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STRUCTURAL AND OPTICAL INSIGHTS INTO A PHENOTHIAZINE-DERIVED CHALCONE SYNTHESIZED VIA ECO-FRIENDLY METHODS

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ABSTRACT. A novel chalcone containing (hetero)aromatic units was obtained by the Claisen-Schmidt condensation of phenothiazinyl-3-carbaldehyde with 4-hydroxy-acetophenone under three experimental approaches based on classical, mechanochemical and ultrasound-assisted procedures. A comparison between the experimental outcomes emphasizes shorter reaction time and lower energy usage in the case of mechanochemical and ultrasound-assisted procedures, recommending these methods as more environmentally friendly synthetic options. Moreover, the use of the sonochemical method resulted in the formation of the reaction product in crystalline state thus simplifying the purification process. The optical properties of the new chalcone were assessed by UV-vis spectroscopy. (E)-1-(4-hydroxyphenyl)-3-(10-methyl-10H-phenothiazin-3-yl)prop-2-en-1-one exhibited an intense absorption in the UV region (λ_{max}=408 nm) and low intensity fluorescence emission in DMSO solution (λ_{em}=560 nm, Stokes shift 7558 cm^{-1}). The X-ray diffraction on monocrystal technique which was employed to ascertain the solid-state structure of the new chalcone revealed strong O-H···O hydrogen bonds and weak dispersive van der Waals interactions such as π···π and C-H···π contacts ensuring its supramolecular architecture, crystal cohesion and stability.

Keywords: *Phenothiazine; Chalcone; Sonochemistry; Mechanochemistry, UV-vis spectroscopy, XRD.*

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INTRODUCTION

In the last decades, the chemistry of methine dyes has received increasing interest because of the wide range of uses and applications of these dyes in many sectors such as technology, engineering, pharmacology, and medicine [1]. Cyanines, a classes of methine dyes, found various applications such as acid-base indicators in analytical chemistry, anti-tumor and anti-cancer agents, bactericidal and fungicidal agents in medicine [2]. Additionally, they are exploited in nucleic acids or protein detection, biomolecules labeling and laser technology [2].

Besides cyanines, another intensively studied class of methine dyes is represented by chalcones. Chalcones are biologically active diaryl-α,β-unsaturated carbonyl compounds, being precursors of various heterocyclic compounds [3]. Chalcones serve as a pharmacophore in a variety of physiologically active compounds and have multiple uses, including anticancer [4], anti-inflammatory and antioxidant compounds [5]. Some chalcone-isoflavone dimers have shown promising results in being used as anti-inflammatory, and some phenyl coumarins and chalcones were proposed as suppressors of LTR-dependent transcription in 2007–in an attempt to find new anti-HIV treatments however, the mechanism of action has not been fully characterized [6]. Keeping the same principles as the previous anti-HIV active compounds, in 2016, Cole et al. synthesized some butein- and xanthohumol-based chalcones that have shown promising results in anti-HIV studies [7].

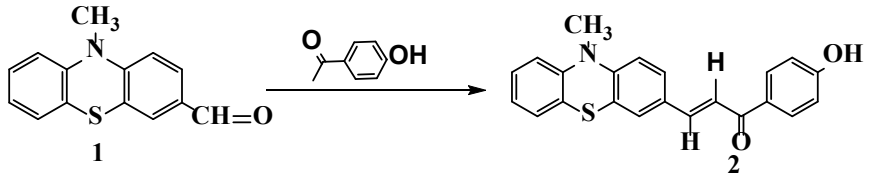
Despite the fact that chalcones have great promise as medicinal agents, this class of compounds is also renowned for their photochemical, optical, and non-linear optical characteristics (NLO), being employed as fluorophores in Organic Light-Emitting Diodes (OLED) [8], chemosensors, and fluorescent nano-probes [9]. By integrating optical characteristics with biocompatibility and biological functionality, chalcones can effectively serve as fluorescent labels in cellular imaging for the identification of tumor tissue (B16-F10 murine melanoma) [10] and specific staining of cellular organelles [11], cell nucleus and the intercalation between the nitrogenous bases of the nucleus [12]. High selectivity towards mitochondria was observed with fluorescent probes based on polyfluorinated cyanine dyes, polymethine dyes, and the cyanine-benzothiazole hybrid system, which emitted predominantly in the near infrared region [10]. Selective labelling was performed on the lysosomes of various cell lines, including HF-P4, BLM, U-2 OS, and A-2058, using fluorescent Coumarin Troger's base derivatives with cyanine substituents [13]. The cytoplasm was selectively stained through the use of a variety of organic dyes. As an illustration, boron-based Schiff bases complexes were effectively utilized to stain the cytoplasm

