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Artigo

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N- e *O*-Reação de 5,10-Dióxido de 2-Amino e 2-Hidroxifenazina Via Irradiação de Micro-ondas

Resumo: Fenazina 5,10 dióxidos *N*-*e O*-substituídos foram sintetizadas utilizando a irradiação de micro-ondas a partir dos correspondentes amino e hidroxi-derivados. As tentativas de *N*- ou *O*-substituição em condições de aquecimento convencionais, quando ocorreu, conduziu à formação dos produtos desejados em rendimentos extremamente baixos e em tempos de reação muito longos. A fim de obter as fenazinas 5,10-dióxidos *N*-ou-*O*-substituídas em melhores rendimentos e em menor tempo, foi estudada a reação em condições de irradiação de micro-ondas. Nestes estudos, obteve-se com êxito novos derivados 2-benziloxi, benzilamino e 2-sulfonamino phenazine 5,10-dióxidos, em baixos rendimentos, porém, significativamente maiores que aqueles obtidos através de aquecimento convencional. Estudos teóricos sugerem que a baixa nucleofilicidade das 2-amino and 2-hidroxifenazinas estudas esteja relacionada à presença da subunidade *N,N*-dióxido.

Palavras-chave: Micro-ondas; 5,10 fenazina-dióxidos; de substituição nucleofílica.

Abstract

Phenazine 5,10 dioxides *N*- and *O*-substituted were synthesized using microwave irradiation from the corresponding amino- and hydroxy-derivatives. Attempts to obtain phenazine 5,10-dioxides *N*- and *O*-substituted in conventional heating conditions, when it occurred, conducted to the desired products in extremely low yields with very long reaction times. In order to obtain phenazine 5,10-dioxide *N*- and *O*-substituted in good yields and lower reaction times, microwave irradiation was studied. In these experiments, we obtained new 2-benzyloxy, 2-benzylamino and 2-sulphonamino phenazine 5,10-dioxide derivatives successfully, in low yields, however, significantly higher than those obtained with conventional heating. Theoretical studies suggest that low nucleophilicity of 2-amino and 2-hydroxy phenazines studied is related to the presence of the moieties *N*,*N*-dioxide.

Keywords: Microwave; phenazine 5,10-dioxides; nucleophilic substitution.

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N- and *O*-Reaction of 2-Amino and 2-Hydroxyphenazine 5,10-Dioxide Via Microwave Irradiation

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1. Introduction

It is well known the existence of hypoxic and necrotic regions in solid tumours as consequence of the rapid growth of the cancerous cells and their deficient vascularisation that produce a decrease in molecular oxygen diffusion.[1] This common feature of cancerous cells, hypoxia, has been used for the development of a distinct therapy for treating cancer: the use of bioreductive antitumour agents.[2,3] Phenazine 5,10-dioxides were described as bioreductive antitumour prodrug pharmacophores that potentially interact with DNA after the corresponding bioreduction in hypoxic conditions (i.e. **1-4** in Figure 1a).[4-11] Furthermore, recently phenazine dioxides with improve DNA interaction capacity features an increased planar π-conjugation or flexible moieties that add to DNA-stacking.[12,13]

