

TDMQ20, a specific copper chelator, reduces memory impairments in AD-mouse models

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Supporting Information

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Duplicate of the novel object recognition (NOR) test by icv-CuA β and hippo-CuA β non-transgenic mice, after TDMQ20 or CQ treatment.

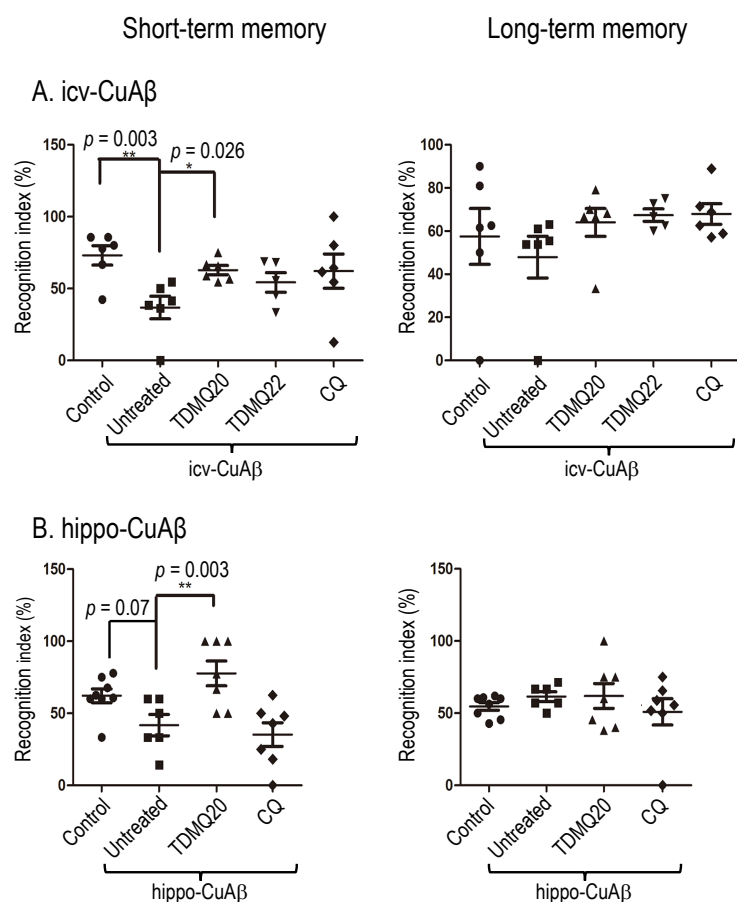


Figure S1. Declarative memory evaluated by the recognition index $[(n_Y/n_{X+Y}) \times 100]$ for short term memory or $[(n_Z/n_{X+Z}) \times 100]$ for long-term memory in the NOR task in the icv-CuA β (panel A, n=6) and the hippo-CuA β (panel B, n=7) non-transgenic mouse models. See SI Appendix, and Figure 2 for full details. Each mark represents the result obtained with a single mouse; horizontal lines represent mean values \pm SEM. ** $p < 0.01$, * $p < 0.05$ vs. the untreated AD group in the ANOVA.

Raw data and statistical analysis

Figure 2 (A) - Short-term recognition index of icv-CuA β mice in the NOR test

Raw data

Recognition index for short-term memory				
Control	AD	TDMQ20	Clioquinol	
72.73	56.52	75.00	60.00	
45.45	33.33	57.14	50.00	
50.00	0.00	50.00	44.44	
50.00	42.86	53.33	46.67	
58.82	20.00	64.29	40.00	
80.00	30.00	71.43	50.00	
58.33	50.00	80.00		

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.252	6	0.2*	0.886	6	0.299
AD	0.157	6	0.2*	0.987	6	0.979
TDMQ20	0.181	6	0.2*	0.938	6	0.646
CQ	0.247	6	0.2*	0.946	6	0.709

* This is a lower bound of the true significance.

a. Rielly's significance correction

ANOVA

		quadratic sum	degree freedom	mean square	F	significance
VAR00002	inter-group	3990.668	3	1330.223	7.289	0.001
	intra-group	4197.497	23	182.5		
	amount to	8188.165	26			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	signific ance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	26.08857*	7.22100	.001	11.1508	41.0263
	TDMQ20	-5.12286	7.22100	.485	-20.0606	9.8149
	CQ	10.81452	7.51585	.164	-4.7332	26.3623
AD	Control	-26.08857*	7.22100	.001	-41.0263	-11.1508
	TDMQ20	-31.21143*	7.22100	.000	-46.1492	-16.2737
	CQ	-15.27405	7.51585	.054	-30.8218	.2737
TDMQ20	Control	5.12286	7.22100	.485	-9.8149	20.0606
	AD	31.21143*	7.22100	.000	16.2737	46.1492
	CQ	15.93738*	7.51585	.045	.3897	31.4851
CQ	Control	-10.81452	7.51585	.164	-26.3623	4.7332
	AD	15.27405	7.51585	.054	-.2737	30.8218
	TDMQ20	-15.93738*	7.51585	.045	-31.4851	-.3897

*. The mean difference of the post-test was significant at the 0.05 level.

Figure 2 (A) - Long-term recognition index of icv-CuA β mice in the NOR test**Raw data**

Recognition index for long-term memory				
Control	AD	TDMQ20	Clioquinol	
60.00	56.25	64.29	66.67	
60.00	66.67	50.00	50.00	
50.00	100.00	53.33	53.33	
52.63	50.00	59.09	50.00	
57.14	66.67	50.00	55.56	
78.57	40.00	72.73	62.50	
54.55	0.00	100.00		

Normality test

	Kolmogorov-Sminov(V) ^a			Shapiro-wilk		
	statistics	degree of freedom	significance	statistics	degree of freedom	significance
Control	0.322	6	0.051	0.845	6	0.142
AD	0.268	6	0.2*	0.912	6	0.452
TDMQ20	0.207	6	0.2*	0.899	6	0.369
CQ	0.212	6	0.2*	0.886	6	0.3

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

		quadratic sum	degree of freedom	mean square	F	significance
VAR00002	inter-group	385.274	3	128.425	0.359	0.783
	intra-group	8235.942	23	358.084		
	amount to	8621.216	26			

VAR00002

LSD

Multiple comparisons (LSD)

(I) VAR00001	(J) VAR00001	Mean difference (I-J)	Standard error	significance	95% confidence interval	
					Lower limit	Upper limit
Control	AD	-2.02	1.12	0.091	-4.39	0.36
	TDMQ20	0.11	1.12	0.926	-2.27	2.48
	CQ	0.42	1.12	0.714	-1.96	2.79
AD	Control	2.02	1.12	0.091	-0.36	4.39
	TDMQ20	2.12	1.12	0.076	-0.25	4.50
	CQ	2.43*	1.12	0.045	0.06	4.81
TDMQ20	Control	-0.11	1.12	0.926	-2.48	2.27
	AD	-2.12	1.12	0.076	-4.50	0.25
	CQ	0.31	1.12	0.784	-2.06	2.69
CQ	Control	-0.42	1.12	0.714	-2.79	1.96
	AD	-2.43*	1.12	0.045	-4.81	-0.06
	TDMQ20	-0.31	1.12	0.784	-2.69	2.06

*The mean difference of the post-test was significant at the 0.05 level.

