

Novel Neuroprotective Role Of Dichamanetin Against Hydrogen Peroxide-Mediated Cytotoxicity *In-Vitro* Alzheimer's Disease Model

0303

Muhammad-Safuan Zainuddin¹, Ang Chee Wei², Saatheevavaane Bjuvanendran Pillai¹, Adzzie-Shazleen Azman¹

¹Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, ²School of Science, Monash University Malaysia

Dichamanetin was successfully synthesized with a good yield (50%) with high purity (95-97%) | Had low antioxidative activity | Non-cytotoxic to cholinergic neurons below 3.125µg/mL at 24, 48, and 72 hours | At 0.781µg/mL of dichamanetin conferred the highest neuroprotection in 6-hour co-staggered treatment against H₂O₂-insult | Dichamanetin has a potent neuroprotective effect against H₂O₂.

INTRODUCTION

Alzheimer's disease (AD) is a major neurodegenerative disorder with limited therapeutic options.

Oxidative stress is an early hallmark of AD and contributes to neuronal damage, neuroinflammation, and disease progression¹.

Flavonoids are promising therapeutic candidates owing to their antioxidant and neuroprotective activities².

Dichamanetin (C₂₉H₂₄O₆), a rare C-benzylated flavanone with reported anti-AD activity, remains poorly characterized regarding its antioxidant and neuroprotective mechanisms³.

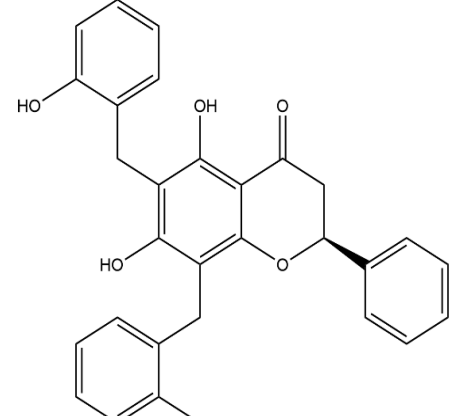


Fig 1. Two-dimensional (2D) structure of dichamanetin

METHODS

Dichamanetin Synthesis

Microwave Synthesiser → MPLC Purification → ¹H NMR Characterisation → Dichamanetin

In-Vitro Antioxidant Panel

Dichamanetin → 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) & Ferric Reducing Power (FRAP) Assay

RESULTS

Dichamanetin was afforded as a light-yellow solid (22.5mg, 50%, 95-97% purity) with 467.32 m/z (calc. for [C₂₉H₂₄O₆]⁺ = 468.5). The dichamanetin was characterised using ¹H NMR (300 MHz, Acetone-d₆) (Table 1).

	Assign	Shift	Range	H's	Integral	Class	J's
1	A (s)	9.14	9.17-9.11	2	1.58	s	
2	B (m)	7.46	7.50-7.41	2	2.10	m	
3	C (m)	7.30	7.38-7.23	3	2.68	m	
4	D (d)	7.09	7.14-7.05	1	0.92	d	7.47
5	E (d)	6.95	6.99-6.91	1	0.81	d	7.58
6	F (m)	6.87	6.91-6.81	2	2.11	m	
7	G (dd)	6.70	6.75-6.66	2	2.09	dd	4.99, 7.87
8	H (m)	6.59	6.66-6.51	2	2.00	m	
9	I (dd)	5.48	5.54-5.43	1	0.99	dd	3.16, 12.70
10	K (d)	3.77	3.81-3.74	4	3.96	d	2.24
11	L (m)	3.06	3.14-2.98	1	1.08	m	
12	M (d)	2.76	2.79-2.74	1	0.55	d	3.14

RESULTS

In-Vitro CCK-8 Cytotoxicity Assay

SH-SY5Y Plating (1.5x10⁵ cell/mL) → 7-Day Differentiation → Dichamanetin, Quercetin, Donepezil (0-100µg/mL) → CCK-8 (24-, 48-, & 72-Hour Cytotoxicity Assay) → 3-Hour

In-Vitro CCK-8 Neuroprotective Assay

SH-SY5Y Plating (1.5x10⁵ cell/mL) → 7-Day Differentiation → Pre-Treatment (Dichamanetin, Quercetin, Donepezil) → 24-Hour Exposure (Hydrogen Peroxide) → CCK-8 (3-Hour)

Co-Treatment: Dichamanetin, Quercetin, Donepezil + H₂O₂ → CCK-8 (24-Hour Co-Treatment + H₂O₂ Exposure) → 3-Hour

Staggered Co-Treatment: Dichamanetin, Quercetin, Donepezil + H₂O₂ → CCK-8 (1-3-6-Hour Pre-Treatment, 24-Hour Co-Treatment + H₂O₂ Exposure) → 3-Hour

Compound	Concentration
Dichamanetin	0.195-6.25µg/mL
Quercetin	3.125µg/mL
Donepezil	3.125µg/mL
Hydrogen Peroxide	62.5µM

RESULTS

Figure 2. The relative percentage of reducing power using the FRAP assay was measured at 592nm. (A) The standard curve of Trolox using a range of concentration (0-200µg/mL). (B) The relative percentage of reducing power of dichamanetin using a range of concentrations (0-200µg/mL). The data is represented by mean ± SD of pentaplicates.

Figure 3. In-vitro cytotoxicity study of dichamanetin (A), quercetin (B), and donepezil (C) at different concentrations (0-100µg/mL) on cholinergic neurons at 24-, 48-, and 72-hour carried out using CCK-8 cell viability assay. The results are represented by mean ± SEM (n=3); Two-Way ANOVA followed by Tukey's post-hoc analysis (A*p<0.05, B**p<0.01, C***p<0.001, D****p<0.0001).

CONCLUSIONS

Dichamanetin was successfully synthesised with **50% yield** and **95-97% purity**. Although it exhibited low antioxidant activity, it was **non-cytotoxic** and demonstrated **potent neuroprotection** against H₂O₂-induced oxidative stress. These findings highlight dichamanetin as a promising neuroprotective candidate for Alzheimer's disease and support further mechanistic investigation

REFERENCES

- Cummings, Jeffrey L et al. "Alzheimer's disease drug development pipeline: 2025." *Alzheimer's & dementia (New York, N. Y.)* vol. 11,2 e70098. 3 Jun. 2025, doi:10.1002/trc2.70098
- Maher, Pamela. "The Potential of Flavonoids for the Treatment of Neurodegenerative Diseases." *International journal of molecular sciences* vol. 20,12 3056. 22 Jun. 2019, doi:10.3390/ijms20123056
- Quimque, Mark Tristan et al. "Potential Cancer- and Alzheimer's Disease-Targeting Phosphodiesterase Inhibitors from *Uvaria alba*: Insights from *In Vitro* and Consensus Virtual Screening." *ACS omega* vol. 6,12 8403-8417. 16 Mar. 2021, doi:10.1021/acsomega.1c00137

CONTACT

Email: Muhammad.Zainuddin@monash.edu