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Short Note

(*R*,*S*)-5-(4-Chlorophenyl)-3-{(*E*)-2-[(*R*,*S*)-2,6,6-trimethylcyclohex-2-en-1-yl]vinyl}-4,5-dihydro-1*H*-pyrazole-1-carboximidamide Hydrochloride

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Abstract: Pyrazoline and amidine motifs are important in medicinal chemistry due to their broad spectrum of bioactivities. This work's goal was to synthesize a new hybrid amidino pyrazoline from terpenyl chalcone. The chosen method consists of making the terpenyl chalcone react with aminoguanidine hydrochloride in the presence of potassium hydroxide using ethanol as solvent. The reaction was carried out under ultrasonic irradiation. The resulting terpenyl amidino pyrazoline was isolated after separation in a silica-gel chromatographic column in 86% of yield. The product structure was confirmed by the analysis of the high resolution mass, ^{1}H and ^{13}C-NMR spectra. The data was consistent with the expected structure. In summary, the method was efficient for the synthesis of a new hybrid terpenyl amidino pyrazolines under sonochemical conditions.

Keywords: cyclocondensation reaction; amidino pyrazoline; terpenyl pyrazoline; ultrasound

1. Introduction

The role of pyrazole derivatives in medicinal chemistry is well established. In this direction, pyrazolines occupy a pivotal role in the development of new bioactive compounds due to the wide range of activities that were attributed to them [1,2]. Recently, terpenyl pyrazolines have been prepared and evaluated for their in vitro and in vivo antileishmanial activities against *Leishmania donovani* [3]. The reported synthetic route started with the preparation of terpenyl chalcones from ionones and aromatic aldehydes by the classical aldol condensation reaction. Afterward, the terphenyl pyrazolines were synthesized by the cyclocondensation reaction between terpenyl chalcones and phenylhydrazine.

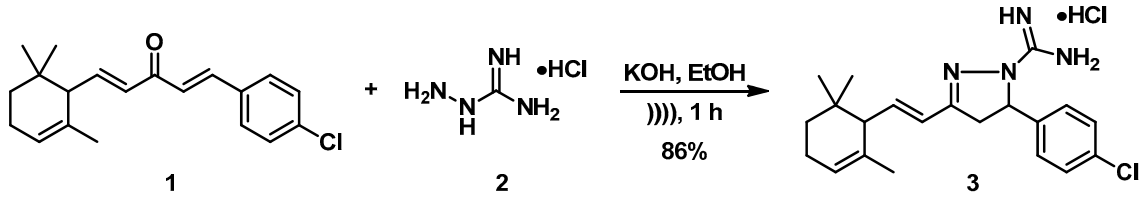
At the same time, amidine-containing derivatives have appeared as promising molecules with important activities such as antimicrobial [4], antithrombotic [5], anticancer [6], and antifungal [7]. Pentamidine is included on the WHO's List of Essential Medicines for the basic health system. It is indicated for the treatment of first stage human African tripanosomiasis and as a second-line therapy for leishmaniasis [8].

Recently, we have reported that ultrasonic irradiation was valuable in promoting the cyclocondensation reaction of chalcones and aminoguanidine hydrochloride for the synthesis of amidino pyrazolines [9–11]. This method prompted us to synthesize series of pyrazolines in short reaction times and good yields using ethanol as green solvent, despite the low solubility of aminoguanidine in such medium.

As part of the continuation to this program, in this work, we report the synthesis of an amidine-containing terpenyl pyrazoline by the cyclocondensation reaction of terpenyl chalcone and aminoguanidine hydrochloride.

2. Results and Discussion

The terpenyl chalcone **1** was prepared from α-ionone and 4-chlorobenzaldehyde according to reported methodology [12]. For the synthesis of the amidine-containing terpenyl pyrazoline **3**, the terpenyl chalcone **1** was sonicated with aminoguanidine hydrochloride **2** in the presence of potassium hydroxide and ethanol as solvent (Scheme 1). The product was obtained in 86% of yield after extraction with chloroform and recrystallization from ethyl acetate-methanol (10:1).