Figure 2 (B) - Short-term recognition index of hippo-CuA β mice in the NOR test**Raw data**

Recognition index for short-term memory				
Control	AD	TDMQ20	Clioquinol	
75.00	30.00	50.00	57.14	
77.78	66.67	50.00	50.00	
75.00	46.15	50.00	0.00	
71.43	50.00	73.91	0.00	
50.00	45.00	77.78	50.00	
71.43	33.33	60.00	50.00	
63.64	52.38	80.00	50.00	

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.307	7	0.045	0.821	7	0.066
AD	0.175	7	0.2*	0.954	7	0.77
TDMQ20	0.257	7	0.178	0.814	7	0.056
CQ	0.415	7	0.001	0.675	7	0.002

*. This is a lower bound of the true significance.

a. Rielly's significance correction

ANOVA

		quadratic sum	degree freedom	mean square	F	significance
VAR00002	inter-group	4702.685	3	1567.562	5.866	0.004
	intra-group	6413.098	24	267.212		
	amount to	11115.783	27			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	significance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	22.96429*	8.73764	.015	4.9307	40.9979
	TDMQ20	6.08429	8.73764	.493	-11.9493	24.1179
	CQ	32.44857*	8.73764	.001	14.4150	50.4822
AD	Control	-22.96429*	8.73764	.015	-40.9979	-4.9307
	TDMQ20	-16.88000	8.73764	.065	-34.9136	1.1536
	CQ	9.48429	8.73764	.289	-8.5493	27.5179
TDMQ20	Control	-6.08429	8.73764	.493	-24.1179	11.9493
	AD	16.88000	8.73764	.065	-1.1536	34.9136
	CQ	26.36429*	8.73764	.006	8.3307	44.3979
CQ	Control	-32.44857*	8.73764	.001	-50.4822	-14.4150
	AD	-9.48429	8.73764	.289	-27.5179	8.5493
	TDMQ20	-26.36429*	8.73764	.006	-44.3979	-8.3307

*. The mean difference of the post-test was significant at the 0.05 level.

Figure 2 (B) - Long-term recognition index of hippo-CuA β mice in the NOR test**Raw data**

Recognition index for long-term memory				
Control	AD	TDMQ20	Clioquinol	
81.82	35.71	73.33	68.75	
62.50	55.00	87.50	75.00	
42.86	47.06	62.50	0.00	
57.14	54.17	72.73	33.33	
50.00	61.11	60.00	50.00	
66.67	45.45	100.00	62.50	
60.00	62.50	50.00	50.00	

Normality test

	Kolmogorov-Smno(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.157	7	0.2*	0.975	7	0.929
AD	0.179	7	0.2*	0.945	7	0.687
TDMQ20	0.19	7	0.2*	0.965	7	0.857
CQ	0.238	7	0.2*	0.901	7	0.335

*. This is a lower bound of the true significance.

a. Rielly's significance correction

ANOVA

		quadratic sum	degree freedom	mean square	F	significance
VAR00002	inter-group	2381.358	3	793.786	2.681	0.07
	intra-group	7106.179	24	296.091		
	amount to	9487.536	27			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	significance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	8.56849	9.19768	.361	-10.4146	27.5516
	TDMQ20	-12.15368	9.19768	.199	-31.1368	6.8294
	CQ	11.62926	9.19768	.218	-7.3538	30.6123
AD	Control	-8.56849	9.19768	.361	-27.5516	10.4146
	TDMQ20	-20.72217*	9.19768	.034	-39.7053	-1.7391
	CQ	3.06078	9.19768	.742	-15.9223	22.0439
TDMQ20	Control	12.15368	9.19768	.199	-6.8294	31.1368
	AD	20.72217*	9.19768	.034	1.7391	39.7053
	CQ	23.78294*	9.19768	.016	4.7999	42.7660
CQ	Control	-11.62926	9.19768	.218	-30.6123	7.3538
	AD	-3.06078	9.19768	.742	-22.0439	15.9223
	TDMQ20	-23.78294*	9.19768	.016	-42.7660	-4.7999

*. The mean difference of the post-test was significant at the 0.05 level.

Figure 2 (C) - Short-term recognition index of 5XFAD mice in the NOR test**Raw data**

Recognition index			
Control	AD	TDMQ20	CQ
70.0	62.5	79.2	60.0
58.3	61.4	58.8	66.7
50.0	57.1	69.2	46.7
61.1	65.8	68.4	63.2
72.2	83.3	50.0	70.0
62.5	74.1	100.0	66.7
50.0	66.7	0.0	20.0
66.7	53.3		44.4
76.5			

Normality test

	Kolmogorov-Smirnov(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.173	7	0.2	0.919	7	0.46
AD	0.24	7	0.2	0.924	7	0.501
20	0.222	7	0.2	0.906	7	0.37
CQ	0.3	7	0.057	0.777	7	0.024

*. Lower limit of significance.

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic sum	degree freedom	mean square	F	significance
inter-group	517.311	3	172.437	0.528	0.667
intra-group	9148.464	28	326.731		
amount to	9665.775	31			

VAR00002

LSD

Multiple comparisons (LSD)

(I) VAR00001	(J) VAR00001	Mean difference (I-J)	Standard error	significance	95% confidence interval	
					Lower limit	Upper limit
Control	AD	-2.49	8.78	0.779	-20.48	15.50
	TDMQ20	2.23	9.11	0.808	-16.43	20.89
	CQ	8.32	8.78	0.352	-9.67	26.31
AD	Control	2.49	8.78	0.779	-15.50	20.48
	TDMQ20	4.73	9.36	0.617	-14.44	23.89
	CQ	10.81	9.04	0.242	-7.70	29.33
TDMQ20	Control	-2.23	9.11	0.808	-20.89	16.43
	AD	-4.73	9.36	0.617	-23.89	14.44
	CQ	6.09	9.36	0.521	-13.08	25.25
CQ	Control	-8.32	8.78	0.352	-26.31	9.67
	AD	-10.81	9.04	0.242	-29.33	7.70
	TDMQ20	-6.09	9.36	0.521	-25.25	13.08

Figure 3 (A) - Spontaneous alternation ratio of hippo-CuA β mice in the Y-maze test**Raw data**

Figure 6B. Spontaneous alternation ratio			
Control	AD	TDMQ20	Clioquinol
0.8	0.8	0.73	0.22
0.87	0.52	0.8	0.62
0.58	0.41	0.5	0.63
1	0.75	0.76	0.45
0.75	0.41	0.70	0.71

Normality test

	Kolmogorov-Smirnov(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.173	5	0.2	0.991	5	0.983
AD	0.222	5	0.2	0.84	5	0.164
TDMQ20	0.307	5	0.14	0.84	5	0.164
CQ	0.285	5	0.2	0.891	5	0.362

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

		quadratic sum	degree freedom	mean square	F	significance
VAR00002	inter-group	0.227	3	0.076	2.738	0.078
	intra-group	0.442	16	0.028		
	amount to	0.669	19			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	signific ance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	0.22	0.11	0.051	0.00	0.44
	TDMQ20	0.10	0.11	0.346	-0.12	0.32
	CQ	.274*	0.11	0.019	0.05	0.50
AD	Control	-0.22	0.11	0.051	-0.44	0.00
	TDMQ20	-0.12	0.11	0.27	-0.34	0.10
	CQ	0.05	0.11	0.628	-0.17	0.27
TDMQ20	Control	-0.10	0.11	0.346	-0.32	0.12
	AD	0.12	0.11	0.27	-0.10	0.34
	CQ	0.17	0.11	0.121	-0.05	0.39
CQ	Control	-.274*	0.11	0.019	-0.50	-0.05
	AD	-0.05	0.11	0.628	-0.27	0.17
	TDMQ20	-0.17	0.11	0.121	-0.39	0.05

* . The mean difference of the post-test was significant at the 0.05 level.

