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Unexpected reduction of ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide derivatives by amines

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Abstract: The unexpected tendency of amines and functionalized hydrazines to reduce the ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide (**1**) to a quinoxaline (**1c**) and mono-oxide quinoxalines (**1a** and **1b**) is described. The experimental conditions were standardized in the use of 2 equivalents of amine in ethanol under reflux for 2 hours, with the aim of studying the distinct reductive profile of the amines and the chemoselectivity of the process. With the exception of hydrazine hydrate, which reduced compound **1** to a 3-phenyl-2-quinoxalinecarbohydrazide derivative, the amines only acted as reducing agents.

Keywords: quinoxaline *N*-oxides, reduction, carboxylate, amines.

Introduction

Quinoxaline and quinoxaline 1,4-di-*N*-oxide are heterocycles that are usually used in the synthesis of biologically active compounds[1-13]. The former is described as a bioisoster of quinoline, naphthyl, benzothienyl and other aromatic rings[14], and it can be found in the structure of anti-inflammatory, anti-cancer, and anti-bactericide agents[1,11-13]. The latter is found in the structure of anti-chagas, anti-malarial, anti-cancer and anti-tuberculosis drug candidates[2-10], and its widespread activity can be associated with the generation of free radicals[15].

In our continuing efforts to find quinoxaline-1,4-di-*N*-oxide derivatives with anti-malarial activity[16], the interconversion of compound (**1**) into hydrazides (**2**) and amide derivatives (**3**) was carried out be means of hydrazinolysis and aminolysis reactions; unexpected results were obtained. In this work, we describe the potential reductive profile of amine derivatives when they reduce the ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide derivative (**1**).

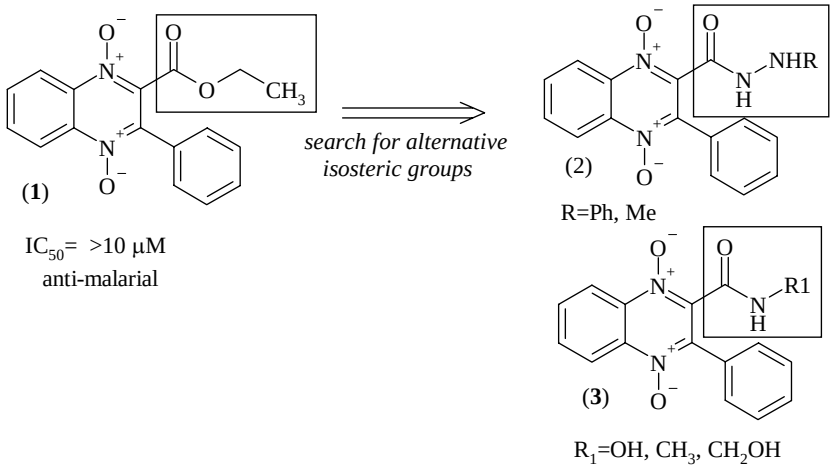
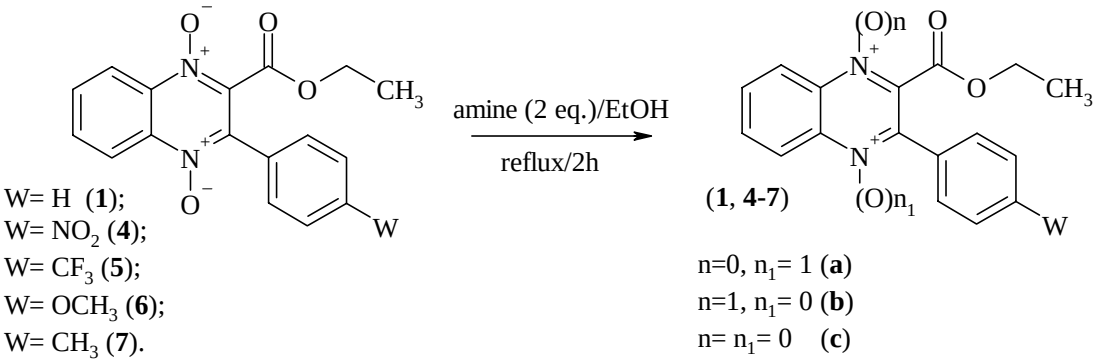


