Structural Derivatization of Clusianone and In Vitro Cytotoxicity Evaluation Targeting Respiratory Carcinoma Cells

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Abstract

Clusianone (1) was isolated from Garcinia parvifolia and structurally modified using semisynthetic methods to obtain compounds 2–4. The structural derivatization included methylation (2), hydrogenation (3), and the addition of a methylamine group [(4) to (1)]. Cytotoxic effects of these compounds were assessed on MRC5 fibroblasts, A549 lung adenocarcinoma, HK1 squamous nasopharynx carcinoma, and NP69 normal nasopharyngeal epithelial cell lines. Clusianone (1) showed cytotoxic activity against A549 cells with an IC_{50} value of 3.06 µM. Compound (4) showed cytotoxic activities against both A549 and HK1 cells with IC_{50} values of 4.09 µM and 3.43 µM, respectively. The results of the cytotoxicity assay provide a correlation on the structure activity relationship of clusianone against HK1 and A549 cells, which can be further investigated as a potential antiproliferative compound.

Key words
Garcinia parvifolia · Clusiaceae · clusianone analogues · semisynthetic methods · cytotoxic activity

Supporting information available online at http://www.thieme-connect.de/products

Natural clusianone belongs to compounds classified as type B polyprenylated polycyclic acylphloroglucines (PPAPs) that are found in plants of the Clusiaceae (Guttiferae) family [1,2]. To date, natural clusianone has been found in various parts of the targeted Clusiaceae plants, including fruits [3], flowers [4,5], leaves [6], roots [7], stems, and bark [8,9]. Naturally occurring 1 has shown potential anticancer activity [9] and further investigations of clusianone-treated HepG2 hepatocarcinoma cells revealed cytotoxic effects by mitochondrial impairment [10]. In the last decade, clusianone (1) has gained substantial interest from the synthetic community leading to a breakthrough in total synthesis of this compound [11–14]. Synthetic clusianone was tested for cytotoxicity in several cancer cell lines: HeLa (cervix carcinoma), MiaPaCa-2 (pancreatic carcinoma), and MCF7 (breast adenocarcinoma). The IC_{50} values ranged from 3.0 µM to 8.3 µM for the three cancer cell lines [15,16].

In our studies, we extracted clusianone using a previously published method [6]. The isolation and abundance of clusianone from the leaves of Garcinia parvifolia (Miq.) Miq. has made chemical modifications readily attainable as compared to clusianone isolated from the dried stem bark of Garcinia assiugu [9] and the roots of Hypericum hypericoides (L.) Crantz [7]. X-ray crystallography was identical in previous reports, and a detailed comparison of our and previously reported NMR data [8] was established. This method provided a quantity of over 0.5 g (0.05% yield) of 1 to be used as starting material for synthesizing sufficient quantities of clusianone derivatives (2–4). All products were purified via column chromatography. The products 1–4 exhibited the predicted fragmentation and mass m/z when analyzed by ESI-MS (Fig. 1). In addition, further characterizations of 1–4 were conducted utilizing 1H NMR and 13C NMR spectroscopy to characterize both tautomers (a/b) present. Data on clusianone and analogues are available as Supporting Information. Furthermore, clusianone derivatives (2–4) were produced in less than two reactions steps, with yields over 75% (Fig. 2).

A bioassay involving the (MTT) tetrazolium dye reduction assay revealed that the cytotoxic effects of all four compounds (1–4) against normal (MRC5 & NP69) and carcinoma (A549 & HK1) respiratory cell lines were profound. The IC_{50} values after 48 h were averaged from three sets of experiments (Table 1). The IC_{50} values were determined from the resulting dose response plot by using the interpolation methods in GraphPad Prism 6. Cytotoxicity screening tests were also performed against normal cell lines to validate the compound inhibitory activity in cancer cells without significantly affecting the viability of normal cells. Based on these results, several findings can be summarized: The methylation of OH has decreased the cytotoxic effects of 2 on both cancer and normal cells by 30-fold. Compound 2 is also less toxic to normal cells in terms of concentration, which is lethal to 50% of the cells. Upon hydrogenation, the tetrarenylated group of 2 leads to a more lipophilic domain of this compound, which has indeed decreased the cytotoxic effect of 3 by 20-fold. The cytotoxicity result of compound 4 is rather significant since the IC_{50} value is in the range of 3.0 to 5.0 µM. Interestingly, compound 4 suppressed the growth rate of MRC5 and NP69 at concentrations above 10.0 µM. Compound 4 showed significant cytotoxic activities against both A549 and HK1 cells with IC_{50} values of 4.09 µM and 3.43 µM, respectively. Apart from this, clusianone (1) showed a significant IC_{50} value of 3.06 µM against A549 cells and thus affects less MRC5 cells. Cytotoxicity results prevail that a hydroxyl group on clusianone is crucial in preventing the cell division progress to occur. Similarly, compound 4 possessing both hydroxyl and amine is postulated to exert a greater interaction with carcinoma cells since 4 suppressed carcinoma cell growth better than 1. This indicates that compounds 1 and 4 both exhibit anti-proliferative characteristics. The antimicrotubube drug docetaxel was used as a control since type B PPAP compounds have been demonstrated to exhibit an inhibitory activity of the tubulin assembly of carcinoma cells [17]. In conclusion, 1 and 4 have potential for development as anticancer candidates, and future works to study their modes of action as antimicrotubule agents should be emphasized.

