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Artigo

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Reação de Beirut e sua Aplicação na Síntese de Compostos Quinoxalina-*N,N'*-Dióxidos Bioativos

Resumo: Descrita por Haddadin and Issidorides em 1965, a cicloadição entre benzofuroxano (*i.e.* óxido de benzofurazano ou 2,1,3-benzoxadiazola-1-óxido) com enaminas, cetonas α,β-insaturadas, 1,3-dinitrilas ou enolatos, produzindo quinoxalina-*N,N'*-dióxidos, é conhecida na literatura como reação de Beirut. A possibilidade de se obter, em etapa única, heterociclos nitrogenados aromáticos oxidados, contendo múltiplo e distinto perfil de funcionalização, tornou a reação de Beirut opção versátil para a síntese de compostos com diferentes aplicações industriais, a exemplo do carbadox, cyadox e olaquindox, disponíveis como antibióticos utilizados como promotores do crescimento em produtos de origem animal, permitindo a identificação do sistema quinoxalina-*N,N'*-dióxidos como importante unidade estrutural para a obtenção de compostos bioativos.

Palavras-chave: Reação de Beirut; quinoxalina; fenazina; benzimidazola; dióxido; compostos bioativos.

Abstracts

Described by Haddadin and Issidorides in 1965, the cycloaddition between benzofuroxan (*i.e.* benzofurazan oxide or 2,1,3-benzoxadiazole-1-oxide) with enamines, α,β-unsaturated ketones, 1,3-dinitriles, or enolates to produce quinoxaline-*N*-,N'-dioxides is referred in chemical literature as the Beirut reaction. The feasibility to synthesize, in a single step, heterocyclic aromatic amine oxides with different levels of functionalization makes the Beirut reaction a central tool for synthesize compounds with several industrial applications, like carbadox, cyadox and olaquindox, that became available as antimicrobial growth promoters in food animal production, allowing the identification of quinoxaline-*N,N'*-dioxides system as an important scaffold for obtaining new bioactive compounds.

Keywords: Beirut reaction; quinoxaline; phenazine; benzimidazole; dioxide; bioactive compounds.

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Beirut Reaction and its Application in the Synthesis of Quinoxaline-*N,N'*-Dioxides Bioactive Compounds

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1. Definition, application and mechanism

Described by Haddadin and Issidorides in 1965 (Figure 1), working at the American University of Beirut, Lebanon, the cycloaddition between benzofuroxan (BFO, *i.e.* benzofurazan oxide or 2,1,3-benzoxadiazole-1-oxide; **1**) with enamines, α,β-unsaturated ketones, 1,3-dinitriles, or enolates to produce quinoxaline-*N,N'*-dioxides is referred in chemical literature as the Beirut reaction, in honor of the city in which it was discovered (Scheme 1).[1-3]

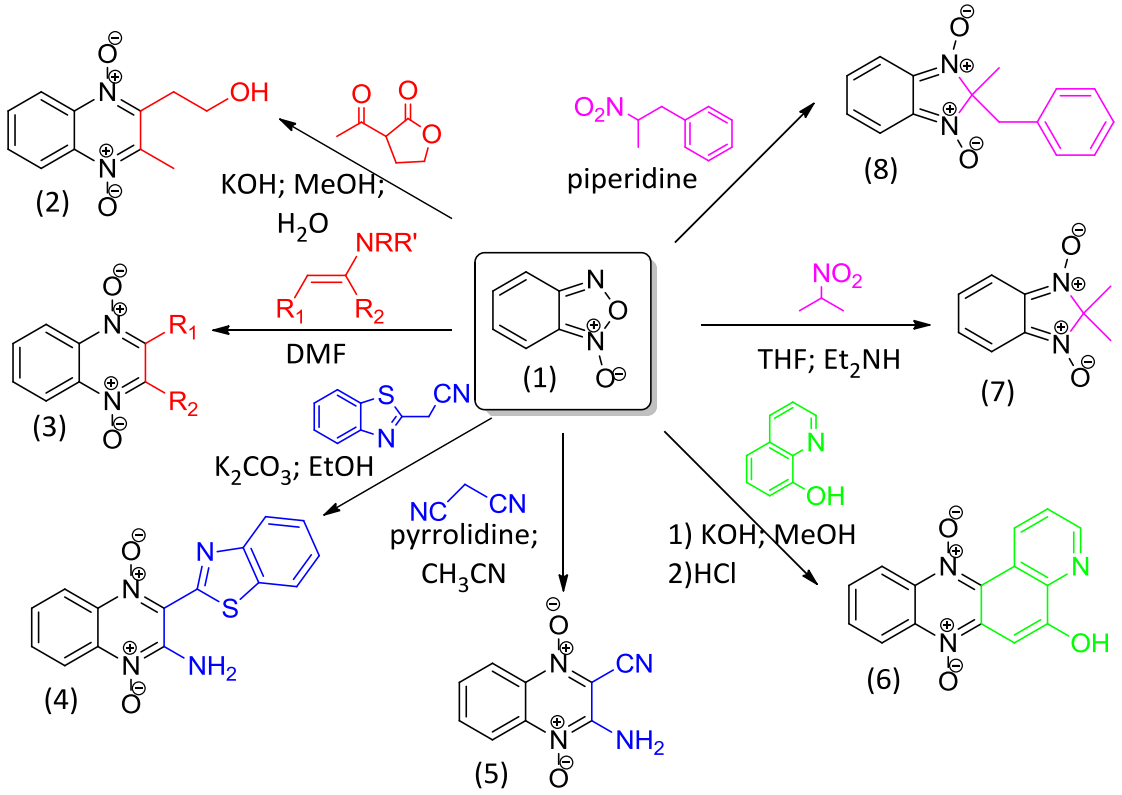
Issidorides, C
1920-2010

Haddadin, M J

Figure 1. Pictures of the inventor and co-inventor of the Beirut Reaction

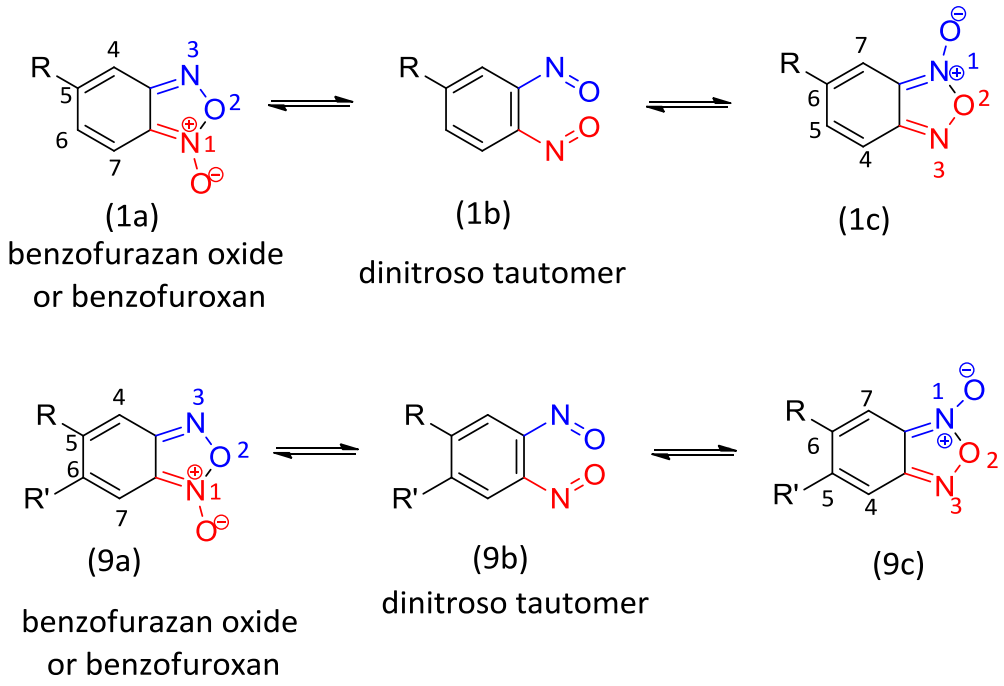
The Beirut reaction also apply to the synthesis of phenazine-*N,N'*-dioxides and benzimidazole-1,3-dioxides. In fact, phenolic enolates from phenol, resorcinol, hydroquinone or benzoquinone undergo similar reaction with benzofuroxan (**1)** to give phenazine-*N,N'*-dioxide derivatives, while carbanions from nitro-alkyl derivatives reacts with BFO to afford benzimidazole-1,3-dioxides (Scheme 1).[4]

Classically, ketones, β-diketones, β-ketoesters, β-ketonitriles, β-ketoacids and β-ketoamides are the common sources of enolates for the Beirut reaction, which is performed using a base as catalyst in the presence of protic or aprotic solvents.