of B16-F10 murine melanoma cells in vitro [14]. Similarly, live mouse embryonic fibroblasts were selectively stained with fluorophores that contained thiophene moieties [15].

In our quest for more environmentally benign methods applicable in the synthesis of heterocyclic compounds, previous studies focused on the condensation of 10-methyl-10*H*-phenothiazine-carbaldehyde under microwave assisted conditions pointed out superior reaction rates and product yields in the preparation of Schiff bases [16], acetals [17] and chalcones [18-20] containing phenothiazine units, as well as in the synthesis of their oxidation [21] or cyclization [22] products. Building upon our prior research regarding the application of other greener methodologies in the syntheses of (phenothiazinyl)vinyl dyes [10,23,24], in this work we developed two alternative experimental procedures for the condensation of phenothiazinyl-3-carbaldehyde with acetophenone derivatives under mechano- and sonochemical conditions. The structure of the novel phenothiazinyl-chalcone was assigned unambiguously by NMR and XRD, while its optical absorption/ emission properties were assessed by UV-vis spectroscopy.

RESULTS AND DISCUSSION

(*E*)-1-(4-hydroxyphenyl)-3-(10-methyl-10*H*-phenothiazin-3-yl)prop-2-en-1-one **2**, was obtained by the condensation of 10-methyl-10*H*-phenothiazine-3-carbaldehyde **1** with 4-hydroxy acetophenone according to Scheme 1.



a) Ethanol, KOH, 60 °C, 24h 33%
b) Grinding, KOH, 30min 20%
c) Ethanol, KOH, 3h,)) 47%

Scheme 1.

Three alternative experimental procedures were tested to optimize the reaction conditions: a) classical convective heating in a homogeneous ethanol solution, b) solvent-free mechanochemical conditions and c) ultrasound irradiation conditions. Notwithstanding the fact that the reaction yields achieved

through the implementation of mechano- and sonochemical conditions were lower (20% mechanochemical) or not too much higher (47% sonochemical) than those achieved through conventional methods (33%), these alternative processes can be deemed "greener" due to their significantly reduced energy consumption and shorter reaction time (30-180 minutes *versus* 24 hours). Furthermore, the ultrasound-assisted procedure yielded a crystalline reaction product that required only simple purification and finally provided single crystals suitable for X-ray diffraction.

The *E* configuration of the C=C bond present in the chalcone **2** structure was suggested by high resolution NMR based on a large vicinal coupling constant between the protons attached to the vinyl unit (^{3}J=15.48 Hz) and conclusively confirmed by the recorded XRD pattern in the monocrystal.

The optical properties of **2** displayed a weak solvatochromism as it may be seen from the data listed in **Table 1** emphasizing the absorption/emission wavelength maxima in various solvents. **Figure 1a** reveals the slight bathochromic shift (806.7 cm^{-1}) recorded in the UV-vis absorption spectrum of **2** when transitioning from ethyl acetate or toluene to dimethylsulfoxide (DMSO)- a solvent recognized for its stabilizing capacity *via* hydrogen bonding. A broad fluorescence emission band was recorded upon excitation of **2** with its longest absorption maxima (**Figure 1b**); the most intense fluorescence emission and the highest value of the Stokes shift were recorded for chalcone **2** in the polar aprotic solvent acetone, while the fluorescence emission was quenched in DMSO solvent which apparently favored the non-radiative relaxation processes.

