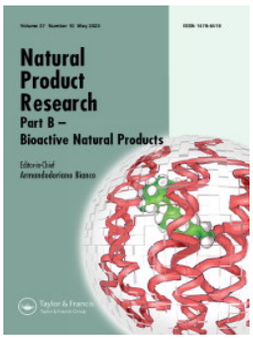

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Isolation of a new cytotoxic colchinoid from *Gloriosa superba* roots

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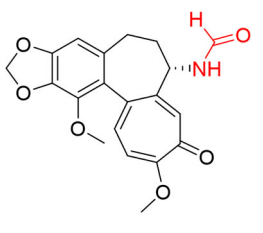
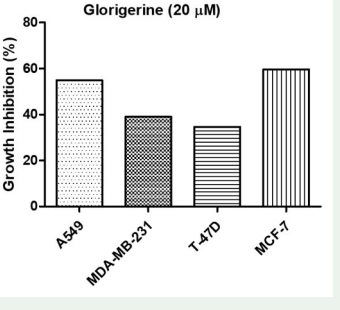
ABSTRACT

A new colchinoid compound, identified as *N*-deacetyl-*N*-formylcornigerine (**1**), named glorigerine was isolated from the roots of *Gloriosa superba*, along with two known compounds. The structures of isolated compounds were elucidated by 1 D and 2 D NMR and HRMS experiments. Glorigerine (**1**) differed from cornigerine (**6**) by the presence of an *N*-formyl group instead of the *N*-acetyl group. Glorigerine (**1**) was found to have moderate cytotoxicity when tested against four human cancer cell lines.



Glorigerine (**1**)



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KEYWORDS
Gloriosa superba; glorigerine; colchinoid; cytotoxicity; natural product

1. Introduction

Colchinoids are the natural products having a special chemical skeleton consisting of three rings: a trimethoxyphenyl ring, a cycloheptane ring, and a tropolone ring. This type of compounds occur naturally in the family Colchicaceae. Colchinoids have been isolated from *Gloriosa* and *Colchicum* species, belonging to the family Colchicaceae (Nett et al. 2020). *Gloriosa superba* is a rich source of colchinoids and has been used for the commercial production of colchicine. Traditionally, *G. superba* roots are used for the treatment of gout, rheumatic disorders, skin diseases, etc. (Jana and Shekhawat 2011). Colchicine has been used for the treatment of gout, familial Mediterranean

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fever, and Behc¸et's disease. Its semisynthetic derivative thiocolchicoside is used as a skeletal muscle relaxant. Colchicine is also being evaluated in a large number of clinical studies for the treatment of a wide variety of therapeutic conditions such as cancer, cardiovascular disorders, SARS-COV-2, etc. Apart from colchicine, other colchinoids such as 3-demethyl colchicine, gloriosine, colchicoside have been isolated from *G. superba* (Lockhart and O'Rahilly 2021; Goel et al. 2022).

In our recent work, we have isolated 14 compounds from the roots of *Gloriosa superba*, including colchicine, gloriosine, lumicolchicines, colchicoside, etc. Gloriosine was evaluated for its cytotoxicity potential against a panel of 15 human cancer cell lines for the first time, and was found to have significant activity with IC_{50} value in the nanomolar range (Goel et al. 2022). In continuation of the previous work, herein we report the isolation of a previously undescribed new cytotoxic compound, and 2 known compounds from the roots of *Gloriosa superba*.

2. Results and discussion

In continuation of our previous work (Goel et al. 2022), three compounds (**1**-**3**), including a new previously undescribed colchinoid analogue (**1**), were isolated from the chloroform/methanol (v/v, 1:1) extract of *G. superba* roots. The isolated compounds were characterised by extensive 1 D and 2 D NMR and HRMS analyses. The known compounds were identified as 3-demethyl-β-lumicolchicine (**2**) (Alali et al. 2005) and 3-demethyl-*N*-deacetyl-*N*-formyl-β-lumicolchicine (**3**) (Al-Tel et al. 1991; Figure 1), by comparing the observed NMR spectra with the reported data (supplementary material).

