Supplementary information

3-Isobutyl-5,5,7-tris(3-methylbut-2-en-1-yl)-1-phenyl-1,7-dihydro-4H-indazole-4,6(5H)-dione

José Edmilson Ribeiro do Nascimento, Daniela Hartwig, Raquel Guimarães Jacob * and Márcio Santos Silva *

Laboratório de Síntese Orgânica Limpa, Centrop de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, P.O. Box 354, Pelotas 96010-900, RS, Brazil; jedmilsonrn@gmail.com (J.E.R.d.N.); dani.hartwig@gmail.com (D.H.)
* Correspondence: raquel.jacob@ufpel.edu.br (R.G.J.); silva.ms@ufpel.edu.br (M.S.S.)

Table of Contents

General Information

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 400 MHz on a Bruker Avance III HD spectrometer. Chemical shifts (δ) are reported in ppm, and the abbreviations to denote the multiplicity of a particular signal are s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), sext (sextet), td (triple doublet) and m (multiplet). NMR data are reported as follows: chemical shift (δ), multiplicity, coupling constant (J) in Hertz and integrated intensity. ^{1}H NMR chemical shifts were referenced to the tetramethylsilane (TMS; 0.0 ppm) internal standard. ^{13}C NMR spectra were recorded at 100 MHz and referenced to deuterated solvent ($CDCl_3$; 77.2 ppm). Spectra were recorded in a $CDCl_3$ solution. Mass spectra (MS) were obtained on a gas chromatograph coupled to a Shimadzu GCMS-QP2010 mass spectrometer. Fragments are described by its mass/charge ratio (m/z) with the relative intensity (%) in parentheses. High resolution mass spectra (HRMS) were recorded using a Bruker micrOTOF-QII (source type: APCI). The sample was dissolved in HPLC-grade acetonitrile and injected into the APCI source by means of a syringe pump at a flow rate of 5.0 µL/min. The Compass 1.3 for micrOTOF-Q II software (Bruker daltonics, USA) was used for data acquisition, processing, and isotopic simulations. For the FTIR (Fourier Transform Infrared) in the attenuated total reflection mode (FTIR-ATR), the samples submitted to KI and placed on the crystal surface of a FTIR Bruker Alpha-P spectrometer, obtained from an average of 24 scans at the range of 4000-1500 cm^{-1}. Melting point were determined using a PDF III Marte with 0,1 °C precision. The NMR, IR and Mass analyses were carried out at the Central Analítica – Centro de Ciências Química, Farmacêuticas e de Alimentos – Universidade Federal de Pelotas – UFPel – Pelotas – Brazil. The HRMS analyses were recorded at the Universidade de Caxias do Sul – UCS – Brazil.

The reactions were monitored by thin layer chromatography (TLC) that was performed using Merck silica gel (60 F254), 0.25 mm thickness. For visualization, TLC plates were either placed under UV light, or stained with iodine vapor, or 5% vanillin in 10% H_2SO_4 and heating. The reactions were monitored by TLC, according on the disappearance of starting materials. Aldrich technical grade silica gel (pore size 60 Å, 230–400 mesh) was used for flash chromatography using hexane/ethyl acetate as eluent. All solvents and reagents used are commercially available (Sigma Aldrich®) and were used without any previous treatment. Temperatures above room temperature were maintained by use of a mineral oil bath with an electrically heated coil connected to an adjustable controller.

Lupulone: Extraction and Purification

The lupulone present in the soft resin fraction of hops was extracted according to Taniguchi method.[1] Once obtained the hop resin, it was purified by column chromatography employing a mixture of *n*-hexane/ethyl acetate in a ratio of 98:02, respectively, as eluating, providing the lupulone in a yield of 3 % (m/m).