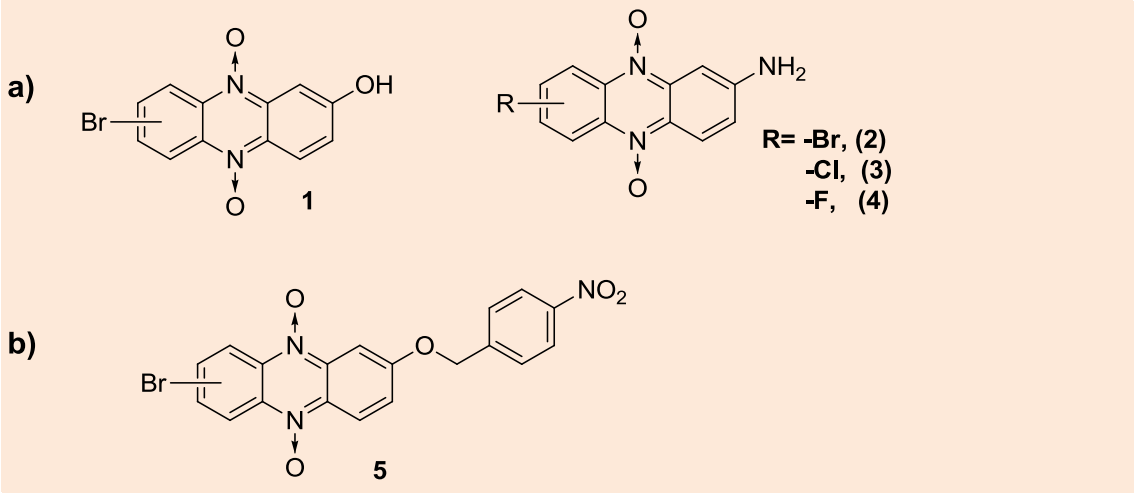
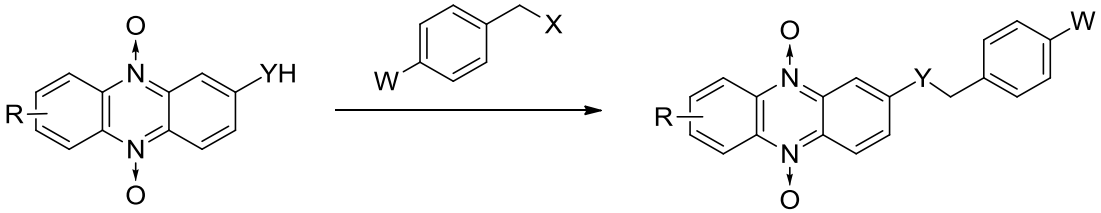


Figure 1. a) Examples of phenazine 5,10-dioxides with bioreductive antitumour properties. **b)** Phenazine 5,10-dioxide with improved DNA-stacking properties

To develop phenazine 5,10-dioxides with increased DNA-stacking properties, like **5** (Figure 1b), we initially[12] used conventional conditions by heating the phenol or amine intermediate in acetonitrile in presence of potassium carbonate as base. In these reaction conditions the yields were extremely low and reaction times were over 120 h (Figure 2a), resulting in decomposition of reactants or formation of several secondary products, mainly de-oxygenated phenazines without alkylation. The absence of reactivity of the amino-derivative **3** (Figure 1a) conducted us to study the reaction with more electrophilic reactant, i.e. sulphonyl chloride derivative, finding generation of the desired product, however, also with poor yield (product **8**, Figure 2b).[13]

a)



Product[a]	R-	-Y-	-W / -X	Reaction conditions	Yield (%)
5	Br-	-O-	$-NO_2$ / -Br	K_2CO_3, CH_3CN , 40 °C, 120 h	5
6	Br-	-O-	-Cl / -Cl	K_2CO_3, CH_3CN , 18-*c*-6[b], 40 °C, 120 h	2
7	Cl-	-NH-	-Cl / -Cl	K_2CO_3, CH_3CN , 18-*c*-6, 40 °C, 120 h	No reaction

[a] The products are obtained as a inseparable mixture of 7(8) isomers. [b] 18-*c*-6: crown ether 18-crown-6.

b)

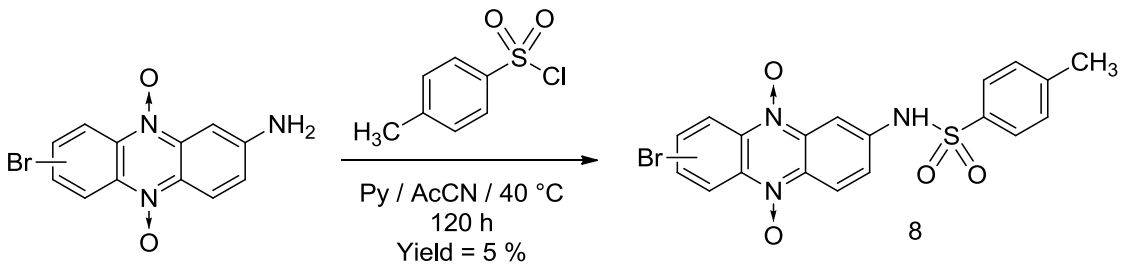


Figure 2. Reaction conditions and results: a) for the procedures used to obtain the benzylamino and benzyloxyphenazine 5,10-dioxides under conventional conditions; b) for the preparation of sulphonamido derivative **8**

The synthesis assisted by microwave irradiations has been previously described as a condition that improves yields and diminishes reaction times.[14,15] For example, the use of microwave irradiations has been used in Williamson etherification where reaction times were reduced from hours to minutes, increasing reaction yields and lowering secondary products generation.[16]

In this sense, microwave assisted synthesis has been explored in the preparation of the phenazine 5,10-dioxides *N*- or *O*-benzylated and sulphonilated compounds trying to increase the reaction rates and to improve yields. Herein, we describe the results of this approach and a theoretical study that could explain the low reactivity of these phenazines.