Chart 1. Design of new quinoxaline 1,4-di-*N*-oxide derivatives

Results and Discussion

In a continuing effort to synthesize new anti-malarial drug candidates, a proposal was made to substitute the carboethoxy moiety, present in lead-compound **1**, with a functionalized hydrazide subunit (**2**) (Chart 1). Therefore, compound **1** was first treated with phenylhydrazine in the presence of ethanol under reflux for 2 hours. After workup, the crude oil was analyzed by ^{1}H RMN, which revealed the presence of four different signals relative to the carboethoxy group. This crude oil was purified by silica gel column chromatography, and the separated products were analyzed by ^{1}H-NMR, IR, mass spectra and elemental analyses. From these analyses, it was observed that the reaction with phenylhydrazine failed to give the functionalized hydrazide derivative (**2**); surprisingly, this reaction gave a mixture of quinoxaline (**1c**), quinoxalines *N*-4 monoxide (**1a**), *N*-1 monoxide (**1b**) and 1,4-di-*N*-oxide (**1**) (table 1). While the reductive profile of hydrazine hydrate[17-18] is well known, the aforementioned information was not clear for phenylhydrazine. In an attempt to determine if the reduction of (**1**) by phenylhydrazine could be influenced by the electronic profile of quinoxaline-ester (**1**), the derivatives (**4-7**), attached to electron-withdrawing and electron-donating groups, were treated with phenylhydrazine in ethanol under reflux for 2 hours; the results are found in table 1 (entries 2, 3, 4 and 5). These results showed no selective reduction for substrates **5-7**, with the exception of compound **4** (4'-nitro, $\sigma_p = 0.81$) that could be selectively reduced to quinoxaline (**4c**) (entry 2). In this particular case, a distinct reductive profile could be traced between phenylhydrazine and hydrazine hydrate because, in the latter, no chemoselectivity was observed, and consequently, the nitro group was also reduced and the hydrazinolysis product was formed (data not shown). Intrigued by these results, the possibility that other amines could be acting as reducing agents of the quinoxaline 1,4-di-*N*-oxide system was then studied. Derivative **1,** used as template, was treated with different amines (2 equivalents) in the presence of ethanol under reflux for two hours (table 1). The results clearly demonstrated the ability of hydroxylamine (entry 9), methylamine (entry 10), ethanolamine (entry 11), methylhydrazine (entry 6), and 2,4-dinitro-phenylhydrazine (entry 7) to reduce compound (**1**) to the mono-oxide derivatives (**1a** and **1b**) and to quinoxaline (**1c**). However, amines, such as triethylamine (entry 12) and aniline (entry 13), were not able to reduce compound **1**, as no significant difference was found when compared to the experiment carried out in the absence of amine (entry 14).

Table 1: Reduction of ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide (**1**). The yields were determined by ^{1}H RMN ($CDCl_3$, 400 MHz) analysis of total crude product mixture, using the integration of the methyl region ($OCH_2C\underline{H}_3$).



Entry	REDUCTOR	W	n=n_1=1 (**1, 4-7**)	n=0, n_1=1 (**1b, 4-7a**)	n=1, n_1=0 (**1c, 4-7b**)	n=n_1=0 (**1a, 4-7c**)
1	$PhNHNH_2$	H	18.1%	30.1%	28.4%	23.4%
2	$PhNHNH_2$	NO_2	16.7%	0%	0%	83.3%
3	$PhNHNH_2$	CF_3	22.6%	30,0%	23.7%	23.7%
4	$PhNHNH_2$	OCH_3	25.7%	28.6%	17.6%	28.1%
5	$PhNHNH_2$	CH_3	15.1%	31.8%	21.9%	31.2%
6	H_3CNHNH_2	H	43.8%	21.8%	22.5%	11.9%
7	2,4-di$NO_2PhNHNH_2$	H	57.4%	8.9%	24.1%	9.6%
8	$N_2H_4.H_2O$	H	0%	0%	0%	100%*
9	$NH_2OH.H_2O$	H	22.6%	8.1%	33.8%	35.5%
10	$NH_2CH_3.H_2O$	H	33.8%	8.0%	49.7%	8.5%
11	$NH_2(CH_2)_2OH$	H	0%	34.2%	36.0%	29.8%
12	Et_3N	H	80.8%	0%	19.2%	0%
13	$PhNH_2$	H	81.2%	0%	18.8%	0%
14	EtOH	H	85.5%	0%	14.5%	0%
15	$P(OCH_3)_3$	H	0%	100%	0%	0%
16	$Na_2S_2O_4$	H	0%	0%	0%	100%

*formation of 3-phenyl-2-quinoxalinecarbohydrazide; absence of chemioselectivity.

The yields were determined by ^{1}H RMN ($CDCl_3$, 400 MHz) analysis of total crude product mixture, obtained after the work-up of each reaction. The methyl ($OCH_2C\underline{H}_3$) resonances for the four compounds **1**, **1a**, **1b** and **1c**, were observed at different chemical shifts, and thus, integration of the methyl region ($OCH_2C\underline{H}_3$) allowed relative molar percentages to be readily ascertained.

