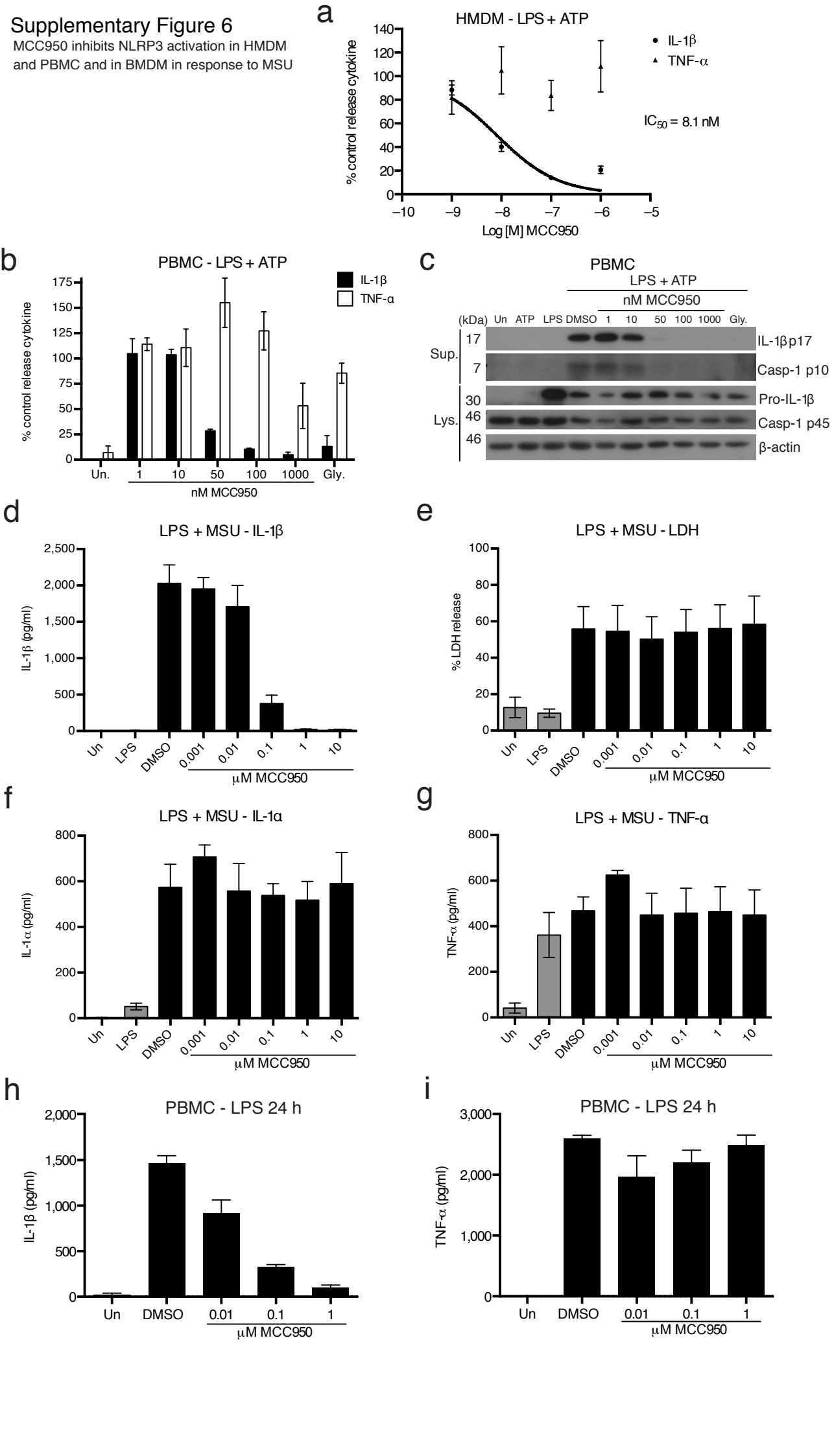
