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# Isolation of brassicasterol, its synthetic prodrug-crystal structure, stereochemistry and theoretical studies

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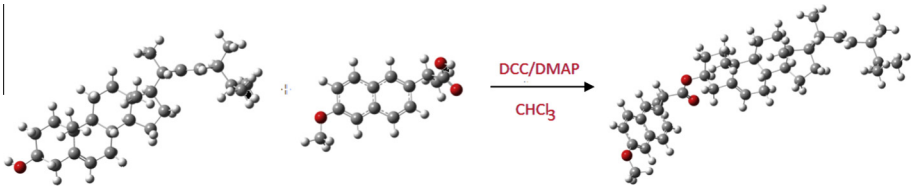
## HIGHLIGHTS

- Brassicasterol (compound **1**) is reported for the first time from *Allamanda violacea*.
- Its structure was further proved by synthesizing its prodrug (**2**) with naproxen by Steglich esterification.
- Single crystal X-ray of (**2**) confirmed structures of (**1**) and (**2**) and stereochemistry of (**2**).
- NBO analysis explained stability of both (**1**) and (**2**) through hyperconjugative interactions.
- HOMO–LUMO energy gaps were calculated with time dependent-DFT.

## GRAPHICAL ABSTRACT

Brassicasterol (compound **1**) was isolated and chemically transformed into 3β-(2-(6-methoxynaphthalene-2yl) propionoxy) 24 methyl cholest-5, 22-dien (compound **2**). Crystal structure of compound **2** not only helped in confirming the structure of compound **1** but it also helped in establishing stereochemistry of both the compounds. DFT studies of both the compounds were carried out using density functional method (DFT/B3LYP) with 6-31G (d, p) basis set.

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## ABSTRACT

In the present study brassicasterol (**1**), was isolated from the chloroform extract of the flowers of *Allamanda violacea* and identified with the help of different spectroscopic techniques like $^{1}H$, $^{13}C$, 2D NMR ($^{1}H$–$^{1}H$ COSY), IR, UV and mass spectrometry. A novel prodrug was synthesized by carrying out esterification of brassicasterol (**1**) with the well known drug naproxen using Steglich esterification to give 3β-(2-(6-methoxynaphthalene-2yl) propionoxy) 24 methyl cholest-5, 22-dien (**2**). Compounds **2** was subjected to single crystal X-ray diffraction technique and crystallized out in monoclinic form having $P2_1$ space group and stabilized by CH–π interactions. Structure and stereochemistry of compound **2** was established with the help of modern spectroscopic techniques like $^{1}H$ NMR, IR, UV, mass spectrometry as well as with single crystal X-ray diffraction.

Molecular geometry and vibrational frequencies of compounds **1** and **2** were calculated by density functional method (DFT/B3LYP) using 6-31G (d, p) basis set, bond parameters and IR frequencies were correlated with the experimental data. $^{1}H$ and $^{13}C$ chemical shifts of compound **1** and $^{1}H$ chemical shifts of compound **2** were calculated with GIAO method and correlated with experimental data. Hyperconjugative interactions were studied with the help of natural bond order analysis (NBO). Electronic properties of both the compounds such as HOMO–LUMO energies were measured with the help of time dependent DFT method.



## Introduction

Phytosterols are one of the most important class of steroid isolated either in their free state or as glycolipids or in the form of their esters from vegetable oils and are thus considered to be one of the essential constituents of our diet. These steroidal compounds are structurally related to cholesterol but differ in the type of substituent at C-24, number and position of the double bond and optical rotations at stereogenic center [1]. Phytosterols like stigmasterol ($\Delta^{22}$-24α-ethylcholesterol), β-sitosterol (24α-ethylcholesterol), campesterol (24α-methylcholesterol), etc. have been reported to

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possess different biological activities like, anti-inflammatory [2], anti-oxidant [3] and have also been found to lower cholesterol level by competing with cholesterol absorption in gut through displacement of cholesterol from micelles [4]. Unsaturated phytosterols, however have lower capability to reduce cholesterol level in comparison to saturated phytosterols [4]. Phytosterols have also been shown to inhibit lung, stomach, ovarian as well as breast cancer [5]. Thus there is increasing interest in extracting these important phytochemical components not only for their nutraceutical applications but also as essential food ingredients [6].

In continuation of our search for newer biologically active steroidal derivatives from plants [7,8], flowers of the plant *Allamanda violacea* were chosen for the isolation of steroidal derivatives. *A. violacea* commonly called purple Allamanda is an ornamental plant of the genus Allamanda belonging to family Apocynaceae. In India the flowers of the plant blooms in months of July–October. Biological screening of the different extracts of the flowers of the plant by the authors suggested the presence of constituents having anti-dyslipidemic, anti-oxidant and anti-diabetic activities [9]. Chloroform extract of the flowers showed most significant anti-dyslipidemic, anti-oxidant and anti-diabetic properties and thus was chosen for the isolation of chemical constituents responsible for these biological activities.

Compound **1** (m.p. 419 K) was isolated for the first time from the chloroform extract of the flowers of the *A. violacea* and its structure was confirmed with the help of different spectroscopic techniques like $^{1}H$, $^{13}C$, 2D NMR ($^{1}H$–$^{1}H$ COSY), IR, UV and mass spectrometry. Isolated compound responded to Liebermann burchard and tetranitromethane test [10] indicating it to be an unsaturated steroidal derivative. Identity of the compound was further confirmed by its chemical transformation into compound **2** (m.p. 401 K) with the well known drug naproxen using Steglich esterification (Scheme 1).

