Research Article

Isolation, Crystal Structure, and In Silico Aromatase Inhibition Activity of Ergosta-5, 22-dien-3\(\beta\)-ol from the Fungus 

\textit{Gyromitra esculenta}

Yerlan Melsuly Suleimen\(^1,2\), Ahmed M. Metwaly\(^3\), Ahmad E. Mostafa\(^3\), Eslam B. Elkaed\(^4\), Hong-Wei Liu\(^5\), Buddha Bahadur Basnet\(^6\), Raigul Nurbekkyzy Suleimen\(^7\), Margarita Yulayevna Ishmuratova\(^8\), Koblandy Muboryakovich Turdybekov\(^9\), and Kristof Van Hecke\(^10\)

\(^1\)Sh. Ualikhanov Kokshetau University, Kokshetau, Kazakhstan
\(^2\)Republican Collection of Microorganisms, Nur-Sultan, Kazakhstan
\(^3\)Pharmacognosy and Medicinal Plants Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt
\(^4\)Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, Ad Diriyah, Riyadh 13713, Saudi Arabia
\(^5\)State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
\(^6\)Faculty of Sciences, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal
\(^7\)L.N. Gumilyov Eurasian National University, Nur-Sultan, Kazakhstan
\(^8\)E. A. Buketov Karagandy University, Karaganda, Kazakhstan
\(^9\)Department of Physics of Academician E.A. Buketov Karagandy University, Karaganda 100028, Kazakhstan
\(^10\)XStruct, Department of Chemistry, Ghent University, Ghent B-9000, Belgium

Correspondence should be addressed to Yerlan Melsuly Suleimen; syerlan75@yandex.kz and Ahmed M. Metwaly; ametwaly@azhar.edu.eg

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Ergosterol derivatives exhibited copious promising biological activities. The fungus \textit{Gyromitra esculenta} is widely distributed in Europe and North America. In order to examine the chemical properties of \textit{Gyromitra esculenta}, a phytochemical study has been preceded and resulted in the isolation of the steroid, ergosta-5, 22-dien-3\(\beta\)-ol (brassicasterol), from its methanol extract. The complete identification and absolute configuration of the isolated compound have been established by X-ray structural analysis to be (22E, 24R)-24-methylcholesta-5, 22-dien-3\(\beta\)-ol. The reported cytotoxicity and the great structural similarity of the isolated compound with the cocrystallized ligand of the aromatase enzyme inspired us to run molecular docking studies against that protein. Ergosta-5, 22-dien-3\(\beta\)-ol occupied the target protein with a binding mode almost the same as the cocrystallized ligand and a binding affinity of \(-33.55\) kcal/mol, which was better than that of the cocrystallized ligand (\(-22.61\) kcal/mol). This promising result encouraged us to conduct in silico ADMET and toxicity studies of ergosta-5, 22-dien-3\(\beta\)-ol against 6 models, and the results expected the likeness of the isolated compound to be a drug. In conclusion, ergosta-5, 22-dien-3\(\beta\)-ol has been isolated from \textit{Gyromitra esculenta}, identified by X-ray structural analysis, and exhibited promising in silico activities against aromatase enzyme.

1. Introduction

Natural products are the key source that humankind relied on to treat diseases and maintain good health [1, 2]. The effective secondary metabolites that are responsible for natural products’ activity could be derived from plants [3, 4] or marines [5, 6]. Furthermore, fungi represent an unlimited origin of bioactive metabolites [7–13]. The isolated bioactive metabolites could be classified regarding their chemical classes to be flavonoids [14–17], saponins
Gyromitra esculenta is a fungus that belongs to the genus Gyromitra which is widely distributed across Europe and North America. It normally fruits in spring and early summer in sandy soils under the coniferous trees. The fruiting body (mushroom) is an irregular brain-shaped dark brown cap that can reach 10 cm in height and 15 cm in width, perched on a stout white stipe up to 6 cm in height [27, 28]. Despite the reported toxicity of G. esculenta, it is still consumed and used in some countries in North America and Europe due to its high nutritive value [29, 30].