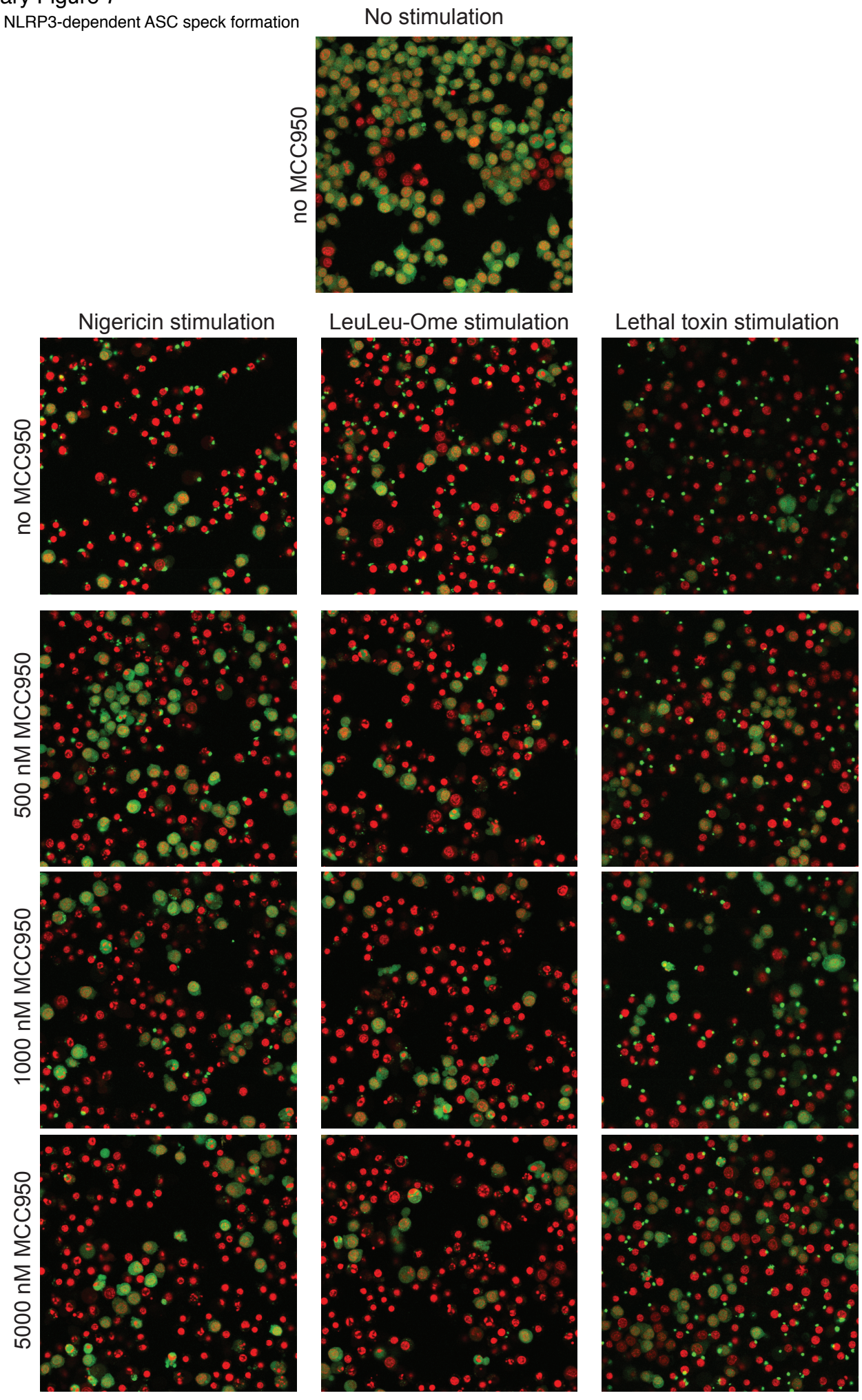
Supplementary Figure 6

