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Article

Tautomerism in Quinoxalines Derived from the 1,4-Naphthoquinone Nucleus: Acid Mediated Synthesis, X-ray Molecular Structure of 5-Chlorobenzo[*f*]quinoxalin-6-ol and Density Functional Theory Calculations

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A reação de *tert*-butil 2-(3-cloro-1,4-dioxo-1,4-di-hidronaftalen-2-ilamino)etilcarbamato com CF_3COOH/CH_2Cl_2 fornece 5-cloro-3,4-di-hidrobenzo[*f*]quinoxalin-6(2H)-ona. Este composto sofre desidrogenação promovida por ácido na presença de água para dar a 5-clorobenzo[*f*]quinoxalin-6-ol, inédita. A estrutura molecular no estado sólido, determinada por um estudo de difração de raios X (XRD), e os dados em solução confirmam que a benzoquinoxalina existe na forma do tautômero enol-imina, tanto no estado sólido, quanto em solução, diferentemente de 5-cloro-3,4-di-hidrobenzo[*f*]quinoxalin-6(2H)-ona que exibe o arranjo ceto-amino. Cálculos de teoria do funcional da densidade (DFT) confirmam a preferência da benzoquinoxalina e dos compostos análogos contendo grupos H ou CH_3 no lugar do Cl pela forma enol-imina. Sugere-se que a preferência da benzoquinoxalina pela estrutura enol-imina se deva ao maior caráter aromático desta estrutura em comparação com a forma ceto-amina. Os cálculos DFT dos dois tautômeros das benzo[*a*]fenazin-5(7H)-onas análogas às benzo[*f*]quinoxalin-6(4H)-onas indicaram que as estabilidades relativas são dominadas por efeitos de solvatação, no primeiro caso, e pelo grau de aromaticidade no segundo.

The reaction of *tert*-butyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino) ethylcarbamate with CF_3COOH/CH_2Cl_2 yields 5-chloro-3,4-dihydrobenzo[*f*]quinoxalin-6(2H)-one which undergoes acid-promoted dehydrogenation in the presence of water to give novel 5-chlorobenzo[*f*]quinoxalin-6-ol. The molecular structure of 5-chlorobenzo[*f*]quinoxalin-6-ol in the solid state, determined by an X-ray diffraction (XRD) study, and the solution data confirm that it exists as the enol-imine tautomer, both in the solid state and in solution, differently from 5-chloro-3,4-dihydrobenzo[*f*]quinoxalin-6(2H)-one, which exhibits the keto-amine arrangement. Density functional theory (DFT) calculations confirmed the preference of 5-chlorobenzo[*f*]quinoxalin-6-ol and of the derivatives containing H and CH_3 groups in place of the Cl atom for the enol-imine tautomer. It is suggested that the enol-imine structure is preferred for 5-chlorobenzo[*f*]quinoxalin-6-ol as a consequence of the higher aromatic character of this structure in comparison with the keto-amine form. DFT calculations carried out on the two tautomers of the benzo[*a*]phenazin-5(7H)-ones analogous to the benzo[*f*]quinoxalin-6(4H)-ones showed that the relative stabilities are dominated by solvation effects in the first case and the degree of aromaticity, in the latter.

Keywords: quinoxalines, 1,4-naphthoquinones, tautomerism, X-ray structure, DFT calculations

Introduction

Quinoxalin derivatives have been the subject of intensive industrial and academic investigation due to their importance as intermediates for the synthesis of pharmaceuticals and new materials. This nucleus is a privileged molecular scaffold, present in a large number of molecules with a wide spectrum of biological activities,[1] *e.g.*, trypanocidal,[2,3] antimalarial,[4,5] antitubercular,[6,7] antimicrobial,[8] antitumoral,[9] anti-proliferative,[10,11] antithrombotic,[12] anti-HIV,[13] among others. Quinoxalines have been investigated as luminescent materials,[14,15] fluorescent probes[16] and as constituents of donor-acceptor type polymers for optoelectronics.[17] Their chelating

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ability has been used for the preparation of molecular sensors[18,19] and hybrid coordination polymers.[20]

Of interest to this paper are the quinoxalines derived from the 1,4-naphthoquinone nucleus which include the benzophenazines reported by Silva Junior *et al.* (Figure 1a, **a**[21] and the dihydrobenzoquinoxalines (Figure 1b), studied extensively by Kallmayer and Seyfang.[25-34] The benzophenazines shown in Figure 1a have been obtained from the reactions of phenylenediamine with 2-hydroxy-3-R-1,4-naphthoquinones in AcOH, under heating, in the presence (**a**-**f**)[21,22] or absence of AcONa (**g**).[23] Unusual dimeric phenazines **h** have been synthesized in high yields by reacting lapachol (2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone) with neat alkylamines.[24] The reactions of ethylene diamines or 1,2-cyclohexyldiamine[34] with 1,4-naphthoquinone (R = H), 2,3-dibromo or dichloro-1,4-naphthoquinone (R = Cl or Br), 2,3-phthalimido-1,4-naphthoquinone (R = NHAc, NH_2)[29] and 2-alkyl or 2-aryl,3-halogen-1,4-naphthoquinones, in CH_2Cl_2/ ethanol,[26] have yielded the dihydrobenzoquinoxalines shown in Figure 1b.