In nature sterols have mostly been found to be esterified with fatty acids or coumaric acid [11]. Besides, these esterified sterols have been found to possess increased oil solubility so that they can be easily incorporated into variety of lipid matrices [12,13].

Naproxen is one of the most widely used non-steroidal anti-inflammatory drug (NSAIDs), used chiefly for getting relief from acute and chronic pain. However, their use is frequently associated with a broad spectrum of adverse effects, related to inhibiting prostaglandin synthesis in tissues where PG's are responsible for physiological homeostasis [14].

As most NSAIDs posses a carboxyl group, hence one of the strategy adopted to avoid gastrointestinal (GI) damage involves carrying out the esterification of the NSAID. It has been reported that esterification of the carboxylic acid moiety of NSAIDs suppress gastro-toxicity without adversely affecting their anti-inflammatory activity [15,16].

Thus in order to increase the biological profile of the isolated sterol and to suppress the gastrointestinal damage by carboxylic group of naproxen, prodrug of **1** was synthesized by carrying out esterification with naproxen. Structure and stereochemistry of the synthesized compound **2**, which was established with the help of $^{1}H$ NMR, IR, UV, mass spectrometry and X-ray crystallography, further helped in confirming the structure of compound **1**. Geometry of compounds **1** and **2** were optimized and vibrational frequencies were calculated using density functional theory (DFT) with the help of B3LYP functional and 6-31G (d, p) basis set. The results were compared with the experimental observations. Further, nuclear magnetic chemical shifts were calculated with the same functional and basis set using GIAO method and results were compared with the experimental data. HOMO–LUMO analysis was also carried out to predict various transitions using time dependent-DFT approach.

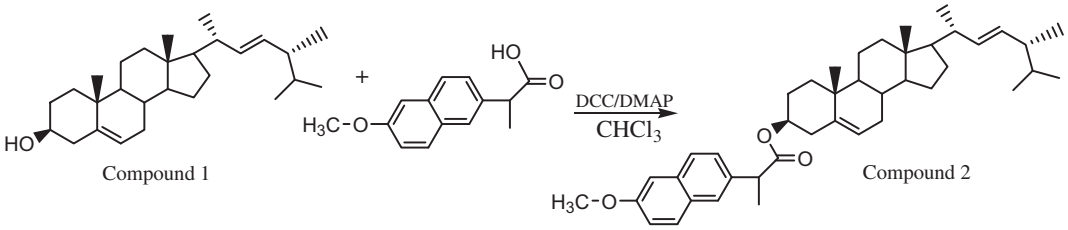
## Experimental

### Materials and methods

The whole plant of the *A. violacea* was collected in the month of October 2010 from Lucknow, India. The identity of the plant was confirmed by Dr. Tariq Hussain, Scientist and Head, Department of Taxonomy and Herbarium, National Botanical Research Institute, Lucknow, India where Voucher specimen, no-97108 was deposited. All the solvents and reagents used were of analytical grade purchased from Merck (India) and dried according to known procedure [17] before use. Thin layer chromatography (TLC) was performed on silica gel G coated plates to detect position of isolated compound and completion of its reaction. $^{1}H$ NMR spectra were recorded on Bruker DRX-300 MHz spectrometer using $CDCl_3$ as the solvent and TMS as internal standard, chemical shifts were reported as δ (ppm). IR spectra were recorded on Perkin Elmer FTIR spectrometer with the range of IR spectrum from 4000 to 400 $cm^{-1}$. The spectra were analyzed using Spectrum™ Software suite. The spectra were measured with 4 $cm^{-1}$ resolution and 1 scan co-addition. ESI-MS was recorded on Agilent 6520 Q-TOF mass spectrometer. Ultraviolet absorption spectra were obtained (in the range of 200–400 nm) using ELICO BL-200 UV–Vis spectrophotometer equipped with a 10 mm quartz cell in chloroform. Melting point was determined using open capillary tube method and uncorrected.

### Extraction and isolation

Extracts were prepared as reported earlier by Sethi et al. [9]. Dry chloroform extract (690 mg) of the flowers of *A. violacea* was subjected to column chromatography using silica gel (60–120 mesh) and n-hexane/ethyl acetate of increasing polarity. n-hexane/ethyl acetate (99:1–95:5) afforded 20 fractions which contained **1** in impure form. Compound **1** (25 mg) was obtained in pure form with repeated column chromatography using n-hexane/ethyl acetate of increasing polarity. Molecular formula $C_{28}H_{46}O$, m.p. [419 K]. ESI-MS: $m/z = 274$ (398-$C_9H_{16}$), $m/z = 318$ (398-$C_4H_6$—$C_2H_2$), $m/z = 262$ (398-$C_4H_6$—$C_6H_{10}$), $m/z = 218$ (398-$C_4H_6$—$C_2H_2$—$C_5H_{12}$). $^{1}H$ NMR ($CDCl_3$, 300 MHz), δ 7.26 (D, s, $CDCl_3$), δ 0.80 (3H, s, C-18), δ 1.01 (3H,s, C-19), δ 0.68 (3H, d, $J = 5.4$ Hz, C-26), δ 0.78 (3H, d, $J = 5.7$ Hz, C-27), δ 0.83 (3H, d, $J = 4.5$ Hz, C-28), δ 1.01 (3H, d, $J = 5.4$ Hz, C-21), δ 1.83 (2H, m, C-21, C-24), δ 2.00 (2H, m, C-7), δ 2.19–2.27 (2H, m, C-4), δ 3.49–3.52 (1H, m, C-3), δ 5.36 (1H, m, C-6), δ 5.15–5.19 (1H, m, C-22), δ 4.97–5.05 (1H, m, C-23).




**Scheme 1.**