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Short Report

Preparation of Phenazine N^5,N^{10}-Dioxides. Effects of Benzofuroxan Substituents in the Outcome of their Expansion Reaction with Phenolates

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Os efeitos mesoméricos do substituinte benzofuroxano, na preparação de N^5,N^{10}-dióxidos de fenazina são descritos. As características eletrônicas do substituinte na posição 5 dos benzofuroxanos determinam a proporção dos isômeros 7 e 8 dos N^5,N^{10}-dióxidos de fenazina, quando fenóis substituídos são usados.

The mesomeric effects of the benzofuroxan substituent in the preparation of phenazine N^5,N^{10}-dioxides are described. The electronic characteristics of the substituent in 5-substituted benzofuroxans determine the ratio of the 7- and 8-isomers of phenazine N^5,N^{10}-dioxides when substituted phenols are used.

Keywords: phenazine N^5,N^{10}-dioxide, benzofuroxan tautomerism, mesomeric effect

Introduction

It is well known that phenolate anions react with benzo[1,2-*c*]1,2,5-oxadiazole *N*-oxides (benzofuroxans) to afford phenazine N^5,N^{10}-dioxide derivatives.[1,2] These are obtained through an heterocycle expansion process by reaction of the corresponding 1,2,5-oxadiazole system with the phenolate carbanion generated in basic medium attacking one of the heterocyclic nitrogen (Figure 1). When substituted benzofuroxans and phenolates are used, the reactions yield a mixture of 6- and 9- or 7- and 8-isomers.[3-5]

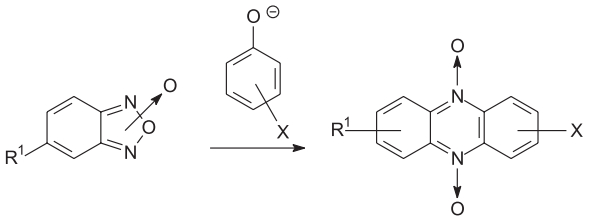

Figure 1. General procedure for the phenazine N^5,N^{10}-dioxide derivatives synthesis.

The formation of the isomers is the result of the well known tautomerism of the benzofuroxans[6,7] (*i.e.* Figure 2), thus the tautomeric forms can react with the carbanion in a non-selective fashion and with similar probability. However, each tautomeric form has different stability and electrophilic capacity.

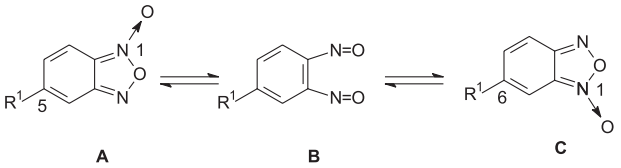

Figure 2. Tautomeric equilibrium of benzofuroxans.

Recently, the synthetic procedure shown in Figure 1 was used to develop a series of phenazine N^5,N^{10}-dioxide derivatives as new hypoxic selective cytotoxic agents.[8] In this synthetic approach, benzofuroxans with different 5-substituents, electron withdrawing and electron releasing moieties, were employed as the starting material. A mixture of the corresponding 7- and 8-substituted-2-amino-phenazine N^5,N^{10}-dioxide was obtained when *p*-amino-phenol was used as the phenolate source and a mixture of 7- and 8-substituted-2-hydroxyphenazine N^5,N^{10}-dioxide was obtained when *p*-hydroquinone was used (Table 1). It was observed that the proportion of isomers was highly dependent on the substituents electronic characteristics.

The existence of benzofuroxan as a mixture of isomers at room temperature (*i.e.* **A**, **B** and **C** tautomers in 5(6)-substituted benzofuroxans, Figure 2) has been intensively studied by different techniques.[9-17] Thermodynamic equilibrium

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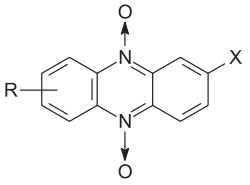
Table 1. Yield and spectroscopic characteristics of precipitated phenazine N^5,N^{10}-dioxide