Following our continuing interest in aminonaphthoquinones[35-38] and their metal complexes[39-41] as potential antimicrobial and anticancer agents, our group has investigated the possibility of synthesizing molecular hybrids from 2,3-dichloro-1,4-naphthoquinone and *N*-Boc-monoprotected diamines ($NH_2(CH_2)_nNHBoc$), whose deprotection would be followed by further extension of the diamine chain with nitrogen or oxygen containing fragments for coordination with metal ions. This tactic has proven successful for the diamines with a linear carbon chain longer than n = 3,[42] and the results will be reported elsewhere. Under the conditions employed for the deprotection of the $NH_2(CH_2)_2NHBoc$ derivative **1**, however, 5-chloro-3,4-dihydrobenzo[*f*]quinoxalin-6(2H)-one **2**[26] was obtained instead, together with the dehydrogenated compound 5-chlorobenzo[*f*]quinoxalin-6-ol **3** (Figure 2). 5-R-3,4-Dihydrobenzo[*f*]quinoxalin-6(2H)-ones (R = CH_3, OCH_3, CN and NHAc, Figure 1b) were reported previously to undergo base and acid promoted oxidation to the respective benzoquinoxalin-6-ol derivatives analogous to **3**, in good yields and trace amounts, respectively.[43] The benzophenazines and dihydrobenzoquinoxalines illustrated in Figure 1 in their keto-amine form could, in principle, exist as the enol-imine tautomer observed for **3** (Figure 2) and analogous benzoquinoxalines.[44]

Herein we describe a novel route to the acid mediated oxidation of **2** and the solution and solid state characterization of 5-chlorobenzo[*f*]quinoxalin-6-ol **3**, including an X -ray diffraction (XRD) analysis. We also report the results of density functional theory (DFT) calculations to determine the relative stabilities of the two tautomers of quinoxalines related to **2** and **3** containing different substituents in place of Cl, and of benzophenazines **b**, **d** and **g** (Figure 1a).

Experimental

General methods

2,3-dichloro-1,4-naphthoquinone (Aldrich), 1,2-ethanodiamine (Aldrich), di-*tert*-butyl-dicarbonate (Aldrich), trifluoroacetic acid (Aldrich), MeCN, EtOAc, hexane, EtOH (Vetec), CH_2Cl_2, Na_2SO_4 and $NaHCO_3$ (Vetec) were used as received. *tert*-Butyl *N*-(2-aminoethyl)carbamate

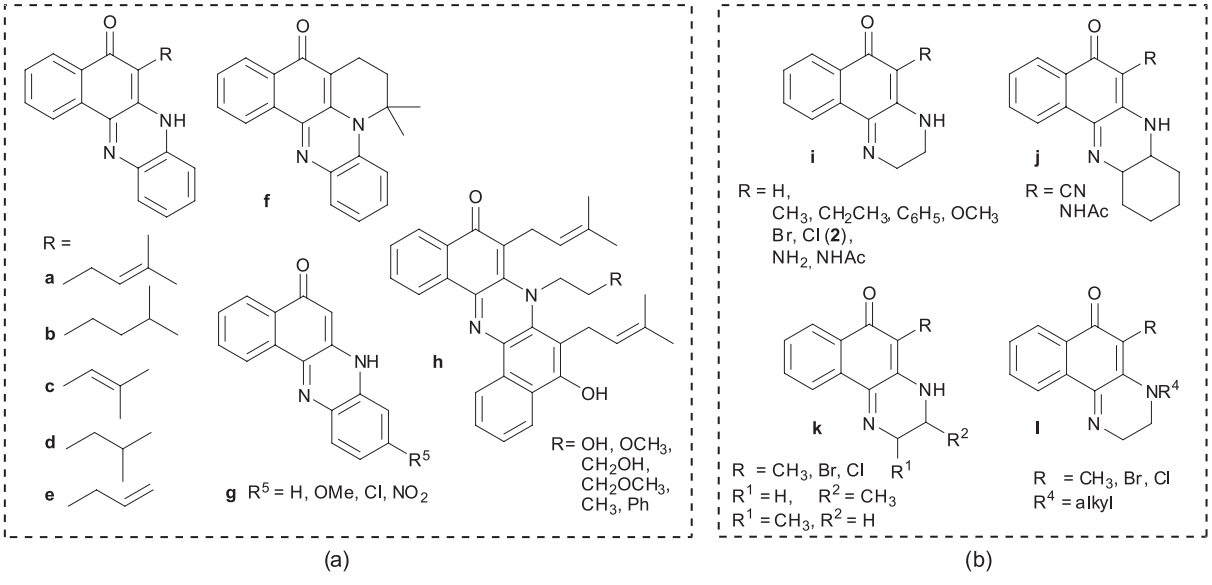


Figure 1. Benzophenazines (a) and dihydrobenzoquinoxalines (b) synthesized from 1,4-naphthoquinones.

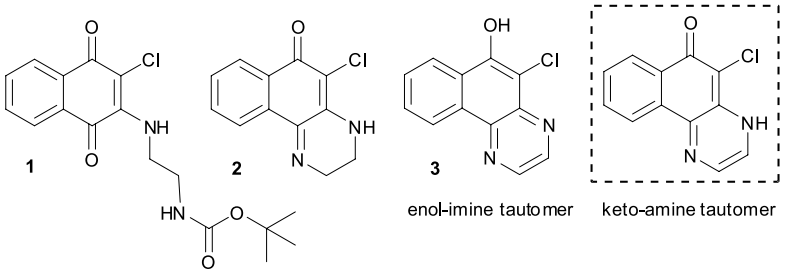


Figure 2. Structures of the compounds synthesized in this study and of the possible tautomers of compound **3**.

was prepared as described in the literature for *tert*-butyl *N*-(3-aminopropyl)carbamate.[45] All solvents were removed under reduced pressure. The reactions were monitored by thin layer chromatography (TLC) analysis on silicagel 60 F_{254} TLC plates with detection by UV absorption (254 nm). Column chromatography (CC) was performed using Acros Organics silica gel (35-70 μm) as the stationary phase and the solvent systems indicated in each experiment. Melting points (mp) were obtained on a ThermoFisher Scientific Digital Melting Point IA9100 apparatus. Infrared spectra were recorded as thin films using a Varian 660 FTIR spectrometer equipped with an attenuated total reflectance (ATR) sampling accessory, and the spectral data are reported in wavenumbers (cm^{-1}). Nuclear magnetic resonance (NMR) spectra were acquired in $CDCl_3$, DMSO-d_6 or CD_3OD as indicated using a Varian VNMRS 300 MHz or Varian VNMRS 500 MHz spectrometer, where ^1H and ^{13}C were measured at 500/300 and 125 MHz, respectively. ^1H NMR spectra are referenced to the $CDCl_3$ (δ 7.26 ppm), DMSO-d_6 (δ 2.50 ppm) and CD_3OD (δ 3.31 ppm) residual solvent peaks. The hydrogen signals were attributed through coupling constant values and $^1H \times {}^1H$ COSY (correlation spectroscopy) experiments. ^{13}C NMR spectra were proton decoupled and referenced to $CDCl_3$ (δ 77.0 ppm) and DMSO-d_6 (δ 39.4 ppm) residual solvents peaks.