Experimental Procedure for the synthesis of Lupulone Derivative 4

The 3-isobutyl-5,5,7-tris(3-methylbut-2-en-1-yl)-1-phenyl-1,3a-dihydro-4H-indazole-4,6(5H)-dione was obtained through the reaction between lupulone (0,5 mmol) and phenyl-hydrazine (0,6 mmol) employing 20 mg of $SiO_2/ZnCl_2$ (30 mol %) as support solid in a solvent-free condition. The reaction media was heated to 60 ºC for 20 h. The conventional heating was removed and, then, the purification was directly carried out, without extraction step. The lupulone derivative was isolated in a moderate yield (75%) by column chromatography, employing a mixture of *n*-hexane/ethyl acetate in a 94:04 ratio. The product is a yellow oil with a pleasant smell. The complete NMR structural characterization of this product was performed by ^{1}H, $^{13}C\{^{1}H\}$, COSY, HSQC, HMBC NMR experiments. The NMR sample was prepared employing 5 mg of the respective lupulone derivative in 600 μL deuterated chloroform. The 90 º pulse width was calibrated and the resolution used in the 2D experiments was 4 K / 512 (*t*2 x *t*1) data points.

[1] Taniguchi, Y.; Taniguchi, H.; Yamada, M.; Matsukura, Y.; Koizumi, H.; Furihata, K.; Shindo, K. Analysis of the components of hard resin in hops (*Humulus lupulus L.*) and structural elucidation of their transformation products formed during the brewing process. *J. Agric. Food Chem.* **2014**, *62*, 11602. DOI: 10.1021/jf504394h.

Lupulone Derivative 4 Characterization

Yield 75%, yellow oil. NMR ^{1}H ($CDCl_3$, 400 MHz) δ 0,98 (d, J = 3,0 Hz, 3H), 0,99 (d, J = 3,0 Hz, 3H), 1,18 (s, 3H), 1,44 (s, 3H), 1,53 (s, 3H); 1,57 (m, 6H); 1,63 (s, 3H); 2.10 (*ddd*, J = 6.3, 6.6 and 14.7 Hz), 2,13-2,18 (m, 1H), 2,50-2,60 (m, 3H), 2,70 (d, J = 7.3 Hz, 2H), 2.90 (d, J = 7.2 Hz, 2H), 3.84 – 3.79 (m, 1H), 4,51 (t, J = 7,2 Hz, 1H), 4,86 (t, J = 7,3 Hz, 2H), 7,47 – 7,37 (m, 3H), 7,55 – 7,47 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17,36; 17,69; 17,96; 22,34; 22,40; 25,71; 25,90; 25,93; 28,07; 30,11; 33,83; 36,32; 39,50. 46,74; 65,65; 118,12; 118,39, 118,58; 119,72; 124,54; 128,70; 129,59; 134,37; 135,05; 135,61; 139,21; 147,14; 153,56; 193,04; 209,92. MS (relative intensity) m/z: 486 (3); 417(39); 349(100); 333(15); 295(4); 251(2); 207(4); 77(5); 69(26); 41(29). IR (cm^{-1}): 3224, 2988, 2220, 1615, 1463, 942, 962, 784. HRMS (ESI): *m/z* $[M + H]^+$ calcd for $C_{32}H_{43}N_2O_2$: 487.33191; found: 487.33183.

