Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information:

Dynamic changes in metabolic profiles of rats subchronically exposed to mequindox

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Supplementary Table 1 Body weights for control and mequindox treated rats

weeks post initial dose	Control	Low-dose	Mid-dose		High-dose	
0	239.6 ± 11.9	240.2 ± 13.5	228.9 ± 12.6	*	232.8 ± 8.4	
1	257.2 ± 13.4	251.5 ± 15.0	237.5 ± 13.0	**	232.9 ± 12.0	***
3	273.1 ± 15.8	268.3 ± 16.3	247.8 ± 15.0	***	241.9 ± 13.1	***
4	290.3 ± 19.4	285.7 ± 18.8	257.5 ± 13.2	***	252.1 ± 15.2	***
5	294.1 ± 22.8	287.3 ± 18.9	259.3 ± 13.3	***	253.1 ± 16.9	***
7	312.0 ± 19.9	306.7 ± 20.2	272.3 ± 16.2	***	265.5 ± 18.5	***
8	313.6 ± 20.8	310.9 ± 21.6	274.1 ± 13.8	***	265.8 ± 18.1	***
10	321.1 ± 21.4	318.2 ± 22.1	278.9 ± 14.3	***	268.8 ± 18.2	***
11	319.5 ± 23.9	316.8 ± 23.6	275.5 ± 14.4	***	267.3 ± 17.5	***
12	330.8 ± 26.6	326.4 ± 25.6	282.4 ± 15.5	***	273.4 ± 16.4	***
13	316.8 ± 24.9	318.0 ± 23.3	272.9 ± 14.4	***	262.9 ± 16.2	***
14	329.3 ± 24.1	326.6 ± 22.6	286.9 ± 17.0	***	280.2 ± 17.0	***
15	321.8 ± 24.4	332.2 ± 24.8	297.3 ± 15.3	**	284.7 ± 16.6	**

All values were presented as mean ± standard deviation. * significant different from control group at $p<0.05$, ** significant different from control group at $p<0.01$, *** significant different from control group at $p<0.001$.

Supplementary Table 2 Serum clinical chemistry data for control and mequindox treated rats

	Control	Low-dose	Mid-dose	High-dose
5 weeks post initial dose				
ALT (IU/L)	37.5±3.8	37.1±19.8	31.6±4.0	36.2±6.1
AST (IU/L)	176.7±22.2	170.2±56.0	154.0±39.2	174.3±29.2
ALP (IU/L)	70.7±12.9	70.1±16.2	65.3±8.4	80.6±9.7
TP (g/L)	68.6±3.7	66.5±3.3	72.1±3.5**	68.9±1.6
ALB (g/L)	45.5±2.2	44.3±2.6	49.2±2.6***	47.4±1.3*
Crea (μmol/L)	54.9±2.7	51.9±3.0*	54.0±5.4	56.3±2.6
TG (mmol/L)	0.57±0.13	0.44±0.09*	0.50±0.10	0.51±0.07
TC (mmol/L)	1.62±0.28	1.58±0.31	1.85±0.33	1.81±0.41
Glc (mmol/L)	4.7±0.5	5.3±0.7	5.1±0.7	5.3±0.4
13 weeks post initial dose				
ALT (IU/L)	35.3±6.8	35.3±10.7	31.9±7.8	29.8±5.3
AST (IU/L)	140.8±11.4	166.5±33.4*	136.5±29.5	130.0±22.1
ALP (IU/L)	57.8±12.7	51.1±7.8	47.7±9.2	69.3±14.6
TP (g/L)	67.2±3.6	68.0±3.0	71.5±3.9**	69.0±2.9
ALB (g/L)	43.4±2.9	44.5±3.4	47.6±3.5***	46.6±2.1*
Crea (μmol/L)	56.5±4.3	55.4±4.7	56.3±2.8	54.3±4.2
TG (mmol/L)	0.42±0.08	0.43±0.16	0.34±0.08	0.32±0.04*
TC (mmol/L)	1.53±0.25	1.42±0.29	1.41±0.41	1.42±0.24
Glc (mmol/L)	6.4±0.9	6.4±0.6	6.2±0.8	5.9±1.9
15 weeks post initial dose				
ALT (IU/L)	30.8±3.9	33.4±2.4	35.6±7.7	30.2±7.9
AST (IU/L)	160.2±30.9	165.6±26.5	186.0±52.1	144.3±42.1
ALP (IU/L)	41.6±4.6	39.4±9.7	44.4±11.1	57.0±9.2***
TP (g/L)	67.9±3.2	68.4±2.8	68.0±2.7	71.2±4.0*
ALB (g/L)	44.3±2.4	44.6±2.6	44.8±2.6	46.0±2.4
Crea (μmol/L)	56.5±2.8	55.6±3.6	54.5±3.4	54.3±2.5
TG (mmol/L)	0.76±0.26	0.53±0.12***	0.52±0.12***	0.42±0.06***
TC (mmol/L)	1.44±0.31	1.55±0.28	1.57±0.30	1.63±0.37
Glc (mmol/L)	5.5±0.4	6.4±0.8***	6.1±0.6*	6.3±0.7*