Synthesis of *tert*-butyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)ethylcarbamate (**1**)

Compound **1** was prepared from 2,3-dichloro-1,4-naphthoquinone and *tert*-butyl *N*-(2-aminoethyl) carbamate according to literature procedures[42,46] to give a red solid, 70%, mp 131 °C; anal. calcd. for $C_{17}H_{19}N_2O_4Cl$: C, 58.21; H, 5.46; N, 7.99; found: C, 58.15; H, 5.70; N, 7.97%; ^1H NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 9H, H13), 3.45 (br q, 2H, *J* 5.0 Hz, H12), 3.97 (br q, 2H, *J* 5.0 Hz, H11), 4.86 (br, 1H, NH), 6.50 (br, 1H, NH), 7.61 (td, 1H, *J* 1.3, 7.6 Hz, H6/H7), 7.71 (td, 1H, *J* 1.3, 7.6 Hz, H6/H7), 8.01 (dd, 1H, *J* 0.9, 7.7 Hz, H5/H8), 8.13 (dd, 1H, *J* 0.9, 7.7 Hz, H5/H8); IR (thin film) ν/cm^{-1} 3373 (N-H, amide), 3338 (N-H, amine), 1669 (C=O) and 1648 (C=O).

Synthesis of 5-chloro-3,4-dihydrobenzo[*f*]quinoxalin-6(2H)-one (**2**)[26]

To a stirred solution of *tert*-butyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)ethylcarbamate (**1**, 0.70 g, 2.0 mmol) in 5 mL of CH_2Cl_2 cooled in an ice bath, was added trifluoroacetic acid (0.77 mL, 1.14 g, 10.0 mmol), and the resulting wine red solution was stirred at room temperature in a closed system for 24 h. After evaporation to dryness a saturated Na_2CO_3 solution was added and the mixture was washed with EtOAc (40 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic extracts were dried (anhydrous Na_2SO_4) and the solvent, evaporated. The residue was purified by CC (hexane/EtOAc, 10/1 to 2/1, v/v) to give an orange solid, 0.40 g, 86%, ; anal. calcd. for $C_{12}H_9N_2OCl.0.25C_4H_8O_2$: C, 61.31; H, 4.35; N, 11.00; found: C, 61.79; H, 4.42; N, 11.02%, mp 128 °C (lit. 135-138 °C, $C_{12}H_9N_2OCl$);[26] ^1H NMR (500 MHz, $CDCl_3$) δ 3.50 (td, 2H, *J* 2.6, 6.5 Hz, H11), 4.19 (t, 2H, *J* 6.5 Hz, H12), 7.57 (m, 2H, H5 e H8), 8.15 (m, 1H, H6/H7), 8.18 (m, 1H, H6/H7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 37.2, 48.4, 109.0, 123.8, 126.1, 131.1, 131.2, 139.5, 152.2, 176.3; IR (thin film) ν/cm^{-1} 3324 (N-H), 1737 (C=O), 1575 (C=C). Solubility: DMSO, acetone, methanol, ethanol, CH_2Cl_2, $CHCl_3$ and hexane; partially soluble in water and EtOAc.

Synthesis of 5-chlorobenzo[*f*]quinoxalin-6-ol (**3**)

Prepared from *tert*-butyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)-ethylcarbamate (**1**, 1.50 g, 4.3 mmol) in 10 mL of $CHCl_2$ and trifluoroacetic acid (1.64 mL, 2.43 g, 21.5 mmol) according to the procedure described above. Evaporation to dryness was followed by addition of EtOAc (2 × 20 mL) and the insoluble residue discarded. The solution was then washed with water (50 mL), the organic layer was separated and the aqueous layer was extracted with EtOAc (4 × 40 mL). The combined organic extracts were dried (anhydrous Na_2SO_4) and the solvent evaporated. The residue was

purified by CC (hexane/EtOAc from 10:1 to 2:1) to give a pale yellow solid, 0.49 g, 46%, mp 216 °C; anal. calcd. for $C_{12}H_7N_2OCl.0.25H_2O$: C, 61.31; H, 4.35; N, 11.00; found: C, 61.79; H, 4.42; N, 11.02%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (m, 2H, H11-H12), 8.37 (m, 1H, H6-H7), 8.83 (d, 1H, *J* 2.1 Hz, H5-H8), 8.93 (d, 1H, *J* 2.1 Hz, H5-H8), 9.18 (m, 1H, H6-H7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.7, 122.5, 127.1, 128.3, 129.2, 129.3, 137.1, 139.9, 141.1, 144.7, 151.0; IR (thin film) ν/cm^{-1} 3199 (O-H), 1596 (C=C). Solubility: DMSO, acetone, methanol, EtOAc, hexane and CH_2Cl_2, partially soluble in H_2O and $CHCl_3$.

Crystallography: molecular and crystal structure of 5-chlorobenzo[*f*]quinoxalin-6-ol (**3**)

XRD data were collected at 150 K on an Agilent Xcalibur Atlas Gemini Ultra diffractometer with Mo Kα radiation (see Table 1). Data processing (including integration, scaling and absorption correction) was performed using CrysAlisPro software.[47] The structure was solved using SHELXS-97 and refined on F2 with SHELXL 97.[48] All the non-hydrogen atoms were refined anisotropically and the hydrogen atoms were refined using a riding model. The aqua hydrogens were located in Fourier difference maps and the benzoquinoxaline hydrogens were generated geometrically.