Scheme 1. Examples of quinoxaline-1,4-dioxides, phenazine-5,10-dioxide and benzimidazole-1,3-dioxide obtained from Beirut reaction[1-5]

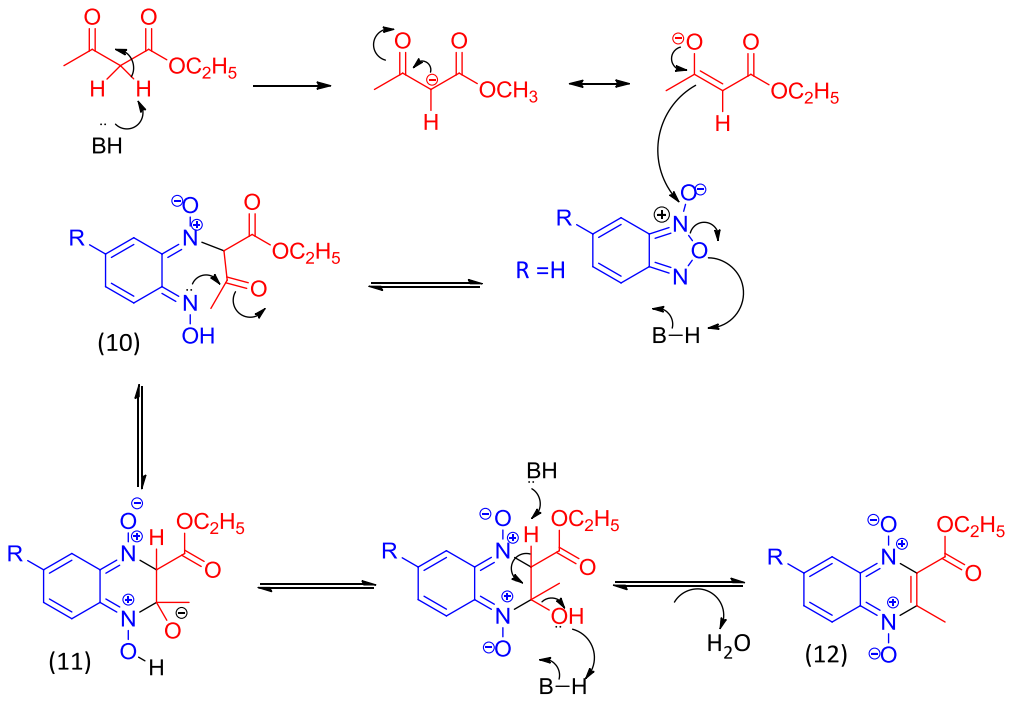
The Beirut reaction using an unsymmetrical monosubstituted or a disubstituted benzofuroxan, carrying different groups at positions 5 and 6, could in thesis, affords two regioisomers, resulting in a mixture of quinoxaline 1,4-dioxides or phenazines *N,N'*-dioxides or benzimidazole-1,3-dioxides.[5] This fact can be explained by the tautomeric relationship observed for benzofuroxan system that exists as a mixture of two distinct isomers, as depicted in Scheme 2. The stability of tautomers (**1a/9a** and **1c/9c**) depends on the nature of the substituent at positions 5 and 6, in other words if the substituent is an electron releasing or electron withdrawing group. Generally, the presence of an electron withdrawing group favors the 6-tautomer over the 5-tautomer, while the opposite occurs with an electron releasing group.[6-8]



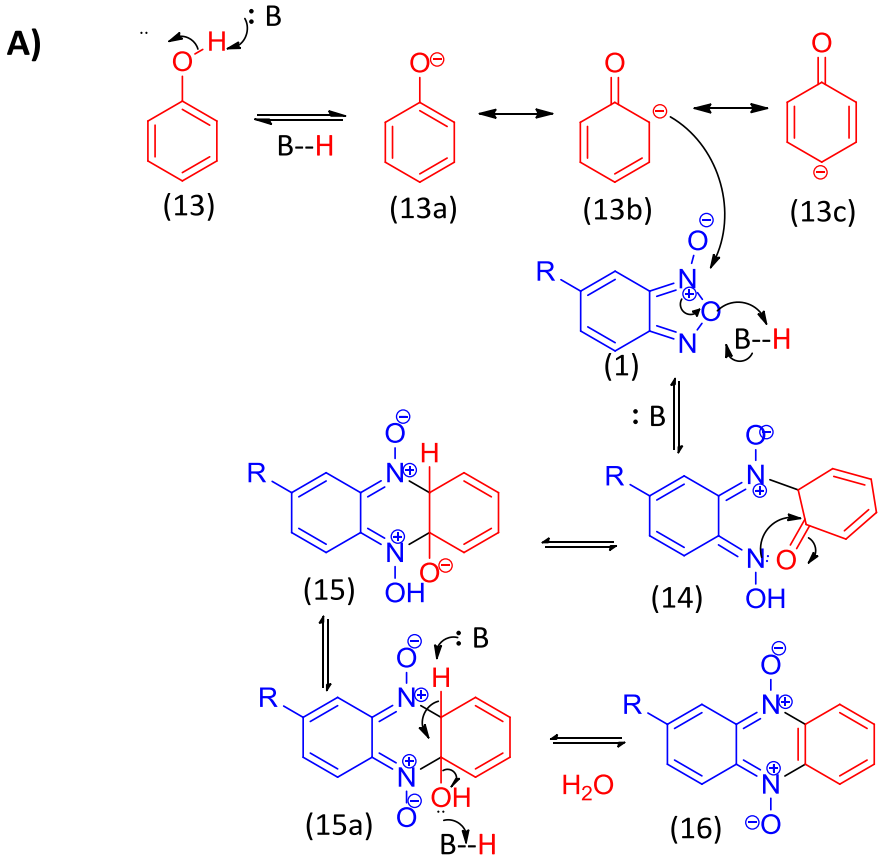
Scheme 2. Mono- and disubstituted benzofuroxan and their tautomers

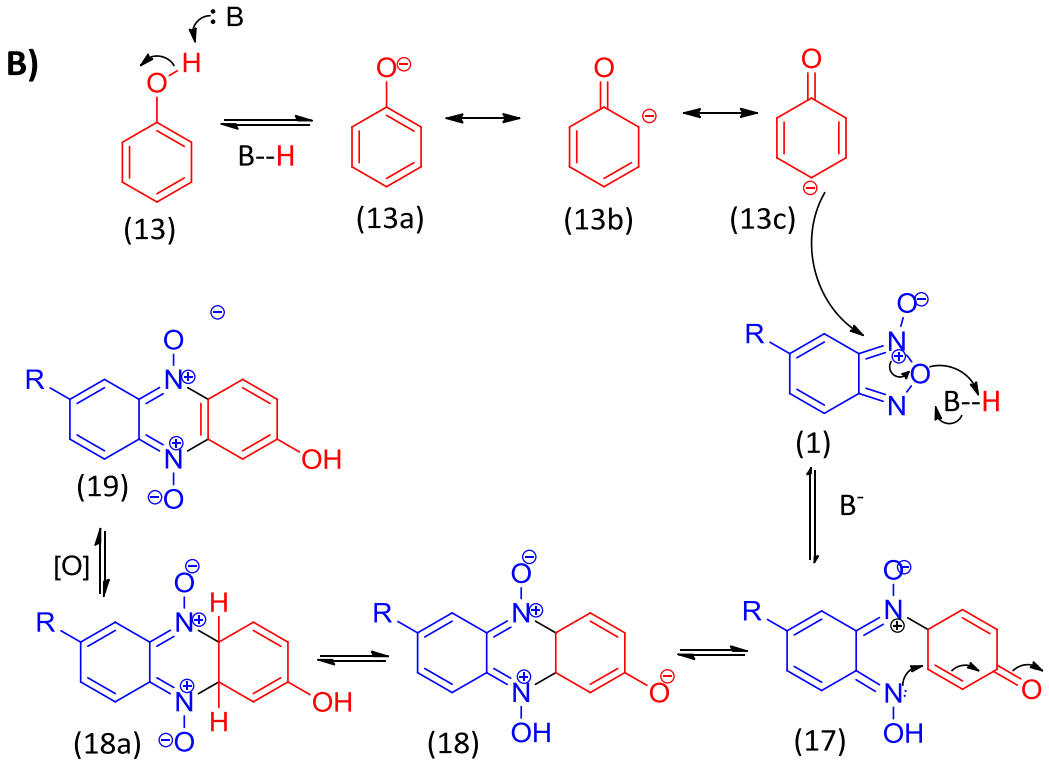
Some proposed mechanisms of the Beirut reaction were reported.[9-12] The general accepted mechanism includes, in the first step, the nucleophilic addition of an enolate ion to the electrophilic nitrogen atom of benzofuroxan (BFO) to form the intermediate **10**. It is not clear which of the electrophilic nitrogens of BFO is the site of nucleophilic attack or even if the reactive species is the dinitroso tautomer (**1b**). However, it´s believed that when R≠H (*i.e.* monosubstituted benzofuroxan) the nucleophilic addition step determine the regioisomer to be formed. Ring closure occurs via condensation of the imino-oxide onto the carbonyl ketone to give dihydroquinoxaline **11**, followed by the β-elimination of water and formation of the quinoxaline-*N,N'*-dioxide system (**12**) (Scheme 3).

