C-Geranylated Flavonoids from *Paulownia tomentosa* Steud. Fruit and Their Antiproliferative and Cytotoxic Activities

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INTRODUCTION

Prenylated or geranylated flavonoids have been studied for their promising antiproliferative and cytotoxic activities. A series of twelve natural geranylated flavonoids (**1**–**12**) was isolated from the fruit of *Paulownia tomentosa* Steud., and their structures were elucidated by interpretation of the spectroscopic data using UV and IR analysis, mass spectrometry, 1D and 2D NMR spectroscopy, and the absolute configurations were determined using circular dichroism. Seven of these compounds was characterized as new geranylated derivatives isolated from a natural source for the first time, namely 3´-*O*-methyl-5´-hydroxyisodiplacone (**3**), paulodiplacone A (**5**), tomentone II (**6**), tomentone B (**7**), tomentodiplacone P (**8**), paulodiplacone B (**9**), and tomentoflavone A (**12**). This investigation was focused on the elucidation of their antiproliferative and cytotoxic potential against human monocytic leukaemia cell line THP-1 in the tested concentration range of 1–30 μM after 24 h of incubation using WST-1 and LDH assays, respectively.

[https://gobotany.newenglandwild.org/species/paulownia/tomentosa/]

EXPERIMENTAL SECTION

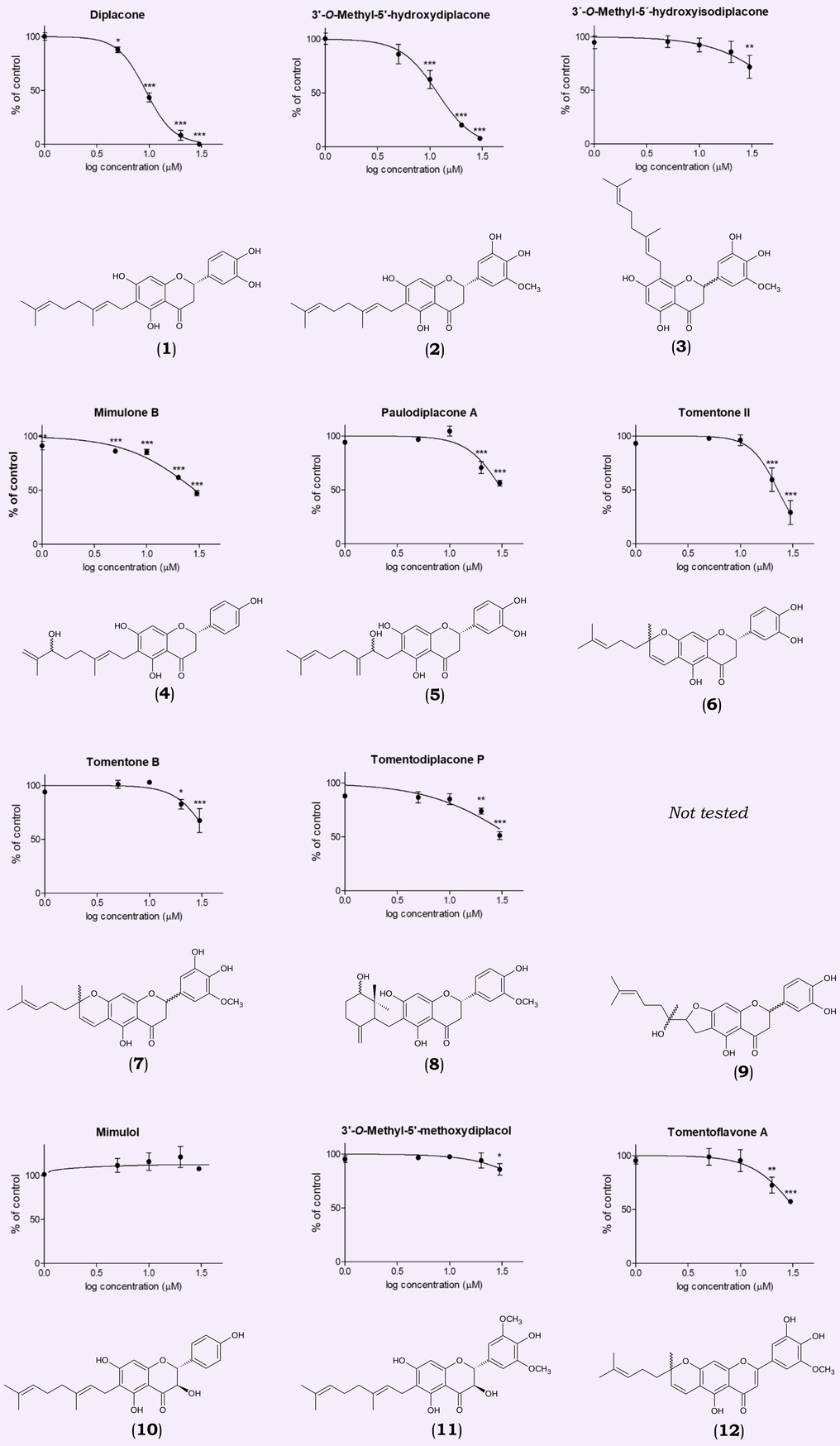
Antiproliferative and cytotoxic activity of geranylated flavonoids *in vitro* were assessed against the human monocytic leukemia cell line THP-1 that was purchased from European Collection of Cell Cultures (Salisbury, UK). The cells were cultured in RPMI 1640 medium supplemented with 10% of foetal bovine serum, antibiotic solution (100 U/ml of penicillin, 100 μg/ml of streptomycin) and 2 mmol/l of L-glutamine, all were obtained from HyClone Laboratories, Inc. (GE Healthcare, Logan, UT, USA). The cells were maintained in an incubator with 5% CO_2 at 37 °C. The tested substances were dissolved in DMSO from Sigma Aldrich (St. Louis, MO, USA); the final concentration of DMSO in experiments did not exceed 0.1%. Compound solutions were prepared fresh each time again before the experiment. The effect of the compounds was evaluated within the concentration range of 1–30 μM after 24 hours of incubation with THP-1 cell line. Antiproliferative activity of isolated compounds was determined by Cell Proliferation Reagent WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) and the cytotoxicity was determined using Cytotoxicity Detection KitPlus (LDH) (both purchased from Roche Diagnostics, Mannheim, Germany), according to the manufacturer's recommended procedure. THP-1 cells (5×10^4 cells/100 μL culture medium per well) were seeded in 96-well plates in triplicate and incubated with tested compounds for 24h. The measurement was taken by Epoch Microplate Spectrophotometer (BioTek, Winooski, VT, USA). The results shown are the average of three independent measurements performed in triplicate. The obtained data were statistically evaluated using a non-parametric one-way ANOVA test in combination with Dunnett's test at significance level $\alpha = 0.05$. The IC_{50} and LC_{50} values were calculated using the nonlinear regression four-parameter logistic model by GraphPad Prism 5.00 software (GraphPad Software, San Diego, CA, USA).