2. Results and Discussion

2.1. Isolation and Identification of Brassicasterol (Compound 1). Brassicasterol (Figure 1 and Table 1) was isolated from G. esculenta using different chromatographic techniques and identified as (22E, 24R)-24-methylcholesta-5, 22-dien-3β-ol (ergosta-5, 22-dien-3β-ol) or brassicasterol. The compound was identified by comparing its 1D and 2D NMR spectroscopic spectra with the published data (supplementary materials, Figures S1–S5) [44, 47].

Ergosterol and its derivatives were reported to have various cytotoxic effects. As an example, ergosterol could inhibit in vitro cancer growth through upregulation of multiple tumor suppressors [31, 32]. Furthermore, ergosterol peroxide exerted promising antitumor activities in colorectal cancer [33] and several other tumor types [34]. Additionally, dehydroergosterol derivatives induced apoptosis in human malignant melanoma cells [35] and inhibited the growth of human breast adenocarcinoma MCF-7 cells [36]. Interestingly, a dehydroergosterol derivative isolated from Ganoderma lucidum inhibited the proliferation of human cervical carcinoma cells with an IC50 value of 8.58 μM through induction of apoptosis [37]. Some ergosterol derivatives displayed anticancer activities through the inhibition of aromatase protein [38, 39].

Compound 1 (brassicasterol) has been isolated before from several plant sources such as the steam distillate of rapeseed oil [40] and Brassica juncea seeds [41]. Moreover, it was found in algae [42] and marine organisms [43]. In addition to the anti-inflammatory effect [44], brassicasterol could inhibit bladder carcinogenesis in an in vivo study [45].

X-ray crystallography is the technique that uses the ability of X-rays to be diffracted by a crystalline structure into different specific directions. The angles and intensities of the diffracted X-ray beams could be used to determine the three-dimensional picture of the electron density of the diffracting crystal. Consequently, the atoms’ positions, chemical bonds, absolute configuration, and several other information can be determined [46].

Herein, we discuss the isolation, crystal structure, and molecular docking of ergosta-5, 22-dien-3β-ol against the aromatase enzyme.

2.2. X-Ray Analysis. In order to confirm the absolute configuration of 1, the crystal structure of its crystalline hydrate was investigated by X-ray diffraction analysis. Compound 1 crystallized in the Sohnke space group P21, with the asymmetric unit consisting of two dehydroergosterol molecules and two hydrate water molecules (Figure 2).

It follows from the data obtained that the bond lengths and bond angles in compound 1 are close to the usual ones [48]. Ring A takes a somewhat distorted chair conformation in the first (1a) and second (1b) crystallographically independent molecules (the minimum parameters of cycle asymmetry [49] and intracyclic torsion angles are given in Table 2). The conformation of cycle B in 1a and 1b, containing the C5=C6 double bond, is close to the slightly distorted 9α, 8-half-chair. The third carbocycle C in molecules 1a and 1b deviates more significantly from the ideal chair. The 5-membered cycle D in molecule 1a takes the conformation of a 13β-envelope, strongly distorted towards the 14α, 13β-half-chair. In the second molecule 1b, this cycle takes the conformation of the 14α, 13β-half-chair, strongly distorted towards the 13β-envelope. In general, distortions of the conformation of A-D cycles are close to those observed in the crystal structures of 3β-hydroxy-Δ5-sterols, for example, in 24 (R), 25-epoxycholesterol [50], stigmast-5-en-3-ol [51], (24E)-26-hydroxydesmosterol [52], and (24E)-26-hydroxydesmosterol monohydrate [53].

![Figure 1: Chemical structure of compound 1.](image-url)
In the crystal, molecules 1a and 1b and molecules of hydration water are linked by intermolecular hydrogen bonds (Table 3), forming infinite ribbons in the (−1 0 2) plane along the b-axis (Figure 3).