All values were presented as mean ± standard deviation. * significant different from control group at $p<0.05$, ** significant different from control group at $p<0.01$, *** significant different from control group at $p<0.001$.

Supplementary Table 3 ^{1}H NMR data for metabolites in rat urine, plasma and liver tissue extracts

Keys	metabolites	moieties	δ^{1}H[a]	sites[b]	
L1	lipid	C**H**$_3$	0.85(m)	P	La
L2	lipid	(C**H**$_2$)$_n$	1.27(m)	P	La
L3	lipid	C**H**$_2$CH$_2$CO	1.57(m)	P	La
L4	lipid	C**H**$_2$CH=CH	2.02(m)	P	La
L5	lipid	CH$_2$C**H**$_2$CO	2.24(m)	P	La
L6	lipid	C=CC**H**$_2$C=C	2.77(m)	P	La
L7	lipid	-C**H**=C**H**-	5.30(m)	P	La
1	bile acids	18-C C**H**$_3$	0.72(s)	U	
		18-C C**H**$_3$	0.74(s)		La
		18-C C**H**$_3$	0.77(s)	U	
		18-C C**H**$_3$	0.78(s)	U	
2	octanoate	C**H**$_3$	0.88(t)	U	
		4~7-C**H**$_2$	1.31(m)		
		3-C**H**$_2$	1.56(m)		
		2-C**H**$_2$	2.18(t)		
3	isovalerylglycine	C**H**$_3$	0.94(d)	U	
		C**H**	2.01(m)		
		C**H**$_2$	2.17(m)		
		NC**H**$_2$	3.76(#)		
4	isoleucine	αC**H**	3.67(d)	P	La
		βC**H**	1.97(m)		
		half γC**H**$_2$	1.26(m)		
		half γC**H**$_2$	1.46(m)		
		γ'C**H**$_3$	1.00(d)		
		δC**H**$_3$	0.93(t)		
5	leucine	αC**H**	3.73(t)	P	La
		βC**H**$_2$	1.72(m)		
		γC**H**	1.69(m)		
		δC**H**$_3$	0.96(d)		
		δ'C**H**$_3$	0.95(d)		
6	valine	αC**H**	3.60(d)	P	La
		βC**H**	2.27(m)		
		γC**H**$_3$	1.04(d)		
		γ'C**H**$_3$	0.98(d)		
7	dihydrothymine	C**H**$_3$	1.06(d)	P	

		CH	2.48(m)			
		half C**H**$_2$	3.53(dd)			
		half C**H**$_2$	3.69(dd)			
8	3-D-hydroxybutyrate	half αC**H**$_2$	2.30(dd)		P	La
		half αC**H**$_2$	2.40(dd)			
		βC**H**	4.15(m)			
		γC**H**$_3$	1.19(d)			
9	lactate	αC**H**	4.13(q)	U	P	La
		βC**H**$_3$	1.34(d)			
10	threonine	αC**H**	3.60(d)	U	P	La
		βC**H**	4.27(m)			
		γC**H**$_3$	1.34(d)			
11	α-hydroxyisobutyrate	C**H**$_3$	1.36(s)	U		
12	alanine	αC**H**	3.79(q)	U	P	La
		βC**H**$_3$	1.49(d)			
13	lysine	αC**H**	3.75(t)		P	La
		βC**H**$_2$	1.90(m)			
		γC**H**$_2$	1.46(m)			
		δC**H**$_2$	1.72(m)			
		εC**H**$_2$	3.02(t)			
14	ornithine	αC**H**	3.77(t)		P	La
		βC**H**$_2$	1.93(m)			
		γC**H**$_2$	1.75(m)			
		δC**H**$_2$	3.05(t)			
15	acetate	C**H**$_3$	1.93(s)	U	P	La
16	*N*-acetyl glycoproteins	C**H**$_3$	1.99(s)	U		
		C**H**$_3$	2.02-2.08(s)	U		
		C**H**$_3$	2.04(s)		P	
17	glutamate	αC**H**	3.76(t)		P	La
		βC**H**$_2$	2.05(m)			
		γC**H**$_2$	2.35(m)			
18	methionine	αC**H**	3.85(m)		P	
		βC**H**$_2$	2.14(m)			
		γC**H**$_2$	2.64(t)			
		δC**H**$_3$	2.13(s)			
19	*O*-acetyl glycoprotein	C**H**$_3$	2.13(s)		P	
20	glutamine	αC**H**	3.76(t)		P	La
		βC**H**$_2$	2.14(m)			
		γC**H**$_2$	2.45(m)			
21	oxidized glutathione	glutamate βC**H**$_2$	2.17(m)			La