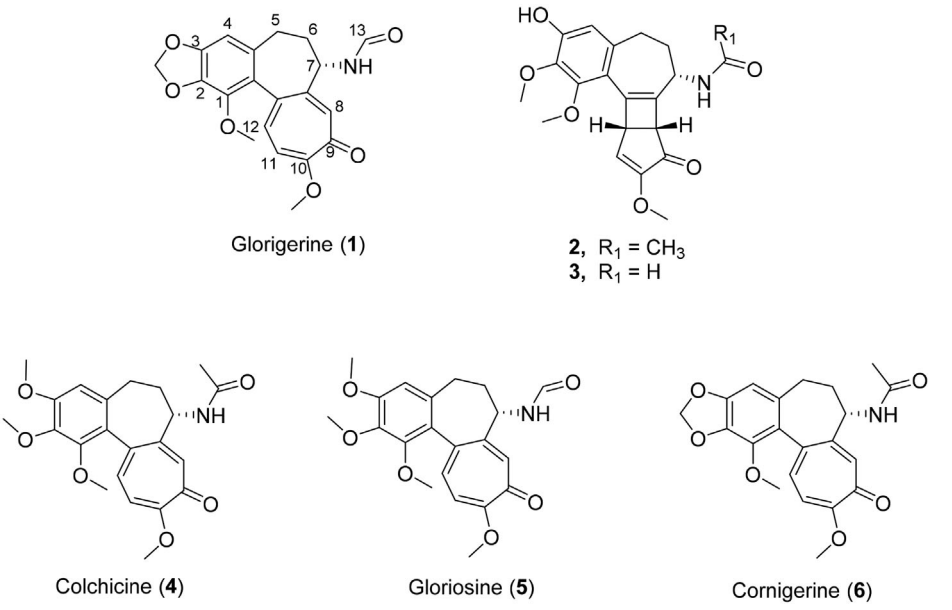

Figure 1. Chemical structure of compounds **1**-**3** isolated from *Gloriosa superba* roots, and related colchinoids.

Compound **1** (5 mg) was obtained as a buff coloured powder. The structure was established by extensive NMR and HRMS experiments. The complete ^{1}H, ^{13}C, DEPT-135, and HMBC data sets are shown in supplementary material (Figures S1–S8 and Table S1). The molecular formula of compound **1** was identified as $C_{20}H_{19}NO_6$ based on the HRMS (observed *m/z* $[M+H]^+$ 370.1282, calcd for $C_{20}H_{20}NO_6^+$, 370.1285). The 1 D NMR data of compound **1** showed the presence of colchinoid nucleus. In ^{1}H NMR spectrum, four aromatic protons were present at δ_H 6.46, 6.84, 7.46, and 7.25, of which two (δ_H 6.84 and 7.25) were in ortho relationship, with $J_o = 10.8$ Hz, while other two appeared as singlet. ^{1}H NMR spectrum also confirmed the presence of four aliphatic protons, two methoxy groups (δ_H 3.81 and 4.01) and two methylene protons (δ_H 6.01). The ^{13}C and DEPT-135 NMR spectra showed the presence of three methylene carbons (δ_C 29.66, 37.25, 101.48), three CH/CH_3 groups (δ_C 50.59, 56.42, 60.56), four olefinic carbons (δ_C 103.45, 112.45, 130.80, 135.63), eight quaternary carbons (δ_C 125.05, 132.80, 136.04, 137.25, 140.91, 149.17, 150.59, 164.05), one aldehydic carbon (δ_C 160.55), and one ketone carbon (δ_C 179.41). Careful examination of 1 D NMR data displayed structural similarities with colchinoids, especially gloriosine (**5**) (Goel et al. 2022). The characteristic peaks for a tropolone ring C, a ketone carbonyl at 179.41 ppm, two adjacent CH doublets at 6.84 and 7.25 ppm (AB pattern, H-11 and H-12 respectively), and a CH singlet at 7.46 ppm (C-8), were present. In particular, compound **1** differed from gloriosine (**5**) by the absence of two methoxy groups. Only two methoxy singlets were present at δ_H 3.81 and 4.01, with HSQC correlations to δ_C 60.56 and 56.42, respectively, and HMBC correlations to C-1 (δ_C 140.91) and C-10 (δ_C 164.05), respectively. Furthermore, one singlet at δ_H 6.01 due to two hydrogen atoms was present, demonstrating HSQC correlation with a methylene carbon at δ_C 101.48 (confirmed by DEPT-135 experiment), as well as HMBC correlations with C-2 (δ_C 137.25) and C-3 (δ_C 149.17). Literature search revealed that this pattern is similar to that of ring A of cornigerine (**6**), in which two methoxy groups at C-2 and C-3 of colchicine (**4**) are condensed to form a methylenedioxy bridge (Alali et al. 2005). However, compound **1** differed from cornigerine (**6**) in the same way that gloriosine (**5**) differed form colchicine (**4**), i.e. presence of an *N*-formyl group rather than the *N*-acetyl group. Thereby, the structure of compound **1** was established as *N*-deacetyl-*N*-formylcornigerine, which on literature search was found to be a new previously undescribed compound and named glorigerine (**1**, Figure 1). On the basis of the well-known biosynthetic pathway for colchinoids, the stereochemistry at C-7 was assumed to be *S* (Maier and Zenk 1997; Alali et al. 2005).