Ref.	-X	-R	Yield (%)[a]	δ of characteristic protons [b]		Ref.	-X	-R	Yield (%)[a]	δ of characteristic protons [b]	
				8-H or *7*-H	*1*-H					*8*-H or *7*-H	*1*-H
1	$-NH_2$	$-OCH_3$	45	7.48 (*8*-H)[c]	7.39	7	-OH	$-OCH_3$	40	7.54 (*8*-H)[c]	7.69
2	$-NH_2$	$-CH_3$	46	7.66 (*8*-H)[c]	7.38	8	-OH	$-CH_3$	42	7.71 (*8*-H)[f]	7.59
3	$-NH_2$		85	7.75 (*8*-H)[c]	7.40	9	OH		47	7.84 (*7*-H)[f]	7.74
4	$-NH_2$	-Cl	40	7.83 (*8*-H)[c]	7.35-7.39 (*1*-H+*3*-H)	10	-OH	-Cl	37	7.91 (*8*-H)[c]	7.72
5	$-NH_2$	-Br	40	8.00 (*8*-H)[d]	7.42-7.47 (*1*-H+*3*-H)	11	-OH	-Br	40	7.95 (*8*-H)[f]	7.70
6	$-NH_2$	$-NO_2$	7	8.44 (*7*-H)[e]	8.46	12	-OH	$-NO_2$	5	8.42 (*7*-H)[e]	8.44

[a] Yield of benzofuroxans to phenazines transformations according to it is described in the text. The yields correspond to precipitated phenazine; [b] For the main isomer; in ppm; [c] In DMSO-d_6; [d] In CD_3OD:D_2O (1:1); [e] In CD_3OD. [f] In DMSO-d_6:D_2O (1:1).

concentrations are dependent on a number of factors, such as solvent, temperature, nature and position of the substituents on the ring.[6,7,18-22] Under the same conditions (solvent and temperature) in 5(6)-substituted benzofuroxans the presence of an electron withdrawing group generally favors the 6-tautomer over the 5-tautomer while the opposite occurs with an electron releasing group.[23-25] In our knowledge, studies related to benzofuroxan tautomerism and reactivity have not been described. On the other hand, in the preparation of phenazine N^5,N^{10}-dioxide derivatives from benzofuroxan,[1-5] the effect of the benzofuroxan substituent on the isomeric proportion of the products has not been studied.[26]

Herein, we present our results on the benzofuroxan tautomerism on the 1,2,5-oxadiazole-expansion process to obtain phenazine N^5,N^{10}-dioxide derivatives. By analysis of the reaction mixture products of 5(6)-nitro-benzofuroxan, it has also been demonstrated that the 1,2,5-oxadiazole heterocycle (tautomer **A** or **C**, Figure 2) is the electrophilic species in this process. Theoretical calculation, using the density functional theory (DFT) approach, were performed in order to determine the electrophilic center in each tautomer. These were in agreement with the experimental results.

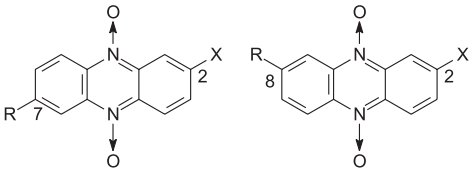
Results and Discussion

The expansion process was performed, as previously reported,[5] with sodium methoxide as the base, the mixture of the benzofuroxan derivative[27-30] and the corresponding phenol in MeOH:THF as the solvent at low temperature. Under these conditions, the products were obtained in moderate yield, except for the highly reactive 5(6)-nitro-benzofuroxan, which produced a complex mixture of products and a low yield of the desired heterocycle (Table 1).

Although, it could be assumed that the carbanion phenolate reacted non regioselectively with each tautomeric form, clear isomeric preferences were observed according to the stability of the tautomers **A** and **C** at the reaction temperature. Under the reaction conditions, the isomeric mixture of phenazine N^5,N^{10}-dioxides precipitated as a mixture which could not be separated neither by crystallization nor by chromatographic methods. The isomeric proportion, was established by ^{1}H NMR analysis of the precipitates (Table 2) analyzing the integrations of *8*-H, in 7-substituted phenazines, and *7*-H, in 8-substituted phenazines, for each isomer. As shown in Figure 3, *7*–H and *8*-H in the 8-substituted isomers result hardly affected, by *2*-NH_2 or *2*-OH electron releasing moieties. This fact together with the chemical shifts of *7*-H and *8*-H (Figure 3b) allowed to analyze the isomeric proportion in derivatives **1-12**.