Selected NMR Spectra

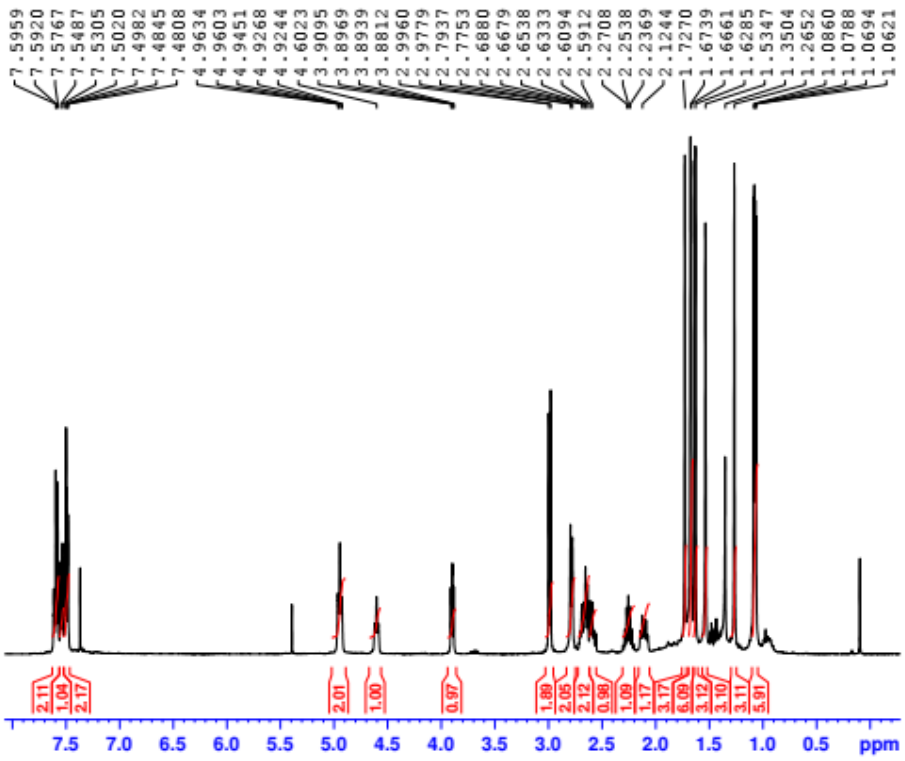

Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of lupulone-indazole.

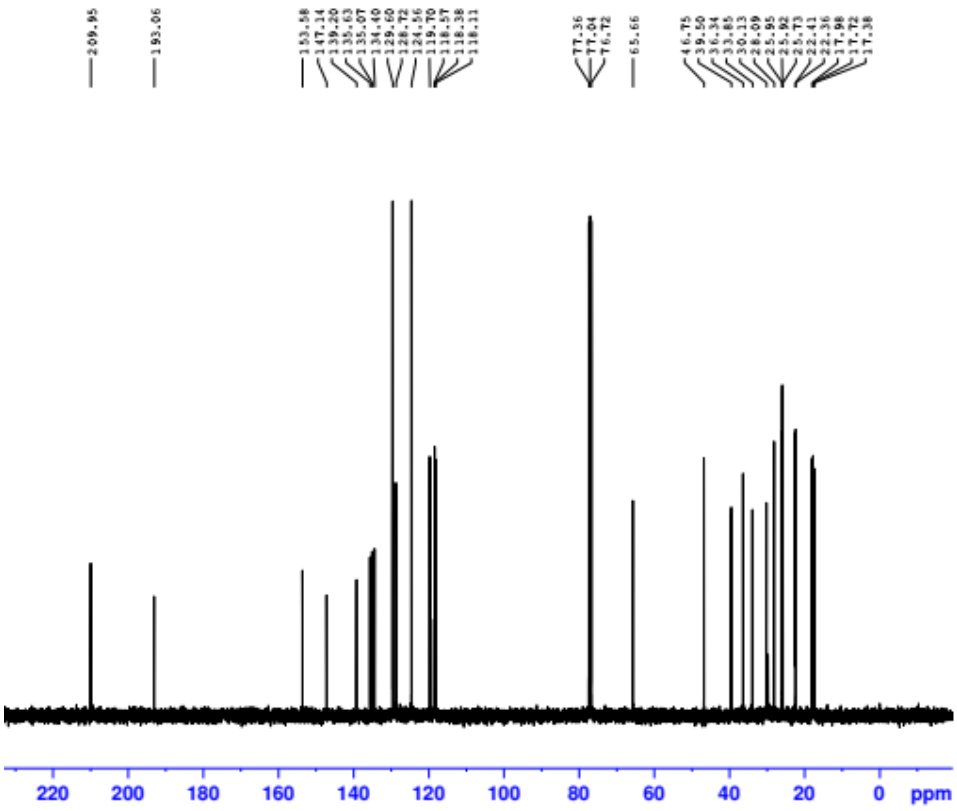

Figure S2. ^{13}C-{^{1}H} NMR spectrum (100 MHz, $CDCl_3$) of lupulone-indazole.