### Table 2: Intracyclic torsion angles (τ, °) and asymmetry parameters (ΔC (min), °) in the structure of hydrate brassicasterol.

| Torsion angles | Crystallographically independent molecule 1a | Crystallographically independent molecule 1b |
|----------------|---------------------------------------------|---------------------------------------------|
|               | Cycle A                                     | Cycle B                                     |
|                | Torsion angles                              | Torsion angles                              |
|                | τ                                           | T                                           |
|                | ΔC^2 (°)                                     | ΔC^2 (°)                                     |
| C10-C1-C2-C3   | −57.9 (4)                                   | C40-C31-C32-C33                            |
|                | ΔC^2 = 2.3                                   | −59.1 (4)                                   |
| C1-C2-C3-C4    | 57.0 (4)                                    | C31-C32-C33-C34                            |
|                | ΔC^2 = 2.7                                   | 57.2 (4)                                    |
| C2-C3-C4-C5    | −54.2 (4)                                   | C32-C33-C34-C35                            |
|                |                                              | −52.2 (5)                                   |
| C3-C4-C5-C10   | 52.2 (4)                                    | C33-C34-C35-C40                            |
|                |                                              | 50.8 (4)                                    |
| C4-C5-C10-C1   | −49.4 (4)                                   | C34-C35-C40-C31                            |
|                |                                              | −50.1 (4)                                   |
| C2-C1-C10-C5   | 51.6 (4)                                    | C32-C31-C40-C35                            |
|                |                                              | 53.3 (4)                                    |
| C10-C5-C6-C7   | 2.2 (5)                                     | C40-C35-C36-C37                            |
|                | ΔC^2 = 1.9                                   | 2.0 (5)                                     |
| C5-C6-C7-C8    | 14.8 (5)                                    | C35-C36-C37-C38                            |
|                |                                              | 15.4 (5)                                    |
| C6-C7-C8-C9    | −45.5 (3)                                   | C36-C37-C38-C39                            |
|                |                                              | −46.5 (4)                                   |
| C7-C8-C9-C10   | 62.2 (3)                                    | C37-C38-C39-C40                            |
|                |                                              | 63.0 (3)                                    |
| C8-C9-C10-C5   | 44.1 (3)                                    | C38-C39-C40-C35                            |
|                |                                              | −45.2 (4)                                   |
| C6-C5-C10-C9   | 12.5 (4)                                    | C36-C35-C40-C39                            |
|                |                                              | 12.8 (4)                                    |
| C14-C8-C9-C11  | −46.0 (3)                                   | C44-C38-C39-C41                            |
|                | ΔC^2 = 9.3                                   | −48.5 (3)                                   |
| C8-C9-C11-C12  | 45.3 (3)                                    | C38-C39-C41-C42                            |
|                | ΔC^2 = 4.7                                   | 48.9 (4)                                    |
| C9-C11-C12-C13 | −52.6 (4)                                   | C39-C41-C42-C43                            |
|                |                                              | −54.4 (4)                                   |
| C11-C12-C13-C14| 58.9 (3)                                    | C41-C42-C43-C44                            |
|                |                                              | 57.5 (3)                                    |
| C12-C13-C14-C8 | −64.2 (3)                                   | C42-C43-C44-C48                            |
|                |                                              | −60.4 (3)                                   |
| C9-C8-C14-C13  | 58.0 (3)                                    | C39-C38-C44-C43                            |
|                |                                              | 56.5 (3)                                    |
| C17-C13-C14-C15| 42.4 (3)                                    | C47-C43-C44-C45                            |
|                | ΔC^2 = 8.0                                   | 46.0 (3)                                    |
| C13-C14-C15-C16| −30.3 (3)                                   | C43-C44-C45-C46                            |
|                | ΔC^2 = 11.8                                  | −35.5 (3)                                   |
| C14-C15-C16-C17| 5.9 (3)                                     | C44-C45-C46-C47                            |
|                |                                              | 10.9 (4)                                    |
| C15-C16-C17-C13| 19.9 (3)                                    | C45-C46-C47-C43                            |
|                |                                              | 17.4 (3)                                    |
| C14-C13-C17-C16| −37.4 (3)                                   | C44-C43-C47-C46                            |
|                |                                              | −37.9 (3)                                   |

### Table 3: Hydrogen-bond geometry for hydrate brassicasterol.

| D-H···A         | D-H···A H···A (Å) D···A (Å) D···A (Å) |
|----------------|-------------------------------------|
| O1-H1···O2W     | 0.84 1.87 2.703 (5) 175.3           |
| O31-H31···O1W   | 0.84 2.09 2.869 (4) 151.5           |
| O1W-H12W···O31W | 0.97 2.875 (6) 148.3               |
| O2W-H21W···O1W  | 0.87 1.95 2.812 (5) 171.2           |
| O2W-H22W···O1W  | 0.87 1.96 2.821 (6) 172.8           |

Symmetry codes: (i) x−1, y, z; (ii) x + 1, y, z; (iii) −x + 2, y + 1/2, −z + 1; −z + 1;
(iv) −x + 1, y−1/2, −z + 1.