Scheme 1. Synthesis of (*R*,*S*)-5-(4-chlorophenyl)-3-{(*E*)-2-[(*R*,*S*)-2,6,6-trimethylcyclohex-2-en-1-yl]vinyl}-4,5-dihydro-1*H*-pyrazole-1-carboximidamide hydrochloride (**3**).

The structure of the terpenyl pyrazoline **3** was confirmed by FTIR, ^{1}H and ^{13}C-NMR and HRMS analysis. The FTIR spectrum shows a series of bands from 3118 to 2865 cm^{-1} attributed to the N–H and C–H stretching vibrations. The C=C and C=N double bond vibrations appear at 1658, 1620 and 1595 cm^{-1}. The ^{1}H-NMR signals are in accordance with previous work reported in the literature for analogous phenyl-substituted terpenyl pyrazolines [3]. The spectrum shows broad signals at 7.95–7.70 ppm attributed to the amidine group hydrogens. The aromatic hydrogens are observed as a singlet at 7.29 ppm. Three multiplets displayed between 6.40 and 5.93 ppm are generated by the vinylic protons. The multiplet attributed to the methine proton of the pyrazoline ring at 5.50–5.48 ppm supports that the cyclization took place near the aromatic ring rather than near the ionone ring. This large chemical shift is observed due to the proximity of the benzene ring. If the cyclization had occurred near the ionone ring, the observed chemical shift would be considerably smaller. The two geminal protons of the pyrazoline ring are observed as two multiplets at 3.74–3.65 ppm and 2.98–2.92 ppm. The ionone ring methine proton is observed as a doublet at 2.31 ppm (J = 9.0 Hz). The signals between 1.57 and 0.81 ppm are attributed to the methylene and methyl groups from the ionone ring. The ^{13}C-NMR spectrum shows nineteen non-equivalent carbons, in accordance with the structure. Some signals are duplicated due to the presence of diastereomers. The DEPT-135 confirms the presence of three methyl groups, three methylene groups, seven methine groups and six quaternary carbons. The FTIR and NMR spectra are given in the Supplementary Material.

The terpenyl pyrazoline **3** was analyzed by HRMS with electrospray ionization (ESI). Spectra were acquired in positive mode ESI(+)-MS and in ESI(+)-MS/MS for evaluation of fragmentation pathway. The exact mass/charge (m/z) of the hydrogen adduct $[M + H]^+$ is present with error equal to 5.7 ppm relative to that theoretically expected for elemental formula (m/z 371.2024 for the formula $[C_{21}H_{27}ClN_4 + H]^+$; theoretical m/z 371.1965). Furthermore, the fit of isotopic ratio (theoretical versus experimental) assist in the confirmation of elementary composition of synthesized compound. Errors of exact m/z below 10 ppm for HRMS with ionization by ESI and mass separation by Quadrupole-Time of Flight (Q-TOF) confirm the elemental composition [13,14]. Moreover, in addition to helping confirmation of elemental formula, the fragmentation pathway has important information about the molecular structure. Figure 1 demonstrates this HRMS tool used to characterize the synthesized pyrazoline.