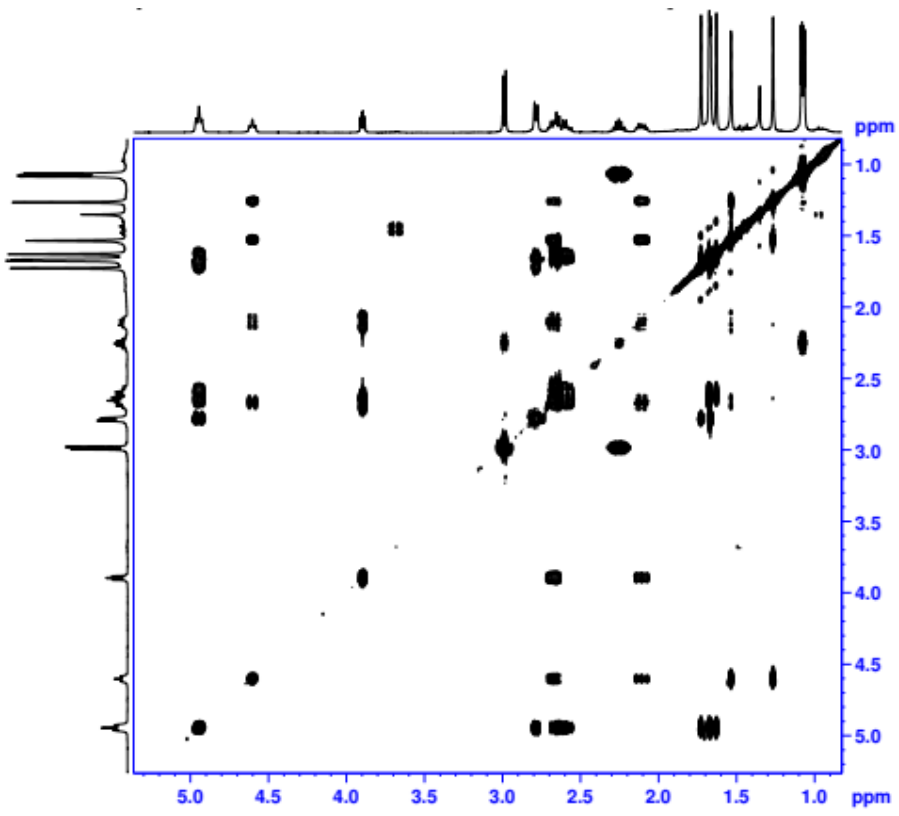

Figure S3. COSY NMR spectrum (400 MHz, $CDCl_3$) of lupulone-indazole.