2. Experimental

2.1. General methods

Products were purified by chromatography column (SiO_2, 230–400 mesh; eluted with mixtures of EtOAC:petroleum ether). The purification and progresses of the reactions were checked by TLC (silica gel 60 F254 layers, EtOAc:petroleum ether, 3:7), visualized under UV light (λ = 254 nm), by exposing to iodine vapor, or by spraying with *p*-anisaldehyde:sulphuric acid reagent and heating at ca. 120 °C. The identities of products were determined using NMR and MS spectra. NMR spectra were acquired at 400 MHz using a Bruker DPX400 instrument with the analyte dissolved in DMSO-d6. Mass spectra were performed on a SHIMADZU GC-MS QP 1100 EX.

2.2. Chemicals

General procedure for the synthesis of phenazine 5,10-dioxides **5**, **6** and **7**. To a solution of **1** (as a mixture of 7 and 8 isomer) or **3** (as a mixture of 7 and 8 isomer), (1 eq.) in acetonitrile (25 $mL.mol^{-1}$), the corresponding benzyl halide (1 eq.), K_2CO_3 (1 eq.), 8-crown-6 ether (1 eq.) and tetrabutylammoniumiodide [$(C_4H_9)_4NI$] (0.1 eq.) were added. The reaction mixture was heated at 120 °C using microwave energy, the potency, pressure and reaction time are indicated in Table 1. After that the crude reaction mixture was partitioned between EtOAc and aqueous HCl (10%). After the work up the organic layer was evaporated *in vacuo* and the residue was purified by column chromatography (SiO_2, petroleum ether : EtOAc (4 : 6)). **5**, **6** and **7** were obtained as an inseparable mixture of 7 (8) isomers.[4-13]

Synthesis of 7(8)-Bromo-2-(4-methylphenylsulfonylamino)phenazine-5,10-

dioxide (**8**). A mixture of **2** (0.16 mmol) and *p*-toluenesulfonyl chloride (0.16 mmol) in acetonitrile (3 mL) was heated at 120 °C using microwave energy at 700 watts of potency and 8 atm of pressure during 20 min. Then the crude reaction mixture was purified by column chromatography (SiO_2, petroleum ether : EtOAc (3 : 7)). **8** was obtained as an inseparable mixture of isomers.[4-13]

2.3. Molecular modeling

Molecular modeling studies were performed using SPARTAN PRO 04 by calculating steroelectronic properties of compounds. These molecular properties were determined using Hartree-Fock level. The geometry of each compound, 7 position isomer was used in all cases, was fully optimized by applying MMFF in gas phase in order to obtain acute results with low time of computational calculi. Then equilibrium geometry energies were determined using STO-3G basis.

3. Results and discussion

3.1. Synthesis of *N*- and *O*-benzylphenazine 5,10-dioxide derivatives

Different phenazine 5,10-dioxides, 7(8)-substituted, were reacted, assisted by microwave irradiation, with *p*-nitrobenzyl bromide and *p*-chlorobenzyl chloride in order to evaluate the effect of the different substituents in positions 7 or 8 of the phenazine moiety on the reactivity of nucleophile atoms. Firstly, the best microwave irradiation conditions were studied using the preparation of product **5** as model. This product was generated when crown ether 18-crown-6 and tetrabutylammonium iodide phase transfer catalysts were used in order to improve the nucleophilic ability of the phenol moiety (Table 1). Clearly, the best nucleophile, anion phenoxide, was formed in presence of the base (i.e. K_2CO_3) and was markedly reactive by the action of the crown ether and the phase transfer catalyst. In this condition the yield was duplicated and the time of reaction was reduced more than 300-fold.