2.3. Molecular Docking Studies. Four reasons motivated us to run molecular docking studies of ergosta-5, 22-dien-3β-ol against the aromatase enzyme: first, the great structural similarity between ergosta-5, 22-dien-3β-ol and the cocrystallized ligand (EXM) of the aromatase receptor (Figure 3); second, the reported cytotoxic effects of ergosterol derivatives; third, the reported anticancer effects of aromatase inhibitors [54]; and finally, the aromatase inhibition reports of some ergosterol derivatives [38, 39]. In this work, molecular docking studies were carried out for ergosta-5, 22-dien-3β-ol against aromatase (PDB ID: 3s7s) using the cocrystallized ligand as a reference...
molecule. We depended on the binding free energy ($\Delta G$) and the correct binding mode between the docked molecules and the active site of aromatase. The binding free energies are summarized in Table 4.

At first, 3D-flexible alignment of ergosta-5, 22-dien-3β-ol with the cocrystallized ligand (EXM) was carried out (Figure 4). From 3D-flexible alignment, it was observed that the structure of ergosta-5, 22-dien-3β-ol has a good overlap with the cocrystallized ligand (EXM).

Then, validation of the docking process was checked through the running of the docking procedure for only the cocrystallized ligand (EXM) against the active pocket of aromatase. It was found that the produced RMSD value between the generated pose of the docked molecule and the original one equals 0.90. This indicates the validity of the docking process (Figure 5).

The binding mode of the cocrystallized ligand (EXM) as a reference molecule showed a binding free energy of $-22.61$ kcal/mol (Table 3). The binding interaction showed that the steroidal nucleus overlapped with the hydrophobic environment of the binding pocket of the aromatase receptor. The methyl group at position-13 formed two hydrophobic interactions with Leu477 and Val370. Besides, the C ring of steroidal moiety was involved in hydrophobic interactions with Ile133. The B ring was engaged in two hydrophobic interactions with Ile133 and Cys437. The A ring formed three hydrophobic interactions with Ile133, Ala306, and Cys437. The methyl group at position-10 formed two hydrophobic interactions with Cys437 and Val370. Finally, the methylene group at position-6 was involved in two hydrophobic interactions with Cys437 and Val373 (Figure 6).

Brassicasterol interacted with the active site of aromatase showing a binding mode almost the same as that of the cocrystallized ligand with extra hydrogen bonding and hydrophobic interactions. Ergosta-5, 22-dien-3β-ol exerted a binding affinity of $-33.55$ kcal/mol, which was higher than that of the cocrystallized ligand. The binding mode revealed the orientation of the steroidal nucleus of the docked molecule toward the hydrophobic pocket of the aromatase receptor. The D ring of the steroidal moiety was involved in hydrophobic interaction with Val370, Val373, and Cys437. The methyl group at position-13 formed two hydrophobic interactions with Trp224 and Ile133. Also, the C ring of the steroidal moiety was involved in hydrophobic interaction with Ile133. The B ring was engaged in two hydrophobic interactions with Ile133 and Cys437. The A ring formed three hydrophobic interactions with Ile133, Ala306, and Cys437. The methyl group at position-10 formed two hydrophobic interactions with Cys437 and Ile133. The hydroxyl group at position-3 was involved in hydrogen bonding interaction with Gly349. Finally, the side chain (5, 6-dimethylhept-3-ene) moiety formed extra hydrophobic interactions with Val369, Val370, Leu477, Phe221, and Trp224 (Figure 7).

2.4. In Silico ADMET Analysis. ADMET studies were carried out for ergosta-5, 22-dien-3β-ol and the cocrystallized ligand. Discovery studio 4.0 was used to predict ADMET descriptors for all compounds. The predicted descriptors are listed in Table 5.