Figure 3 (B) - Spontaneous alternation ratio of 5XFAD mice in the Y-maze test**Raw data**

Figure 5B. Spontaneous alternation ratio				
Control	AD	TDMQ20	Clioquinol	
58.6	58.7	60.7	60.0	
56.8	69.8	71.4	54.5	
67.9	40.9	54.3	67.9	
76.9	59.5	100.0	50.0	
60.0	50.0	66.7	90.0	
72.7	47.8	100.0	44.8	
50.0	51.1	66.7	75.0	
71.4		52.6	80.0	
66.7		71.4		

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree		statistics	degree	
		freedom	significance		freedom	significance
Control	0.206	7	0.2	0.959	7	0.808
AD	0.191	7	0.2	0.967	7	0.879
TDMQ20	0.276	7	0.115	0.83	7	0.08
CQ	0.151	7	0.2	0.958	7	0.801

*. Lower limit of significance.

a. Rielly's significance correction

VAR00002

	ANOVA				
	quadratic sum	degree freedom	mean square	F	significance
inter-group	1225.584	3	408.528	2.23	0.106
intra-group	5312.392	29	183.186		
amount to	6537.975	32			

Multiple comparisons (LSD)

(I) VAR00001	(J) VAR00001	Mean difference (I-J)	Standard error	significance	95% confidence interval	
					Lower limit	Upper limit
Control	AD	10.58	6.82	0.132	-3.37	24.53
	TDMQ20	-6.98	6.38	0.283	-20.03	6.07
	CQ	-0.72	6.58	0.914	-14.17	12.73
AD	Control	-10.58	6.82	0.132	-24.53	3.37
	TDMQ20	-17.56*	6.82	0.015	-31.51	-3.61
	CQ	-11.30	7.00	0.117	-25.63	3.02
TDMQ20	Control	6.98	6.38	0.283	-6.07	20.03
	AD	17.56*	6.82	0.015	3.61	31.51
	CQ	6.26	6.58	0.349	-7.19	19.71
CQ	Control	0.72	6.58	0.914	-12.73	14.17
	AD	11.30	7.00	0.117	-3.02	25.63
	TDMQ20	-6.26	6.58	0.349	-19.71	7.19

*. The mean difference of the post-test was significant at the 0.05 level.

Figure 4 (A) - Travel distance by hippo-CuA β mice in the open-field test**Raw data**

Control	AD	TDMQ20	Clioquinol
6040	1635	5061	3879
4651	3737	5124	3796
4893	3321	4775	3314
4025	4062	5250	3836
5763	3669	5243	4113
4376	3344	4041	4513
3740		3898	3835
7035			

Normality test

Kolmogorov-Sminov(V) ^a			Shapiro-wilk		
statistics	degree freedom	significance	statistics	degree freedom	significance
0.199	6	0.2	0.931	6	0.59
0.346	6	0.024	0.75	6	0.048
0.29	6	0.125	0.782	6	0.041
0.221	6	0.2	0.957	6	0.797

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

	quadratic sum	degree freedom	mean square	F	significance
inter-group	14377868.41	4	3594467.103	6.354	0.001
intra-group	16405867.97	29	565719.585		
amount to	30783736.38	33			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	significance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	1770.71*	406.20	0	939.93	2601.49
	TDMQ20	295.09	389.27	0.455	-501.06	1091.24
	CQ	1167.38*	389.27	0.006	371.23	1963.52
AD	Control	-1770.71*	406.20	0	-2601.49	-939.93
	TDMQ20	-1475.62*	418.45	0.001	-2331.45	-619.78
	CQ	-603.33	418.45	0.16	-1459.17	252.50
TDMQ20	Control	-295.09	389.27	0.455	-1091.24	501.06
	AD	1475.62*	418.45	0.001	619.78	2331.45
	CQ	872.29*	402.04	0.038	50.03	1694.54
CQ	Control	-1167.38*	389.27	0.006	-1963.52	-371.23
	AD	603.33	418.45	0.16	-252.50	1459.17
	TDMQ20	-872.29*	402.04	0.038	-1694.54	-50.03

* . The mean difference of the post-test was significant at the 0.05 level.

Figure 4 (A) - Rearing times by hippo-CuA β mice in the open-field test**Raw data**

Control	AD	TDMQ20	Clioquinol
34	1	41	25
34	30	42	23
32	9	23	26
29	25	29	18
38	15	38	42
25	7	23	42
37			21
35			

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.171	6	0.2	0.968	6	0.882
AD	0.189	6	0.2	0.948	6	0.721
TDMQ20low	0.228	6	0.2	0.847	6	0.148
CQ	0.295	6	0.112	0.837	6	0.124

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic sum	degree freedom	mean square	F	significance
inter-group	1573.537	4	393.384	5.175	0.003
intra-group	2128.524	28	76.019		
amount to	3702.061	32			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	significance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	18.50*	4.71	0.001	8.85	28.15
	TDMQ20	0.33	4.71	0.944	-9.31	9.98
	CQ	4.86	4.51	0.291	-4.39	14.10
AD	Control	-18.50*	4.71	0.001	-28.15	-8.85
	TDMQ20	-18.17*	5.03	0.001	-28.48	-7.86
	CQ	-13.64*	4.85	0.009	-23.58	-3.71
TDMQ20	Control	-0.33	4.71	0.944	-9.98	9.31
	AD	18.17*	5.03	0.001	7.86	28.48
	CQ	4.52	4.85	0.359	-5.41	14.46
CQ	Control	-4.86	4.51	0.291	-14.10	4.39
	AD	13.64*	4.85	0.009	3.71	23.58
	TDMQ20	-4.52	4.85	0.359	-14.46	5.41

* . The mean difference of the post-test was significant at the 0.05 level.

Figure 4 (B) - Grid crossings by 5XFAD mice in the open-field test**Raw data**

Crossing Grids number				
Control	AD	TDMQ20	CQ	
70.	139.	115.	67.	
94.	155.	184.	83.	
128.	209.	42.	100.	
123.	207.	112.	180.	
42.	96.	103.	69.	
110.	76.	96.	92.	
111.	160.	45.	95.	
48.		54.		
90.		18.		

Normality test

	Kolmogorov-Smirnov(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.176	7	0.2	0.953	7	0.76
AD	0.227	7	0.2	0.894	7	0.299
TDMQ20	0.208	7	0.2	0.951	7	0.742
CQ	0.213	7	0.2	0.907	7	0.376

*. Lower limit of significance.

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic sum	degree freedom	mean square	F	significance
inter-group	228.142	3	76.047	0.708	0.555
intra-group	3115.373	29	107.427		
amount to	3343.515	32			

Multiple comparisons (LSD)

Multiple comparisons (LSD)					95% confidence interval	
LSD		Mean difference(I-J)	Standard error	significance	Lower limit	Upper limit
(I) VAR00001	(J) VAR00001					
Control	AD	-58.19*	21.88	0.013	-103.01	-13.37
	TDMQ20	5.22	20.47	0.8	-36.70	47.15
	CQ	-7.33	21.88	0.74	-52.15	37.49
AD	Control	58.19*	21.88	0.013	13.37	103.01
	TDMQ20	63.41*	21.88	0.007	18.59	108.23
	CQ	50.86*	23.21	0.037	3.32	98.40
TDMQ20	Control	-5.22	20.47	0.8	-47.15	36.70
	AD	-63.41*	21.88	0.007	-108.23	-18.59
	CQ	-12.56	21.88	0.571	-57.38	32.27
CQ	Control	7.33	21.88	0.74	-37.49	52.15
	AD	-50.86*	23.21	0.037	-98.40	-3.32
	TDMQ20	12.56	21.88	0.571	-32.27	57.38

*. The mean difference of the post-test was significant at the 0.05 level.

Figure 4 (B) - Rearing times by 5XFAD mice in the open-field test**Raw data**

Rearing time				
Control	AD	TDMQ20	Clioquinol	
15.	30.	17.	9.	
24.	18.	29.	13.	
16.	32.	2.	7.	
28.	15.	15.	15.	
22.	7.	16.	5.	
9.	14.	9.	9.	
11.	12.	8.	15.	
13.		3.	53.	
23.		7.		

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.176	7	0.2	0.953	7	0.76
AD	0.227	7	0.2	0.894	7	0.299
TDMQ20	0.208	7	0.2	0.951	7	0.742
CQ	0.213	7	0.2	0.907	7	0.376

*. Lower limit of significance.