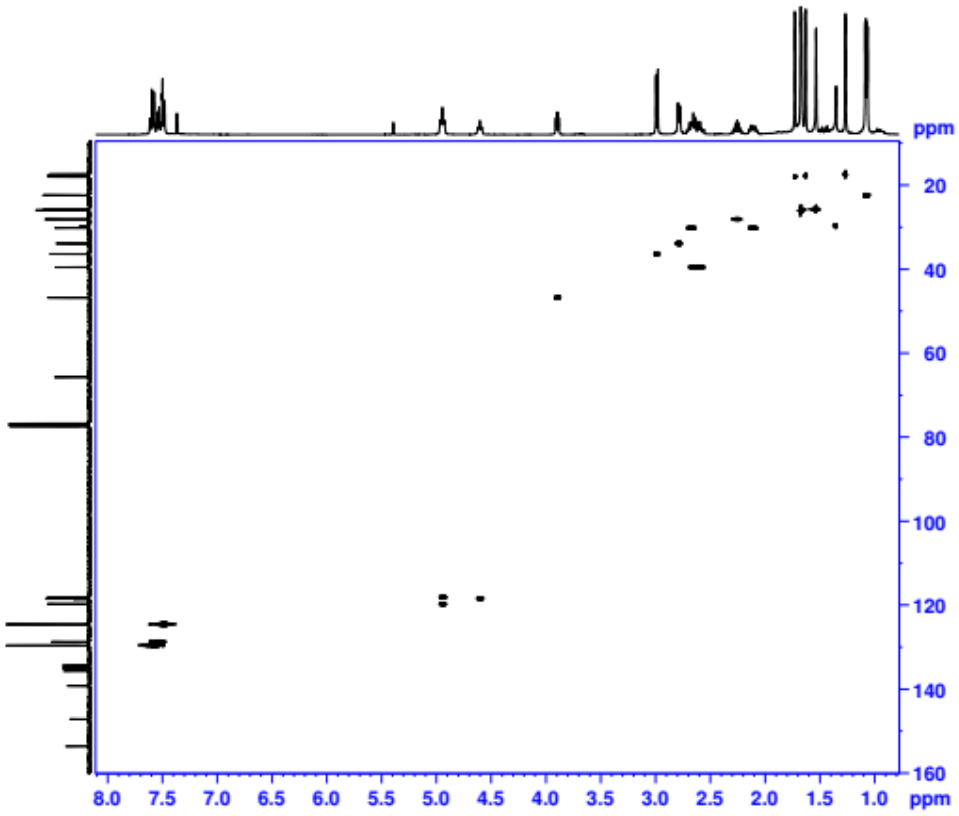

Figure S4. HSQC NMR spectrum (400 MHz, $CDCl_3$) of lupulone-indazole.

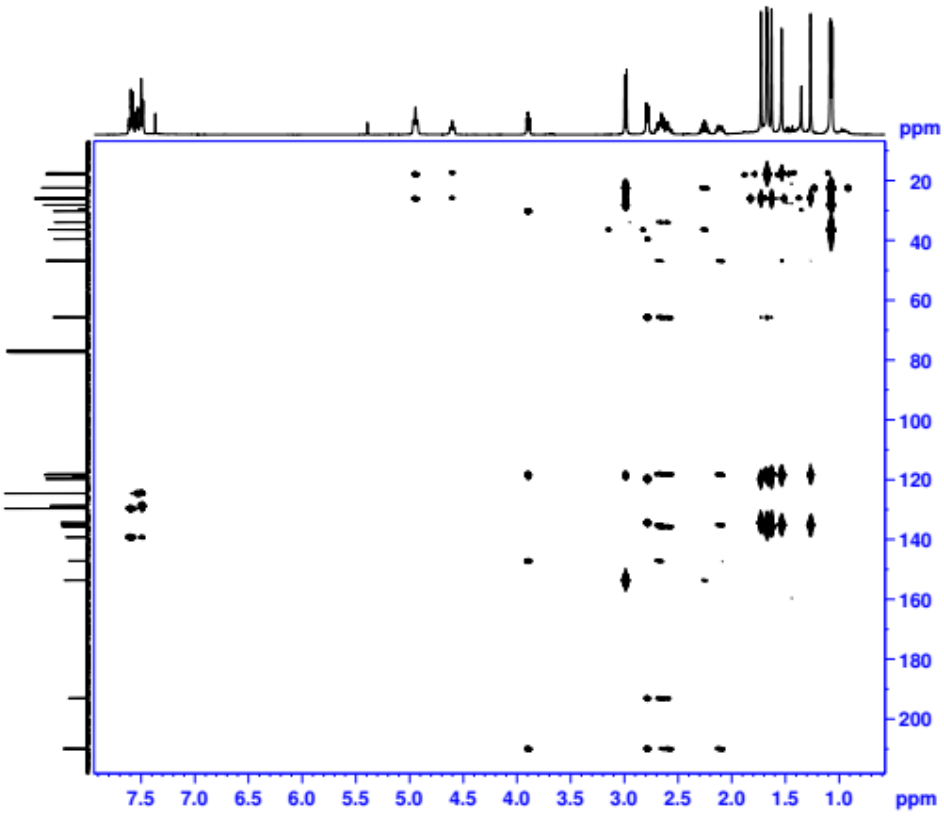

Figure S5. HMBC NMR spectrum (400 MHz, $CDCl_3$) of lupulone-*H*-indazole 2.