Material and Methods

Isolation and optimization of clusianone
Leaves of G. parvifolia (University of Nottingham Malaysia Campus 45) were collected from trees in a reserved forest in Sungai Congkak, Selangor. The plant authenticity was verified and a voucher specimen (herbarium number PID 271210–13) was deposited at the School of Pharmacy of the University of Nottingham Malaysia Campus and Forest Research Institute Malaysia (FRIM). Dried and powdered G. parvifolia leaves (1000 g) were extracted by maceration. A total of 80 g of hexane extract was obtained from the 1000 g of powdered leaves macerated. Successive
column chromatography, including chlorophyll removal steps, was carried out as previously reported in a larger scale to obtain clusianone (1)[6].

Preparation of clusianone derivatives
Three clusianone (1) derivatives [methylated (2), hydrogenated (3), and amine added (4)] were synthesized on a 10 to 100 mg scale and purified using column chromatography. All derivatives were synthesized from clusianone except for 3, which was synthesized from 2 intermediately. Detailed synthesis steps and ESI-MS, 1H NMR, and 13C NMR characterizations of the four compounds are available as Supporting Information.

Compounds
Clusianone (C33H42O4): Yellow crystals; m. p. 90–92 °C; ESI-MS m/z, [M + H]$: 503.3147 calcd. for 503.3167 (rel. int. %) = 503 (100), 435 (23), 379 (4.5), 343 (1.9), 311 (4.6), 235 (1.7). For 1H NMR and 13C NMR of 1 (a/b), see Tables S1 and S2, Supporting Information.
Compound 2 \((C_{34}H_{44}O_4)\): White crystals; m.p. 108–110 °C; ESI-MS \(m/z\); \([M^+ + H]^+\): 517.3316 calc. for 517.3318 (rel. int. %) = 517 (100), 449 (40.6), 393 (5.3), 357 (4.2), 325 (4.0). For \(^1H\) NMR and \(^{13}C\) NMR of \(2\) (a/b), see Tables S3 and S4, Supporting Information.

Compound 3 \((C_{34}H_{32}O_4)\): Brown wax; ESI-MS \(m/z\); \([M + Na]^+\): 545.3607 calc. for 545.3607 (rel. int. %) = 545 (100), 523 (13.4), 429 (2.8), 413 (5.5), 385 (3.4), 341 (3.3), 310 (6.0), 226 (4.0). For \(^1H\) NMR and \(^{13}C\) NMR of \(3\) (a/b), see Tables S5 and S6, Supporting Information.

Compound 4 \((C_{34}H_{45}NO_4)\): Colorless wax; ESI-MS \(m/z\); \([M + H]^+\): 516.3423 calc. for 516.3478 (rel. int. %) = 517 (100), 498 (1.7), 490 (2.0), 448 (1.1). For \(^1H\) NMR and \(^{13}C\) NMR of \(4\) (a/b), see Table S7, Supporting Information.

Cytotoxicity assay
All stock cultures, except for the NP69 cultures, were grown in T25 flasks with Rosewell Park Memorial Institute medium (RPMI) containing 10% fetal bovine serum (FBS). NP69 cultures were grown in keratinocyte-SFM together with a supplement of bovine pituitary extract (BFE) and human recombinant (EGF). Freshly trypsinized cells were seeded into 96-well plates at densities of 10,000 cells in 100 µL medium per well. After one day of culture, each of the wells was subjected to different concentrations of the compounds, from 0–200 µg/mL. The compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted further with RPMI medium for all cultures, except for NP69, which was diluted with keratinocyte-SFM that serves as a stock solution. The DMSO concentration used was not higher than 0.1%. Different concentrations of the compounds (1–4) were prepared through a serial dilution of the stock solution. The plates were then incubated for 48 h to test their cytotoxicity levels. Docetaxol (Fresenius Kabi Oncology Ltd.), with a purity of 99%, was used as a positive control. The absorbance readings were set at 540 nm using a microplate reader. The absorbance was further analyzed as described in the Supporting Information.

Supporting information
A detailed description of the semisynthesis process, spectral data of clusianone and its analogues, and absorbance data are available as Supporting Information.

Acknowledgements
We would like to thank Dr. Alan Khoo Soo-Beng from the Institute of Medical Research and Prof. Tsao George Sai-Wah from the University of Hong Kong for providing the NP69 and HK1 cells. The authors would like to thank the Ministry of Higher Education (MOHE) for providing a grant for the study.


Bibliography

DOI: http://dx.doi.org/10.1055/s-0035-1568332

Published online February 11, 2016


© Georg Thieme Verlag KG Stuttgart · New York · ISSN 2199-157X

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