General mechanism for synthesis of phenazines *N,N'*-dioxides from the Beirut reaction have also been proposed (Scheme 4). The first step embraces the acid-base reaction with formation of a phenolate anion that is stabilized by resonance. As a consequence two carbanions (*ortho* and *para*) are formed and may react with benzofuroxan (**1**) affording the phenazines-*N,N'*-dioxides **16** and **19** (Scheme 4), which products ratios will depend on steric and electronic factors. As described for the synthesis of quinoxaline-1,4-dioxides, a nucleophilic addition to the electrophilic nitrogen atom of benzofuroxan (BFO) occurs, followed by the ring closure via the nucleophilic attack of the imino-oxide onto the carbonyl ketone to give **14**, trailed by elimination of water to restore aromaticity yielding product **16.** From intermediate **17** a Michael-like addition of the imino-oxide onto the α,β-unsaturated carbonyl takes place to give **18**, that is oxidized to furnish the phenazines *N,N'*-dioxide **19**.[6]



Scheme 3. Proposed mechanism for formation of quinoxaline-*N,N'*-dioxide derivatives by Beirut reaction

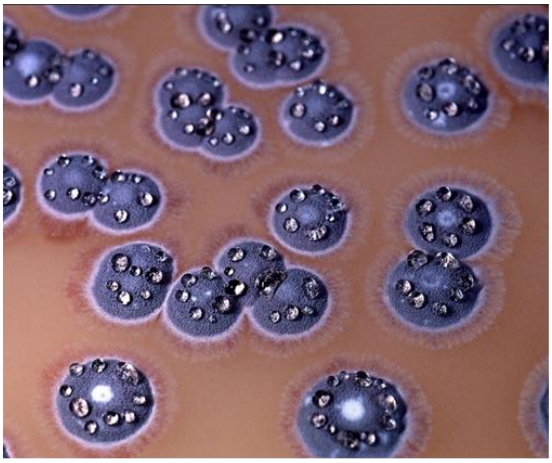




Scheme 4. General proposed mechanism for synthesis of phenazine-5,10-dioxides from the Beirut reaction. **A**) condensation of BFO (**1**) with *ortho* enolate (**13b**); **B**) condensation of BFO (**1**) with *para* enolate (**13c**)

The feasibility to synthesize, in a single step, heterocyclic aromatic amine oxides with different levels of functionalization makes the Beirut reaction an important tool for obtaining compounds with several industrial applications. Among them, the heterocyclic systems prepared from Beirut reaction are of particular interest in the pharmaceutical industry. Numerous quinoxaline-1,4-dioxides, phenazines *N,N'*-dioxides and some benzimidazole-1,3-dioxides were described as anti-tumor, antiparasitic , antimicrobial, and so on.[13-17] The 6-chloro-2-quinoxalinecarboxylic acid 1,4-dioxide (**20**) and the 1,6-phenazinediol-5,10-dioxide (**21**) are examples of naturally occurring heterocyclic aromatic amine oxides, being the first produced from cultures of *Streptomyces ambofaciens* and the later isolated from *Chromobacterium iodinium* and *Pseudomonas iodinum* (Figura 2).[18-19] Besides its bactericidal activity, the clinical use of phenazine-5,10-dioxide (21) was limited by its high toxicity, suggesting the toxicophoric profile of this heterocyclic system, commercially used as a pigment referred as iodinin.[20,-22]

Among the heterocyclic aromatic amine oxides synthetized by the Beirut reaction the quinoxaline-*N,N'*-dioxide is the most important one. For this reason, increased emphasis will be given to illustrate the application of the Beirut reaction in the synthesis of quinoxaline-*N,N'*-dioxides derivatives.



Streptomyces ambofaciens

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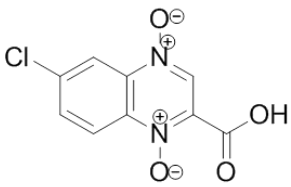
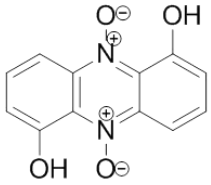


(20)

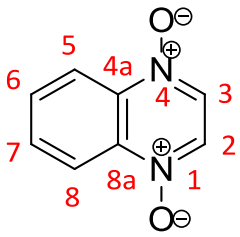


(21)

Figure 2. Examples of heterocyclic aromatic amine oxides from natural sources

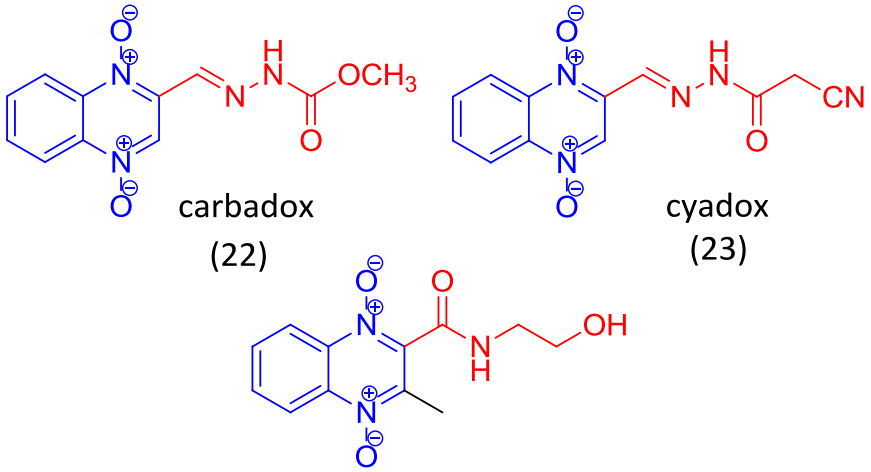
2. Quinoxaline-*N,N'*-dioxide derivatives

The numbering system used for the quinoxaline-*N,N'*-dioxide or quinoxaline 1,4-dioxide nucleus proceeds anticlockwise starting from nitrogen (Scheme 5). Despite the presence of a nominal positive and negative charge in all resonance contributors to such system, it is overall neutral, behaving like an organic substance, soluble in usual organic solvents and considered a fused mesoionic heterocyclic.