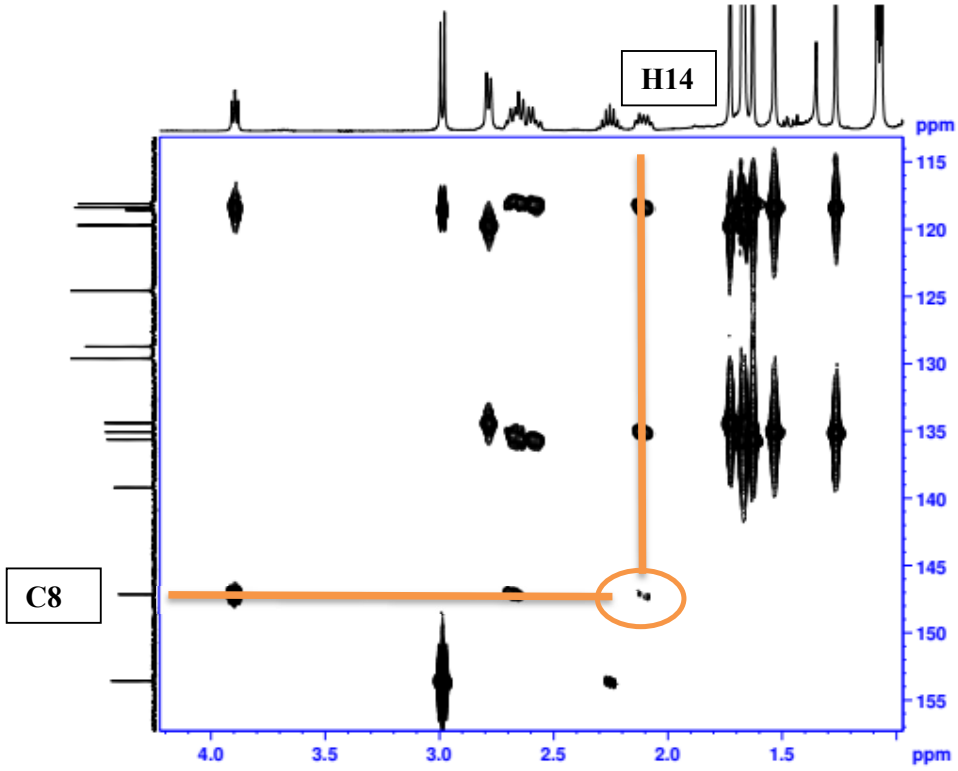

Figure S6. HMBC NMR spectrum (400 MHz, $CDCl_3$) of lupulone-*H*-indazole 2.

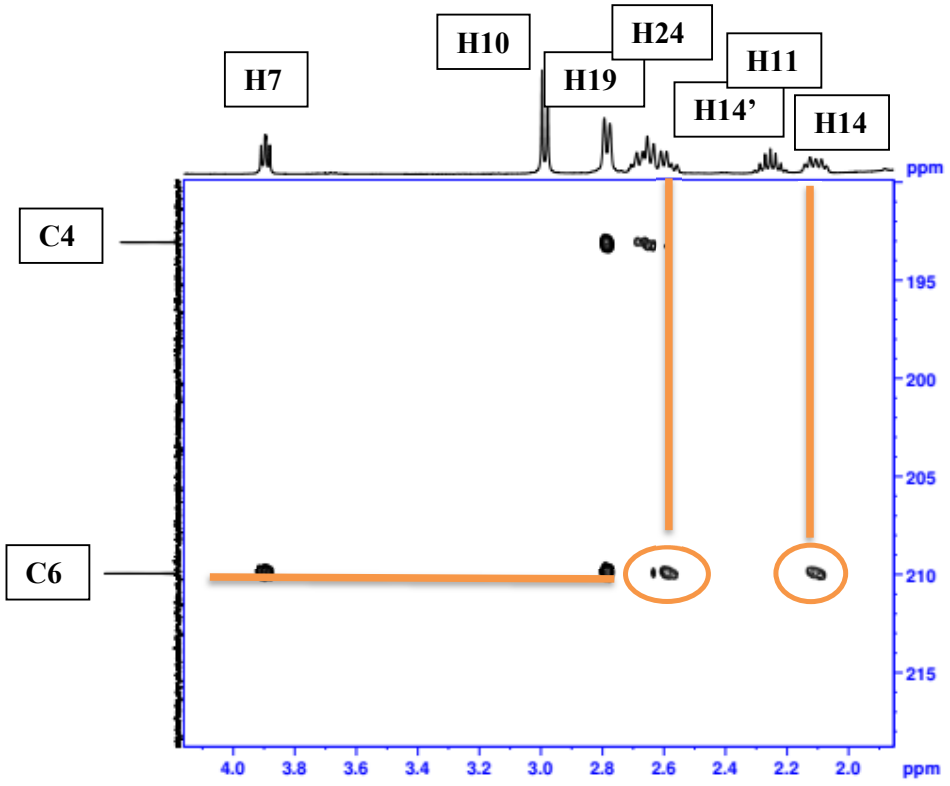

Figure S7. HMBC NMR spectrum (400 MHz, $CDCl_3$) of lupulone-*H*-indazole 2.