Similarly, the optimum conditions for the generation of the benzyloxy derivative **6** (Table 1) involved the use of the crown ether and tetrabutylammoniumiodide as phase transfer catalysts, but in this case higher pressure and potency of the microwave oven were necessary. In this case, the yield increased 5 times (compare figure 1a with table 1, 2% versus 11%) and the time decreased over than 300 times.

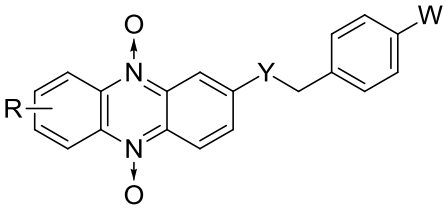
The amino **7**, which was not generated in conventional conditions (Figure 2a), was obtained in the same conditions that those used for prepare the benzyloxy-analogue **6**. Potassium carbonate might not be basic enough to produce the deprotonation of 2-amino-7(8)-chlorophenazine 5,10-dioxide but this was used in accordance with recently described procedures[17].

In absence of phase transfer catalysts, only secondary products were generated, which pointed the need of use of the base combined with such catalysts to obtain the amino derivative **7**.

Using the 7(8)-methoxy-2-hydroxyphenazine 5,10-dioxide (12), 7(8)-methyl (13), and 7(8)-fluoro-2-aminophenazine 5,10-dioxides (14) as starting materials, we were unable to obtain the desired products (9-11). Particularly, the 7(8)-methoxy derivative (12) produced a mixture of secondary products not identified, and the 7(8)-methyl derivative (13) generated in the majority of the conditions the corresponding phenazine, i.e. the deoxygenated product. These results clearly showed an important lack of nucleophilic capacity of the amino moiety (see expected products **7**, **10**, and **11**, Table 1). It was also noticed that electron donor moieties did not improve the reactivity of the phenazine heterocycle as evidenced on the lack of

reaction shown on the attempts to obtain **9**, **10**, and **11** (Table 1).

Table 1. Selected reaction conditions to obtain benzyloxy and benzylaminophenazine 5,10-dioxide derivatives under microwave irradiation conditions. For compounds **5-7** the best reactions conditions are highlighted with bold letters



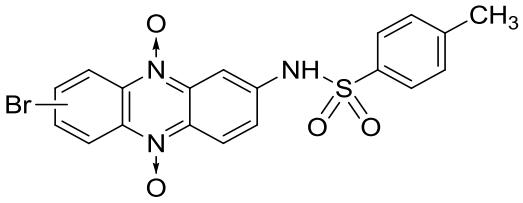
Product	R-	-Y-	-W	Reaction conditions							Yield (%)
				s[a]	b[b]	c[c]	P[d]	T[e]	p[f]	t[g]	
5	Br-	-O-	$-NO_2$	-	-	-	5	50	300	40	N.R.[h]
				CH_3CN	-	-	5	50	300	22	N.R.
				CH_3CN	-	-	5	50	300	22	N.R.
				CH_3CN	K_2CO_3	-	5	50	300	30	N.R.
				CH_3CN	**K_2CO_3**	**18-*c*-6[i] $(C_4H_9)_4NI$**	**5**	**120**	**300**	**22**	10[j]
6	Br-	-O-	-Cl	CH_3CN	K_2CO_3	-	5	50	300	20	N.R.
				CH_3CN	**K_2CO_3**	**18-*c*-6 $(C_4H_9)_4NI$**	**8**	**120**	**700**	**20**	11[j]
7	Cl-	-NH-	-Cl	CH_3CN	K_2CO_3	-	5	50	300	20	S.P.[k]
				CH_3CN	**K_2CO_3**	**18-*c*-6[i] $(C_4H_9)_4NI$**	**8**	**120**	**700**	**20**	7[j]
9	CH_3O-	-O-	$-NO_2$	-	K_2CO_3	18-*c*-6 $(C_4H_9)_4NI$	5	120	700	30	S.P.
10	CH_3-	-NH-	$-NO_2$	CH_3CN	K_2CO_3	-	5	50	200	60	N.R.
				CH_3CN	K_2CO_3	18-*c*-6	5	50	200	15	Deox.[l]
				CH_3CN	K_2CO_3	$(C_4H_9)_4NI$	5	50	200	15	Deox.
				-	-	$(C_4H_9)_4NI$	1	120	1000	25	Deox.
11	F-	-NH-	$-NO_2$	CH_3CN	K_2CO_3	18-*c*-6 $(C_4H_9)_4NI$	5	120	700	30	N.R.