Clearly, the isomeric ratios in the expansion processes were dependent on the benzofuroxan substituent electronic effect. When *p*-hydroquinone is used as the phenolate, the effects resulted clearly important, i.e. an electron releasing group as the methoxy and an electron withdrawing group as the nitro generated only one compound, the 7- and the 8-isomers, respectively.

Table 2. Isomeric proportion of phenazine N^5,N^{10}-dioxide derivatives and Hammett constants used in the correlations



Ref.	-X	-R	Proportion *7*-isomer : *8*-isomers	σ_{pR}	σ^-_{pR}	σ^+_{pR}	Ref.	-X	-R	Proportion *7*-isomer : *8*-isomer
1	$-NH_2$	$-OCH_3$	9.5 : 0.5	-0.27	-0.26	-0.78	7	-OH	$-OCH_3$	10.0 : 0.0
2	-NH2	$-CH_3$	7.0 : 3.0	-0.24	-0.17	-0.31	8	-OH	$-CH_3$	7.6 : 2.4
3	$-NH_2$		6.5 : 3.5	-0.02[a]	-0.00[b]	0.00[b]	9	-OH		3.5 : 6.5
4	$-NH_2$	-Cl	6.3 : 3.7	0.14	0.19	0.11	10	-OH	-Cl	6.6 : 3.4
5	$-NH_2$	-Br	5.4 : 4.6	0.22	0.25	0.15	11	-OH	-Br	6.4 : 3.6
6	$-NH_2$	$-NO_2$	4.0 : 6.0	0.57	1.27	0.79	12	-OH	$-NO_2$	0.0 : 10.0

[a] Sigma inductive of $CH(OEt)_2$ ref. 32; [b] Sigma zero resonance of $CH(OEt)_2$ ref. 33.

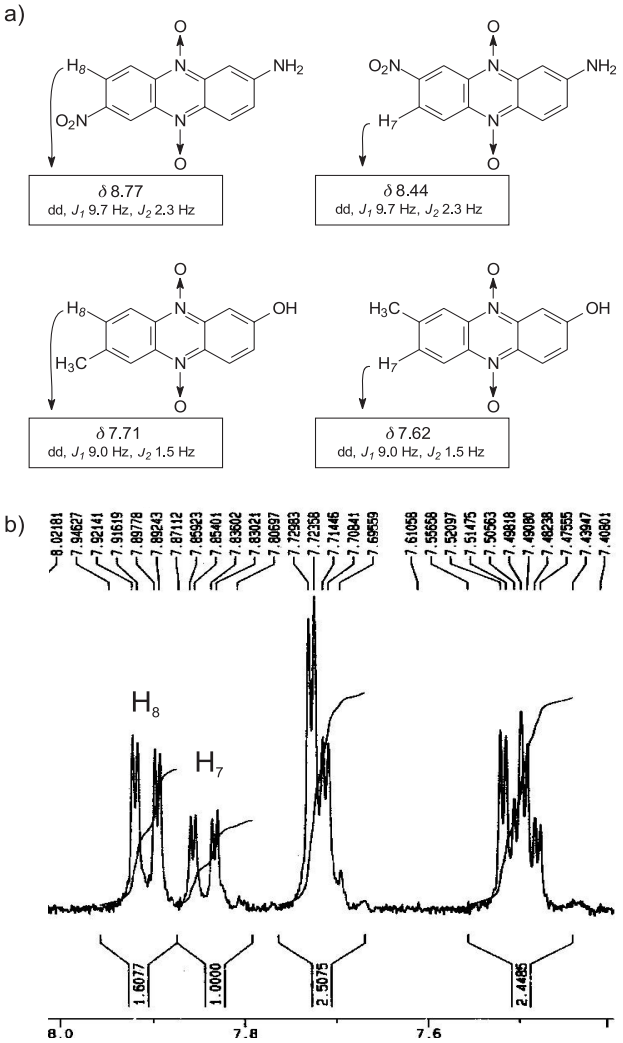


Figure 3. a) *7*- and *8*-Proton chemical shifts in derivatives **6** and **8**; b) Selected region of ^{1}H NMR spectra of derivative **10** showing *7*- and *8*-proton in isomer *8*- and *7*- respectively.