Scheme 5. Numbering system used for the quinoxaline-*N,N'*-dioxides ring

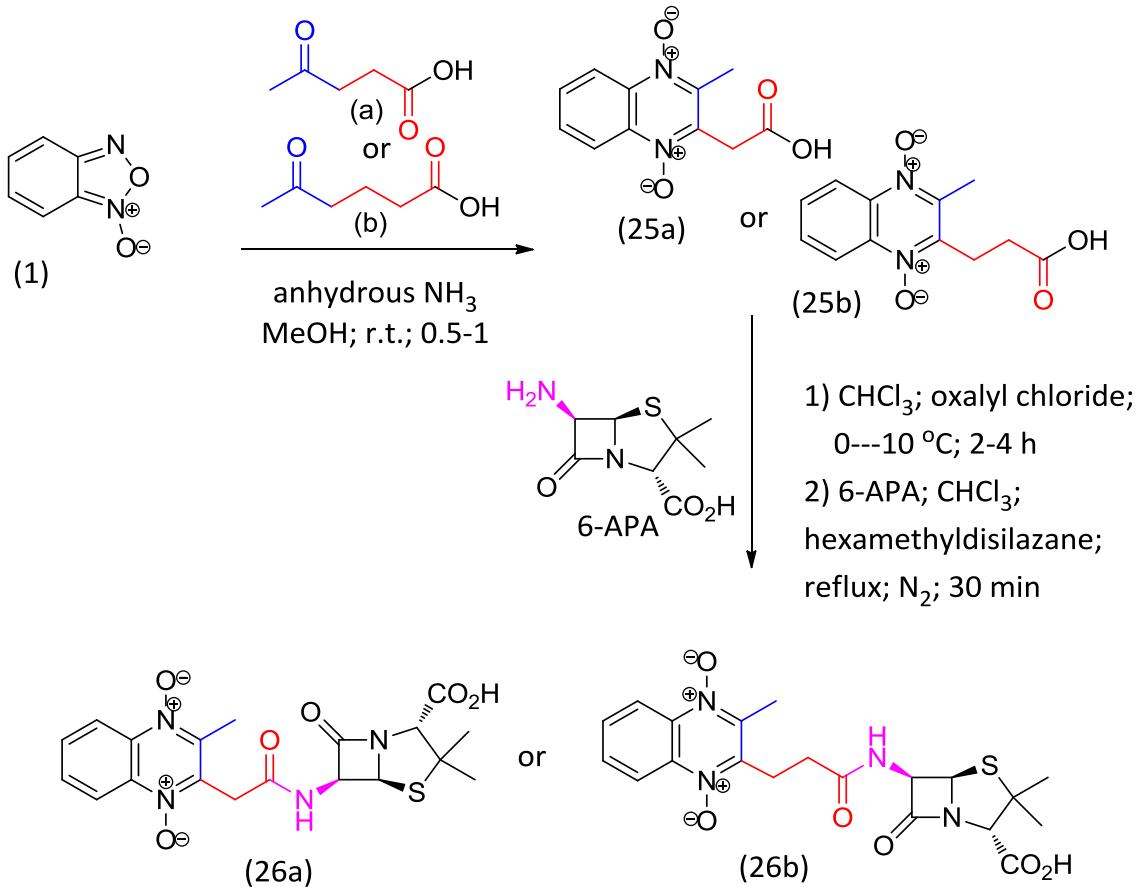
The comprehension of the central role of quinoxaline-*N,N'*-dioxide system as a scaffold for obtaining new bioactive compounds has its milestone in the 1970s, when three quinoxaline 1,4-dioxides derivatives (carbadox, cyadox and olaquindox; **22-24**) became available as antimicrobial growth promoters in food animal production.[23,24]



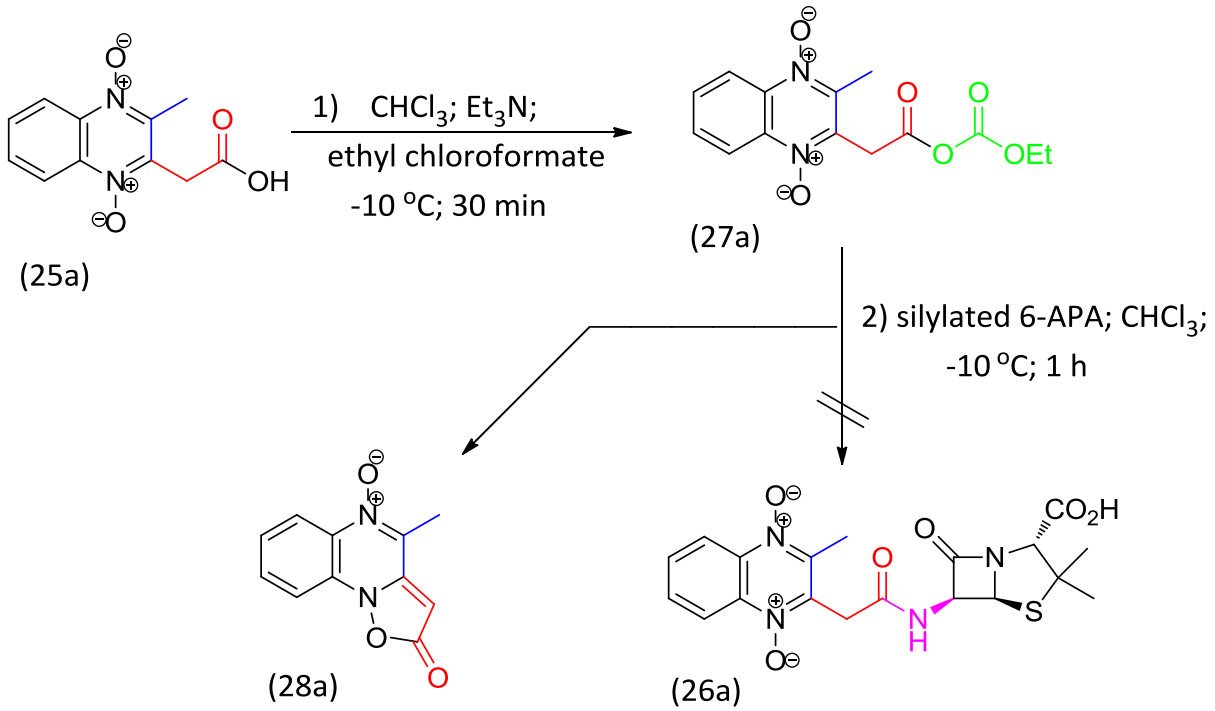
Scheme 6. Antimicrobial quinoxaline-*N,N'*-dioxides with ultraviolet phototoxicity

The occupational exposure of farmworkers to this class of antimicrobials resulted in dermal photosensitivity reactions, revealing the ultraviolet phototoxicity of quinoxaline 1,4-dioxides nucleus.[23] Notwithstanding, several studies have been reporting the quinoxaline-*N,N'*-dioxides scaffold for the discovery of new antimicrobial agents. In 1976, Edwards and coworkers described their attempt to discovery novel β-lactam antibiotics containing the quinoxaline-*N,N'*-dioxide scaffold. These derivatives were synthetized in two steps based on the Beirut reaction and on the condensation from acid chloride intermediate with 6-aminopenicillanic acid (6-APA) (Scheme 7).[24] The quinoxaline-*N,N'*-dioxide carboxylic acids (**25A** and **25B**) were prepared from a mixture of benzofuroxan, methanol and ketones (**A** or **B**, Scheme 7) in the presence of anhydrous NH_3. The construction of C-N bond (CONH) was performed from the interconversion of compounds **25A** and **25B** in the corresponding acid chloride intermediates using oxalyl chloride and then the heated at reflux in the presence of a suspension of 6-APA and hexamethyldisilazane in chloroform.[25]

The attempt to synthetize compounds **26A** and **26B** exploring mixed anhydrides of the quinoxaline-*N,N'*-dioxide carboxylic acids, like compound **27A** (Scheme 8), failed to result in the amide **26A**. In this condition, the 4-methylisoxazolo[2,3-*a*]quinoxalin-2-(2*H*)-one 5-oxide (**28A**) was the only isolable product. [25]



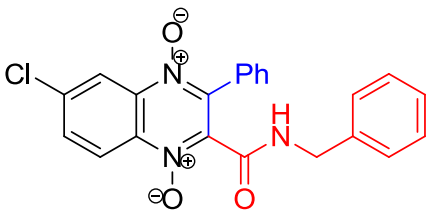
Scheme 7. Synthesis of quinoxaline di-*N*-oxides penicillins **26a** and **26b**



Scheme 8. Synthesis of 4-methylisoxazolo[2,3-*a*]quinoxalin-2-(2*H*)-one 5-oxide (**28a**) from the mixed anhydride **27a**