Table 1. Structural data and refinement parameters for **3**

Chemical formula	$C_{12}H_7ClN_2O.3H_2O$
M_r	284.69
Crystal system, space group	Monoclinic, $P2_1/n$
Temperature / K	150
a, *b*, *c* / Å	13.2632 (8), 6.6446 (5), 14.0610 (9)
β / degree	95.131 (6)
V / Å^3	1234.21 (14)
Z	4
Radiation type	Mo *K*α
μ / mm^{-1}	0.32
Crystal size /mm	0.47 × 0.15 × 0.12
Habit/colour	Plate/yellow
Absorption correction	Multi-scan
T_{min}, T_{max}	0.888, 1
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	7362, 2175, 1538
R_{int}	0.049
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, *S*	0.053, 0.149, 1.13
No. of reflections	2175
No. of parameters	192
No. of restraints	9
H-atom treatment	H atoms treated by a mixture of independent and constrained refinement
$\Delta\rho_{max}$, $\Delta\rho_{min}$ / (e Å^{-3})	0.62, –0.64

Calculations

The structures of the quinoxalines were fully optimized using the B3LYP functional[49,50] and the 6-311++G(d,p) basis set. Solvation effects were included using the polarizable continuum model with the CPCM method.[51,52] All the optimized structures were confirmed as a local minimum on the potential energy surface by calculation of the Hessian matrix force constant (no negative eigenvector). All calculations were performed with the Gaussian 09W software.[53]

Results and Discussion

Synthesis and characterization of compounds **1**-**3**

The reaction of 2,3-dichloro-1,4-naphthoquinone and *tert*-butyl *N*-(2-aminoethyl)carbamate under the conditions described in the literature for other amines[42,46] yielded novel *tert*-butyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)ethylcarbamate **1** (70% after purification by CC) as a red solid, soluble in common organic solvents but insoluble in water. It was characterized by analytical and spectroscopic data (see Experimental section and Figures S1-S3 in the Supplementary Information (SI) section). The ^{1}H NMR spectrum ($CDCl_3$) exhibits the expected peaks due to the carbamate methyl groups at δ 1.44, the methylene hydrogens as two broad quartets at δ 3.97 and 4.86 that result from the couplings with the respective neighboring NH hydrogens. The latter appear as broad peaks at δ 4.86 and 6.50. The four signals in the δ 7.61-8.13 region are due to the naphthoquinone ring hydrogens. The IR spectrum shows the N-H amide (3373 cm^{-1}) and amine (3338 cm^{-1}) bands, a broad intense naphthoquinone carbonyl band (1669 cm^{-1}) and an unusually weak band associated with the carbamate C=O stretching (1648 cm^{-1}) possibly due to the formation of a N-H…O (carbamate) hydrogen bond, as observed in the X-ray structure of the analogous compound derived from *tert*-butyl *N*-(3-aminopropyl)carbamate.[42]

Under the conditions employed for *N*-Boc deprotection (CF_3COOH/CH_2Cl_2), the initial orange solution of compound **1** immediately turned purple. It was left under stirring in a closed flask for 24 h. Depending on the conditions employed for the treatment of the

dark purple solid obtained after solvent evaporation, 5-chloro-3,4-dihydrobenzo[*f*]quinoxalin-6(2H)-one **2** and 5-chlorobenzo[*f*]quinoxalin-6-ol **3** were isolated in varying yields (Scheme 1). Thus, treatment of the dark purple solid with saturated $Na_2CO_{3(aq)}$ resulted in CO_2 evolution and formation of known orange dihydrobenzoquinoxaline **2**,[26] which was extracted with EtOAc, purified by CC and obtained in 86% yield. Under these conditions, trace amounts of quinoxaline **3** were also isolated from the column. When the purple EtOAc solution was treated with water instead, the solution color turned dark yellow. Purification of the organic extracts by CC as before gave pale yellow benzoquinoxaline **3** in up to 46% yield, besides **2**.

Both compounds have been fully characterized by analytical and spectroscopic data (see Experimental and SI sections), that confirmed their identity, and the novel compound **3** was also characterized by a single crystal XRD study.

The ^{1}H NMR data for dihydrobenzoquinoxaline **2** (Figures S5-S7 in the SI section) are in agreement with those previously reported[26] and confirm that the compound exists as the keto-amine tautomer, both in $CDCl_3$ and in CD_3OD. The ^{13}C NMR spectrum of **2** ($CDCl_3$, Figure S8 in the SI section) exhibits all the expected signals, those corresponding to C4 (C=O) and C1 (C=N), at δ 176.3 and 152.2, respectively. The ATR-FTIR (attenuated total reflectance Fourier transform infrared spectroscopy) spectrum of **2** (Figure S4 in the SI section) indicates similar structure in the solid state. It shows the amine $\nu_{N\text{-}H}$ band, at 3324 cm^{-1}, and the quinoxaline ν_{CO} low intensity band, at 1604 cm^{-1}, at lower frequency than previously reported (1635 cm^{1-})[26] probably due to intermolecular hydrogen bonding. The presence of EtOAc (ν_{CO} = 1737 cm^{-1}) was confirmed by elemental analysis.

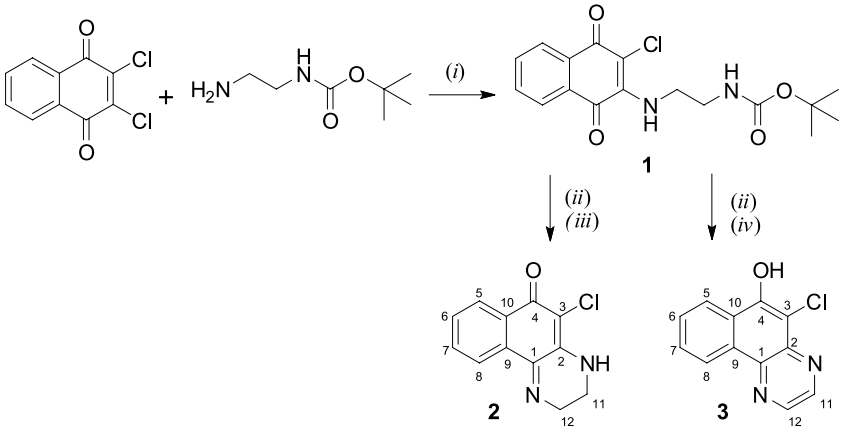
The ^{1}H NMR spectrum of benzoquinoxaline **3** in $CDCl_3$ (Figures S10 and S11 in the SI section) shows hydrogens H11 and H12 as a multiplet which is actually a partially superimposed pair of doublets at δ 7.82; H6/H7 appear at δ 8.37 and 9.18 as multiplets, and H5/H8 appear at δ 8.83 and 8.93 as doublets. A peak around δ 6.7, attributed to the OH hydrogen, is absent in the spectra in CD_3OD and DMSO-d_6 (Figure S12 and S13 in the SI section). The ^{13}C NMR (APT) spectrum of **3** in DMSO-d_6 (Figure S14 in the SI section) shows all the expected peaks and no evidence for the presence of the keto-amine tautomer in solution. The OH bonded to C4 appears at δ 151.0, typically a phenol carbon chemical shift. The sp^2 C1 and C2 carbons bonded to the quinoxaline nitrogens appear at δ 140.0 and 137.0, respectively.