MCC950 inhibits NLRP3 activation in HMDM and PBMC and in BMDM in response to MSU



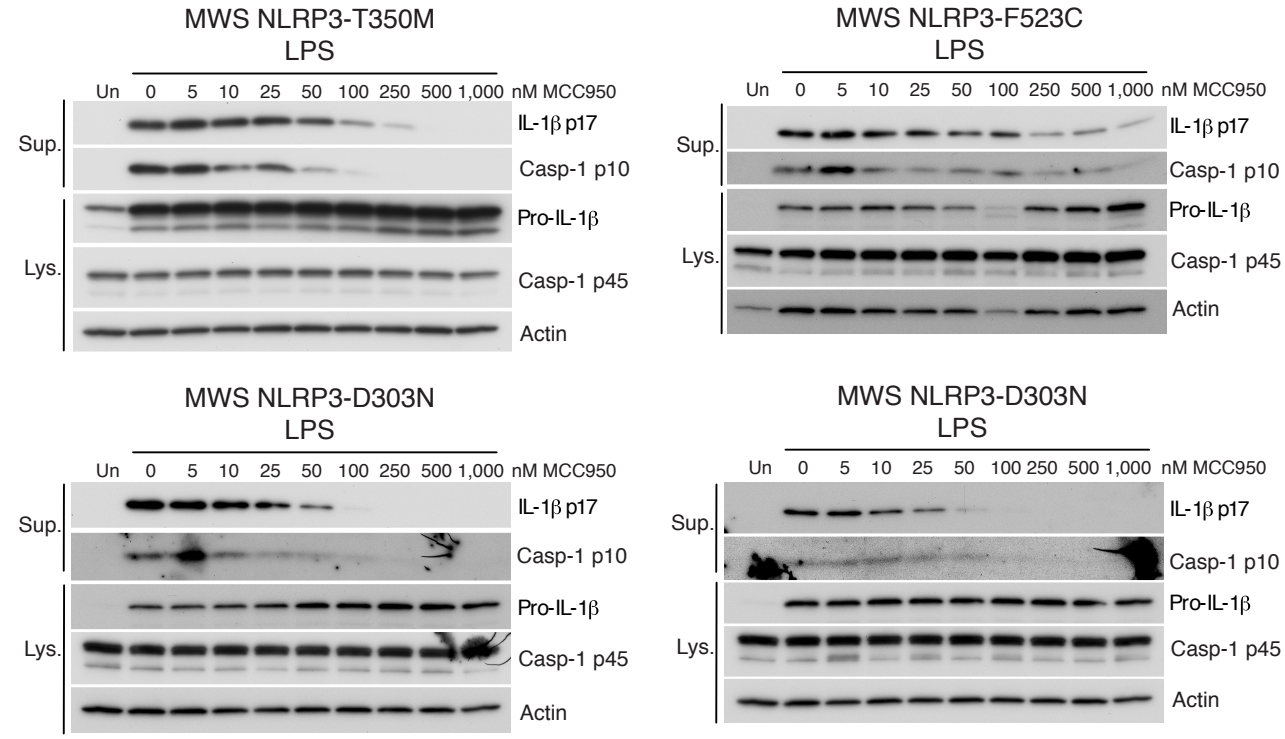
Supplementary Figure 7

MCC950 blocks NLRP3-dependent ASC speck formation



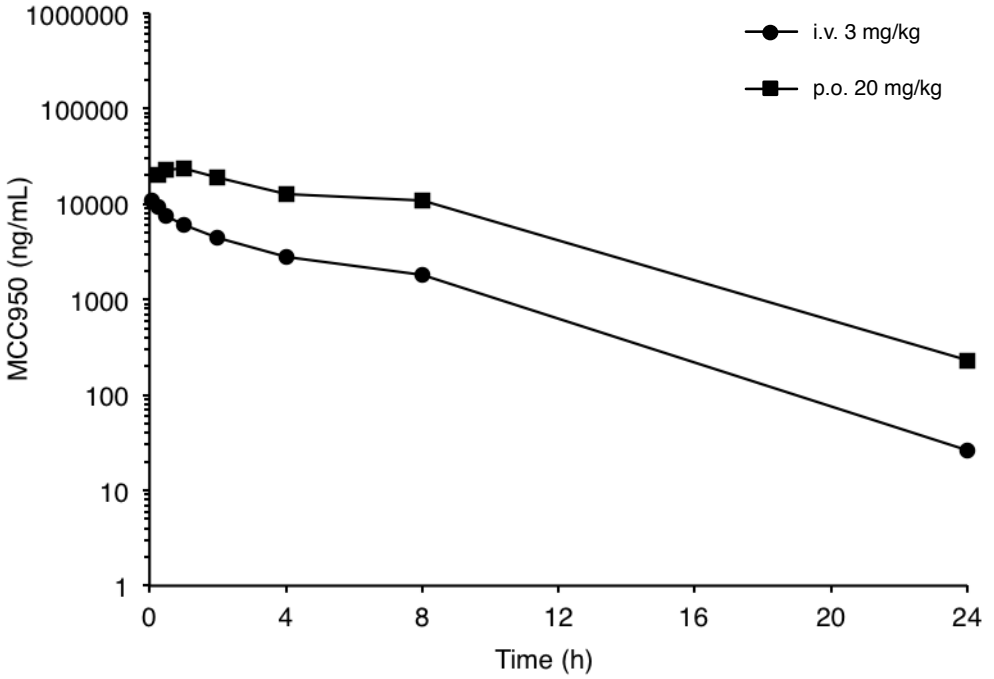
Supplementary Figure 8

MCC950 inhibits NLRP3 activation in PBMC from individuals with MWS *ex vivo*



Supplementary Figure 9

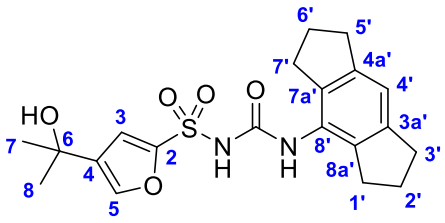
Pharmacokinetics of MCC950



Supplementary Table 1. MCC950 structural assignments

Structural assignments from NMR Data of MCC950 recorded in DMSO-*d6*

position	^{13}C δ_C (ppm)	^{1}H δ_H (mult., *J* (Hz))	HMBC (H to C) (w = weak)
furan			
2	155.7		
3	137.3	7.37 (d, 0.9)	C2, C4, C5,
4	135.7		
5	109.3	6.58 (d, 0.9)	C2, C3, C4, C6 (w),
6	66.6		
7,8	31.1	1.34 (s)	C4, C6, C7/C8
urea CO	157.4		
1,2,3,5,6,7-hexahydro-*s*-indacene			
1',7'	30.6	2.65 (t, 7.3)	C2'/C6', C3'/C5'(w), C3a'/C4a', C7a'/C8a', C8'
2',6'	25.1	1.89 (tt, 7.3, 7.3)	C1'/C7', C3'/C5', C2a'/C6a' (w), C7a'/C8a'(w)
3',5''	32.6	2.74 (t, 7.3)	C2'/C6', C1'/C7'(w), C3a'/C4a', C7a'/C8a', C4'(w)
3a',4a'	142.2		
4'	115.7	6.77 (s)	C3a'/C4a', C3'/C5'
