Effects of Novel Chalcone Derivatives upon H9c2 and MDCK Cell Viability

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Abstract

Many compounds with potential anti-cancer activity fail to reach the latter stages of clinical trials due to adverse effects, often causing cardiac and renal toxicity. Here, we synthesised a group of novel chalcone compounds, thought to have potential anti-cancer activity[4] and assessed their effects upon cardiac and renal cell viability. Data revealed that all compounds produced minimal short term toxicity. Further work will be performed to assess the long term effects of these compounds on cell viability, leading to mechanistic studies and structure activity relationship analyses.

Introduction

The characteristic 3'4'5'-trimethoxy aromatic ring from Combretastatin A-4 has been utilised in many chalcone (1,3-diphenyl-2-propen-1-one) derivatives to produce promising anticancer activity[1] of which one is DMU-135 pro drug. A group of halogenated methoxy based chalcone analogues (Mk 25, Mk 33, Mk 34 and Mk 35) were synthesised and designed based on the DMU-135 pro drug (3,4-Methylenedioxy-3',4',5'-trimethoxy chalcone), with the aim of developing potential new pharmaceutical agents.
Despite their observed efficacy[2], many chalcone-based drug discovery studies have not addressed cardiovascular toxicity. However, this is a significant adverse effect associated with many anticancer drugs. We looked at the effects of novel chalcone derivatives upon MTT reduction using H9c2 and MDCK cell lines as models of cardiac and renal cells, respectively.

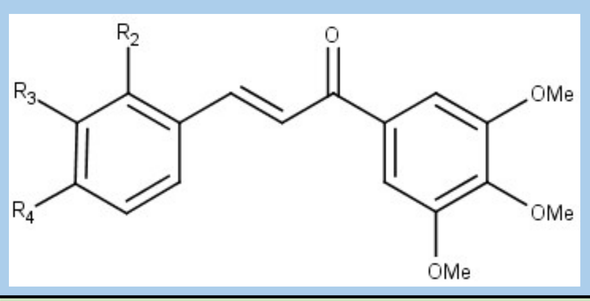

Figure 1: All four compounds are halogenated derivatives of this 3'4'5'-trimethoxy based chalcone analogue from DMU-135.

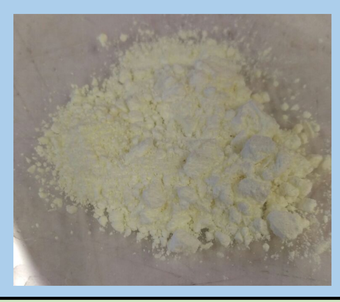

Figure 2: Mk33 compound appearing as a pale yellow powder

Materials and Methods

Synthesis: Potassium Hydroxide 3 ml (20% w/v solution) was added to a mixture of 3'4'5'-trimethoxyacetophenone (5 mmol) and aryl aldehyde (5 mmol) dissolved in 9 ml of ethanol. The mixture was stirred at room temperature for 1 hour monitored by thin layer chromatography. The precipitate formed was filtered off and washed with 2x 30ml water and 2x 30ml cold ethanol. Purification *via* ethanol recrystallization was used to produce pure chalcone which was verified by MS and NMR. Mass spectra were recorded on a Thermo Scientific LTQ Orbitrap XL low resolution triple quadropole mass spectrometer (EPSRC National Mass Spectrometry Service Centre, Swansea UK). The 1H NMR and 13C NMR spectra were recorded on a Bruker Avance AV400 NMR spectrometer at 30oC. Tetramethylsilane was used as an internal standard.

MTT assay: H9c2 and MDCK cells were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM) and maintained in a gassed incubator (37°C, 95% air/5% CO_2). For experiments, cells were seeded into 96-well plates (5000 cells/well) and, 24 hours later, treated with novel compounds (2 hours incubation at 37°C, 3-1000 µM in serum-free DMEM). Cell viability was assessed by the colorimetric conversion of 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) to purple formazan product (measured at 560 nm using a GloMax plate reader, Promega).

Data analysis: Data were analysed using SigmaPlot 13. Statistical significance was determined by ANOVA with a post hoc Tukey's test. $P < 0.05$ was considered statistically significant. All data are presented as means ± S.E.M. The n in the text refers to the number of separate experiments, each performed in triplicate.