The ATR-FTIR spectrum of microcrystaline **3** is shown in Figure S9 in the SI section. The spectrum exhibits a broad band centered around 3200 cm^{-1}, attributed to $\nu_{O\text{-}H}$, but the $\nu_{C=N}$ band(s) could not be unambigously located in the spectrum, suggesting the presence of intermolecular hydrogen bonds. In order to investigate the solid state structure of **3**, an XRD study was carried out and the results are described below.

Molecular and crystal structure of **3**

Crystals suitable for an XRD study were obtained by slow evaporation of an ethanol solution of **3**. The X-ray structure analysis of **3** revealed the enol-imine tautomer, shown in Figure 3a. Selected bond lengths and angles are also given in Figure 3a. As suggested by the IR spectrum, this tautomer is stabilized by hydrogen bonds [O(1)-H(1)...O(1w) and O(2w)-H(1w2)…N(1)] (Figure 3b). To our knowledge, this is the first example of a structurally characterized molecule containing the benzo[*f*]quinoxalin-6-ol nucleus.

The compound crystallizes in the P2$_1$/n space group with three independent water molecules in the asymmetric



Scheme 1. Reactants and conditions: (*i*) K_2CO_3, MeCN, reflux, 5 h, (*ii*) CF_3COOH, CH_2Cl_2, 24 h, room temperature, (*iii*) sat. $Na_2CO_{3(aq)}$/EtOAc extraction, CC and (*iv*) H_2O/EtOAc extraction, CC.

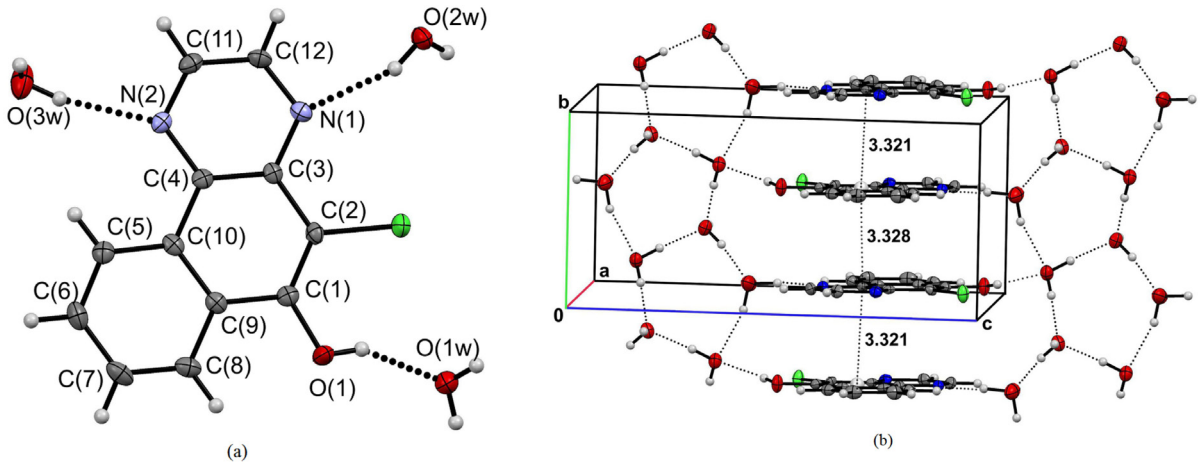


Figure 3. (a) ORTEP view of **3** with the crystallographic atom numbering scheme. Non-hydrogen atom displacement ellipsoids are drawn at the 50% probability level; (b) view showing the hydrogen bond interactions between the water molecules chain and the π-stacked benzoquinoxaline rings. Selected geometric parameters are as follows: bond distances (Å): Cl1-C2 1.729 (3), O1-C1 1.351 (3), C3–N1 1.359 (4), C3-C4 1.412 (4), C3-C2 1.431 (4), C1-C2 1.365 (4), C1-C9 1.442 (4), N1-C12 1.317 (4) C10-C5 1.410 (4), C10-C9 1.410 (4), C10-C4 1.447 (4), N2-C11 1.323 (4), N2-C4 1.358 (3), C8-C7 1.369 (4), C8-C9 1.411 (4), C11-C12 1.387 (4), C5-C6 1.370 (4) C6-C7 1.398 (4) and bond angles (degree): N1-C3-C4 121.2 (2), N1-C3-C2 119.6 (3), O1-C1-C2 125.0 (3), O1-C1-C9 114.4 (2), C12–N1-C3 116.2 (3), C11–N2-C4 116.8 (3), N2-C11-C12 122.1 (3), C1-C2-Cl1 120.1 (2). Tables S1 and S2 (in the SI section) contain the full list of bond distances and angles.

unit. It exhibits a planar structure with all ring atoms sp^2 hybridized and bond distances and angles typical of conjugated π-systems (see Tables S1 and S2 in the SI section).[21,22] The benzoquinoxalines are packed in an ABAB pattern formed by π-π interactions and hydrogen bonds along the *b*-axis (Figure 3b). The interactions between the water molecules result in double chains of packed molecules. These chains are interconnected by benzoquinoxaline molecules along the 2(1) screw axis. The observed distances between the parallel π-stacked benzoquinoxaline rings are shorter than 3.4 Å.[54] Other interactions between benzoquinoxalines and water molecules {[Q…O(1w)…O(2w)$^{½+x,½-y,½+z}$…O(3w)$^{x,y,1+z}$…Q$^{x,y,1+z}$] Figure S15a and [(Q...O(2w)...O(1w)$^{-½+x,½-y,-½+z}$...Q$^{-½+x,½-y,-½+z}$)] Figure S15b and Table S3 in the SI section} result in the packing along the *c*-axis and [101] direction, respectively, and stabilize the 3D crystalline arrangement.