The correct assignment of *N*-4 oxide (**1a**), *N*-1 oxide (**1b**) and quinoxaline (**1c**) derivatives was unequivocally determined by ^{1}H-NMR , IR, mass spectra and elemental analyses. In an attempt to specifically identify mono-oxide quinoxaline derivatives (**1a** *versus* **1b**), a selective monodeoxygenation of compound **1** was performed (entry 14) using trimethylphosphite (entry 15), as previously described by Kluge and coworkers[19]. By this methodology, it was only possible to obtain the *N*-4 oxide derivative (**1a**), which was characterized by ^{1}H RMN ($CDCl_3$, 400 MHz); the data was

used for direct comparison with the *N*-oxides obtained from the reaction with the amines. In a similar way, the quinoxaline di-*N*-oxide (**1**) was totally reduced to quinoxaline (**1c**), using $Na_2S_2O_4$ (entry 16) in a mixture of methanol and water[20]; the chemical shift of compound **1c** was measured by ^{1}H RMN ($CDCl_3$, 400 MHz) and compared with the products obtained from amine reductions.

Compounds **1a**, **1b** and **1c** were evaluated for their ability to inhibit *P. falciparum* (Chloroquine sensitive), and were shown to be inactive as anti-malarial agents (data not shown).

Conclusions

In conclusion, this work clearly demonstrates a tendency of amines and functionalized hydrazines to reduce the ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide (**1**) to a quinoxaline (**1c**) and mono-oxides quinoxalines (**1a** and **1b**). Although the starting material (**1**) was recovered in most of the reactions (entries 1-7, 9-10, 12-14), suggesting that a larger time reaction could be necessary, the experimental conditions were standardized in 2 hours, using 2 equivalents of amine, with the aim of studying the distinct reductive profile of the amine being used and the chemoselectivity of the process. With exception of hydrazine hydrate, a well-known reducing agent, which reduced compound **1** to a 3-phenyl-2-quinoxalinecarbohydrazide derivative, the amines were not able to act as nucleophiles; they acted exclusively as reducing agents. Compounds **1a**, **1b** and **1c** were inactive as antimalarial agents.

Experimental Section

Chemistry

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), infrared (IR), nuclear magnetic resonance (^{1}H-NMR), mass spectra (MS) and elemental microanalysis (CHN). Alugram SIL G/UV254 (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352. D-52313 Düren, Germany) was used for Thin Layer Chromatography and Silica gel 60 (0.040-0.063 mm) for Column flash Chromatography (Merck). The ^{1}H NMR spectra were recorded on a Bruker 400 Ultrashield instrument (400 MHz), using TMS as the internal standard and with DMSO-d^6 and $CDCl_3$ as the solvents; the chemical shifts are reported in ppm (δ) and coupling constants (*J*) values are given in Hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), q (quadruplet), dd (double doublet) and m (multiplet). The IR spectra were performed on a Thermo Nicolet Nexus FTIR (Madison, USA) in KBr pellets; the frequencies are expressed in cm^{-1}. The mass spectra were measured on an Agilent Technologies Model MSD/DS 5973N (mod. G2577A) mass spectrometer with direct insertion probe (DIP) (Waldbronn, Germany) and the ionization method was electron impact (EI, 70 eV). Elemental microanalyses were obtained on an Elemental Analyzer (Leco CHN-900, Tres Cantos, Madrid, Spain) from vacuum-dried samples. The analytical results for C, H, and N, were within ± 0.4 of the theoretical values. Chemicals were purchased from Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma-Aldrich Química, S.A., (Alcobendas, Madrid), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

General procedure for the reduction of ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-N-oxide (1).

1 mmol of the ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide (**1**) was added to 10 mL of ethanol and 1 mmol of the amine derivative. The mixture was refluxed for two hours. After this time had elapsed, the work-up was carried out by adding 50 mL of CH_2Cl_2, followed by extraction with aqueous HCl 10% (4 x 15 mL). The organic layer was dried (Na_2SO_4), filtered, and evaporated to dryness. The yields were determined by ^{1}H RMN ($CDCl_3$, 400 MHz) analysis of total crude product mixture. The residue was later purified by silica gel column chromatography (*n*-hexane: ethyl acetate).