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic sum	degree freedom	mean square	F	significance
inter-group	228.142	3	76.047	0.708	0.555
intra-group	3115.373	29	107.427		
amount to	3343.515	32			

Multiple comparisons (LSD)

VAR00002		Mean difference (I-J)	Standard error	significance	95% confidence interval	
LSD	(I) VAR00001				(J) VAR00001	Lower limit
Control	AD	-0.40	5.22	0.94	-11.08	10.29
	TDMQ20	6.11	4.89	0.221	-3.88	16.10
	CQ	2.14	5.04	0.674	-8.16	12.44
AD	Control	0.40	5.22	0.94	-10.29	11.08
	TDMQ20	6.51	5.22	0.223	-4.17	17.19
	CQ	2.54	5.36	0.64	-8.44	13.51
TDMQ20	Control	-6.11	4.89	0.221	-16.10	3.88
	AD	-6.51	5.22	0.223	-17.19	4.17
	CQ	-3.97	5.04	0.437	-14.27	6.33
CQ	Control	-2.14	5.04	0.674	-12.44	8.16
	AD	-2.54	5.36	0.64	-13.51	8.44
	TDMQ20	3.97	5.04	0.437	-6.33	14.27

Figure 5 - Closed arm entries by 5XFAD mice in the elevated plus maze test**Raw data**

Number of close arm entries				
Control	AD	TDMQ20	CQ	
4.	14.	9.	7.	
5.	21.	9.	8.	
2.	15.	3.	8.	
6.	5.	4.	9.	
3.	11.	6.	11.	
4.	11.	4.	4.	
7.	4.	7.	7.	
4.	6.	8.		
6.		3.		

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	.360	7	.007	.689	7	.003
AD	.195	7	.200*	.865	7	.168
TDMQ20	.504	7	.000	.453	7	.000
CQ	.206	7	.200*	.861	7	.155

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

	quadratic sum	degree freedom	mean square	F	significance
inter-group	188.1	3	62.7	5.486	0.004
intra-group	331.415	29	11.428		
amount to	519.515	32			

Multiple comparisons (LSD)

VAR00002		LSD			95% confidence interval	
(I) VAR00001	(J) VAR00001	Mean difference (I-J)	Standard error	significance	Lower limit	Upper limit
Control	AD	-6.32*	1.64	0.001	-9.68	-2.96
	TDMQ20	-1.33	1.59	0.41	-4.59	1.93
	CQ	-3.16	1.70	0.074	-6.64	0.33
AD	Control	6.32*	1.64	0.001	2.96	9.68
	TDMQ20	4.99*	1.64	0.005	1.63	8.35
	CQ	3.16	1.75	0.081	-0.42	6.74
TDMQ20	Control	1.33	1.59	0.41	-1.93	4.59
	AD	-4.99*	1.64	0.005	-8.35	-1.63
	CQ	-1.83	1.70	0.293	-5.31	1.66
CQ	Control	3.16	1.70	0.074	-0.33	6.64
	AD	-3.16	1.75	0.081	-6.74	0.42
	TDMQ20	1.83	1.70	0.293	-1.66	5.31

* . The mean difference of the post-test was significant at the 0.05 level.

Figure 5 - Time spent in closed arms by 5XFAD mice in the elevated plus maze test**Raw data**

Time spend in closed arm (s)				
Control	AD	TDMQ20	CQ	
91.3	78.0	83.0	84.3	
88.3	68.7	86.7	57.7	
89.0	75.0	91.7	81.3	
91.7	86.7	92.7	72.0	
95.3	75.0	77.3	97.7	
93.3	73.0	95.0	92.3	
85.3	81.3	90.0	67.0	
92.0	86.7	75.0		
90.0		92.7		

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree		statistics	degree	
		freedom	significance		freedom	significance
Control	0.154	7	0.2	0.986	7	0.982
AD	0.193	7	0.2	0.971	7	0.909
TDMQ20	0.195	7	0.2	0.936	7	0.607
CQ	0.139	7	0.2	0.977	7	0.941

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic sum	degree freedom	mean square	F	significance
inter-group	954.938	3	318.313	4.658	0.009
intra-group	1981.704	29	68.335		
amount to	2936.642	32			

VAR00002

Multiple comparisons (LSD)

LSD

		Mean difference (I-J)	Standard error	significance	95% confidence interval	
(I) VAR00001	(J) VAR00001				Lower limit	Upper limit
Control	AD	12.64*	4.02	0.004	4.42	20.85
	TDMQ20	3.57	3.90	0.368	-4.40	11.54
	CQ	11.79*	4.17	0.008	3.27	20.31
AD	Control	-12.64*	4.02	0.004	-20.85	-4.42
	TDMQ20	-9.07*	4.02	0.032	-17.29	-0.86
	CQ	-0.85	4.28	0.844	-9.60	7.90
TDMQ20	Control	-3.57	3.90	0.368	-11.54	4.40
	AD	9.07*	4.02	0.032	0.86	17.29
	CQ	8.22	4.17	0.058	-0.30	16.74
CQ	Control	-11.79*	4.17	0.008	-20.31	-3.27
	AD	0.85	4.28	0.844	-7.90	9.60
	TDMQ20	-8.22	4.17	0.058	-16.74	0.30

* . The mean difference of the post-test was significant at the 0.05 level.

Figure 6 (A) - MDA level in the cortex of icv-CuA β mice.

Raw data

Cortex (nmol/mgprot)				
Control	AD	TDMQ20	Clioquinol	
4.17	6.62	6.07	4.76	
5.51	6.92	5.08	5.33	
6.49	5.34	5.23	6.01	
5.25	6.55	5.71	4.06	
4.31	5.34	3.36	4.51	

Normality test

	Kolmogorov-Smirnov(V) ^a			Shapiro-wilk		
	statistics	degree of freedom	significance	statistics	degree of freedom	significance
Control	0.211	5	0.2*	0.931	5	0.604
AD	0.299	5	0.165	0.81	5	0.097
20	0.298	5	0.169	0.875	5	0.289
CQ	0.192	5	0.2*	0.975	5	0.904

*This is a lower bound of the true significance

a. Rielly's significance correction

ANOVA

VAR00002		quadratic	degree	mean square	F	significance
		sum	freedom			
	inter-group	4.63	3.00	1.54	1.97	0.159
	intra-group	12.54	16.00	0.78		
	amount to	17.17	19.00			

Multiple comparisons (LSD)

VAR00002

LSD

(I) VAR00001	(J) VAR00001	Mean difference(I-J)	Standard error	significance	95% confidence interval	
					Lower limit	Upper limit
Control	AD	-1.008	0.560	0.091	-2.195	0.178
	TDMQ20	0.054	0.560	0.924	-1.133	1.241
	CQ	0.210	0.560	0.713	-0.977	1.397
AD	Control	1.008	0.560	0.091	-0.178	2.195
	TDMQ20	1.063	0.560	0.076	-0.124	2.250
	CQ	1.218*	0.560	0.045	0.031	2.405
TDMQ20	Control	-0.054	0.560	0.924	-1.241	1.133
	AD	-1.063	0.560	0.076	-2.250	0.124
	CQ	0.156	0.560	0.784	-1.031	1.343
CQ	Control	-0.210	0.560	0.713	-1.397	0.977
	AD	-1.218*	0.560	0.045	-2.405	-0.031
	TDMQ20	-0.156	0.560	0.784	-1.343	1.031

*The mean difference of the post-test was significant at the 0.05 level.