Results

Mk 25:, pale yellow powder, yield 82%, m.p 106-110°C, TLC: Rf. = 0.25 (Hexane/Ethyl Acetate 8:2); M_w 332.5; MS, m/z [EI+, %] 333.0890 (M+1, 100%) ; ^{1}H NMR (DMSO-d6, 400 MHz): δ 3.78 (3H, s, OCH3), 3.90 (6H, s, OCH3), 7.44 (2H, s, ArH), 7.46-7.50 (2H, m, ArH), 7.56-7.60 (1H, m, ArH), 7.97 (1H, d, J = 15.7 Hz, C=CH), 7.85 (1H, d, J = 15.7 Hz, C=CH), 8.21-8.24 (1H, m, ArH); ^{13}C NMR (DMSO-d6) δ 56.2, 60.2, 106.3, 124.6, 127.6, 128.7, 129.9, 131.9, 132.3, 132.6, 134.6, 138.4, 142.2, 152.9, 187.8.

Mk 33: pale yellow powder, yield 81%, m.p 136-139°C, TLC: Rf. = 0.29 (Hexane/Ethyl Acetate 8:2); M_w 367.0; MS, m/z [EI+, %] 367.0501 (M+1, 100%) ; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.78 (3H, s, OCH_3), 3.91 (6H, s, OCH_3), 7.44 (2H, s, ArH), 7.71 (1H, d, *J* = 15.2 Hz, C=CH), 7.74 (1H, d, *J* = 7.1 Hz, ArH), 7.91 (1H, dd, *J* = 7.1, 2.0 Hz, ArH), 8.02 (1H, d, *J* = 15.2 Hz, C=CH), 8.25 (1H, d, *J* = 2.0 Hz, ArH); ^{13}C NMR (DMSO-d_6) δ 56.3, 60.2, 106.4, 123.6, 129.1, 130.3, 130.4, 131.8, 132.6, 132.7, 135.5, 141.1, 142.2, 152.9, 187.7.

Mk 34: pale yellow powder, yield 83%, m.p 110-115°C, TLC: Rf. = 0.25 (Hexane/Ethyl Acetate 8:2); M_w 332.5; MS, m/z [EI+, %] 333.0882 (M+1, 100%) ; ^{1}H NMR (DMSO-d6, 400 MHz): δ 3.77 (3H, s, OCH3), 3.91 (6H, s, OCH3), 7.44 (2H, s, ArH), 7.49-7.52 (2H, m, ArH), 7.72 (1H, d, J = 16.0 Hz, C=CH), 7.84-7.87 (1H, m, ArH), 8.01 (1H, d, J = 16.0 Hz, C=CH), 8.05 (1H, s, ArH); ^{13}C NMR (DMSO-d6) δ 56.3, 60.2, 106.3, 123.3, 127.8, 128.1, 130.1, 132.7, 133.7, 136.9, 142.2, 142.3, 152.9, 187.8.

Mk 35: pale yellow powder, yield 70% m.p 115-119°C, TLC: Rf. = 0.25 (Hexane/Ethyl Acetate 8:2); M_w 332.5; MS, m/z [EI+, %] 333.0882 (M+1, 100%) ; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.77 (3H, s, OCH_3), 3.90 (6H, s, OCH_3), 7.43 (2H, s, ArH), 7.53 (2H, d, *J* = 8.5 Hz, ArH), 7.73 (1H, d, *J* = 15.3 Hz, C=CH), 7.95 (2H, d, *J* = 8.5 Hz, ArH), 7.96 (1H, d, *J* = 15.3 Hz, C=CH); ^{13}C NMR (DMSO-d_6) δ 56.2, 60.2, 106.2, 122.5, 128.9, 130.7, 132.8, 133.6, 135.1, 142.0, 142.4, 152.9, 187.8.

MTT assay:
All four chalcone compounds produced concentration-dependent inhibition of cell viability, as measured by MTT reduction in Hc92 (n=4) and MDCK (n=5). * indicates significance ($P < 0.05$) vs. control (i.e. untreated cells, 100%.viability). See figure 3A-D.