Mechanism to access Lupulone Derivatives 1 and 3

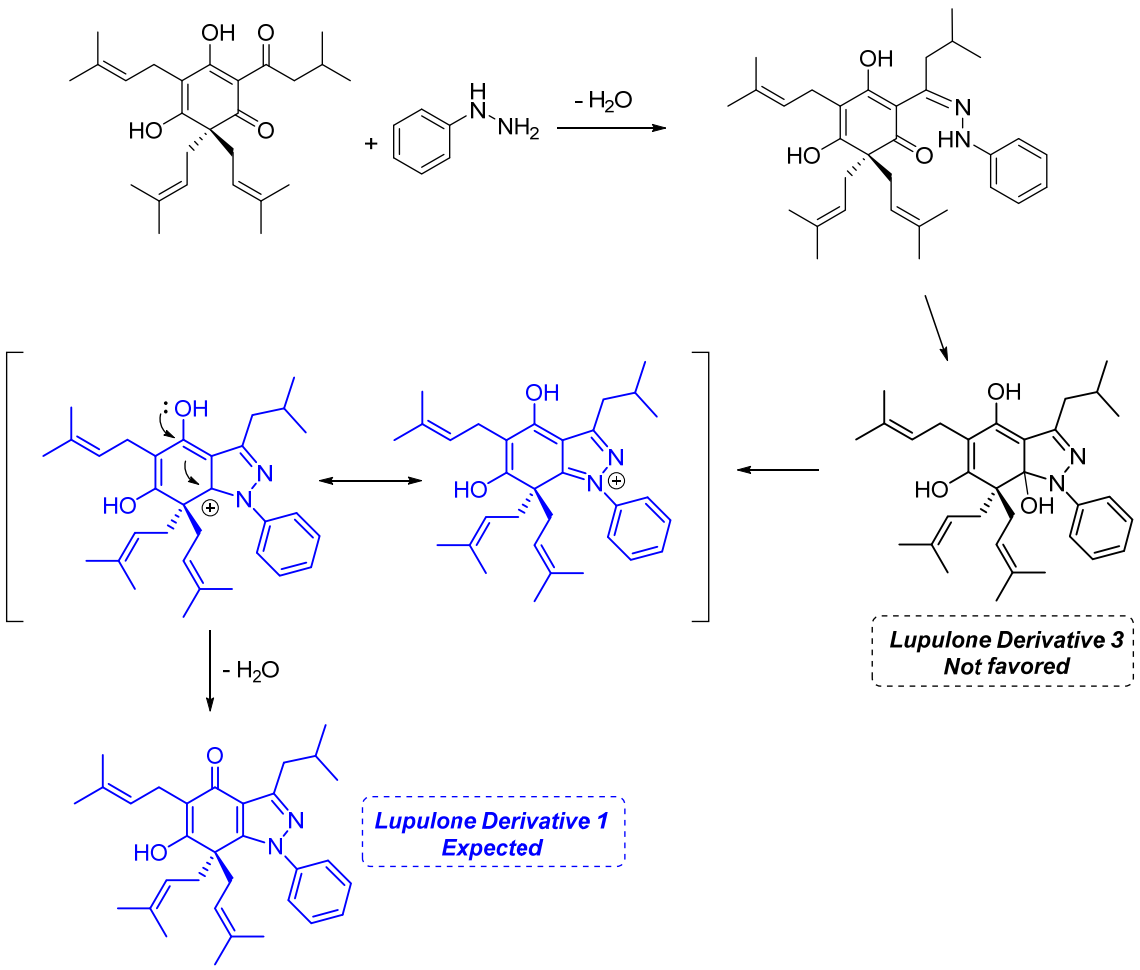

Figure S8. Mechanism to access the Lupulone Derivative 1 (Expected) and Lupulone Derivative 3 (not favored).