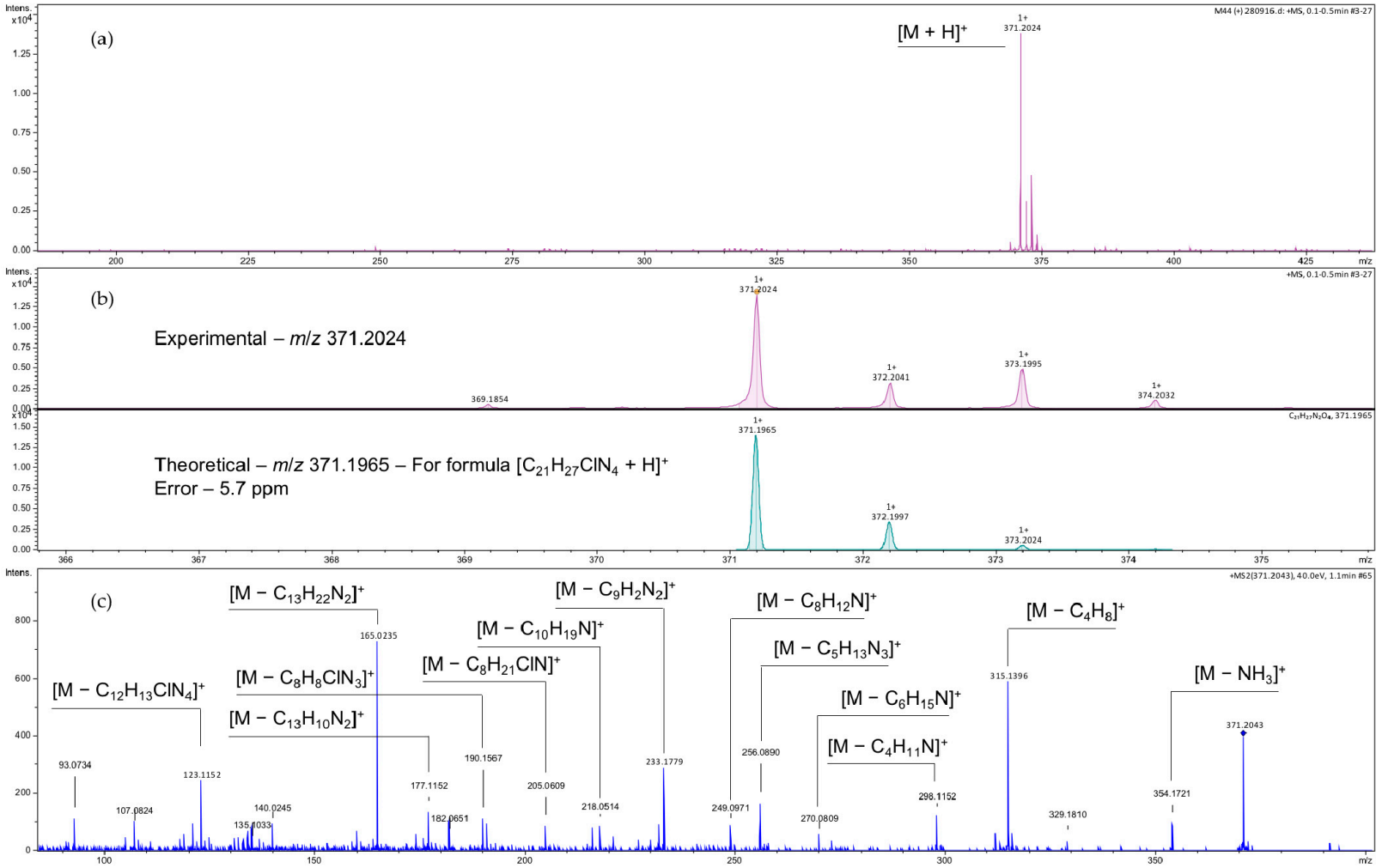


Figure 1. The HRMS analysis of compound **3**. (**a**) the full spectra; (**b**) the expansion between 356 and 376 u.a.m. highlighting the exact mass and isotopic ratio; (**c**) the analysis in MS–MS mode (fragmentation pathway).