[a] s: solvent; [b] b: base; [c] c: catalyst; [d] P: pressure in atmosphere; [e] T: temperature in °C; [f] p: potency in Watt; [g] t: time in minute; [h] N.R.: not reaction; [i] 18-*c*-6: crown ether 18-crown-6; $(C_4H_9)_4NI$: phase transfer catalyst. [j] The leftover of material corresponded to unreacted starting material; [k] S.P.: secondary products; [l] Deox.: products of deoxygenation.

3.2. Synthesis of sulfonamide derivative 8

The synthesis assisted by microwave irradiations was also assayed in the preparation of sulfonamide **8** (Figure 2b). This was obtained under microwave irradiation, in presence of potassium carbonate as base and 18-*c*-6 crown ether, with better yields and in lower reaction time than in conventional heating conditions (Table 2).

Table 2. Comparison of conventional and microwave irradiation conditions assayed to obtain sulfonamide derivative **8**



Reaction conditions	Yield (%)
Pyridine, CH_3CN, 40 °C, 120 h	5
MW, CH_3CN, K_2CO_3, 18-*c*-6[a], 120 °C, 8 atm, 20 min	15[b]

[a] 18-*c*-6: crown ether 18-crown-6; [b] The leftover of material corresponded to unreacted starting material.

3.3. Theoretical studies

Given the different reactivity of the phenazine 5,10-dioxide derivatives, theoretical studies were performed on the phenazines and the corresponding electrophiles, *p*-nitrobenzyl bromide, *p*-chlorobenzyl chloride, and *p*-toluenesulphonyl chloride. For the phenazine 5,10-dioxides we paid attention on the energies of the entities, the energies of the HOMO and the Mulliken charges on oxygen or nitrogen atom, for both the neutral and anionic forms. For the electrophiles, we looked the corresponding energies of the LUMO.

For the bromophenazine **1** (Figure 1a) which produces compound **5**, we could observe that the phenoxide is the most stable form in the equilibrium and also the most nucleophilic, featuring the most negative oxygen (Figure 3a). Additionally, the process presents an adequate GAP (E_{HOMO} of the nucleophile – E_{LUMO} of the electrophile). However, the same analysis for phenazine **3** (Figure 1a) to obtain compound **7** showed different result. The amide ion of phenazine **3** is less stable and nucleophilic than the neutral form (Figure 3b) but its HOMO energy is closer to electrophile E_{LUMO} than those the neutral form, i.e. GAP = 5.69 eV *versus* GAP = 10.57 eV.

Clearly, these theoretical approaches could explain the better results in the synthesis of phenazine **5** in basic milieu, where the nucleophilicity of the compound increases greatly in the most stable form, i.e. phenoxide form, and the reactants have adequate energies of frontier orbitals to allow the process. In the case of phenazine **3** the basic milieu, and the concomitant deprotonation, improves the E_{HOMO} of the reactant, however this intermediate is less stable and less nucleophilic. It could hypothesize that product **7** was obtained only assisted by microwave energy because it allows the displacement of the equilibrium to the amide.