Therefore, the electronic effects of the substituent define, in the reaction medium, the position of the benzofuroxan tautomeric equilibria so they define the predominant electrophilic species. In these species the electrophilic atom is the nitrogen 1 in each tautomer (Figure 4a). These electrophilic preferences could be explained via a substituent mesomeric process (*i.e.* Figure 4b). Thus, electron releasing substituents decrease the electrophilic characteristic of the benzofuroxan nitrogen at position 3 in the favored 5-substituted tautomers. However, electron withdrawing substituents increase the electrophilic characteristic of benzofuroxan nitrogen at position 1 in the mesomerism-stabilized 6-substituted tautomers. The relevance of the electronic properties of the substituent in the isomeric ratio was tested studying the relationships between the proportion of 7-isomer in each reaction and the substituent inductive or mesomeric effect (expressed as Hammett constants, σ, or Swain-Lupton constants, $\mathcal{R}$ and $\mathcal{F}$). Thus, phenazine N^5,N^{10}-dioxide isomeric distribution, expressed as the proportion of the 7-isomer, was linearly related to the Hammett benzofuroxan substituent σ_p (Table 2, Figure 5, inset graphics).[31-33] In the amino-derivatives a better correlation was observed between σ_p and the proportion of 7-isomer (r= -0.9032, s= 0.1514, p=0.0136) than for hydroxy-derivatives (r= -0.8254, s= 0.1990, p= 0.0431). Because "through-conjugation" could be operative in the expansion processes, the correlations between σ_p^- and σ_p^+ substituent constants and the proportion of the 7-isomer were analyzed.[34] For the amine derivatives the substituent resonance-electron-withdrawing effects (expressed as σ_p^-) did not correlate adequately to the proportion of the 7-isomers as did σ_p constants (r= -0.8463, s= 0.3300, p=

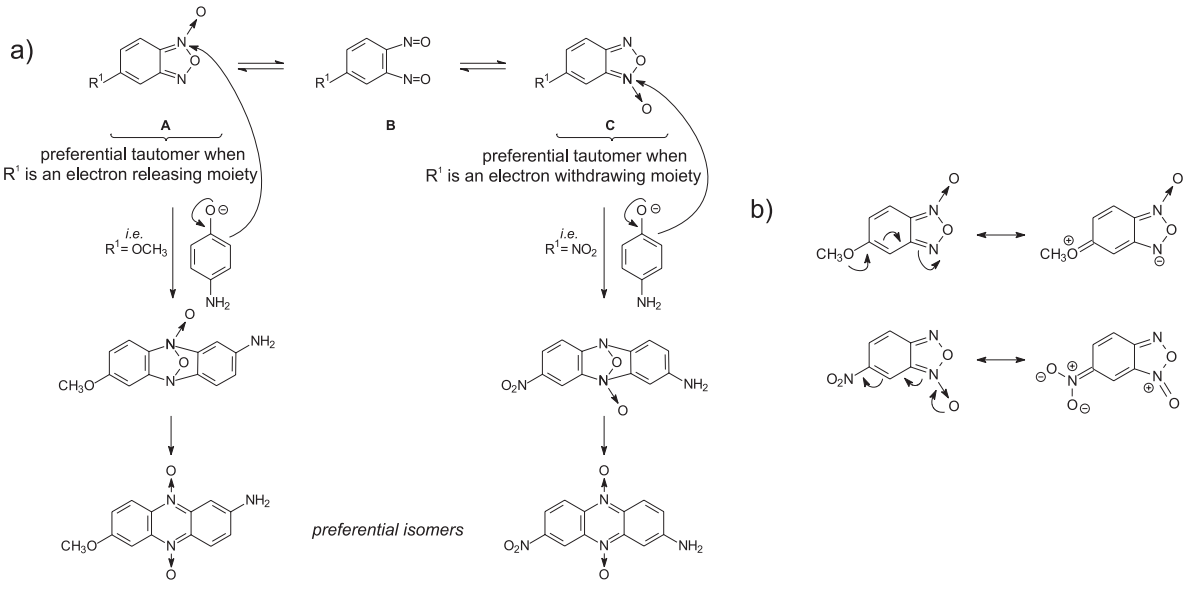


Figure 4. a) Proposed nucleophilic attack on different substituted benzofuroxans; b) Substituted benzofuroxan mesomeric processes.