CONCLUSIONS

Almost all compounds induced a concentration-dependent decrease of metabolic activity of THP-1 cells and a concentration-dependent decrease of cell viability. The most potent antiproliferative as well as cytotoxic effect showed diplacone (**1**) (IC_{50} 9.31 ± 0.72 μM, LC_{50} 18.01 ± 1.19 μM), whereas 3´-*O*-methyl-5´-hydroxydiplacone (**2**) (IC_{50} 12.61 ± 0.90 μM, LC_{50} > 30 μM) exhibited the widest range between the decrease of cell proliferation and viability indicating that it may serve as potential lead compound for further testing.

RESULTS

Tested compound	IC_{50} ± SD (μM)	LC_{50} ± SD (μM)
Diplacone (**1**)	9.31 ± 0.72	18.01 ± 1.19
3´-*O*-Methyl-5´-hydroxydiplacone (**2**)	12.61 ± 0.90	> 30
3´-*O*-Methyl-5´-hydroxyisodiplacone (**3**)	> 30	--
Mimulone B (**4**)	20.91 ± 1.00	> 30
Paulodiplacone A (**5**)	> 30	--
Tomentone II (**6**)	19.99 ± 0.24	> 30
Tomentone B (**7**)	> 30	--
Tomentodiplacone P (**8**)	> 30	--
Mimulol (**10**)	> 30	--
3´-*O*-Methyl-5´-methoxydiplacol (**11**)	> 30	--
Tomentoflavone A (**12**)	> 30	--



Antiproliferative activity of flavonoids from *P. tomentosa* against THP-1 cell line after 24h incubation determined by WST-1 assay. P < 0.05, ** P < 0.01, *** P < 0.001, significantly different from drug-free control.