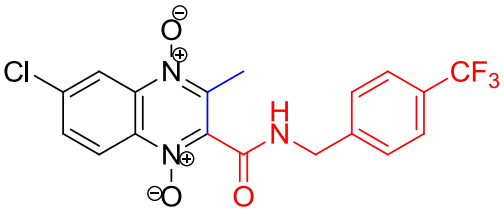
In vitro evaluation against penicillinase-producing *Staphylococcus aureus* revealed very weak bactericidal activity for compounds **26A** and **26B**, with MIC = 100 μg/mL, and inactivity against *Salmonella schottmuelleri.*[25]

More recently, Moreno and coworkers described the synthesis of forty-three quinoxaline-*N,N'*-dioxide carboxamides and their antimicrobial activity *against Mycobacterium tuberculosis*. The compounds showing values of ≤ 10μg/mL were considered active and therefore selected to the secondary screening assay aiming to determine IC_{90}. The CC_{50} was established from VERO cells and the ratio between the IC_{90} and CC_{50} values were used to determine the Selectivity Index (SI). Compounds **29** and **30** were identified as the most interesting with SI higher than 10 (Scheme 9).[26]



(29)

IC_{90}= 3.39 mg/mL;
CC_{50}= > 40 mg/mL;
SI > 11.79



(30)

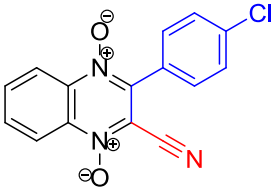
IC_{90}= 3.38 mg/mL;
CC_{50}= > 40 mg/mL;
SI > 11.82

Scheme 9. Examples of antimicrobial quinoxaline-*N,N'*-dioxides derivatives

In the search for new antimalarial agents, several 2-carbonitrile-3-quinoxaline-1,4-dioxide derivatives were synthesized and evaluated against chloroquine-resistant (K1) and chloroquine-sensitive (3d7) strains of *Plasmodium falciparum*. The cytotoxicity to mammalian cells was also investigated using KB cells. The most interesting results were obtained from evaluating the quinoxaline-*N,N'*-dioxides compounds against K1 strain (CQ-resistant) and KB cells. Compounds bearing an electron withdrawing group on phenyl substituent (W), like **31** and **32**, or bearing a CF_3 group on the quinoxaline ring, like **33**, showed superior activity for K1 strain in comparison with chloroquine and good selectivity index (Scheme 10).[27]

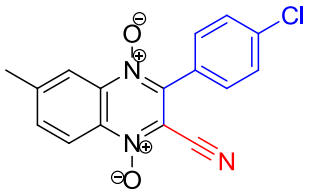
The demonstration that the fluorine atom in fluoroarenes (F-Ar) can form C-**F---H**-N hydrogen bonds to heterocyclic bases of DNA stimulated the synthesis of several fluorinated benzodiazines.[28] With this idea in mind, Chupakhin and coworkers reported the synthesis of 7-mono- and 6,7-difluorinated derivatives of quinoxaline-*N,N'*-dioxide and the furo[3,4-*b*]- and pyrrolo[3,4-*b*]annelated systems derived from them.[28,29] These heterocyclic systems were synthetized by Beirut reaction from 5,6-difluorobenzofuroxan (**34**) with β-ketoesters or β-ketoamides (Scheme 11) in ethanol or dioxane at room temperature, using triethylamine as base, obtaining the 6,7-difluoroquinoxaline-*N,N'*-dioxides (**35a-c**) in minor yields. Best results were achieved using triethylamine as solvent and catalyst simultaneously (60-72%). The substitution of Et_3N by cyclic amines, as morpholine, using anhydrous ethanol as solvent, was accompanied by the selective substitution of the fluorine atom at C6 by the saturated nitrogen of the cyclic amine (Scheme 12). The easiness replacement of the fluorine atom is

explained by the activating effect of the carbonyl group at C2, which acting as electron withdrawing group, decreases the electron density of the carbons C6 and C7 (Figure 3) and consequently decreases the energy of the LUMO. Therefore, favoring electrophilic aromatic substitution reactions on such carbons.[28]



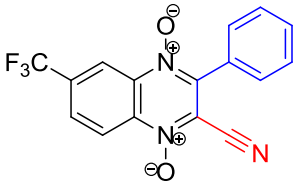
(31)

IC_{50} (K1) = 0.41 μM;
IC_{50} (3d7) = 1.01 μM;
IC_{50} (KB cell) = 9.73 μM



(32)

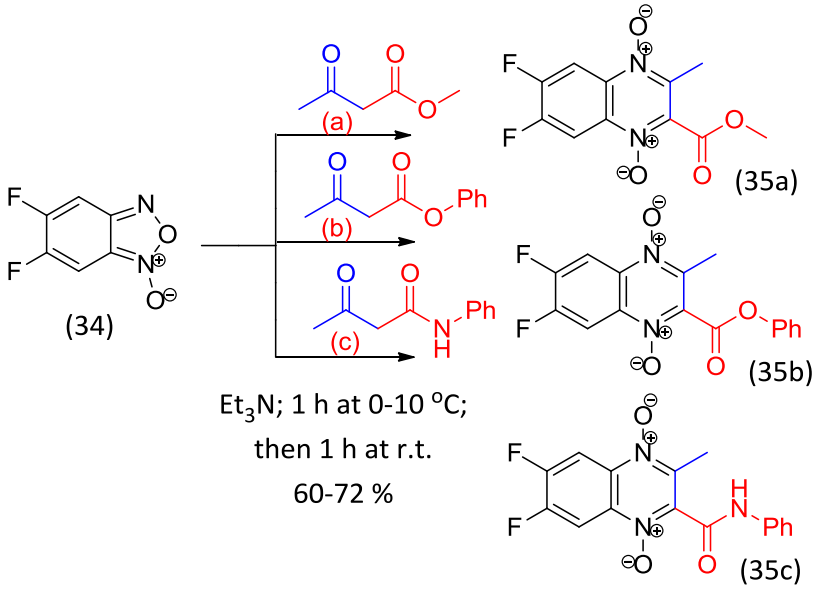
IC_{50} (K1) = 0.19 μM;
IC_{50} (3d7) = 0.48 μM;
IC_{50} (KB cell) = 9.25 μM



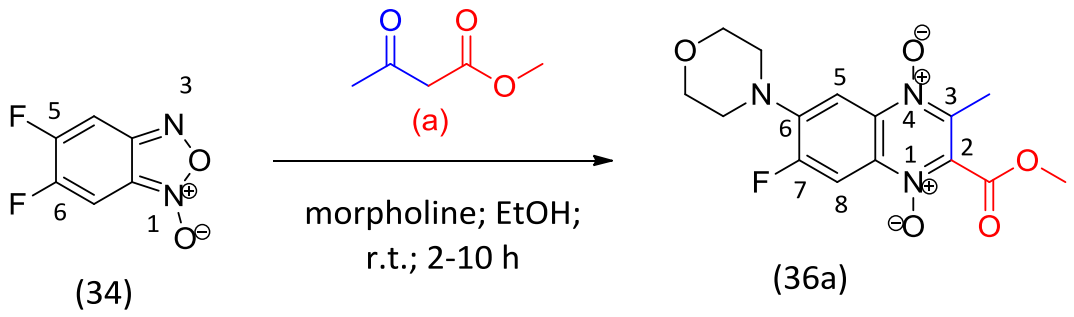
(33)