0.0336). For the hydroxyl-derivatives, improvements in the statistical parameters were obtained in the correlation between σ_p^- and the 7-isomer proportions (r= -0.8610, s= 0.3151, p= 0.0276). However, the 7-isomer proportions correlate better with the σ_p^+ parameters than with the σ_p (see Figure 5). This could be indicative that the substituent resonance-electron-donating effect is operative in the benzofuroxan expansion processes.

The participation of tautomer **B** (Figure 2) as the electrophilic species in the reactions was rejected according to the results obtained in the yield optimization studies of the reaction involving 5(6)-nitrobenzofuroxan. Attempts to improve the yield in the preparation of derivative **6** were done using Et_3N as base under different conditions (Table 3). The use of Et_3N at room temperature was optimal for the preparation of **6**. In all cases a complex mixture of products was obtained.

Compound **13** and 4-amino-2-nitrophenol were also isolated, together with compound **6** (Figure 6). Compound **13** was the S_NAr product of the reaction between the hard electrophilic *6*–carbon, due to the presence of the nitro moiety, and the phenolate. The 4-amino-2-nitrophenol could be regarded as the product of the nucleophilic attack of the carbanion at the nitrogen in position 1 in the cyclic tautomer producing intermediate **(X)** (Figure 6) followed by a fragmentation process.

In order to corroborate the electrophilic capacity of the nitrogen atoms of the three tautomers of 5(6)-substituted benzofuroxan (**A**, **B**, and **C**, Figure 2), atomic charges (as natural charges) on oxadiazole's nitrogen atoms were calculated for nitro and methoxy derivatives.

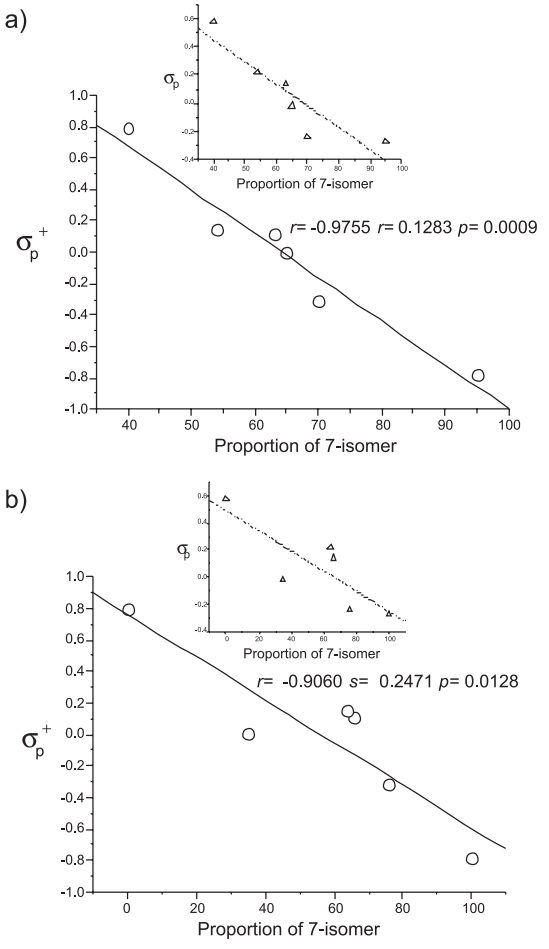
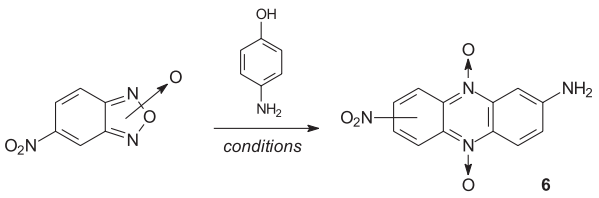


Figure 5. a) Isomeric proportion *vs* substituent σ_p^+ constant (inset: isomeric proportion *vs* substituent σ_p) for reaction between benzofuroxan derivatives and *p*-aminophenol; b) Isomeric proportion *vs* substituent σ_p^+ constant (inset: isomeric proportion *vs* substituent σ_p) for reaction between benzofuroxan derivatives and *p*-hydroquinone.