Ethyl 3-phenylquinoxaline-2-carboxylate 1,4-di-*N*-oxide (**1**). **^{1}H NMR** ($CDCl_3$): δ 1.08 (t, *J*= 7.2 Hz, 3H, $OCH_2C\underline{H}_3$); 4.25 (t, *J*= 7.2 Hz, 2H, $OC\underline{H}_2CH_3$); 7.53 (m, 3H, H3'-H5'); 7.61 (m, 2H, H2' and H6'); 7.91 (m, 2H, H6 and H7); 8.65 (m, 2H, H5 and H8) ppm. **^{13}C NMR** ($CDCl_3$): δ 13.98 ($OCH_2\underline{C}H_3$), 63.65 ($O\underline{C}H_2CH_3$), 120.89 (C5), 121.08 (C8), 127.84 (C1'), 129.16 (C3' and C5'), 130.15 (C2' and C6'), 131.26 (C4'), 132.51 (C6), 132.53 (C7), 136.53 (C2), 137.73 (C10), 138.81 (C3), 140.08 (C9), 159.66 ($\underline{C}O_2Et$) ppm. **IR** (KBr): 2978 (ArC-H), 1746 (C=O), 1352 (*N*-oxide), 701 and 666 (mono-substituted phenyl) cm^{-1}. **MS**: 310 (m/z, 100%), 294 (M^+, 6%), 249 (M^+, 51%), 221 (M^+, 46%), 77 (M^+, 46%).*Anal*. Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.52; N, 9.03. Found: C, 65.65; H, 4.57; N, 8.98.

Ethyl 3-phenylquinoxaline-2-carboxylate 4-*N*-oxide (**1a**). **RMN ^{1}H** (400 MHz, $CDCl_3$) δ: 1.04 (t, *J*=7.2 Hz; 3H, $OCH_2C\underline{H}_3$); 4.20 (q, *J*=7.2 Hz; 2H, $OC\underline{H}_2CH_3$); 7.56 (m, 3H, H3'-H5'); 7.61 (m, 2H, H2'and H6'); 7.88 (m, 2H, H6 and H7); 8.26 (dd, *J*= 1.2, 8.2 Hz; 1H, H5); 8.65 (dd, *J*= 1.2, 7.6 Hz; 1H, H8). **IR** (KBr): 2981 (ArC-H), 1742 (C=O), 1359 (*N*-oxide), 701 and 666 (mono-substituted phenyl) cm^{-1}. **MS**: 294 (m/z, 65%), 265 (M^+, 13%), 249 (M^+, 26%), 221 (M^+, 100%), 77 (M^+, 18%). *Anal*. Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.37; H, 4.77; N, 9.51.

Ethyl 3-phenylquinoxaline-2-carboxylate 1-*N*-oxide (**1b**). **RMN ^{1}H** (400 MHz, $CDCl_3$) δ: 1.25 (t, *J*=7.2 Hz; 3H, $OCH_2C\underline{H}_3$); 4.42 (q, *J*=7.2 Hz; 2H, $OC\underline{H}_2CH_3$); 7.54 (m, 3H, H3'-H5'); 7.80 (m, 3H, H2'and H6' and H7); 7.89 (dt, *J*= 8.4, 7.6 Hz; 1H, H6); 8.20 (d, *J*= 8.4 Hz; 1H, H5); 8.61 (d, *J*= 8.0 Hz; 1H, H8). **IR** (KBr): 2979 (ArC-H), 1735 (C=O), 1363 (*N*-oxide), 701 and 671 (mono-substituted phenyl) cm^{-1}. **MS**: 294 (m/z, 60%), 265 (M^+, 9%), 249 (M^+, 31%), 221 (M^+, 100%), 77 (M^+, 26%). *Anal*. Calcd. for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.39; H, 4.80; N, 9.51.

Ethyl 3-phenylquinoxaline-2-carboxylate (**1c**). **RMN ^{1}H** (400 MHz, $CDCl_3$) δ: 1.19 (t, *J*=7.2 Hz; 3H, $OCH_2C\underline{H}_3$); 4.34 (q, *J*=7.2 Hz; 2H, $OC\underline{H}_2CH_3$); 7.53 (m, 3H, H3'-H5'); 7.76 (m, 2H, H2'and H6'); 7.85 (m, 2H, H6 and H7); 8.20 (d, *J*= 8.0 Hz; 1H, H5); 8.24 (d, *J*= 7.6 Hz; 1H, H8). **IR** (KBr): 2990 (ArC-H), 1730 (C=O), 711 and 669 (mono-substituted phenyl) cm^{-1}. **MS**: 278 (m/z, 33%), 249 (M^+, 26%), 234 (M^+, 15%), 206 (M^+, 100%), 77 (M^+, 33%). *Anal*. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.35; H, 5.08; N, 10.05.

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