		glutamate γC**H**$_2$	2.54(m)			
		glutamate αC**H**	3.78(t)			
		cysteine half βC**H**$_2$	2.98(dd)			
		cysteine half βC**H**$_2$	3.32(dd)			
		cysteine αC**H**	4.74(#)			
		glycine C**H**$_2$	3.78(s)			
22	acetone	C**H**$_3$	2.22(s)		P	
23	acetoacetate	C**H**$_3$	2.27(s)		P	
		C**H**$_2$	3.43(s)			
24	*p*-cresol glucuronide	2,6-C**H**	7.05(d)	U		
		3,5-C**H**	7.22(d)			
		C**H**$_3$	2.30(s)			
		6'-C**H**	5.08(d)			
		5'-C**H**	3.61(#)			
		4'-C**H**	3.89(#)			
25	pyruvate	C**H**$_3$	2.36(s)		P	
26	malate	half C**H**$_2$	2.39(dd)	U		
		half C**H**$_2$	2.68(dd)			
		C**H**	4.31(dd)			
27	succinate	C**H**	2.41(s)	U		La
28	2-oxoglutarate	βC**H**$_2$	3.02(t)	U		
		γC**H**$_2$	2.45(t)			
29	3-(3-hydroxyphenyl)propionate	C**H**$_2$COO	2.48(t)	U		
		C**H**$_2$	2.84(t)			
		6-C**H**	6.76(d)			
		2-C**H**	6.81(s)			
		4-C**H**	6.88(d)			
		5-C**H**	7.25(t)			
30	citrate	half C**H**$_2$	2.55(d)	U	P	
		half C**H**$_2$	2.68(d)			
31	methylamine	C**H**$_3$	2.61(s)	U		
32	hypotaurine	C**H**$_2$S	2.66(t)			La
		C**H**$_2$N	3.36(t)			
33	aspartate	αC**H**	3.90(dd)			La
		half βC**H**$_2$	2.67(dd)			
		half βC**H**$_2$	2.81(dd)			
34	dimethylamine	C**H**$_3$	2.72(s)	U		La
35	asparagine	half βC**H**$_2$	2.84(dd)		P	
		half βC**H**$_2$	2.94(dd)			
		αC**H**	3.98(dd)			
36	trimethylamine	C**H**$_3$	2.88(s)			La
37	*N,N*-dimethylglycine	C**H**$_3$	2.93(s)	U		