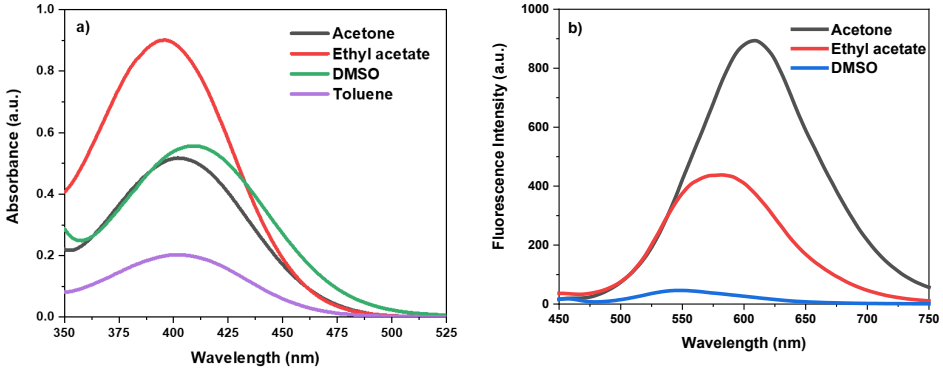


Figure 1. UV-vis spectra of chalcone **2** in various solvents: a) absorption spectra (C_M=4x10^{-5}M), b) fluorescence emission spectra (C_M=8x10^{-7}M).

Table 1. UV-vis absorption/emission wavelength maxima, molar extinction coefficients and Stokes shifts of chalcone **2** in various solvents.

Solvent	**λ_{abs} (nm)**	**ε ($cm^{-1}M^{-1}$)**	**λ_{em} (nm)**	**Stokes Shift (cm^{-1})**
Acetone	400	$1.3x10^4$	607	8526
Ethyl acetate	395	$2.2x10^4$	577	7986
DMSO	408	$1.3x10^4$	560	7558
Toluene	400	$0.5x10^4$	-	-

The crystal structure of **2** within solid state was completely elucidated by single crystal X-ray diffraction technique. It crystallizes centrosymmetrically in the body-centered I2/a monoclinic space group. The structure is embedding isopropyl alcohol molecules in the lattice in a stoichiometric ratio of 1:1 between chalcone **2** and alcohol. The molecular perspective of the asymmetric unit along with the atom numbering scheme is presented in **Figure 2a**.

In the formation of supramolecular architectures, crystal cohesion and stability are involved strong classical O-H···O hydrogen bonds and other weak dispersive van der Waals interactions such as π···π and C-H···π contacts (see table 2). An isopropyl alcohol molecule is bounded within the lattice by O-H···O hydrogen bonds, the hydroxyl group of alcohol molecule serving both as donor and acceptor. O2-H2A···O3 interaction is characterized by a separation distance of 1.951 Å linking the hydroxyl group of the alcohol with the carbonyl group of the chalcone. Further the alcohol molecule is involved in hydrogen bonds with the hydroxyl substituent of the aromatic ring, the recorded O1-H1···O2 distance being 1.863 Å. Both phenyl rings of phenothiazine moiety participate in mutual π···π interactions characterized by intermolecular C4···C7 distances of 3.365 Å. These are completed by C-H···π interactions linking isopropanol molecule to chalcone **2** adjacent molecules. An extended packing diagram of compound **2** is illustrated in Figure 2b.

Table 2. Intermolecular interactions in compound **2** (Å).