Figure 6 (B) - MDA level in the cortex of 5XFAD mice.**Raw data**

Cortex (nmol/mgprot)			
Control	AD	TDMQ20	Clioquinol
2.42	3.32	2.00	2.82
2.59	3.31	2.91	2.47
2.85	2.34	2.04	2.75
2.18	2.66	1.70	2.70
2.33	2.55	1.86	2.15

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.18	5.00	0.20*	0.97	5	0.902
AD	0.25	5.00	0.2* ^c	0.85	5	0.202
TDMQ20	0.35	5.00	0.04	0.81	5	0.097
CQ	0.27	5.00	0.20*	0.88	5	0.324

*This is a lower bound of the true significance

a. Rielly's significance correction

ANOVA

		quadratic sum	degree freedom	mean square	F	significance
VAR00002	inter-group	1.39	3.00	0.46	3.269	0.049
	intra-group	2.27	16.00	0.14		
	amount to	3.658	19			

Multiple comparisons (LSD)

VAR00002

LSD

(I) VAR00001	(J) VAR00001	Mean difference(I-J)	Standard error	significance	95% confidence interval	
					Lower limit	Upper limit
Control	AD	-0.36	0.24	0.148	-0.867	0.143
	TDMQ20	0.37	0.24	0.138	-0.133	0.877
	CQ	-0.10	0.24	0.668	-0.609	0.401
AD	Control	0.36	0.24	0.148	-0.143	0.867
	TDMQ20	0.73*	0.24	0.007	0.229	1.239
	CQ	0.26	0.24	0.295	-0.247	0.763
TDMQ20	Control	-0.37	0.24	0.138	-0.877	0.133
	AD	-0.73*	0.24	0.007	-1.239	-0.229
	CQ	-0.48	0.24	0.063	-0.981	0.029
CQ	Control	0.10	0.24	0.668	-0.401	0.609
	AD	-0.26	0.24	0.295	-0.763	0.247
	TDMQ20	0.48	0.24	0.063	-0.029	0.981

*The mean difference of the post-test was significant at the 0.05 level.

Figure S1 (A) - Short-term recognition index of icv-CuA β mice in the NOR test. Duplicate.**Raw data**

Recognition index for short-term memory				
Control	AD	TDMQ20	TDMQ22	Clioquinol
80	38.4	66.6	33.3	54.5
77.7	0	54.5	68.1	80
42.3	54.5	58.8	55.5	12.5
85.7	41.3	65.6	45.4	61.5
85.7	50	75	68.7	100
66.6	36.3	56.5	nd	64.2

Normality test

statistics	Kolmogorov-Smirn(V) ^a		statistics	Shapiro-wilk		significance
	degree freedom	significance		degree freedom	significance	
Control	0.374	5	0.021	0.713	5	0.013
AD	0.329	5	0.082	0.817	5	0.11
20	0.176	5	0.2	0.971	5	0.884
22	0.22	5	0.2	0.915	5	0.499
CQ	0.213	5	0.2	0.968	5	0.86

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

quadratic sum	degree freedom	mean square	F	significance
inter-group	4	1093.288	3.003	0.038
intra-group	24	364.022		
amount to	28			

Multiple comparisons (LSD)

(I)	(J)	Mean difference (I-J)	Standard error	significance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	36.25*	11.02	0.003	13.52	58.98
	TDMQ20	10.17	11.02	0.365	-12.57	32.90
	CQ	10.88	11.02	0.333	-11.85	33.62
	TDMQ22	18.80	11.55	0.117	-5.04	42.64
AD	Control	-36.25*	11.02	0.003	-58.98	-13.52
	TDMQ20	-26.08*	11.02	0.026	-48.82	-3.35
	CQ	-25.37*	11.02	0.03	-48.10	-2.63
	TDMQ22	-17.45	11.55	0.144	-41.29	6.39
TDMQ20	Control	-10.17	11.02	0.365	-32.90	12.57
	AD	26.08*	11.02	0.026	3.35	48.82
	CQ	.72	11.02	0.949	-22.02	23.45
	TDMQ22	8.63	11.55	0.462	-15.21	32.48
CQ	Control	-10.88	11.02	0.333	-33.62	11.85
	AD	25.37*	11.02	0.03	2.63	48.10
	TDMQ20	-.72	11.02	0.949	-23.45	22.02
	TDMQ22	7.92	11.55	0.5	-15.93	31.76
TDMQ22	Control	-18.80	11.55	0.117	-42.64	5.04
	AD	17.45	11.55	0.144	-6.39	41.29
	TDMQ20	-8.63	11.55	0.462	-32.48	15.21
	CQ	-7.92	11.55	0.5	-31.76	15.93

* . The mean difference of the post-test was significant at the 0.05 level.

Figure S1 (A) - Long-term recognition index of icv-CuA β mice in the NOR test. Duplicate.

Raw data

Recognition index for long-term memory				
Control	AD	TDMQ20	TDMQ22	Clioquinol
80.9	55.5	66.6	66.6	62.5
50	53.8	33.3	72.7	68.7
62.5	63.1	70	62.5	58.8
90	53.8	79.1	60	57.1
61.5	0	68.1	75	88.8
0	61.1	66.6	nd	71.42857

Normality test

	Kolmogorov-Smirn(V) ^a			Shapiro-wilk		
	statistics	degree freedom	significance	statistics	degree freedom	significance
Control	0.256	5	0.2	0.938	5	0.653
AD	0.431	5	0.003	0.685	5	0.007
20	0.372	5	0.023	0.796	5	0.075
22	0.197	5	0.2	0.935	5	0.632
CQ	0.253	5	0.2	0.829	5	0.138

* . Lower limit of significance .

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic sum	degree freedom	mean square	F	significance
inter-group	1642.846	4	410.712	0.994	0.43
intra-group	9914.954	24	413.123		
amount to	11557.8	28			

Multiple comparisons (LSD)

(I)	(J)	Mean difference(I-J)	Standard error	signific ance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	9.60	11.73	0.421	-14.62	33.82
	TDMQ20	-6.47	11.73	0.587	-30.69	17.75
	CQ	-10.40	11.73	0.384	-34.62	13.81
	TDMQ22	-9.88	12.31	0.43	-35.28	15.53
AD	Control	-9.60	11.73	0.421	-33.82	14.62
	TDMQ20	-16.07	11.73	0.184	-40.29	8.15
	CQ	-20.00	11.73	0.101	-44.22	4.21
	TDMQ22	-19.48	12.31	0.127	-44.88	5.93
TDMQ20	Control	6.47	11.73	0.587	-17.75	30.69
	AD	16.07	11.73	0.184	-8.15	40.29
	CQ	-3.94	11.73	0.74	-28.16	20.28
	TDMQ22	-3.41	12.31	0.784	-28.81	21.99
CQ	Control	10.40	11.73	0.384	-13.81	34.62
	AD	20.00	11.73	0.101	-4.21	44.22
	TDMQ20	3.94	11.73	0.74	-20.28	28.16
	TDMQ22	0.53	12.31	0.966	-24.87	25.93
TDMQ22	Control	9.88	12.31	0.43	-15.53	35.28
	AD	19.48	12.31	0.127	-5.93	44.88
	TDMQ20	3.41	12.31	0.784	-21.99	28.81
	CQ	-0.53	12.31	0.966	-25.93	24.87

Figure S1 (B) - Short-term recognition index of hippo-CuA β mice in the NOR test. Duplicate.**Raw data**

Control	AD	TDMQ20	Clioquinol
62.5	60	100	62.5
60.71429	60	100	18.2
60	33.3	50	50
75	50	77.3	0
60	14.3	50	25
67.7	33.3	100	48
33.3		66.6	42.8
77.7			

Normality test

	Kolmogorov-Smirnov(V) ^a			Shapiro-wilk		
	statistics	degree		statistics	degree	
		freedom	significance		freedom	significance
Control	0.286	6	0.136	0.799	6	0.058
AD	0.181	6	0.2	0.902	6	0.387
TDMQ20	0.298	6	0.104	0.772	6	0.033
CQ	0.225	6	0.2	0.951	6	0.747

*. Lower limit of significance.

a. Rielly's significance correction

ANOVA

VAR00002

	quadratic	degree	mean square	F	significance
	sum	freedom			
inter-group	8250.655	4	2062.664	5.206	0.003
intra-group	11886.363	30	396.212		
amount to	20137.017	34			