Mechanistic aspects of the dehydrogenation of **2**

Pure dihydrobenzoquinoxaline **2** is air-stable in CH_2Cl_2 solution in the absence of acid. Nevertheless, it undergoes slow dehydrogenation when chromatographed on a silica gel column with the same solvent system used for its purification (see Experimental section and Figure 4).

Kallmayer and Seyfang[43] reported in the eighties that related dihydrobenzo[*f*]quinoxalin-6(2H)-ones (with R = CH_3, OCH_3, CN and NHAc in place of Cl) also undergo dehydrogenation to the respective benzoquinoxalin-6-ol compounds analogous to **3**. When promoted by base, the products were obtained in high yields. The acid promoted reaction (aqueous HCl/EtOH solution), however, gave only trace amounts of the benzoquinoxalin-6-ol derivatives besides the respective hydrolysis products, 2-hydroxy-3-R-1,4-naftoquinones. The mechanism for the acid promoted dehydrogenation was suggested to involve, as the first step, reversible protonation of the carbonyl group, followed by proton migration and, presumably, H_2 elimination (see Figure S16 in the SI section).[43]

Herein, we suggest that the dark purple product formed under the conditions used for the *N*-Boc deprotection of **1** is the protonated form of the dihydrobenzoquinoxaline **2** proposed by Kallmayer and Seyfang [43] (see Scheme 2). In other words, nucleophilic attack of the deprotected amine at the carbonyl carbon atom to yield **2** is a facile process. Moreover, purple protonated **2** is stable in CH_2Cl_2/CF_3COOH. This would account for the fact that when this purple species is treated with base, the dihydrobenzoquinoxaline **2** is formed in good yields (see Scheme 1), but under acid conditions, in the presence of water, the reaction proceeds to give the dehydrogenated product **3**.

Relative energies of a series of dihydrobenzoquinoxalines and benzoquinoxalines tautomers related to **2** and **3**

Benzophenazines (*e.g.*, Figure 1a) and dihydrobenzoquinoxalines can exist in either the keto-amine form, such as **2** (see Figure 1b), or in the enol-imine form exhibited by **3**. In view of our continuing interest in the tautomerism of naphthoquinone derived compounds,[38,55,56] we decided to undertake a detailed investigation of the

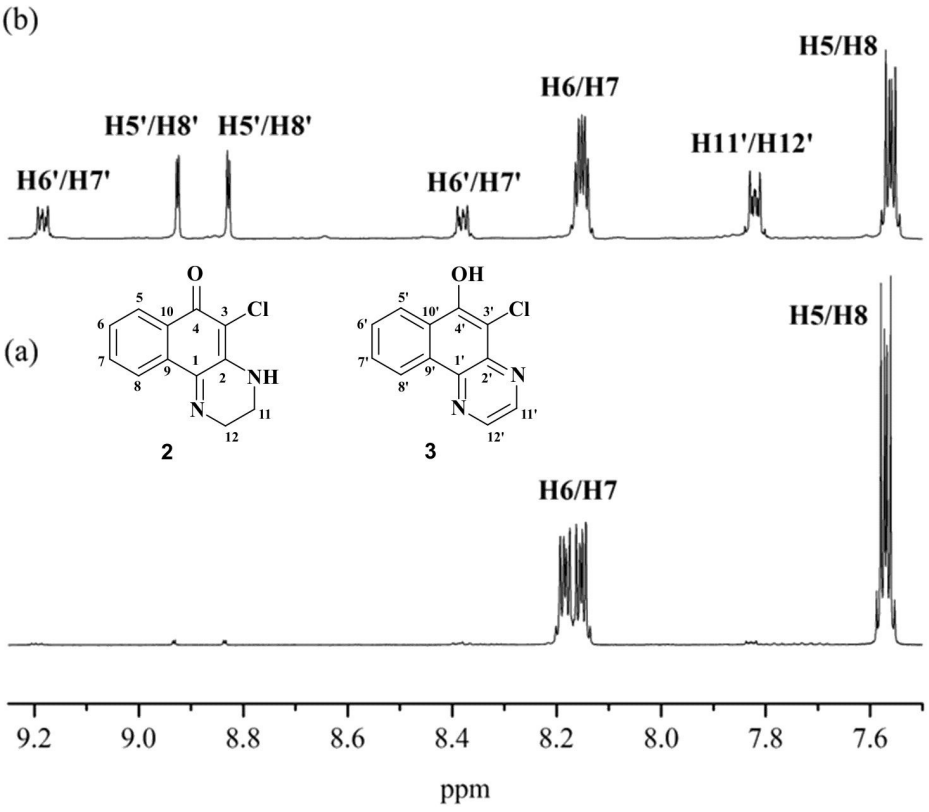
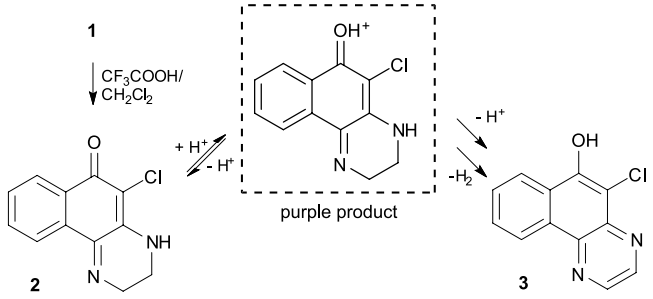


Figure 4. (a) ^{1}H NMR (500 MHz) spectrum of **2** in $CDCl_3$ (obtained after neutralization of the crude solid with saturated $Na_2CO_{3(aq)}$, extraction with EtOAc and purification by flash chromatography-hexane/EtOAc, 10/1 to 2/1, v/v); (b) ^{1}H NMR (500 MHz) spectrum of **2** in $CDCl_3$ after flash chromatography showing formation of **3**.