7a',8a'	136.7		
8'	132.4		
8'-NH		7.61 (br s)	C7a'/C8a' (w)



MCC950

Supplementary Table 2 Microsomal stability of MCC950

	Human liver microsomes		Mouse liver microsomes	
	T1/2 (min)	**Remaining (T=60min)**	**T1/2 (min)**	**Remaining (T=60min)**
MCC950	108.3	70.8%	>145	79.1%
Testosterone	9.7	1.3%	2.3	0.5%
Diclofenac	7.2	0.4%	39.6	39.1%
Propafenone	3.9	0.0%	1.0	0.0%

Supplementary Table 3 CYP substrate preparation

CYP	Substrate	Methanol Stock Conc.	Working Conc.	Final Assay Conc.	Vol.
1A2	Phenacetin	20 mM	100 µM	10 µM	15 µL
2C9	Diclofenac	10 mM	50 µM	5 µM	15 µL
2C19	S-mephenytoin	30 mM	300 µM	30 µM	30 µL
2D6	Dextromethorphan	10 mM	50 µM	5 µM	15 µL
3A4	Midazolam	10 mM	20 µM	2 µM	6 µL
2919 µL Phosphate buffer added to give total volume of 3 mL					

Supplementary Table 4 Effect of MCC950 on CYP

CYP	**Metabolite**	**control cpd (3 μM) % inhibition**		**MCC950 (10 μM) %inhibition**
1A2	Acetominophen	α-Naphthoflavone	93.8	5.2
2C9	4'-hydroxydiclofenac	Sulfaphenazole	87.4	14.7
2C19	4'-hydroxymephenytoin	N-3-benzylnirvanol	93.5	5.0
2D6	Dextrorphan	Quinidine	96.1	4.2
3A4	1'-hydroxymidazolam	Ketoconazole	98.8	3.0