Multiple comparisons (LSD)

(I)	(J)	Mean	Standard	signific	95% confidence interval	
VAR00001	VAR00001	difference (I-J)	error	ance	Lower limit	Upper limit
Control	AD	20.30	10.75	0.069	-1.66	42.25
	TDMQ20	-15.59	10.30	0.141	-36.62	5.45
	CQ	26.90*	10.30	0.014	5.86	47.94
AD	Control	-20.30	10.75	0.069	-42.25	1.66
	TDMQ20	-35.88*	11.07	0.003	-58.50	-13.27
	CQ	6.60	11.07	0.556	-16.01	29.22
TDMQ20	Control	15.59	10.30	0.141	-5.45	36.62
	AD	35.88*	11.07	0.003	13.27	58.50
	CQ	42.49*	10.64	0	20.76	64.21
CQ	Control	-26.90*	10.30	0.014	-47.94	-5.86
	AD	-6.60	11.07	0.556	-29.22	16.01
	TDMQ20	-42.49*	10.64	0	-64.21	-20.76

*. The mean difference of the post-test was significant at the 0.05 level.

Figure S1 (B) - Long-term recognition index of hippo-CuA β mice in the NOR test. Duplicate.**Raw data**

C Long-term memory			
Control	AD	TDMQ20	Clioquinol
60	57.1	100	51.7
60.8	71.4	38.1	55.5
56.2	66.6	45.4	75
42.8	50	60	50
61.9	66.6	75	0
60	57.1	75	65.5
50		40	58.8
45.4			

Normality test

	Kolmogorov-Smirnov(V) ^a			Shapiro-wilk		
	statistics	degree of freedom	significance	statistics	degree of freedom	significance
Control	0.331	6	0.039	0.712	6	0.008
AD	0.239	6	0.2	0.929	6	0.569
TDMQ20	0.172	6	0.2	0.956	6	0.789
CQ	0.339	6	0.03	0.824	6	0.095

* . Lower limit of significance.

a. Rielly's significance correction

ANOVA

	quadratic sum	degree of freedom	mean square	F	significance
inter-group	604.495	4	151.124	0.575	0.683
intra-group	7882.435	30	262.748		
amount to	8486.93	34			

Multiple comparisons (LSD)

(I)	(J)	Mean difference(I-J)	Standard error	significance	95% confidence interval	
VAR00001	VAR00001				Lower limit	Upper limit
Control	AD	-6.83	8.75	0.441	-24.71	11.05
	TDMQ20	-7.29	8.39	0.392	-24.42	9.84
	CQ	3.71	8.39	0.662	-13.42	20.84
AD	Control	6.83	8.75	0.441	-11.05	24.71
	TDMQ20	-0.46	9.02	0.959	-18.88	17.96
	CQ	10.54	9.02	0.252	-7.88	28.96
TDMQ20	Control	7.29	8.39	0.392	-9.84	24.42
	AD	0.46	9.02	0.959	-17.96	18.88
	CQ	11.00	8.66	0.214	-6.69	28.69
CQ	Control	-3.71	8.39	0.662	-20.84	13.42
	AD	-10.54	9.02	0.252	-28.96	7.88
	TDMQ20	-11.00	8.66	0.214	-28.69	6.69

ADME-Tox data of TDMQ20

7.1. ADME-Tox: *In Vitro* Absorption

7.1.1. Test Compound Results

Compound I.D.	Client Compound I.D.	Test Concentration	Permeability (10^{-6} cm/s)			Percent Recovery(%)		
			1 st	2 nd	Mean	1 st	2 nd	Mean
A-B permeability (Caco-2, pH 6.5/7.4)								
100042200-1	TDMQ5	1.0E-05 M	15.24	15.13	15.2	40	35	37
100042200-2	TDMQ20	1.0E-05 M	8.32	8.47	8.4	36	35	35
100042200-3	TDMQ22	1.0E-05 M	19.87	19.04	19.5	55	47	51
B-A permeability (Caco-2, pH 6.5/7.4)								
100042200-1	TDMQ5	1.0E-05 M	17.55	17.29	17.4	63	66	65
100042200-2	TDMQ20	1.0E-05 M	9.61	10.56	10.1	77	72	74
100042200-3	TDMQ22	1.0E-05 M	27.30	21.91	24.6	73	79	76

7.2. ADME-Tox: *In Vitro* Metabolism

7.2.1. Test Compound Results

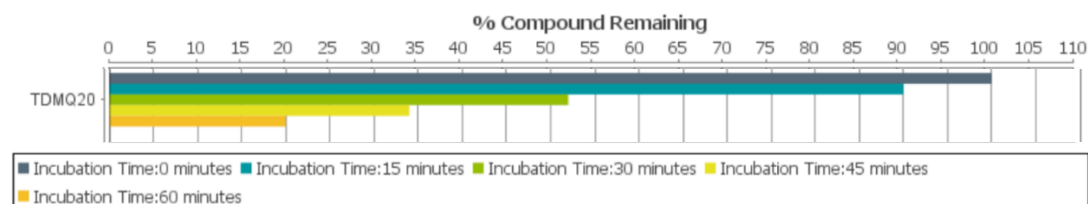


Figure 1. Histogram for Intrinsic clearance (liver microsomes- human)

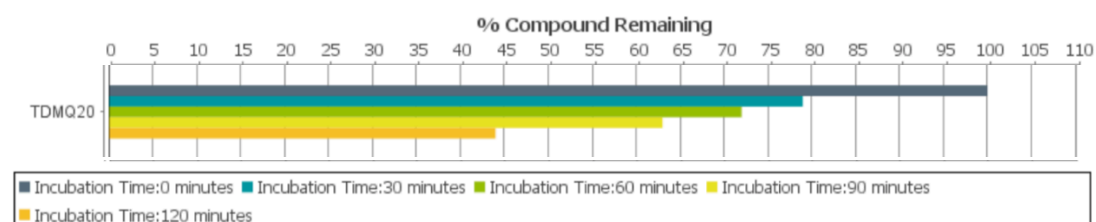


Figure 2. Histogram for Intrinsic clearance (cryopreserved hepatocytes, human)