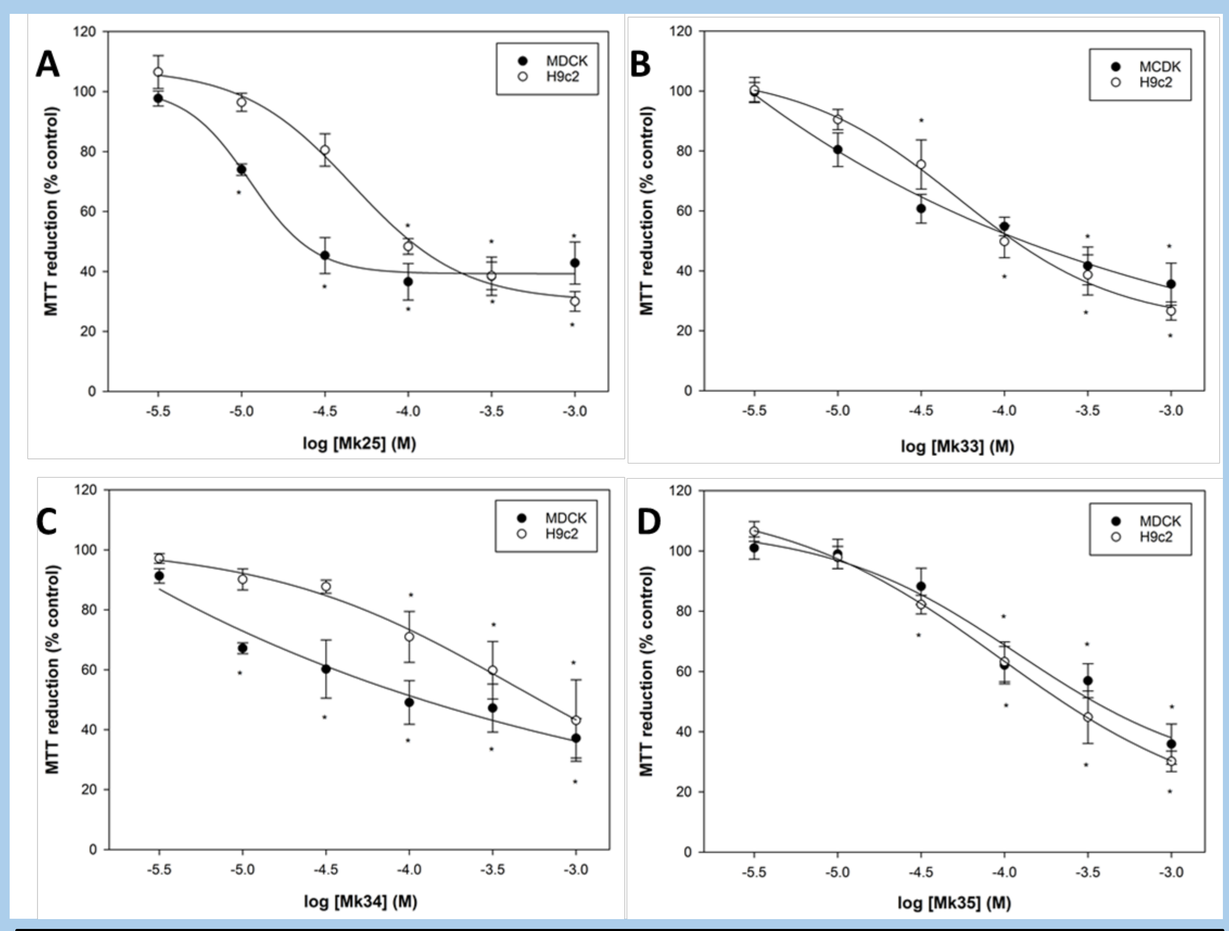

Figure 3: MDCK and H9c2 cells were exposed to novel compounds Mk25 (A), Mk 33 (B), Mk 34 (C) or Mk35 (D) for 2 hrs, then cell viability was measured using the MTT assay. Data shown as mean ± SEM. For MDCK data n=5, for H9c2 cells n=4; each experiment performed in triplicate * = $P < 0.05$ vs. untreated controls (100% viability).

Conclusion

Data revealed that despite all four compounds showing concentration dependent inhibition of cell viability, significant inhibition was only observed at very concentrated level of 10 uM or higher. This suggests that these compounds exert minimal short term toxicity upon these cell lines. Further long term and mechanistic studies on cancer and endothelial cell lines will help to uncover any anti-cancer activity these compounds may possess.

Acknowledgements

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