Glorigerine (**1**) was evaluated for its cytotoxicity potential against four human cancer cell lines viz. A549 (lung epithelial carcinoma), MDA-MB-231 (breast adenocarcinoma), T-47D (breast carcinoma), and MCF-7 (breast carcinoma) at the concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 20 μM for 48 h, using MTT assay. It exhibited moderate cytotoxic activity against all the cell lines tested (Figure 2). It is well established that trimethoxyphenyl (TMP) and tropolone rings are essential for binding of colchinoids to the colchicine binding site (CBS) of tubulin protein. Any modification of ring A results in complete loss of activity (Gracheva et al. 2020). The decreased activity of glorigerine (**1**) may be due to condensation of two methoxy groups into methylenedioxy bridge. The Loss of TMP ring may led to decreased binding of glorigerine to tubulin protein,

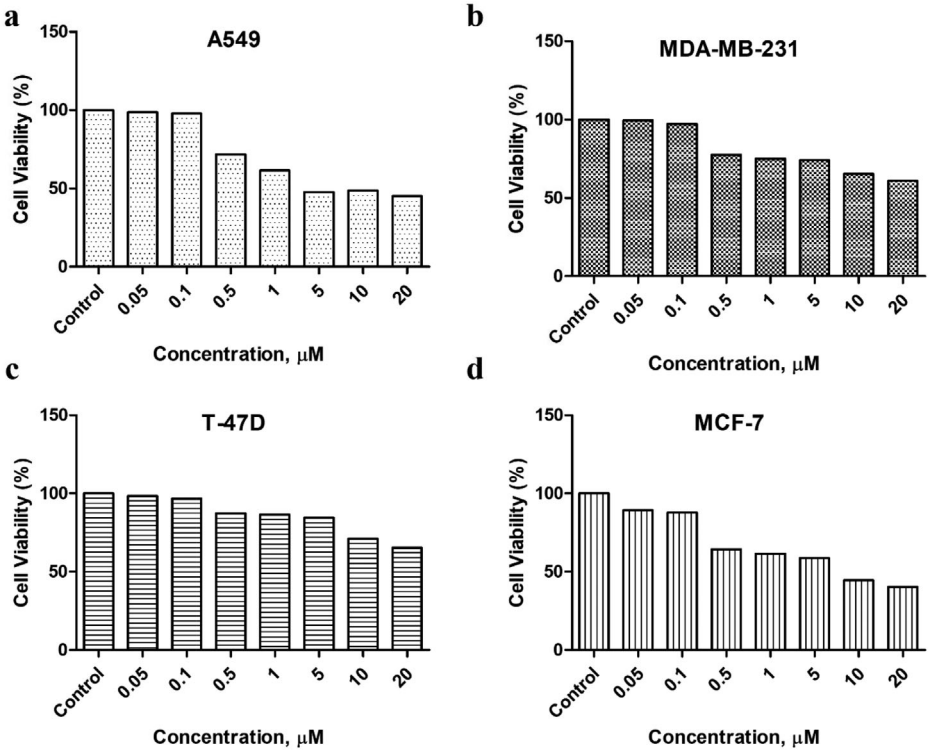

Figure 2. Percentage cell viability of A549, MDA-MB-231, T-47D, and MCF-7 cells on treatment with different concentrations of glorigerine (**1**) for 48 h.

accounting for its reduced activity. However, a detailed molecular study is still required to elucidate the mechanism of action.