The ADMET-Blood-Brain Barrier (BBB) penetration study predicted that ergosta-5, 22-dien-3β-ol has a very low level of penetration indicating that ergosta-5, 22-dien-3β-ol is safe against the central nervous system (CNS). ADMET aqueous solubility of ergosta-5, 22-dien-3β-ol was very low, and andergosta-5, 22-dien-3β-ol was predicted to have a poor level of intestinal absorption. CYP2D6 is involved in the metabolism process of a broad range of metabolites inside the liver [55]. Ergosta-5, 22-dien-3β-ol was predicted as a noninhibitor of CYP2D6. Consequently, the liver dysfunction reaction is not expected upon administration of brassicasterol. The plasma protein binding model predicts whether a xenobiotic is highly bound (>90% bound) to carrier proteins in the blood [56]. Ergosta-5, 22-dien-3β-ol was expected to bind plasma protein over than 90% (Figure 8).

2.5. In Silico Toxicity Studies. Toxicity prediction was carried out based on the validated and constructed models in Discovery studio software [57, 58] as follows: (i) FDA rodent carcinogenicity, (ii) rat maximum tolerated dose, (iii) rat oral median lethal dose (LD$_{50}$), (iv) rat chronic lowest
observed adverse effect level (LOAEL), (v) ocular irritancy in the Draize test [59], and (vi) skin irritancy in a rabbit skin irritancy test [59]. As shown in Table 6, ergosta-5, 22-dien-3β-ol showed in silico low adverse effects and toxicity against the tested models. Regarding FDA rodent carcinogenicity, ergosta-5, 22-dien-3β-ol was predicted to be noncarcinogenic. Regarding the rat maximum tolerated dose model, ergosta-5, 22-dien-3β-ol showed a maximum tolerated dose value of 0.028 g/kg body weight which was higher than that of the cocrystallized ligand (0.026 g/kg body weight). Ergosta-5, 22-dien-3β-ol showed a rat oral LD50 value of 1.071 mg/kg body weight/day, which was less than of
the cocrystallized ligand (2.015 mg/kg body weight/day). For the rat chronic LOAEL model, ergosta-5, 22-dien-3β-ol showed a LOAEL value less than the cocrystallized ligand. Moreover, ergosta-5, 22-dien-3β-ol was predicted to be irritant and moderately irritant against ocular irritancy and skin irritancy models, respectively.
3. Experimental

3.1. Isolation of Brassicasterol (1). The raw material of G. esculenta was collected in summer in the vicinity of the city of Karkaraly, Karaganda Region, Kazakhstan. G. esculenta was extracted via adding 300 mL of MeOH to 207.5 g of a semidried powder and sonicated at 40–50°C for 3 hrs. The procedure was repeated 3 times per day. The obtained extracts were combined and evaporated under reduced pressure. Total weight of the obtained extract was 62.9 g.

The total extract was subjected to a SiO₂ column (400 g) using hexane–EtOAc and CH₂Cl₂–MeOH as mobile phases in a manner of increasing polarity. Pure white-colored crystal of 1 was obtained from fraction 54 (hexane–EtOAc 1:10).

3.2. X-Ray Analysis. X-ray intensity data for the compound C₂₈H₄₆O·H₂O were collected at 100 K, on a Rigaku Oxford Diffraction Supernova Dual Source (Cu at zero) diffractometer equipped with an Atlas CCD detector using ω scans and CuKα (λ = 1.54184 Å) radiation. The images were interpreted and integrated with the program CrysAlisPro [60]. Using Olex2 [61], the structure was solved by direct methods using the ShelXT structure solution program and refined by full-matrix least squares on F [2] using the ShelXL program package [62, 63]. Nonhydrogen atoms were anisotropically refined, and the hydrogen atoms in the riding mode were with isotropic temperature factors fixed at 1.2 times U (eq) of the parent atoms (1.5 times for methyl and hydroxyl groups). The absolute configuration was established showing a refined Flack parameter of 0.0 (2).

CCDC-2060747 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/structures.

Details of the X-ray crystal structure data collection and refinement are given in Table 7.

3.3. In Silico Studies

3.3.1. Molecular Docking. The used software was Molecular Operating Environment (MOE) [64–67]. For more details, see the supplementary materials.