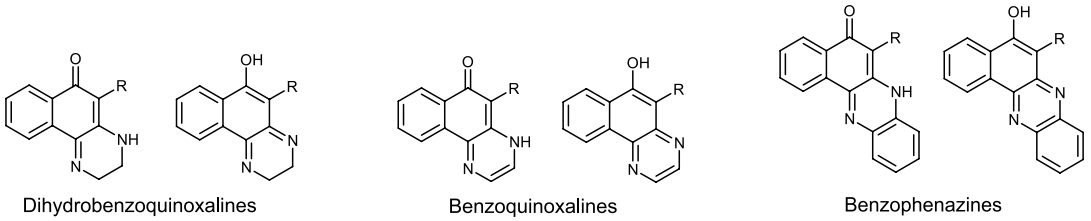
Scheme 2. Proposed path for the formation of **2** and **3** from *tert*-butyl 2-(3-chloro-1,4-dioxo-1,4-dihydronaphthalen-2-ylamino)ethylcarbamate **1**.[30]

relative energies of the tautomers of **2** and tautomers of **3** and of several related compounds. In addition, it was also investigated possible correlations, *e.g.*, with the nature of the substituent at position 3 and the extension of aromaticity. Solvation effects using the polarizable continuum solvation approach were included to investigate the effect of non-polar ($CHCl_3$), polar protic (CH_3OH) and polar aprotic (DMSO) solvents.

From the two alternative conformations for the hydroxyl hydrogen of the enol-imine tautomer of compounds **2** and **3** and of related compounds containing H or CH_3 in place of the Cl atom at position 3, the most stable is that in which the hydroxyl hydrogen atom is directed towards the substituent. This conformation is the one adopted in the solid state structure of **3** (see Figure 3a and Table S4 in the SI section). Therefore, all computational results reported below are for this conformation. Calculated bond lengths and angles for **3** are in good agreement with the experimental (XRD) data (see Tables S1 and S2 in the SI section).

The relative energies calculated at the B3LYP/6-311++G(d,p) level for the tautomers of the dihydrobenzoquinoxalines, benzoquinoxalines and benzophenazines are given in Table 2. All values reported were obtained by subtracting the energy of the keto-amine form from that of the enol-imine one, *i.e.*, the more negative the value, the more stable the enol-imine tautomer and *vice-versa*.

Table 2. B3LYP/6-311++G(d,p) relative energies (kcal mol^{-1}) calculated for the two tautomers of dihydrobenzoquinoxalines, benzoquinoxalines and benzophenazines. The energy values were calculated as $E_{enol\text{-}imine} - E_{keto\text{-}amine}$ forms



Solvent (ε)[a]	R =								
	H	Cl (**2**)	CH_3	H	Cl (**3**)	CH_3	H	Cl	CH_3
Gas phase	11.70	12.13	12.53	−9.78	−8.15	−8.56	−2.69	−1.61	−1.06
$CHCl_3$ (4.7)	13.50	14.35	14.21	−5.20	−3.66	−4.48	0.37	1.56	1.21
CH_3OH (36.2)	14.31	15.25	14.92	−3.46	−1.96	−2.92	1.52	2.71	2.21
DMSO (46.8)	14.36	15.31	14.96	−3.35	−1.86	−2.83	1.59	2.78	2.27

[a]In parenthesis is given the value of the dielectric constant (ε) of each solvent.

For the dihydrobenzoquinoxaline series (Table 2), the keto-amine tautomer is by far the most stable form, independent of the substituent and solvent. These results are in agreement with the experimental data, both in the solid state and in solution (see Experimental and reference 26).

For the benzoquinoxaline series, the relative energy of the enol-imine tautomer is lower than that of the keto-amine tautomer. For the tautomers of the structurally related benzophenazines, which contain an additional benzene fused to the pyrazine ring, the gas phase relative energies also indicate the enol-imine tautomer as the most stable one, although less so than in the benzoquinoxaline series (Table 2). The different substituents (R = H, Cl or CH_3) have only marginal effect on the relative energies. However, in all cases, the Cl and CH_3 substituents decrease the relative stabilities of the enol-imine forms as compared to R = H.

Solvation has a strong effect on the relative stabilities, with the more polar DMSO and CH_3OH solvents having a higher effect than the less polar $CHCl_3$. The three solvents preferentially stabilize the keto-amine tautomers, mainly because these tautomers have higher dipole moments than the enol-imine tautomers (see Table S5 in the SI section, *e.g.*, $|\mu|$ (D) = 5.45 × 1.61 (**2**) and 5.95 × 1.19 (**3**), respectively). The preferential stabilization of the keto-amine form by the solvent is enough to reverse the stability order in the case of the benzophenazines. For the latter compounds, the keto-amine form, which is the least stable in the gas phase, becomes the most stable in all solvents. The relative effect of the solvents follows the corresponding dielectric constant order (Table 2), with $CH_3Cl < CH_3OH \sim$ DMSO. In summary, in the gas-phase, dihydrobenzoquinoxalines are most stable as the keto-amine tautomer, while for the benzoquinoxalines and benzophenazines the enol-imine forms are the most stable. The three solvents studied reverse the stability order for the benzophenazines, making them more stable in the keto-amine form.

These results agree with the ^{1}H and ^{13}C NMR spectra of **3** obtained in the three (deuterated) solvents (see Figures S10-S14 in the SI section) and the ^{1}H NMR spectrum of the CH_3 substituted derivative reported in $CDCl_3$.[43] They indicate, therefore, that the relative stabilities of the two tautomers are dominated by the extent of aromaticity in the benzoquinoxaline series. However, this aspect seems to be less important for the benzophenazines, making the solvent more relevant in this case.