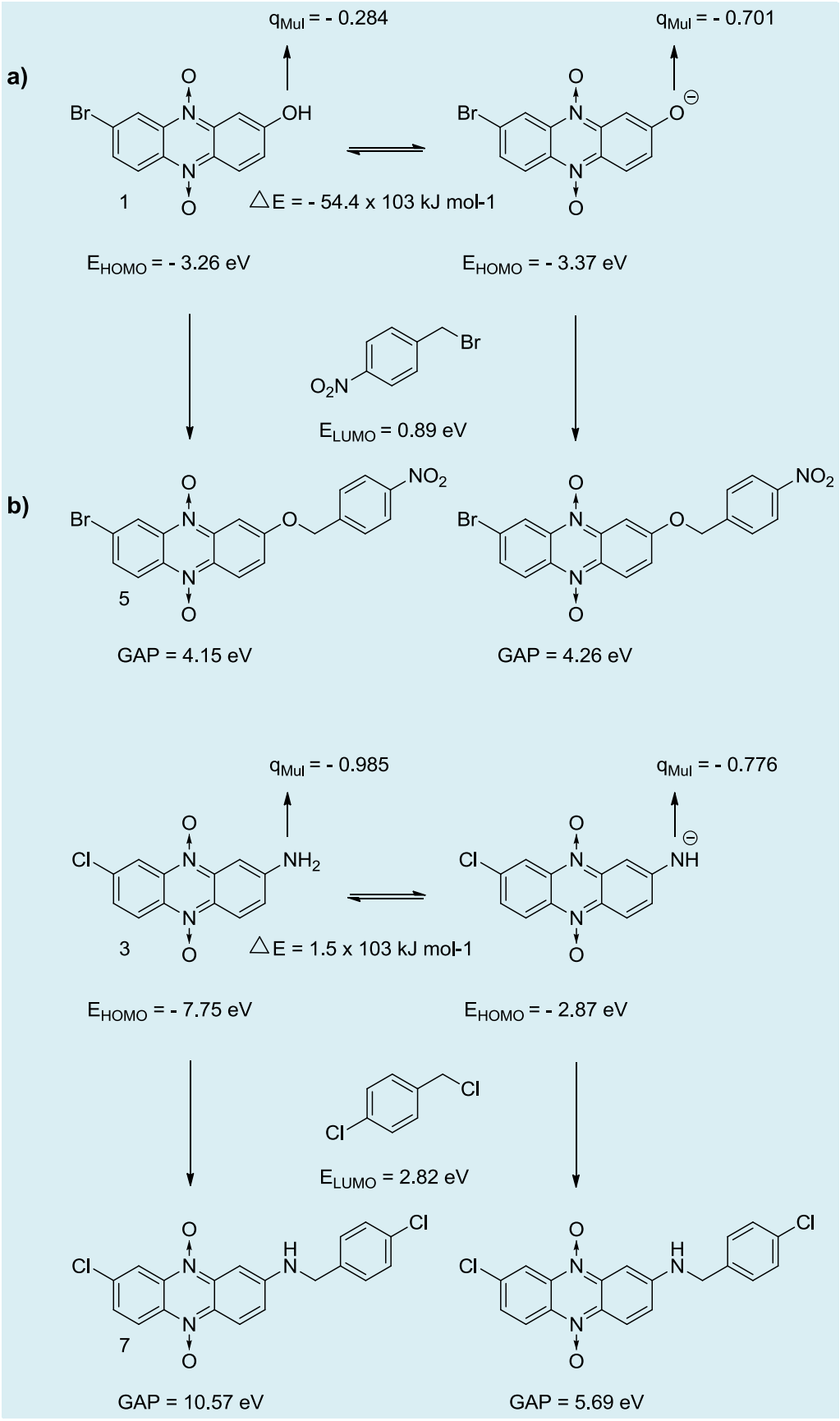
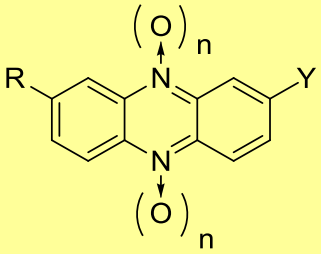


Figure 3. Theoretical studies performed on the processes to obtain products **5** and **7**

The amino and phenol low reactivity as nucleophiles, and thus the generation of undesirable products from deoxygenation and other secondary products, could be explained by the presence of the *N*-oxide moieties in the phenazine nucleus with their capability as mesomeric electron withdrawing groups.[18] To confirm this phenomenon, we studied for parent compounds **1** and **3**, the corresponding anionic forms, and the corresponding reduced phenazines, the theoretical properties that illustrate the reactivity, E_{HOMO}, and nucleophilicity, Mulliken charge on the reacting atoms (Table 3).

For compound **1**, the absence of *N*-oxide moieties increases slightly the negative charge on the phenol oxygen atom but decreases the ability to react with the electrophiles, i.e. the E_{HOMO} decreases. However, according to previous conclusion being the most probable reacting form the phenoxide (Figure 3), the reactivity and nucleophilicity of the anion increases when the *N*-oxides are not present (compare **1 anion** versus **1 anion-reduced**, Table 3). Graphically, it could be also visualized, analyzing the merged maps of electronic density and HOMO frontiers orbitals, that the phenoxide-oxygen negative charge is more delocalized in the dioxide form than in the reduced one (Figure 4) being the values of the merged properties on these atoms in agreement. In the case of compound **3** and the corresponding amide (**3 anion**, Table 3) the absence of *N*-oxide groups clearly becomes more negative the amino nitrogens and more positive the E_{HOMO}, in both forms. Once more, the mesomeric electron withdrawing effect of the *N*-oxide moieties could be explaining the cause of the low reactivity as nucleophiles via the amino group of this reactant.