IC_{50} (K1) = 0.18 μM;
IC_{50} (3d7) = 0.33 μM;
IC_{50} (KB cell) = 2.82 μM

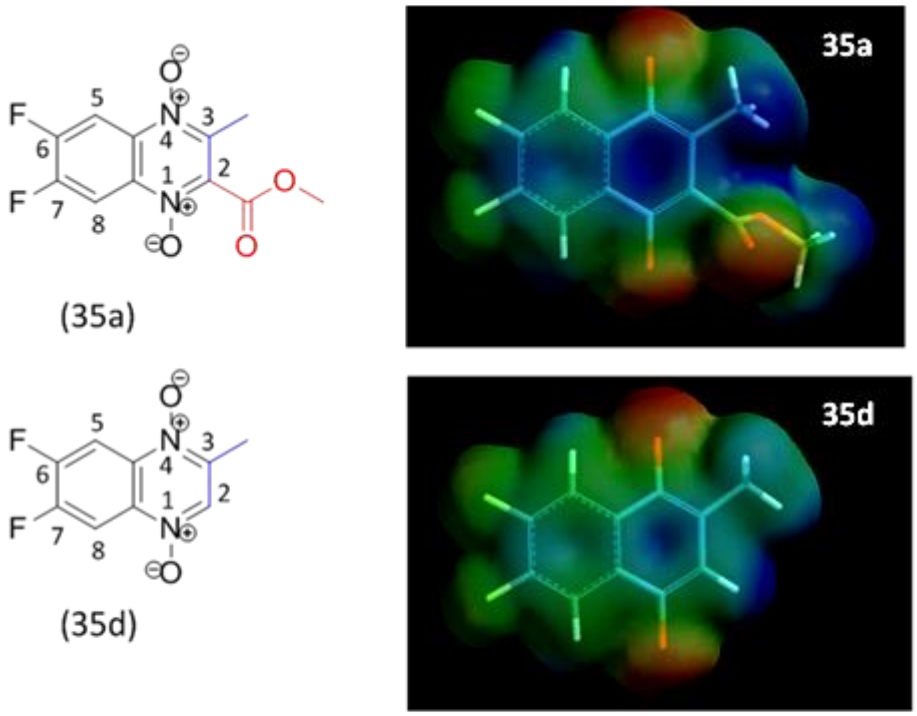
Scheme 10. Examples of antimalarial quinoxaline-*N,N'*-dioxides derivatives



Scheme 11. Synthesis of 6,7-difluoro-quinoxaline-*N,N'*-dioxides (**35a-c**)



Scheme 12. Selective substitution of fluorine atom by cyclic amine in 6,7-difluoro-quinoxaline-1,4-dioxides derivatives

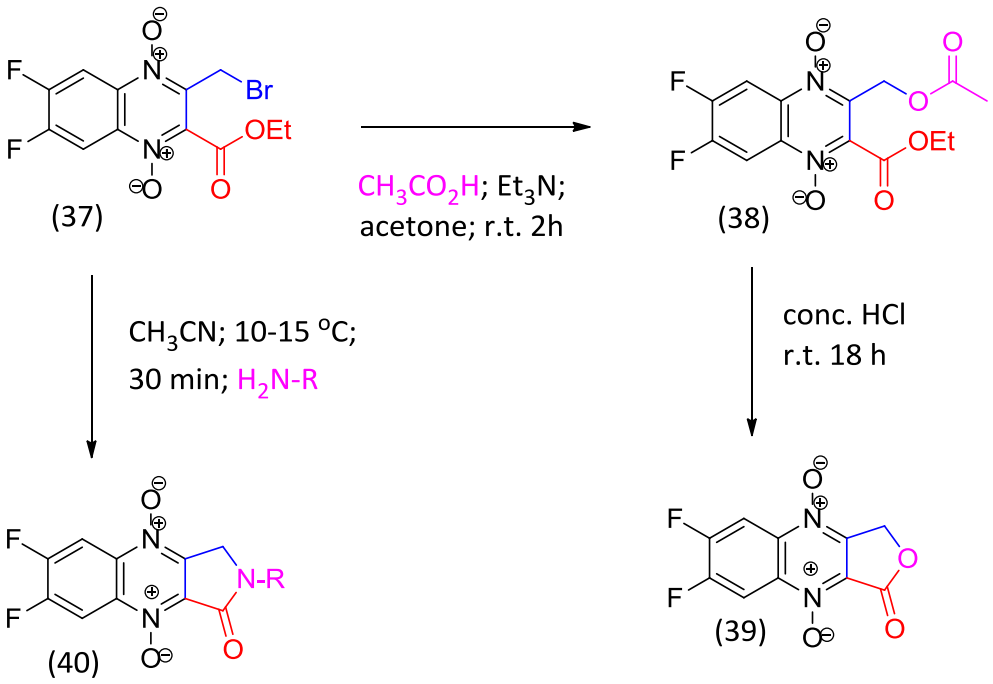


	Compound 35a		Compound 35d	
Charges	C6	C7	C6	C7
electrostatic	0,348	0,345	0,378	0,371

Figure 3. Electrostatic potential surface map calculated for compounds **35a** and **35d** and the electrostatic charge of carbons C6 and C7 (Program Spartan 8.0, Wavefunction Inc)

The dihydrofuro[3,4-*b*]- and dihydropyrrolo[3,4-*b*]quinoxaline 4,9-dioxides (**39** and **40**) were prepared exploring the 3-bromomethyl-2-ethoxycarbonyl-6,7-difluoroquinoxaline 1,4-dioxide (**37**) as key intermediate (Scheme 13). The nucleophilic substitution of the bromine atom by CH_3COO^- produced the acetoxymethyl derivative (**38**), which was hydrolyzed by concentration HCl, resulting in the spontaneous cyclization of intermediate hydroxymethyl derivative in the lactone **39**. The treatment of **37** with ammonia or alkyl-amines resulting in the synthesis of dihydropyrrolo[3,4-*b*]quinoxaline

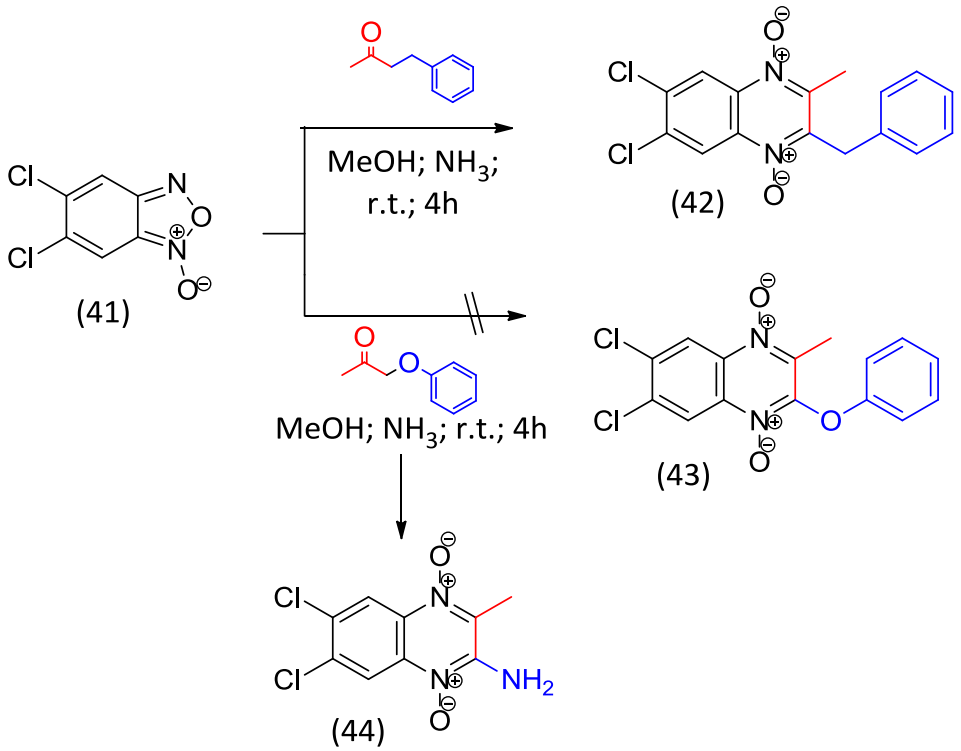
4,9-dioxide (**40**; Scheme 13).[29]