7.2.3. Test Compound Results

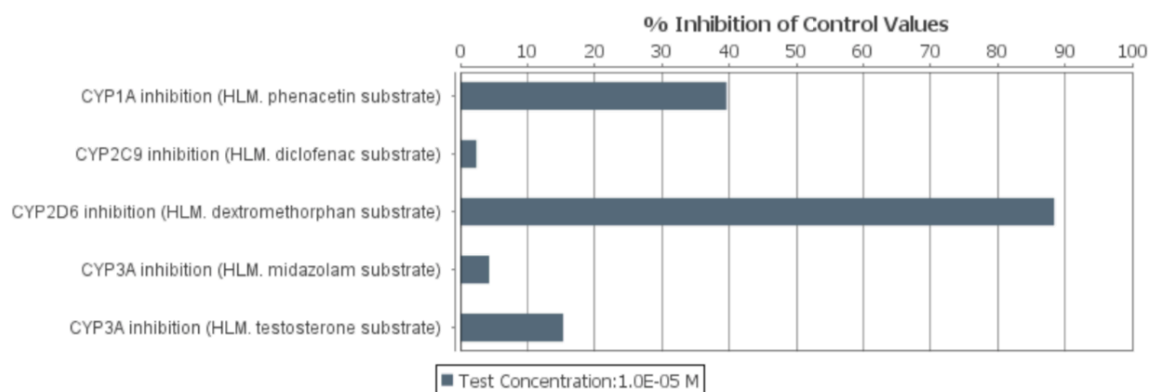


Figure 4. Histogram for TDMQ20

7.3. ADME-Tox: Genetic Toxicity

7.3.1. Test Compound Results

Compound I.D.	Client Compound I.D.	Test Concentration	1 st	2 nd	%Effect 3 rd	Mean %Effect	Cytotoxicity (% of control)	1 st	Flags 2 nd	3 rd
Bacterial cytotoxicity (TA98 - S9)										
100042200-2	TDMQ20	6.0E-07 M	106.5	102.2	102.2	103.6	104			
100042200-2	TDMQ20	1.2E-06 M	99.6	102.2	97.8	99.9	100			
100042200-2	TDMQ20	2.5E-06 M	82.3	97.8	92.6	90.9	91			
100042200-2	TDMQ20	5.0E-06 M	95.2	97.8	103.0	98.7	99			
100042200-2	TDMQ20	1.0E-05 M	90.0	88.3	98.7	92.4	92			
100042200-2	TDMQ20	2.5E-05 M	87.4	91.8	93.5	90.9	91			
100042200-2	TDMQ20	5.0E-05 M	93.5	73.6	112.6	93.2	93			
Bacterial cytotoxicity (TA100 - S9)										
100042200-2	TDMQ20	6.0E-07 M	100.8	94.8	101.8	99.2	99			
100042200-2	TDMQ20	1.2E-06 M	96.8	96.8	93.8	95.8	96			
100042200-2	TDMQ20	2.5E-06 M	97.8	89.7	82.7	90.1	90			
100042200-2	TDMQ20	5.0E-06 M	88.7	84.7	76.6	83.4	83			
100042200-2	TDMQ20	1.0E-05 M	83.7	72.6	70.6	75.6	76			
100042200-2	TDMQ20	2.5E-05 M	86.7	79.7	75.6	80.7	81			
100042200-2	TDMQ20	5.0E-05 M	93.8	82.7	81.7	86.1	86			
Bacterial cytotoxicity (TA1535 - S9)										
100042200-2	TDMQ20	6.0E-07 M	102.1	92.0	106.1	100.1	100			
100042200-2	TDMQ20	1.2E-06 M	92.0	95.0	101.1	96.0	96			
100042200-2	TDMQ20	2.5E-06 M	97.0	94.0	97.0	96.0	96			
100042200-2	TDMQ20	5.0E-06 M	106.1	95.0	101.1	100.7	101			
100042200-2	TDMQ20	1.0E-05 M	112.2	108.1	119.3	113.2	113			
100042200-2	TDMQ20	2.5E-05 M	112.2	112.2	110.2	111.5	112			
100042200-2	TDMQ20	5.0E-05 M	114.2	114.2	117.2	115.2	115			
Bacterial cytotoxicity (TA1537 - S9)										
100042200-2	TDMQ20	6.0E-07 M	99.4	102.3	97.4	99.7	100			
100042200-2	TDMQ20	1.2E-06 M	98.4	95.5	98.4	97.4	97			
100042200-2	TDMQ20	2.5E-06 M	110.1	106.2	106.2	107.5	107			
100042200-2	TDMQ20	5.0E-06 M	87.7	85.7	87.7	87.0	87			
100042200-2	TDMQ20	1.0E-05 M	74.0	79.9	77.9	77.3	77			
100042200-2	TDMQ20	2.5E-05 M	87.7	83.8	71.1	80.8	81			
100042200-2	TDMQ20	5.0E-05 M	87.7	79.9	76.0	81.2	81			

Notes:

1. Cytotoxicity is presented as % of control growth.
2. A cytotoxicity value of less than 60 % is flagged, and the compound is considered as toxic at the respective concentration.

NSOL: Not Soluble. Test compound is not completely soluble in the working solution.

∅: That replicate was excluded from the calculation

7.3.5. Test Compound Results

Compound I.D.	Client Compound I.D.	Test Concentration	Count (# of wells)	Positive Significance (- to +++)	Fisher Exact Test (p-value)	Count Flag
Ames fluctuation test (TA98 - S9)						
100042200-2	TDMQ20	5.0E-06 M	0	-	1.0000	
100042200-2	TDMQ20	1.0E-05 M	0	-	1.0000	
100042200-2	TDMQ20	5.0E-05 M	0	-	1.0000	
100042200-2	TDMQ20	1.0E-04 M	0	-	1.0000	
Ames fluctuation test (TA98 + S9)						
100042200-2	TDMQ20	5.0E-06 M	0	-	0.5000	-
100042200-2	TDMQ20	1.0E-05 M	0	-	0.5000	-
100042200-2	TDMQ20	5.0E-05 M	0	-	0.5000	-
100042200-2	TDMQ20	1.0E-04 M	0	-	0.5000	-
100042200-2	TDMQ20	5.0E-06 M	0	-	0.5000	-
100042200-2	TDMQ20	1.0E-05 M	0	-	0.5000	-
100042200-2	TDMQ20	5.0E-05 M	0	-	0.5000	-
100042200-2	TDMQ20	1.0E-04 M	0	-	0.5000	-
Ames fluctuation test (TA100 + S9)						
100042200-2	TDMQ20	5.0E-06 M	3	-	0.5000	-
100042200-2	TDMQ20	1.0E-05 M	3	-	0.5000	-
100042200-2	TDMQ20	5.0E-05 M	0	-	0.0586	-
100042200-2	TDMQ20	1.0E-04 M	0	-	0.0586	-
Ames fluctuation test (TA1535 - S9)						
100042200-2	TDMQ20	5.0E-06 M	0	-	1.0000	
100042200-2	TDMQ20	1.0E-05 M	0	-	1.0000	
100042200-2	TDMQ20	5.0E-05 M	0	-	1.0000	
100042200-2	TDMQ20	1.0E-04 M	0	-	1.0000	
Ames fluctuation test (TA1535 + S9)						
100042200-2	TDMQ20	5.0E-06 M	0	-	0.5000	-
100042200-2	TDMQ20	1.0E-05 M	0	-	0.5000	-
100042200-2	TDMQ20	5.0E-05 M	0	-	0.5000	-
100042200-2	TDMQ20	1.0E-04 M	0	-	0.5000	-
Ames fluctuation test (TA1537-S9)						
100042200-2	TDMQ20	5.0E-06 M	0	-	1.0000	-
100042200-2	TDMQ20	1.0E-05 M	0	-	1.0000	-
100042200-2	TDMQ20	5.0E-05 M	0	-	1.0000	-
100042200-2	TDMQ20	1.0E-04 M	0	-	1.0000	-
Ames fluctuation test (TA1537 + S9)						
100042200-2	TDMQ20	5.0E-06 M	0	-	1.0000	-
100042200-2	TDMQ20	1.0E-05 M	0	-	1.0000	-
100042200-2	TDMQ20	5.0E-05 M	0	-	1.0000	-
100042200-2	TDMQ20	1.0E-04 M	0	-	1.0000	-

Notes:

- Weak positive, if $p < 0.05$, denoted as "+"
Strong positive, if $p < 0.01$, denoted as "++"
Very strong positive, if $p < 0.001$, denoted as "+++"
- When possible, compounds which score significantly below background are flagged.
This may indicate low level cytotoxicity undetectable by the growth assay.
The compounds are flagged as described below.
if $p < 0.05$, flagged as "<"
if $p < 0.01$, flagged as "<<"
if $p < 0.001$, flagged as "<<<"
- Hyphens (-) indicate negative results.

Materials and methods for ADME-Tox evaluation of TDMQ20

Minor variations to the experimental protocol described below may have occurred during the testing, they have no impact on the quality of the results obtained.