The ^{13}C NMR spectrum of the unsubstituted benzophenazine (R = H) in DMSO-d_6 (δ 206.4 ppm) has been interpreted in terms of the keto-amine tautomer in agreement with the relative energies reported above (Table 2).[23] In $CDCl_3$/CF_3COOD (δ 168.7 ppm), however, the enol-imine tautomer was proposed as the most stable, differently from the prediction based on the relative energies.[44] In $CHCl_3$ the energy difference between the two tautomers for this derivative is very low, only 0.37 kcal mol^{-1} in favor of the keto-amine tautomer, certainly below the accuracy of the theoretical method employed. To see whether this could be accounted for by the entropic and thermal contributions to the equilibrium, it was also calculated the relative Gibbs free energies of the two tautomers at 298 K (Table S6 in the SI section). The Gibbs free energy difference between the two tautomers is reduced to 0.22 kcal mol^{-1}, still favoring the keto-amine tautomer, thus suggesting that both tautomers may be present under equilibrium conditions.

To look for the origin of the difference between the computed relative energies and the main tautomer found

in solution for this benzophenazine, we simulated the ^{13}C NMR spectra of the two tautomers of this compound (Tables S7-S11 in the SI section). For the dihydrobenzoquinoxaline **2**, which exists mainly as the keto-amine tautomer (δ 176.3 ppm), and the benzoquinoxaline **3**, which exists mainly as the enol-imine tautomer (δ 151.0 ppm), the C4 carbon chemical shifts (in $CDCl_3$) are good probes to distinguish between the two tautomers in solution.

The calculated C4 carbon chemical shift of the benzophenazine keto-amine tautomer (δ 188.6 ppm) agrees (to within 8.6%) with the experimental value in DMSO-d_6 (Table 3). However, the C4 carbon chemical shift measured in $CDCl_3/CF_3COOD$ (δ 168.7 ppm) has a much lower value, indicating the prevalence of the enol-imine tautomer in this solvent. Indeed, the computed value for the C4 carbon chemical shift of this tautomer agrees with the experimental data to within 2.7%. Nevertheless, the experimental spectrum in $CDCl_3$ was obtained in the presence of CF_3COOD,[44] which could protonate either the keto-amine or the enol-imine tautomer, leading in each case to the same structure with an OH bond, typical of the enol-imine tautomer. Calculation of the C4-carbon chemical shift for the protonated structure (optimized from the protonation of both tautomers, see data in Table 3) confirms that in $CDCl_3/CF_3COOD$, the experimental value is close to that found for the enol-imine tautomer. Therefore, in this solvent system, the experimental data accounts for either the protonated benzophenazine or the enol-imine tautomer.

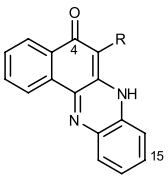
The reported ^{13}C NMR data for benzophenazine derivatives substituted at position 15 (see Figure 1a, **g**, R^5 = OMe, Cl and NO_2) in DMSO-d_6 (δ 206.4 for all compounds)[23] also agree with the proposed keto-amine tautomer.

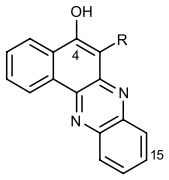
Likewise, the same keto-amine structure has been proposed in the literature for the 3-isopentyl- and 3-isobutyl-substituted benzophenazines[22] (see Table 3) in spite of the very different C4-carbon chemical shifts observed in the spectra of the two compounds in DMSO-d_6 (R = isopentyl, δ 198.6; R = isobutyl, δ 152.6 ppm). As discussed above, chemical shifts in the 160-170 ppm range are typical for the enol-imine tautomer. It is suggested, therefore, that the observed chemical shift of δ 152 ppm for the isobutyl substituted derivative indicates prevalence of the enol-imine tautomer for this derivative (see Tables S12-S15). This unexpected result might arise from intermolecular association which has been reported to lead to protomeric equilibrium shift in similar compounds.[57,58] Studies of concentration effects on C4-carbon chemical shifts of this class of compounds would shed some light on the problem.

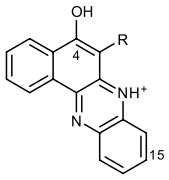
Conclusions

In the present study, we report on the tautomeric equilibrium in benzo[*f*]quinoxalin-6(4H)-ones derived from the 1,4-naphthoquinone nucleus. XRD analysis and DFT calculations confirm the enol-imine tautomer as the most stable form of these benzoquinoxalines. The higher stability of the enol-imine tautomer for these derivatives has been attributed to enhanced aromaticity in the enol-imine tautomeric form. For the enol-imine tautomer of dihydrobenzoquinoxalines, aromatization of the additional ring does not occur, which makes the keto-amine tautomer much more stable. This fact was also confirmed by means of the C4-carbon atom ^{13}C chemical shift. The chemical shift of this carbon was employed as a probe to conclude that benzo[*a*]phenazin-5(7H)-one exists mainly in the

Table 3. ^{13}C NMR experimental and calculated C4 chemical shifts (δ in ppm) for selected benzophenazines (see Tables S8-S16 in the SI section)







R	Keto-amine δ calc. (exp.) / ppm	Enol-imine δ calc. (exp.) / ppm	Protonated structure δ calc. (exp.) / ppm
H[a,23]	188.6 (206.4)	165.4	
H[b,44]		(168.7)	176.3 (168.7)
H[c]	187.9	164.2	
isobutyl[a,22]	187.6	162.3 (152.6)	
isopentyl[a,22]	187.3 (198.6)	162.9	

[a]In DMSO (d_6-DMSO); [b]in $CHCl_3$ ($CDCl_3/CF_3COOD$); [c]in $CHCl_3$.

keto-amine form in DMSO, whereas in $CHCl_3/CF_3COOH$, it exists mainly as the enol-imine form, although the B3LYP/6-311++G(d,p) calculations with inclusion of the solvent effects give a slight (0.22 kcal mol^{-1}) preference for the keto-amine tautomer.

Supplementary Information

Supplementary information associated with this paper contains IR and NMR spectra (^1H and ^{13}C), X-ray structural parameters of **3** and the results of the theoretical calculations. These data are available free of charge at http://jbcs.sbq.org.br as PDF file. Crystallographic data for the structural analysis of compound **3** have been deposited with the Cambridge Crystallographic Data Center, CCDC No. 908496. Copies of this information may be obtained free of charge from CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK.

Acknowledgments

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