3. Experimental

3.1. General experimental procedures

All the chemicals were obtained from Sigma-Aldrich and used as received. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker-Avance III HD 500 MHz and 125 MHz NMR instruments using tetramethylsilane (TMS) as the internal standard. HRMS spectra were recorded on Sciex X500R QTOF system. All chromatographic purifications were carried out using silica gel (#60 – 120 or #100 – 200) obtained from Merck. The thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 GF_{254} aluminum sheets (Merck) and observed under UV light (254 nm).

3.2. Plant material

The dried roots and tubers of *Gloriosa superba* were purchased from the local market of Varanasi, India, in January 2020 and authenticated by Dr. Bikarma Singh. A specimen sample (accession number: RRLH57502) was preserved in Janaki Ammal Herbarium at the CSIR-IIIM, Jammu, India.

3.3. Extraction and isolation

The dried roots of *G. superba* (2 kg) were coarsely powdered and extracted three times (4 L, each) with chloroform: methanol (v/v, 1:1) at room temperature. The crude extract was filtered and evaporated to dryness *in vacuo* to yield 163 g of semisolid material. The dried extract was chromatographed over silica gel (#100-200) and eluted with *n*-hexane-ethyl acetate step gradient system (v/v, 1:0 → 0:1) and then with ethyl acetate: methanol (v/v, 1:0 → 9:1). In continuation with the previous work (Goel et al. 2022), three compounds (**1**-**3**) were isolated after repeated silica gel column chromatography. The isolated compounds were characterised by 1 D and 2 D NMR and MS experiments.

3.3.1. Glorigerine; N-deacetyl-N-formylcornigerine (1)

6 mg; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.17 (s, 1H), 7.46 (s, 1H), 7.25 (d, $J = 10.7$ Hz, 1H), 6.93 (d, $J = 7.5$ Hz, 1H), 6.85 (d, $J = 10.9$ Hz, 1H), 6.46 (s, 1H), 6.01 (s, 2H), 4.74 (dt, $J = 12.1$, 6.9 Hz, 1H), 4.01 (s, 3H), 3.81 (s, 3H), 2.50 (m, 1H), 2.36 (m, 1H), 2.27 (m, 1H), 1.87 − 1.78 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 179.41, 164.05, 160.55, 150.59, 149.17, 140.91, 137.25, 136.04, 135.61, 132.80, 130.80, 125.05, 112.44, 103.43, 101.48, 60.56, 56.42, 50.59, 37.25, 29.66; HRMS *m/z* 370.1282 $[M + H]^+$ (calcd for $C_{20}H_{20}NO_6^+$, 370.1285).

3.4. Cell culture and growth conditions

All the cells lines were purchased from National Cancer Institute (NCI), Bethesda USA, and cultured according to the provided protocol. The cells were grown in a CO_2 incubator (Esco) at 37 °C in a humidified atmosphere (98% humidity) of 95% air and 5% CO_2.

3.5. In-vitro cytotoxicity studies

The cytotoxicity study was performed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5, ditetrazolium bromide (MTT) assay. In this assay, the cells were plated in a 96-well plate for 12 h. Then, the cells were treated for 48 h with different concentrations (0.05, 0.1, 0.5, 1, 5, 10, 20 μM) of glorigerine (**1**). Following incubation, 10 μL of MTT dye (2.5 mg/mL in PBS) was added to each well and incubated at 37 °C for 4 h. The supernatant was then aspirated and purple MTT-formazan crystals obtained were dissolved in 150 μL DMSO. The absorbance of this colored solution was measured at a wavelength of 570 nm (Goel et al. 2021).

4. Conclusion

In conclusion, a new colchinoid derivative, glorigerine (**1**), along with two known compounds were isolated from the extract of *Gloriosa superba* roots. The isolated compounds were characterised by 1 D and 2 D NMR and HRMS experiments. Glorigerine (**1**) was evaluated for cytotoxic activity against four human cell lines and found to have moderate cytotoxic activity. In comparison to gloriosine (**5**), the reduced activity

of glorigerine may be due to condensation of two trimethoxy groups into methylenedioxy bridge.

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Disclosure statement

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