8.1.1. ADME-Tox: *In Vitro* Absorption

Assay	Source	Incubation	Detection Method	Bibl.
ADME				
A-B permeability (Caco-2, pH 6.5/7.4)	Caco-2 cell line	0 and 60 min 37°C	HPLC-MS/MS	1101
B-A permeability (Caco-2, pH 6.5/7.4)	Caco-2 cell line	0 and 40 min 37°C	HPLC-MS/MS	1101

8.1.2. ADME-Tox: *In Vitro* Metabolism

Assay	Source	Substrate	Incubation	Measured Component	Detection Method	Bibl.
ADME						
CYP1A inhibition (HLM, phenacetin substrate)	Human liver microsomes (0.1 mg/mL)	Phenacetin (10 µM)	10 min 37°C	Acetaminophen	HPLC-MS/MS	732
CYP2C9 inhibition (HLM, diclofenac substrate)	Human liver microsomes (0.1 mg/mL)	Diclofenac (10 µM)	10 min 37°C	4'-hydroxydiclofenac	HPLC-MS/MS	732
CYP2D6 inhibition (HLM, dextromethorphan substrate)	Human liver microsomes (0.1 mg/mL)	Dextromethorphan (5 µM)	10 min 37°C	Dextrorphan	HPLC-MS/MS	732
CYP3A inhibition (HLM, midazolam substrate)	Human liver microsomes (0.1 mg/mL)	Midazolam (5 µM)	10 min 37°C	1-hydroxymidazolam	HPLC-MS/MS	732
CYP3A inhibition (HLM, testosterone substrate)	Human liver microsomes (0.1 mg/mL)	Testosterone (50 µM)	10 min 37°C	6beta-hydroxytestosterone	HPLC-MS/MS	732
Intrinsic clearance (liver microsomes- human)	Human liver microsomes (0.1 mg/mL)	Test compound	0, 15, 30, 45, 60 min 37°C	Test compound	HPLC-MS/MS	828
Intrinsic clearance (cryopreserved hepatocytes, human)	Human cryopreserved hepatocytes (0.7 million viable cells/mL)	Test compound	0, 0.5, 1, 1.5, 2 hr 37°C	Test compound	HPLC-MS/MS	828

8.1.3. ADME-Tox: Genetic Toxicity

Assay	Source	Technique	Incubation	Detection Method	Bibl.
Toxicity					
Bacterial cytotoxicity (TA98 - S9)	Reverted Salmonella typhimurium (TA98)	Optical density	96 hr 37°C	Photometry	341
Bacterial cytotoxicity (TA100 - S9)	Reverted Salmonella typhimurium (TA100)	Optical density	96 hr 37°C	Photometry	341
Bacterial cytotoxicity (TA1535 - S9)	Reverted Salmonella typhimurium (TA1535)	Optical density	96 hr 37°C	Photometry	341
Bacterial cytotoxicity (TA1537 - S9)	Reverted Salmonella typhimurium (TA1537)	Optical density	96 hr 37°C	Photometry	341
Ames fluctuation test (TA98 - S9)	<i>Salmonella typhimurium</i> (TA98)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA98 + S9)	<i>Salmonella typhimurium</i> (TA98) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA100 - S9)	<i>Salmonella typhimurium</i> (TA100)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA100 + S9)	<i>Salmonella typhimurium</i> (TA100) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1535 - S9)	<i>Salmonella typhimurium</i> (TA1535)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1535 + S9)	<i>Salmonella typhimurium</i> (TA1535) and rat liver S9	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1537-S9)	<i>Salmonella typhimurium</i> (TA1537)	Fluctuation test	96 hr 37°C	Photometry	341
Ames fluctuation test (TA1537 + S9)	<i>Salmonella typhimurium</i> (TA1537)	Fluctuation test	96 hr 37°C	Photometry	341

Analysis and expression of the results for ADME-Tox evaluation of TDMQ20

8.2.1. ADME-Tox: *In Vitro* Absorption

Permeability

The apparent permeability coefficient (P_{app}) of the test compound was calculated as follows:

$$P_{app}(\text{cm/s}) = \frac{V_R \cdot C_{R, \text{end}}}{\Delta t} \cdot \frac{1}{A \cdot (C_{D, \text{mid}} - C_{R, \text{mid}})}$$

where V_R is the volume of the receiver chamber. $C_{R, \text{end}}$ is the concentration of the test compound in the receiver chamber at the end time point, Δt is the incubation time and A is the surface area of the cell monolayer. $C_{D, \text{mid}}$ is the calculated mid-point concentration of the test compound in the donor side, which is the mean value of the donor concentration at time 0 minute and the donor concentration at the end time point. $C_{R, \text{mid}}$ is the mid-point concentration of the test compound in the receiver side, which is one half of the receiver concentration at the end time point. Concentrations of the test compound were expressed as peak areas of the test compound.

Recovery of the Test Compound from the Permeability Assay

The recovery of the test compound was calculated as follows:

$$\text{Recovery}(\%) = \frac{V_D \cdot C_{D, \text{end}} + V_R \cdot C_{R, \text{end}}}{V_D \cdot C_{D0}} \cdot 100$$

where V_D and V_R are the volumes of the donor and receiver chambers, respectively. $C_{D, \text{end}}$ is the concentration of the test compound in the donor sample at the end time point. $C_{R, \text{end}}$ is the concentration of the test compound in the receiver sample at the end time point. C_{D0} is the concentration of the test compound in the donor sample at time zero. Concentrations of the test compound are expressed as peak areas of the test compound.

Fluorescein assessment for Permeability assays

Fluorescein was used as the cell monolayer integrity marker. Fluorescein permeability assessment (in the A-B direction at pH 7.4 on both sides) was performed after the permeability assay for the test compound. The cell monolayer that had a fluorescein permeability of less than 1.5×10^{-5} cm/s for Caco-2 and MDR1-MDCKII cells and 2.5×10^{-5} cm/s for MDCKII cells was considered intact, and the permeability result of the test compound from intact cell monolayer is reported.

8.2.2. ADME-Tox: *In Vitro* Metabolism

Cytochrome P450 Inhibition (HPLC-UV/VIS and HPLC-MS/MS detection)

Peak areas corresponding to the metabolite of each substrate were recorded. The percent of control activity was then calculated by comparing the peak area obtained in the presence of the test compound to that obtained in the absence of the test compound. Subsequently, the percent inhibition was calculated by subtracting the percent control activity from 100 for each compound. IC_{50} values (concentration causing a half-maximal inhibition of control values) were determined by non-linear regression analysis of the concentration-response curve using Hill equation curve fitting.

Intrinsic Clearance (microsomes, S9, cryopreserved hepatocytes, recombinant CYP, recombinant UGT)

Metabolic stability, expressed as percent of the parent compound remaining, was calculated by comparing the peak area of the compound at the time point relative to that at time-0. The half-life ($T_{1/2}$) was estimated from the slope of the initial linear range of the logarithmic curve of compound remaining (%) vs. time, assuming the first-order kinetics. The apparent intrinsic clearance (CL_{int} , in $\mu\text{L}/\text{min}/\text{pmol}$, $\mu\text{L}/\text{min}/\text{mg}$ or $\mu\text{L}/\text{min}/\text{Mcell}$) was calculated according to the following formula:

$$CL_{int} = \frac{0.693}{T_{1/2} \cdot (\text{mg protein}/\mu\text{L or million cells}/\mu\text{L or pmol CYP isozyme}/\mu\text{L})}$$

8.2.3. ADME-Tox: Genetic Toxicity

Bacterial Cytotoxicity

The results for cytotoxicity are expressed as percent of control growth (OD_{600}). Compounds with growth of less than 60 % of control are flagged and considered cytotoxic.

Ames Tests

Wells that displayed bacteria growth due to the reversion of the histidine mutation (as judged by the ratio of OD_{430}/OD_{570} being greater than 1.0) are counted and recorded as positive counts. The significance of the positive counts between the treatment (in the presence of test compound) and the control (in the absence of test compound) are calculated using the one-tailed Fisher's exact test.

Three significance levels are reported as follows:

Weak positive, if $0.01 \leq p < 0.05$, denoted as "+"

Strong positive, if $0.001 \leq p < 0.01$, denoted as "++"

Very strong positive, if $p < 0.001$, denoted as "+++"

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