		$C\mathbf{H}_2$	3.73(s)			
38	creatine	$C\mathbf{H}_3$	3.03(s)		P	La
		$C\mathbf{H}_2$	3.93(s)			
39	creatinine	$C\mathbf{H}_3$	3.05(s)	U		
		$C\mathbf{H}_2$	4.06(s)			
40	ethanolamine	$C\mathbf{H}_2NH_2$	3.15(t)			La
		$C\mathbf{H}_2OH$	3.84(t)			
41	choline	$N(C\mathbf{H}_3)_3$	3.20(s)		P	La
		$NC\mathbf{H}_2$	3.51(m)			
		$OC\mathbf{H}_2$	4.06(m)			
42	phosphocholine	$N(C\mathbf{H}_3)_3$	3.21(s)		P	La
		$NC\mathbf{H}_2$	3.59(m)			
		$OC\mathbf{H}_2$	4.16(m)			
43	glycerophosphocholine	$N(C\mathbf{H}_3)_3$	3.22(s)		P	La
		$NC\mathbf{H}_2$	3.63(m)			
		$OC\mathbf{H}_2$	4.30(m)			
44	β-glucose	1-C**H**	4.64(d)		P	La
		2-C**H**	3.24(dd)			
		3-C**H**	3.49(t)			
		4-C**H**	3.40(dd)			
		5-C**H**	3.46(ddd)			
		half6-$C\mathbf{H}_2$	3.72(dd)			
		half6-$C\mathbf{H}_2$	3.89(dd)			
45	α-glucose	1-C**H**	5.23(d)		P	La
		2-C**H**	3.53(dd)			
		3-C**H**	3.71(dd)			
		4-C**H**	3.41(dd)			
		5-C**H**	3.83(m)			
		half6-$C\mathbf{H}_2$	3.76(dd)			
		half6-$C\mathbf{H}_2$	3.84(dd)			
46	betaine	$C\mathbf{H}_3$	3.27(s)	U	P	La
		$C\mathbf{H}_2$	3.90(s)			
47	trimethylamine *N*-oxide	$C\mathbf{H}_3$	3.27(s)	U		
48	taurine	$C\mathbf{H}_2S$	3.28(t)	U	P	La
		$C\mathbf{H}_2N$	3.43(t)			
49	methanol	$C\mathbf{H}_3$	3.36(s)			La
50	glycogen	1-C**H**	5.40-5.43(m)			La
		2~6 C**H**	3.43-4.00(m)			
51	glycerol	half $C\mathbf{H}_2$	3.56(dd)		P	
		half $C\mathbf{H}_2$	3.65(dd)			
		C**H**	3.79(m)			

52	glycine	α**CH**$_2$	3.57(s)	U	P	La
53	phenylacetylglycine	**CH**$_2$CO	3.68(s)	U		
		CH$_2$COO	3.76(d)			
		m-C**H**	7.42(m)			
		o,p-C**H**	7.36(m)			
		N**H**	8.03(m)			
54	guanidoacetate	**CH**$_2$	3.80(s)	U		
55	hippurate	**CH**$_2$NH	3.97(d)	U		
		m-C**H**	7.56(t)			
		p-C**H**	7.64(t)			
		o-C**H**	7.84(d)			
		N**H**	8.55(bs)			
56	cytidine	2'-C**H**(ribose)	4.30(#)			La
		1'-C**H**(ribose)	5.92(d)			
		5-C**H**(ring)	6.07(d)			
		6-C**H**(ring)	7.85(d)			
57	uridine	2'-C**H**(ribose)	4.35(dd)			La
		5-C**H**(ring)	5.91(d)			
		1'-C**H**(ribose)	5.92(d)			
		6-C**H**(ring)	7.88(d)			
58	adenosine	3'-C**H**(ribose)	4.45(t)			La
		1'-C**H**(ribose)	6.10(d)			
		8-C**H**(ring)	8.24(s)			
		2-C**H**(ring)	8.35(s)			
59	1-methylnicotinate	**CH**$_3$	4.44(s)	U		
		5-C**H**	8.08(m)			
		4-C**H**	8.83(d)			
		6-C**H**	8.85(d)			
		2-C**H**	9.13(s)			
60	mannose	1-C**H**	5.19(d)			La
61	allantoin	C**H**	5.39(s)	U		
		N**H**	7.40(#)			
62	urea	N**H**$_2$	5.82(bs)	U		
63	uracil	5-C**H**	5.81(d)	U		La
		6-C**H**	7.54(d)			
64	fumarate	C**H**	6.53(s)	U		La
65	tyrosine	3,5-C**H**	6.90(d)		P	La
		2,6-C**H**	7.19(d)			
66	histidine	5-C**H**	7.06(s)		P	La
		2-C**H**	7.79(s)			
67	phenylalanine	2,6-C**H**	7.32(m)		P	La
		4-C**H**	7.37(m)			