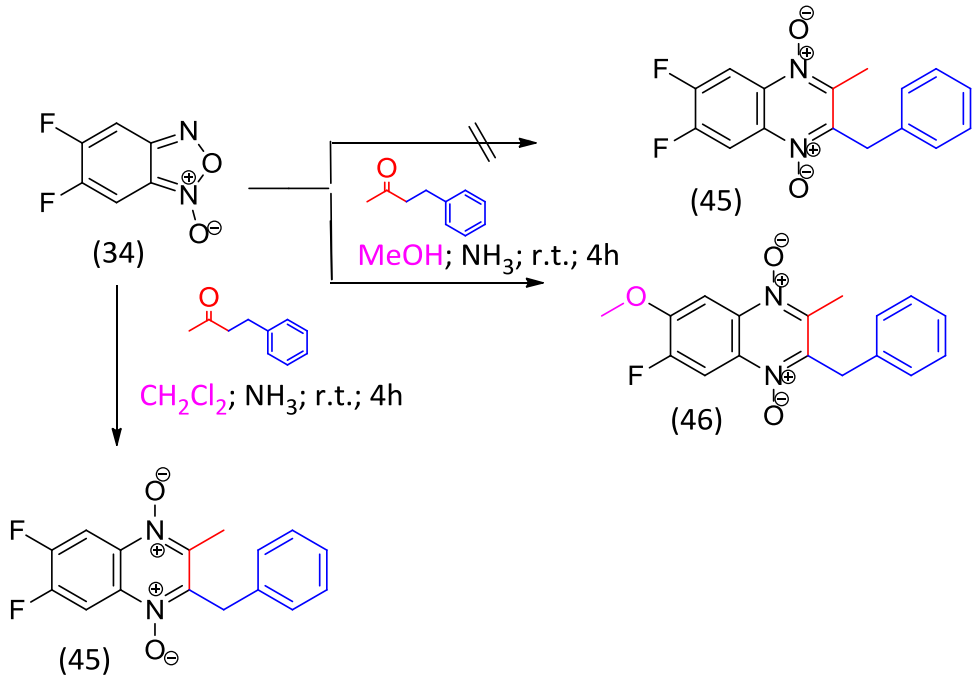
Scheme 13. Synthesis of fluorinated dihydrofuro[3,4-*b*]- and dihydropyrrolo[3,4-*b*]quinoxaline 4,9-dioxides (**39** and **40**, respectively)

Aiming the identification of new antitubercular prototypes Vicente and coworkers reported the synthesis of 2-benzyl- and 2-phenoxy-3-methylquinoxaline-1,4-dioxides derivatives based on the classical Beirut reaction, using halogenated benzofuroxan with benzylacetone or phenoxyacetone, in methanol and gaseous ammonia as solvent and catalyst, respectively (Scheme 14).[30] In these conditions, a different pattern of reaction was observed between the isosters benzylacetone and phenoxyacetone. The latter failed to give the functionalized 2-phenoxy derivative (**43**), being isolated the 2-amino-6,7-dichloro-3-methylquinoxaline-1,4-dioxide (**44**) (Scheme 14). This unexpected resulted could be explained by the favorable leaving group nature of phenoxy subunit and therefore could be avoided modulating the nucleophilic profile of the base. Thus, the authors performed the replacement of ammonia by piperidine in chloroform obtaining the 2-phenoxy-6,7-dichloro-3-methylquinoxaline-1,4-dioxide (**43**) in satisfactory yields.

Moreover, as observed by Chupakhin and coworkers, the use of difluorobenzofuroxan (**34**) as the electrophilic component of the Beirut reaction revealed the high electrophilic nature of C6, and the attempt to synthesize the 2-benzyl-6,7-difluoro-3-methylquinoxaline-1,4-dioxide (**45**) failed, when the authors performed the reaction in the presence of ammonia in methanol (Scheme 15). The exchange of methanol by dichloromethane circumvented the nucleophilic aromatic substitution, yielding the desired compound **45** (Scheme 15).[29,30]



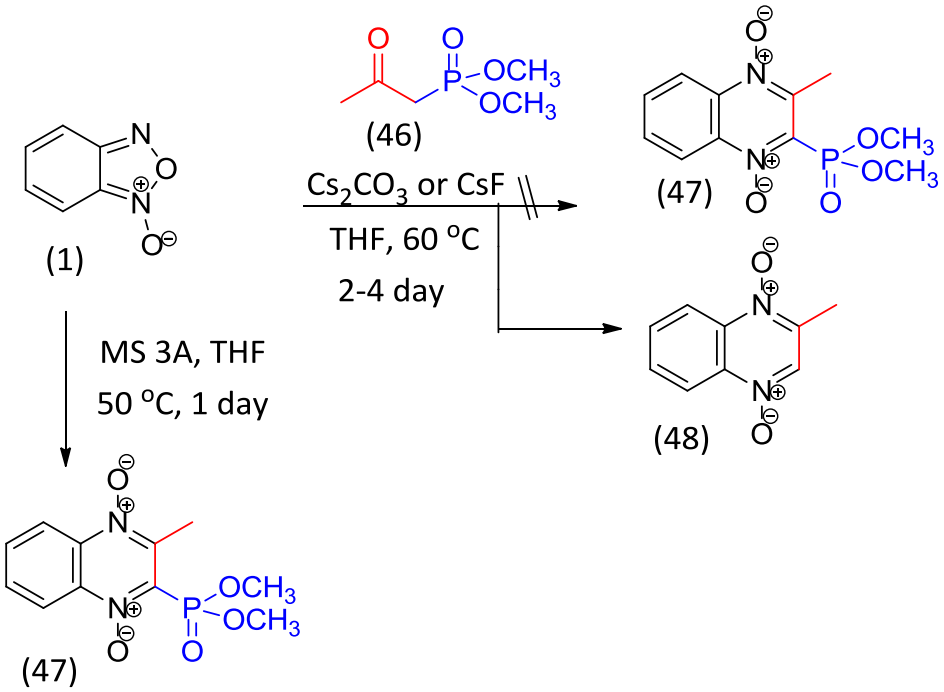
Scheme 14. Attempts to synthesize 2-benzyl- and 2-phenoxy-6,7-dichloro-3-methylquinoxaline-1,4-dioxides (**42** and **43**) and the formation of the secondary product **44**



Scheme 15. Synthesis of the 2-benzyl-6,7-difluoro-3-methylquinoxaline-1,4-dioxide (**45**)

Considering the isosteric relationship between carboxylates, phosphonates and sulphonates, and their role as pharmacophoric group in medicinal

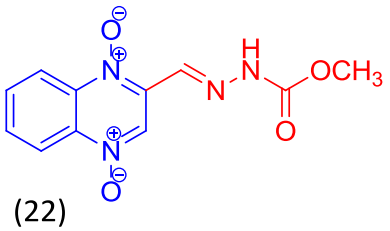
chemistry, Dahbi and coworkers (2010) described for the first time the synthesis of 2-phosphonylated quinoxaline-1,4-dioxides derivatives.[30] The attempts to promote the Beirut reaction between the benzofuroxan (**1**) and dimethyl-2-oxopropyl phosphonate (**46**), using cesium carbonate or cesium fluoride as base in the presence of THF, failed to produce the phosphonylated quinoxaline (**47**), being isolated the 2-methylquinoxaline-1,4-dioxide (**48**) in good yield (Scheme 16). In order to avoid the phosphonate elimination, the authors replaced the base by molecular sieves 3 Å in THF at 50°C, obtaining compound **47** in 77% of yield, with traces of the dephosphorylated compound **48** (6%) (Scheme 16).



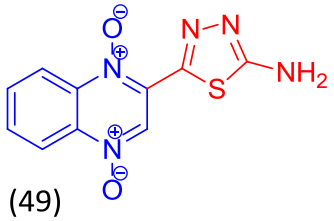
Scheme 16. Synthesis of the 2-phosphonylated quinoxaline-*N,N'*-dioxide derivative (**47**)

Large amounts of quinoxaline-*N,N'*-dioxides were synthesized aiming to achieve new antibiotic agent. These searches were originally instigated by carbadox (**22**), a licensed antibiotic used therapeutically in the United States for the treatment and prevention of colibacillosis, diarrhea, dysentery and enteric salmonellosis in swine. The structure pattern of modification includes the replacement of hydrazone subunit present in **22** by a heterocyclic ring, amide, tioeter, methylnitrone and so on (Scheme 17).[32]