D-H···A	D-H	H…A	D…A	<(D-H…A)
O1-H1···O2	0.820	1.863(3)	2.765(4)	170.2(4)
O2-H2···O3	0.820	1.951(2)	2.271(1)	177.8(4)
π ···π				
C4···C7			3.365(4)	
C-H···π				
C22-H22···C1	0.930	2.859(3)	3.546(2)	131.7(2)
C24-H24···C19	0.980	2.837(4)	3.731(2)	151.8(4)

(a)

(b)

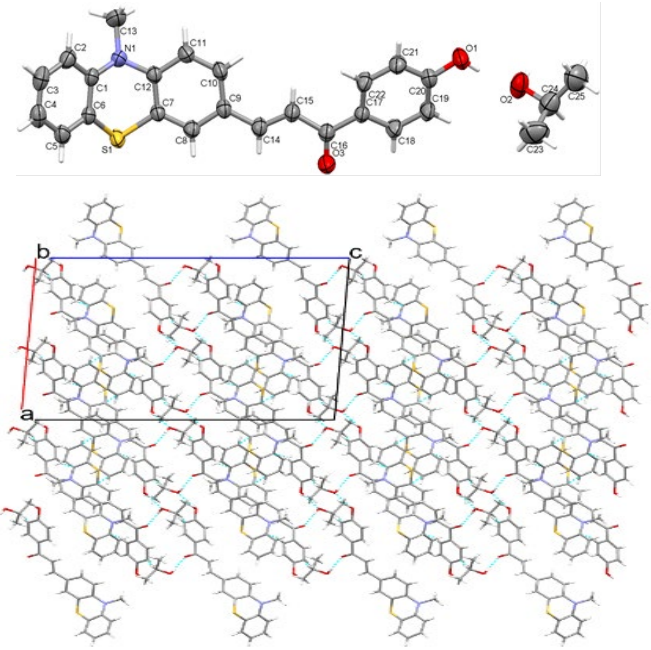


Figure 2. Chalcone **2** a) ORTEP illustration of the asymmetric unit presenting the atoms as ellipsoids at 50% probability level; b) packing perspective along ab-axis.

CONCLUSIONS

This work described three alternative experimental procedures applicable in the synthesis of (*E*)-1-(4-hydroxyphenyl)-3-(10-methyl-10H-phenothiazin-3-yl)prop-2-en-1-one **2**, based on i) the conventional convective heating in homogeneous solution, ii) the solventless mechanochemical grinding and iii) the ultrasound irradiation techniques. The mechano- and sonochemical procedures may be qualified as greener synthetic approaches requiring much shorter reaction times and lower energy inputs. A major advantage of the sonochemical procedure is its capacity to induce the precipitation of the reaction product in a solid microcrystalline state, much simplifying its purification. The electronic properties of **2** displayed a strong absorption peak in the UV region (408 nm) and fluorescence emission in the visible range (560 nm) of low intensity but large Stokes shift 7558 cm^{-1}).

EXPERIMENTAL SECTION

Materials and Methods

All the materials for experiments, reagents, and solvents were obtained from commercial suppliers and used without further purification unless otherwise noted. UV-vis absorption respective emission spectra in a solvent were recorded with Perkin Elmer Lambda 35 and Perkin Elmer LS55 spectrophotometers. NMR spectra were recorded on Brucker NEO-1 600 MHz instrument. Single crystals of compound **2** were successfully grown and analyzed by X-Ray diffraction. A suitable single crystal of **2** was mounted on the goniometer of a SuperNova diffractometer using inert Paratone oil and a nylon loop. The collection of diffraction intensities was carried out by CrysAlis PRO software at room temperature using CuKα radiation. The diffractometer is equipped with an X-Ray tube operating at X-ray 50 kV and 0.8 mA and an Eos CCD detector. The crystal structure was solved by the SHELXT [25] solution program via Intrinsic Phasing and further was refined by the least-squares minimization method with the SEHLXL [26] refinement package in Olex2 software [27]. H and O atoms were treated as riding considering an isotropic displacement parameter $U_{iso}(H)=1.2U_{eq}(C)$ for CH [C-H=0.93 Å], $U_{iso}(H)=1.5U_{eq}(C)$ for CH_3 groups [C-H=0.96 Å] and OH groups [O-H=0.82 Å]. The CIF files of 2 have been deposited with the Cambridge Crystallographic Data Centre, having the associated deposition number 2304437. A copy can be obtained free of charge on written application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44-12-2333-6033); on request via e-mail to deposit@ccdc.cam.uk or by access to http://www.ccdc.cam.ac.uk.