		3,5-C**H**	7.42(m)			
68	indoxyl sulfate	2-C**H**	7.36(s)	U		
		4-C**H**	7.71(d)			
		7-C**H**	7.51(d)			
		5-C**H**	7.21(t)			
		6-C**H**	7.28(t)			
69	nicotinamide	5-C**H**	7.59(ddd)			La
		4-C**H**	8.24(dd)			
		6-C**H**	8.70(dd)			
		2-C**H**	8.93(dd)			
70	pseudouridine	C**H**NH	7.68(s)	U		
71	xanthine	8-C**H**	7.90(s)			La
72	hypoxanthine	2-C**H**	8.20(s)			La
		8-C**H**	8.22(s)			
73	nicotinamide mononucleotide	5-C**H**	8.30(t)			La
		4-C**H**	9.00(d)			
		6-C**H**	9.31(d)			
		2-C**H**	9.64(s)			
74	formate	C**H**	8.46(s)	U	P	La

[a] multiplicity for ^{1}H resonances: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; ddd, doublet of doublets of doublets. #: signals were not determined. [b]U: urine; P: plasma; La: liver extract.

Supplementary Table 4 Summaries of OPLS-DA and corresponding PLS-DA models derived from all the ^{1}H NMR spectra acquired for urine, plasma and liver tissue extracts from control and mequindox treated rats. Keys: C, control group; L, low-dose group; M, mid-dose group; H, high-dose group. Time: week 0, 2, 5, 7, 9, 11, 13: 0, 2, 5, 7, 9, 11, 13 weeks post initial dose; week 15, 2 weeks after the ceasing of mequindox treatment

time post initial dose /week	comparison	OPLS-DA			PLS-DA
		R^2X	Q^2Y	CV-ANOVA *p* value	permutation test*
Urine					
0	L vs. C	0.245	0.096	7.32E-01	n.s.
	M vs. C	0.254	-0.073	1.00E+00	n.s.
	H vs. C	0.287	0.209	3.22E-01	n.s.
2	L vs. C	0.273	0.809	1.32E-06	s.
	M vs. C	0.322	0.772	2.68E-05	s.
	H vs. C	0.287	0.911	9.95E-10	s.
5	L vs. C	0.258	0.845	1.87E-07	s.
	M vs. C	0.247	0.896	4.34E-09	s.
	H vs. C	0.247	0.939	2.82E-11	s.
7	L vs. C	0.289	0.767	3.14E-05	s.
	M vs. C	0.323	0.775	1.16E-05	s.
	H vs. C	0.301	0.907	1.42E-08	s.
9	L vs. C	0.304	0.800	1.93E-06	s.
	M vs. C	0.289	0.805	3.43E-06	s.
	H vs. C	0.332	0.925	1.92E-10	s.
11	L vs. C	0.249	0.677	2.69E-04	s.
	M vs. C	0.268	0.767	1.63E-05	s.
	H vs. C	0.316	0.893	4.68E-08	s.
13	L vs. C	0.233	0.604	1.02E-03	s.
	M vs. C	0.207	0.715	5.09E-05	s.
	H vs. C	0.296	0.921	3.14E-10	s.
15	L vs. C	0.282	0.287	1.50E-01	n.s.
	M vs. C	0.271	0.470	1.73E-02	n.s.
	H vs. C	0.281	0.306	1.61E-01	n.s.
Plasma					
5	L vs. C	0.318	0.268	1.83E-01	n.s.
	M vs. C	0.306	0.518	5.80E-03	s.
	H vs. C	0.336	0.734	2.72E-05	s.
13	L vs. C	0.282	0.179	4.16E-01	n.s.
	M vs. C	0.294	0.839	2.67E-07	s.
	H vs. C	0.299	0.792	2.82E-06	s.
15	L vs. C	0.308	0.413	3.12E-02	n.s.
	M vs. C	0.363	0.462	1.49E-02	n.s.
	H vs. C	0.390	0.739	2.28E-05	s.

Liver extract					
5	L vs. C	0.342	0.179	4.15E-01	n.s.
	M vs. C	0.230	0.638	4.58E-04	s.
	H vs. C	0.438	0.767	8.04E-06	s.
13	L vs. C	0.322	-0.046	1.00E+00	n.s.
	M vs. C	0.511	0.581	1.70E-03	s.
	H vs. C	0.457	0.804	1.60E-06	s.
15	L vs. C	0.483	0.406	3.46E-02	n.s.
	M vs. C	0.479	0.164	4.68E-01	n.s.
	H vs. C	0.362	0.471	1.28E-02	n.s.

*: n.s: non-significant; s: significant.