3.3.2. ADMET. ADMET descriptors of brassicasterol were computed by Discovery studio 4.0.20. More details are given in the supplementary materials.
Table 7: Crystal data and details of the data collection and structure refinement for hydrate brassicasterol.

| Parameters                          | Hydrate brassicasterol |
|-------------------------------------|------------------------|
| Empirical formula                   | C_{28}H_{46}O·H_2O     |
| Formula weight (g/mol)              | 416.66                 |
| Temperature (K)                     | 100 (1)                |
| Crystal system                      | Monoclinic             |
| Space group                         | P2_1                   |
| a (Å)                               | 9.8621 (2)             |
| b (Å)                               | 7.5289 (2)             |
| c (Å)                               | 34.4423 (5)            |
| β (°)                               | 93.926 (1)             |
| Volume (Å³)                         | 2551.37 (9)            |
| Z                                   | 4                      |
| \(\rho_{\text{calc}}\) (g/cm³)    | 1.085                  |
| \(\mu\) (mm⁻¹)                     | 0.495                  |
| \(F(000)\)                          | 928.0                  |
| Crystal size (mm³)                  | 0.351 × 0.129 × 0.072 |
| Radiation                           | CuKα (λ = 1.54184)     |
| 2Θ range for data collection (°)    | 7.718 to 150.484       |
| \(T_{\text{min}}, T_{\text{max}}\)| 0.760, 1.000           |
| Index ranges                        | −12 ≤ h ≤ 12, −8 ≤ k ≤ 9, −40 ≤ l ≤ 43 |
| Reflections collected               | 47210                  |
| Independent reflections            | 9920 [R_{int} = 0.0542, R_{eigen} = 0.0442] |
| Data/restraints/parameters          | 9920/1/561             |
| Goodness-of-fit on \(F^2\)         | 1.037                  |
| Final R indexes [I ≥ 2σ (I)]       | R_I = 0.0738, wR_I = 0.1944 |
| Final R indexes [all data]         | R_I = 0.0773, wR_I = 0.1994 |
| Largest diff. peak/hole (e·Å⁻³)    | 0.54/−0.34             |
| Flack parameter                     | 0.0 (2)                |

3.3.3. *In Silico Toxicity.* Discovery studio 4.0 was used to calculate the toxicity parameters of brassicasterol [25]. For more details, see the supplementary materials.

4. Conclusions

Ergosta-5, 22-dien-3β-ol (1) was isolated from a methanol extract of the fungus *Gyromitra esculenta*. The absolute configuration of 1 was determined by X-ray structural crystallography. Ergosta-5, 22-dien-3β-ol occupied the binding site of the aromatase enzyme with a binding mode very similar to that of the cocrySTALLized ligand and a binding affinity of −33.55 kcal/mol, which was higher than that of the cocrySTALLized ligand (−22.61 kcal/mol). *In silico* ADMET and toxicity studies against 6 models have been conducted, and the results expected the safety of 1 with a disadvantage of poor water solubility and absorption.

Data Availability

Details of the *in silico* experimental part and NMR data of compound 1 are available in the supplementary data. Also, CCDC-2060747 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors’ Contributions

Yerlan Meluly Suleimen was responsible for collection of raw material, extraction of raw material, isolation of compounds, and identification of spectra. Ahmed M. Metwaly contributed to identification of spectra and doking studies of the compound. Ahmad E. Mostafa and Eslam B. Elkaeed contributed to doking studies of the compound. Hong-wei Liu and Buddha Bahadur Basnet took part in isolation of compound. Raigul Nurbekkyzy Suleimen was responsible for identification of spectra. Margarita Yulayevna Ishmuratova contributed to collection of raw material and identification of mushroom. Koblandy Muboryakovich Turydymbekov took part in interpretation of X-ray analysis. Kristof Van Hecke was responsible for X-ray analysis and interpretation of results of it.

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Supplementary Materials

Figure S1: 1H-NMR (500 MHz, CDCl3) spectrum of 1. Figure S2: 13C-NMR (125 MHz, CDCl3) spectrum of 1. Figure S3: DEPT (125 MHz, CDCl3) spectrum of 1. Figure S4: 1H–1H Cosy (500 MHz, CDCl3) spectrum of 1. Figure S5: HMBC spectrum of 1. Figure S6: HMQC spectrum of 1. (Supplementary Materials)

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