Starting material, 10-methyl-10*H*-phenothiazine-3-carbaldehyde **1**, was prepared by Vilsmeier-Haack Formylation with trichlorophosphate in N,N,-dimethyl-formamide and dichloroethane as following the previously reported methods [28].

Experimental procedures for the synthesis of (E)-1-(4-hydroxy-phenyl)-3-(10-methyl-10H-phenothiazin-3-yl)prop-2-en-1-one 2

a) Classical synthesis

In a three-necked flask fitted with a reflux condenser, thermometer and septum were introduced 10-methyl-10*H*-phenothiazine-3-carbaldehyde **1** 0.73g (3.02 mmol) of and a saturated solution of potassium hydroxide in ethanol. The mixture was stirred and heated to 60°C until the raw material **1** was completely dissolved and further it was added 4-hydroxy acetophenone 0.72 g (5.29 mmol). The reaction mixture was stirred for 24 hours at 60°C.

After the completion of the reaction, the solvent was partially removed by vacuum distillation. The orange-red precipitate obtained was filtered and recrystallized from 2-propanol and acetonitrile (10:2 v/v), affording 0.36 g product (yield 33%).

b) Mechanochemical synthesis

In a mortar, 10-methyl-10*H*-phenothiazine-3-carbaldehyde **1** (0.73g, 3.02 mmol) and KOH (0.34g, 6 mmol) were added and the mixture was ground with a pestle until a homogeneous mixture was obtained. Over this mixture, 4-hydroxy acetophenone 0.72 g (5.29 mmol) was added in portions, the blend being homogenized after each added portion. After adding the last portion, the mixture was grounded with the pistil for another 30 minutes. The orange paste was recrystallized from 2-propanol and acetonitrile (10:2 v/v), affording 0.22 g pure product (yield of 20%).

c) Ultrasound assisted synthesis

10-methyl-10*H*-phenothiazine-3-carbaldehyde **1** (0.73g, 3.02 mmol), 50 mL of ethanol and KOH (0.34g, 6 mmol) were introduced into a pear-shaped flask. After 10 min of ultrasonating, 4-hydroxy acetophenone 0.72 g (5.29 mmol) was added and the mixture was ultrasonated for another 3 hours in an ultrasonic bath. After the completion of the reaction, small red crystals appeared in the reaction mass. After filtration, 0.51 g pure product (yield 47%) was obtained after recrystallization from 2-propanol.

2 Melting point (from 2-propanol): 185°C

^{1}H-NMR (600 MHz, acetone-d_6) δ (ppm): 8.11(d, 2H, J=8.76 Hz); 7.8(d, 1H, J=15.48 Hz); 7.68(d, 1H, J=7.62 Hz); 7.67(d, 1H, J=15.48 Hz); 7.63(dd, 1H, J=8.34 Hz); 7.24(dd, 1H, J=7.32 Hz); 7.18(dd, 1H, J=2.56 Hz); 7.02(m, 1H); 7.00(m, 1H); 6.99 (m, 1H); 6.97(m, 2H); 3.45(s, 3H).

^{13}C-NMR (150 MHz, acetone-d_6) δ (ppm): 34.5, 114.4, 114.7, 115.2 (2C), 119.0, 122.3, 122.9, 123.5, 126.0, 126.8, 127.8, 129, 130.4, 131.5 (2C), 143.0, 145.0, 147.5, 148.0, 161.2, 187.5.

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