Table 3. Conditions for the preparation of derivative **6**



Conditions	Yield (%) derivative **6**	Other products (Yield / %)
MeONa/MeOH/-10 ºC	7	4-amino-2-nitrophenol [a]
Et_3N/0 ºC	9	4-amino-2-nitrophenol, **13**
Et_3N/room temperature	15	4-amino-2-nitrophenol (22), **13** [b] (13)
Et_3N/0 ºC to 60 ºC	Decomposition	

[a] Characterized by NMR spectroscopy and X-ray crystallographic studies; [b] According to ^1H NMR and MS data (see Figure 6).

The results, Figure 7, showed that the most positive nitrogen was nitrogen 1 on tautomer **A** for nitro derivative and nitrogen 1 on tautomer **C** for methoxy derivative, being nitroso nitrogens marginal in their electrophilic capacities (tautomers **B**).

In conclusion, the mesomeric effects of the 5-substituent of the benzofuroxan determine the 7:8 isomeric proportion of the isolated phenazine N^5,N^{10}-dioxide when *p*-aminophenol or *p*-hydroquinone are used as the nucleophilic agents.

Experimental

General

All starting materials were commercially available research-grade chemicals and were used without further purification. All solvents were dried and distilled prior to

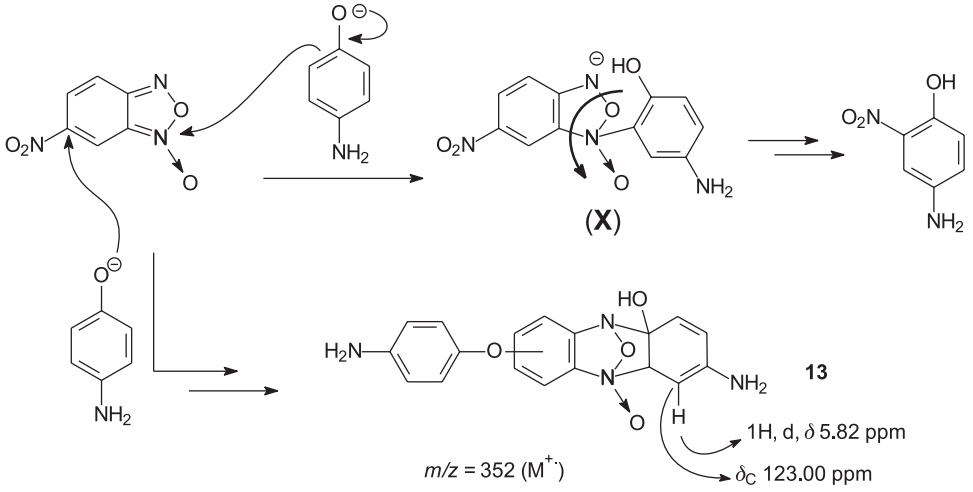


Figure 6. Proposed 4-amino-2-nitrophenol generation from 5-nitrobenzofuroxan and *p*-aminophenol.