Supplementary Table 5 Effect of MCC950 on the hERG channel

Sample	IC50(μM)	SD (μM)	HillSlope	N
Amitriptyline	3.55	0.028	0.81	2
MCC950	>30		0.21	3

Supplementary Methods

Microsomal stability

MCC950 or control compound DMSO stock solution (10 mM) was diluted with 50% aq methanol to a concentration of 100 µM. A 50 µL aliquot of this solution was diluted using potassium phosphate buffer to a concentration of 10 µM. MCC950, compound control or blank solution (10 µL) was incubated at 37 °C with human or mouse liver microsomes (80 µL of 0.7 mg protein/mL phosphate buffer) for 10 min before addition of NADPH regenerating system (10 µL) to start the reaction. Reactions were stopped at 0, 5, 10, 20, 30 and 60 min time-points by addition of 300 µL cold acetonitrile (containing tolbutamide as internal standard at 100 ng/mL) and centrifuged at 4,000 rpm for 20 min. The supernatant (100 µL) was added to water (300 µL) and analyzed by LC/MS/MS. The ratio of peak area of test compound remaining/internal standard was used to determine reduction in concentration of test compound over time. The half-life (T1/2) and % compound remaining after 60 min were then calculated.

Cytochrome P450 inhibition

A working stock solution of MCC950 (10 mM in DMSO) was diluted using phosphate buffer to a concentration of 100 µM. Five inhibitor stock solutions were prepared at a concentration of 3 mM in DMSO: α-naphthoflavone, sulfaphenazole, *N*-3-benzylnirvanol, quinidine and ketoconazole. The inhibitor stocks were diluted using phosphate buffer to a concentration of 30 µM. A cocktail of substrates (phenacetin, diclofenac, S-mephenytoin, dextromethorphan and midazolam) for 5 major CYP isozymes (1A2, 2C9, 2C19, 2D6 and 3A4) was prepared by dilution of methanol stock solutions using phosphate buffer as shown in Supplementary Table 3. MCC950, known inhibitor or blank solution (20 µL of working stock solution) and substrate cocktail solution (20 µL) were incubated at 37 °C with human liver microsomes (140 µL of 0.286 mg/mL in phosphate buffer) for 10 min prior to addition of the co-factor NADPH (20 uL of 10 mM solution in 33 mM $MgCl_2$). Incubation was continued and aliquots were removed at 0, 5, 10, 20, 30 and 60 min then mixed with cold acetonitrile (containing tolbutamide as internal MS standard at 200 ng/mL) and centrifuged at 4,000 rpm for 20 min. The supernatant was analyzed by LC/MS/MS to determine the peak area of metabolite and internal standard. Peak areas determined in the presence and absence of test compound were used to determine % inhibition.

hERG channel assay

Automated patch clamp method ($QPatch^{HTX}$) was used at WuXi AppTech to evaluate the effects of MCC950 on the hERG potassium channel. CHO cells that stably express hERG potassium channels from Aviva Biosciences were used. The IC_{50} of MCC950 and amitriptyline as positive control on whole cell hERG currents were determined.

Pharmacokinetics

Single dose pharmacokinetic parameters were determined at WuXi AppTech using fasted male C57BL/6 mice (3 mice per group, 2 groups). Groups were dosed i.v. (3 mg/kg) or p.o. (20 mg/kg) and blood samples taken *via* submandibular or saphenous vein at the following time points post dosing: 0.083 (i.v. only), 0.25, 0.5, 1, 2, 4, 8 and 24 h. An aliquot of 7 μL of blood plasma was protein precipitated with 70 μL internal standard solution (100 ng/mL diclofenac & 100 ng/mL tolbutamide in MeOH), the mixture was vortex-mixed for 1 min and centrifuged at 13,000 rpm for 15 min. The supernatant (70 μL) was then mixed with 210 μL water/methanol (v:v, 50:50), vortex-mixed for 15 min and centrifuged at 4,000 rpm for 10 min at 4 °C. The concentration of MCC950 in the plasma samples was determined using LC-MS/MS with the peak areas compared to a calibration curve determined using 3-3,000 ng/mL MCC950 in male C57BL/6 mouse plasma.