3. Materials and Methods

3.1. General

All of the chemicals were used without purification as purchased from commercial suppliers. The sonicated reaction was carried out with a microtip probe connected to a 500 W Sonics Vibracell ultrasonic processor (Sonics, Newtown, CT, USA) operating at 20 kHz at 20% of the maximum power output. Infrared spectrum (FTIR) was acquired on a JASCO-4100 spectrophotometer (JASCO, Easton, MD, USA). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were acquired on a Bruker Avance III HD instrument (Bruker, Rheinstetten, Germany, 300 MHz for ^{1}H and 75 MHz for ^{13}C) in 5 mm sample tubes at 298 K in $CDCl_3$ using tetramethylsilane (TMS) as an internal reference. A hybrid high-resolution and high accuracy microTof (Q-TOF) (Bruker® Scientific, Billerica, MA, USA) was used for detection, with electrospray ionization (ESI) source (MicrOTOF-QII Bruker® Scientific) in positive mode. The compound **3** was dissolved in a solution of 50% (v/v) chromatographic grade acetonitrile (Tedia, Fairfield, OH, USA), 50% (v/v) deionized water and 0.1% formic acid. The solution was infused directly into the ESI source by means of a syringe pump (Harvard Apparatus, Holliston, MA, USA) at a flow rate of 180 μL min^{-1}. The drying temperature was 200 °C and nitrogen was used for drying gas, in a 10 L min^{-1} flow. The ESI(+)-MS and tandem ESI(+)-MS/MS were acquired under the following conditions: capillary and cone voltages were set to +3500 V and +40 V, respectively, with a de-solvation temperature of 100 °C. The software Compass DataAnalysis version 4.3 (Bruker® Scientific) was used for spectrum analysis. The diagnostic ions were identified by the comparison of their ESI(+)-MS/MS dissociation patterns with compounds with theoretical mass for the respective elementary formula.

3.2. Procedure for the Synthesis of Terpenyl Pyrazoline **3**

In a 50 mL Falcon tube containing 20 mL of absolute ethanol, it was added the terpenyl chalcone (0.31 g, 1 mmol), the aminoguanidine hydrochloride (0.33 g, 3 mmol) and pellets of KOH (0.17 g, 3 mmol). The mixture was sonicated for 60 min. After cooling in an ice bath, the excess of aminoguanidine that precipitated was removed by filtration. The resulting solution was acidified with 10% HCl (15 mL) and extracted with chloroform (3 × 30 mL). The combined organic layer was dried with anhydrous magnesium sulfate. The solvent was removed under vacuum and the residue was purified by recrystallization from ethyl acetate-methanol (10:1) to afford the title compound (**3**) as yellowish solid.

3.3. Characterization and Experimental Data of Terpenyl Pyrazoline **3**

Yellowish solid, mp 210–212 °C, 86%; FTIR (ATR): v (cm^{-1}) 3118, 3065, 3031, 2955, 2925, 1658, 1646, 1620, 1595, 1490; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm) 7.95–7.70 (bs, NH), 7.29 (s, 4H, Ar-H), 6.40–6.35 (m, 1H, =CH), 6.15–6.12 (m, 1H, =CH), 6.01–5.93 (m, 1H, =CH), 5.50–5.48 (m, 1H), 3.74–3.65 (m, 1H), 2.98–2.92 (m, 1H), 2.31 (d, 1H, J = 9.0 Hz), 1.57 (s, 3H, CH_3), 1.40–1.31 (m, 2H), 1.26–1.18 (m, 2H), 0.92 (s, 3H, CH_3), 0.81 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ* (ppm) 158.2 (158.2), 153.3, 145.9, 137.4 (137.4), 134.7 (134.7), 132.2 (132.1), 129.6, 127.4 (127.4), 122.9 (122.8), 122.6, 59.8, 55.0, 43.6 (43.6), 32.8 (32.7), 31.2 (31.2), 28.1 (28.1), 26.8 (26.8), 23.1, 23.0. *The δ of the duplicated signals are given in brackets.

Supplementary Materials: The FTIR and NMR spectra are available online.

Author Contributions: Conceptualization, L.P.; Methodology, M.C.C. and L.P.; Validation, M.C.C.; Investigation, M.C.C; Resources, L.P.; Writing—Original Draft Preparation, M.C.C and L.P.; Writing—Review and Editing, L.P.; Supervision, L.P.; Funding Acquisition, L.P.

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