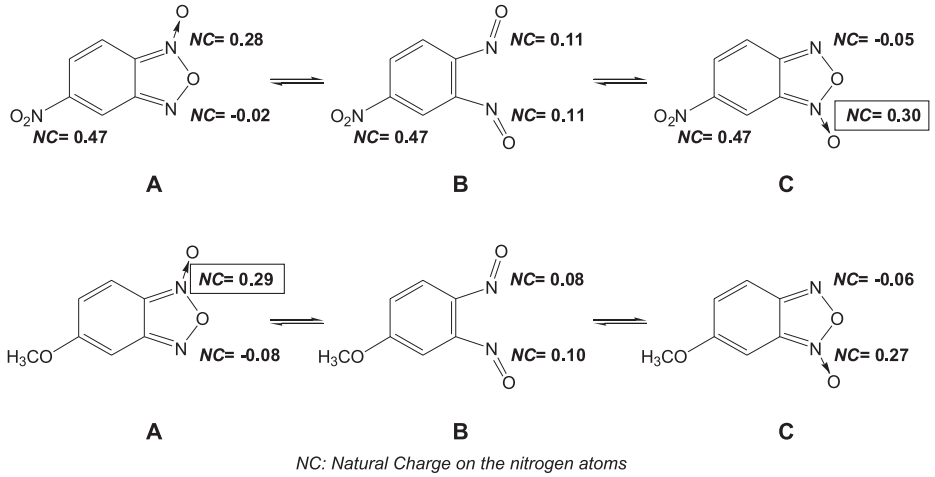


Figure 7. Atomic charges, as natural charge (in electron), theoretically calculated for the three tautomers of 5(6)-nitrobenzofuroxan and 5(6)-methoxybenzofuroxan. The most positive nitrogen in each compound is marked.

use. All the reactions were carried out under a nitrogen atmosphere. The typical work-up included washing with brine and drying the organic layer with sodium sulfate. Infrared spectra were recorded on a Perkin Elmer 1310 apparatus, using potassium bromide tablets, the frequencies are expressed in cm^{-1}. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3-4 mm Hg, 24 h at room temperature) and performed on a Fisons EA 1108 CHNS-O analyzer, and were within ± 0.4% of theoretical values. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 (at 400 MHz and 100 MHz) instrument, with tetramethylsilane as the internal reference and in the indicated solvent; the chemical shifts are reported in ppm. The ^{1}H and ^{13}C NMR signals reported were obtained at room temperature. Bruker software was used to perform HMQC, and HMBC experiments. Mass spectra were recorded on a Shimadzu GC-MS QP 1100 EX instrument (electronic impact, 70 eV).

General procedure for the synthesis of phenazine N^5,N^{10}-dioxide derivatives

A suspension of sodium methoxide was prepared (Na, 89.7 mg, 3.9 mmol, in 25.0 mL of anhydrous methanol) at –5 ºC under nitrogen. Then a mixture of benzofuroxan (20.0 mmol), the corresponding phenol (*p*-aminophenol or *p*-hydroquinone) (20.0 mmol), anhydrous methanol (5.0 mL) and anhydrous THF (30.0 mL) was added at –5 ºC during 15 minutes. The mixture was stirred at room temperature for 24 h. It was left stay at –20 ºC for 24 h. The resulting precipitate was collected by filtration and washed with small portions of methanol.

2-Hydroxy-8-nitrophenazine N^5,N^{10}*-dioxide,* ***12***

Green solid; mp > 240 ºC; IR ν_{max} / cm^{-1}:3400, 1525, 1340, 1330; MS, *m/z* (%): 273 ($M^{+\cdot}$, 1), 256 (4), 239 (4), 223 (5); Anal. ($C_{12}H_7N_3O_5$) C, H, N. ^{1}H NMR (CD_3OD, 400MHz) δ 9.27 (1H, d, H_9, J 2.2 Hz), 8.78 (1H, d, H_6, J 9.5 Hz), 8.46 (1H, d, H_4, J 9.6 Hz), 8.44 (1H, s, H_1), 8.42 (1H, dd, H_7, J_1 9.6 Hz, J_2 2.3 Hz), 7.34 (1H, d, H_3, J 9.9 Hz), 6.63 (1H, s, OH). ^{13}C NMR (HMQC-HMBC) (CD_3OD, 100MHz) δ 121.19, 115.54 (two carbons), 169.10, 149.80, 135.66, 134.00, 133.50, 131.74, 130.90, 127.80, 122.60.

Computational methods[35-37]

Calculations were performed with the Spartan'04 suit of programs, version 1.0.1.[38] First, the compounds were built with standard bond lengths and angles. A detailed conformational distribution study was performed, by using mechanics force field (MMFF). The geometry of each minimum energy conformer was fully optimised by applying PM3 in gas phase. Then the tautomers were fully optimized using the gradient-corrected density functional methodology. Becke's exchange functional (B) and Becke's three-parameter adiabatic connection (B3) hybrid exchange functional were used in combination with the Lee-Yang-Parr correlation functional. The standard 6-31G* basis set of DZP quality was used for orbital expansion to solve the Kohn-Sham equations for first and second-row elements.

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