Table 3. Comparison of theoretical properties for parent compounds **1** and **3** and the corresponding reduced phenazines



derivative	-R	-Y	n	E_{HOMO} (eV)	$q_{Mulliken}$
1	-Br	-OH	1	-3.26	-0.284
1-reduced	-Br	-OH	0	-5.67	-0.285
1 anion	-Br	$-O^-$	1	-3.37	-0.701
1 anion-reduced	-Br	$-O^-$	0	-2.77	-0.741
3	-Cl	$-NH_2$	1	-7.75	-0.985
3-reduced	-Cl	$-NH_2$	0	-7.69	-0.986
3 anion	-Cl	$-NH^-$	1	-2.87	-0.776
3 anion-reduced	-Cl	$-NH^-$	0	-2.19	-0.812

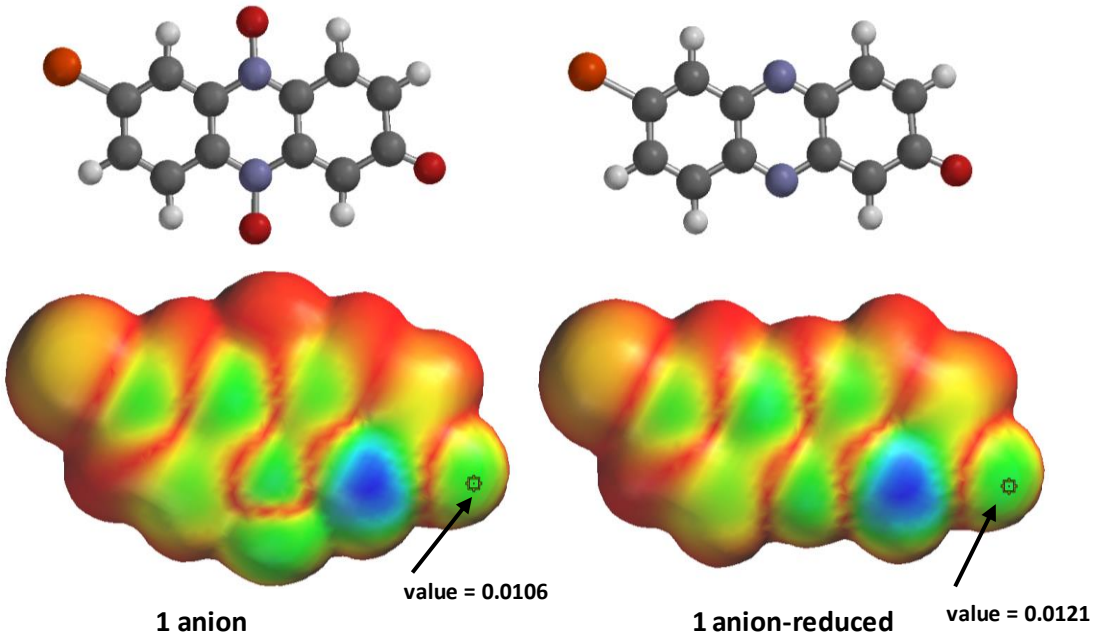


Figure 4. HOMO frontiers orbitals merged to electronic density (isovalue: 0.002) for the anionic for of the hydroxyphenazine 5,10-dioxide **1** (**1 anion**) and the corresponding hydroxyphenazine analogue (**1 anion reduced**).

4. Conclusions

The yields and the reaction times of *N*- and *O*-reaction were successfully improved when using assistance of microwave irradiation. Reaction conditions were optimized in order to achieve best yields and improve those obtained using conventional conditions. The results with 2-hydroxyphenazine 5,10-dioxides were better in basic milieu, in presence of crown ether and tetrabutylammoniumiodide as phase transfer catalysts possible as a result of improving the nucleophilicity and reactivity, according to the results of the theoretical studies. The lack of reactivity via the hydroxy or amino moieties could be the result of the electron-withdrawing effects of the *N*-oxides moieties present in the studied molecules.

Acknowledgments

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