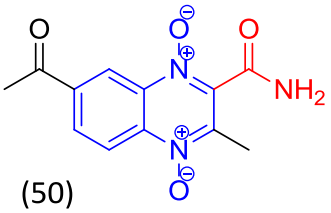
Bearing in mind the ability of quinoxaline-*N,N'*-dioxide act as a hypoxia selective redox activated DNA-cleaving agent (Scheme 18),[33] several authors investigated cytotoxicity to hypoxic cell of quinoxaline-*N,N'*-dioxide derivatives. An interesting example emerged from Monge's research group, that described the 7-chloro-3-(dimethylaminopropyl)amino-2-quinoxalinecarbonitrile 1,4-dioxide (**54**) as potent and selective water soluble derivative with high hypoxic cytotoxicity ratio (Scheme 19).[34,35] The ketones (**55-57**) described by Gali-Muhtasib and coworkers are others good examples of quinoxaline-*N,N'*-dioxides able to inhibit cell growth and induce cycle changes in human tumorogenic epithelial cell lines under hypoxic condition (Scheme 19).[36]



(22)
carbadox

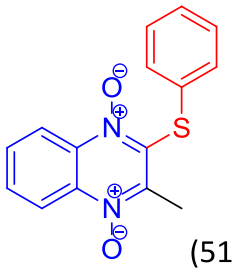


(49)
Escherichia coli



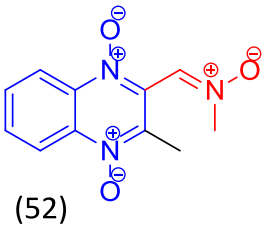
(50)

Escherichia coli, Pasteurella multocida and Streptococcus pyogenes



(51)

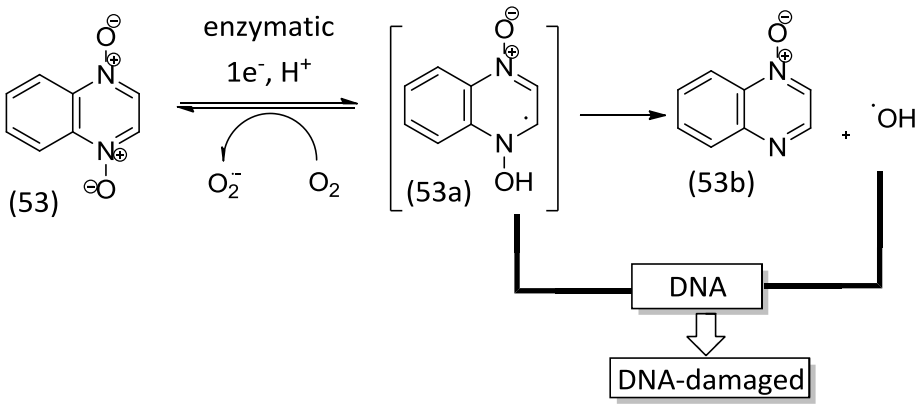
Enterococcus faecalis and Enterococcus faecium



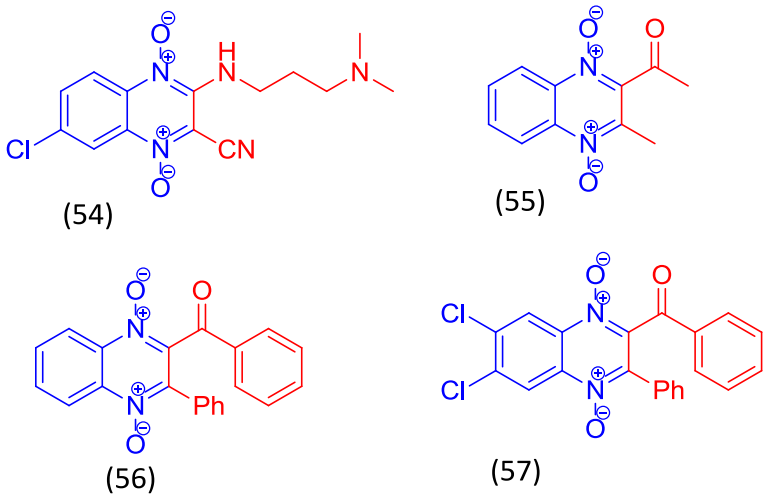
(52)

Proteus mirabilis and Salmonella schottmeullery

Scheme 17. Examples of quinoxaline-*N,N'*-dioxides with antibiotic activity

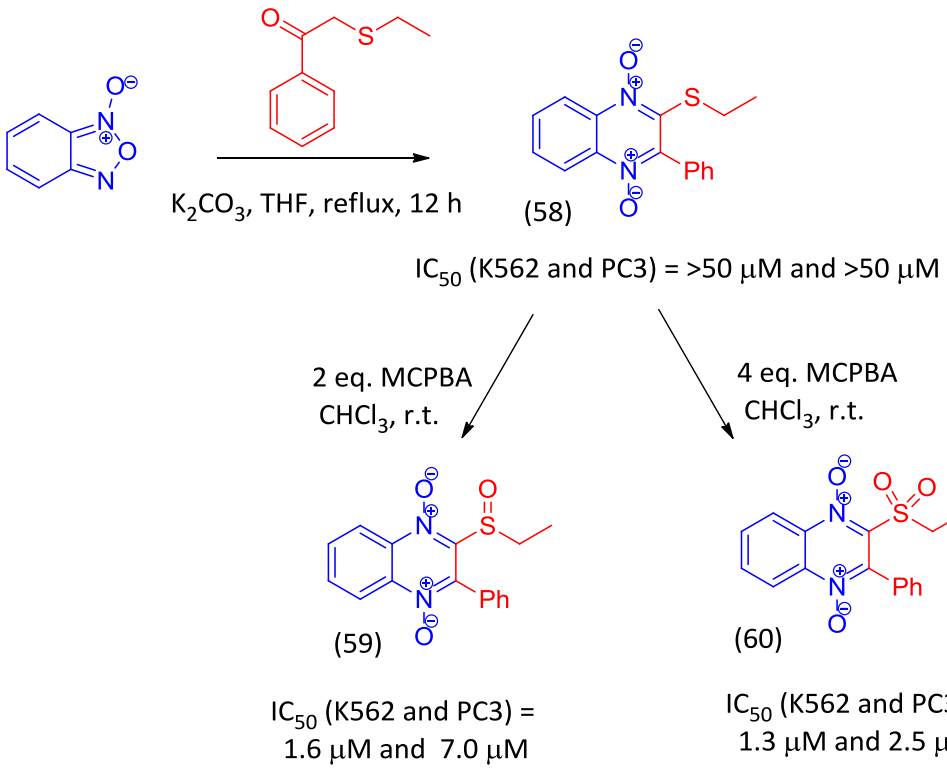


Scheme 18. Enzymatic reduction of quinoxaline-*N,N'*-dioxide (**53**)



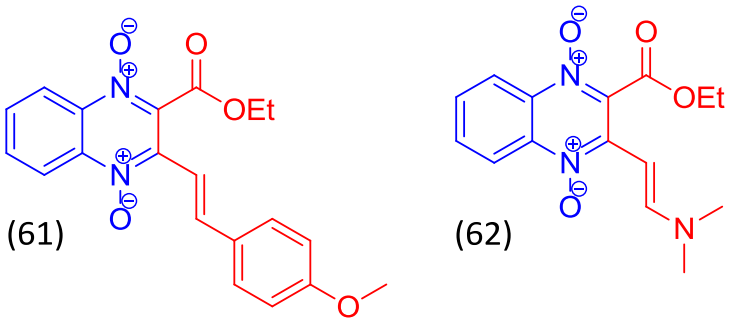
Scheme 19. Examples of cytotoxic quinoxaline-*N,N'*-dioxide derivatives (**54-57**)

More recently, Sheng and coworkers described the synthesis and the cytotoxic activity against human tumor cell lines, in hypoxia and in normoxia conditions, of a series of 3-phenyl-2-thio-quinoxaline-*N,N'*-dioxides derivatives (Scheme 20). Remarkable difference was found in the cytotoxic activity of quinoxaline-*N,N'*-dioxide derivatives bearing a sulfide (**58**), a sulfoxide (**59**) or sulfone (**60**) group at C2 position of heterocyclic nucleus, against all tested cancer cell lines under hypoxic condition (Scheme 20). Compounds **59** and **60** also showed high activity in normoxia condition, being de sulfone derivative the most promising cytotoxic agent.[37]



Scheme 20. Examples of cytotoxic quinoxaline-*N,N'*-dioxide derivatives (**58-60**) against human leukemia cell line (K562) and human prostate cancer cell (PC3)

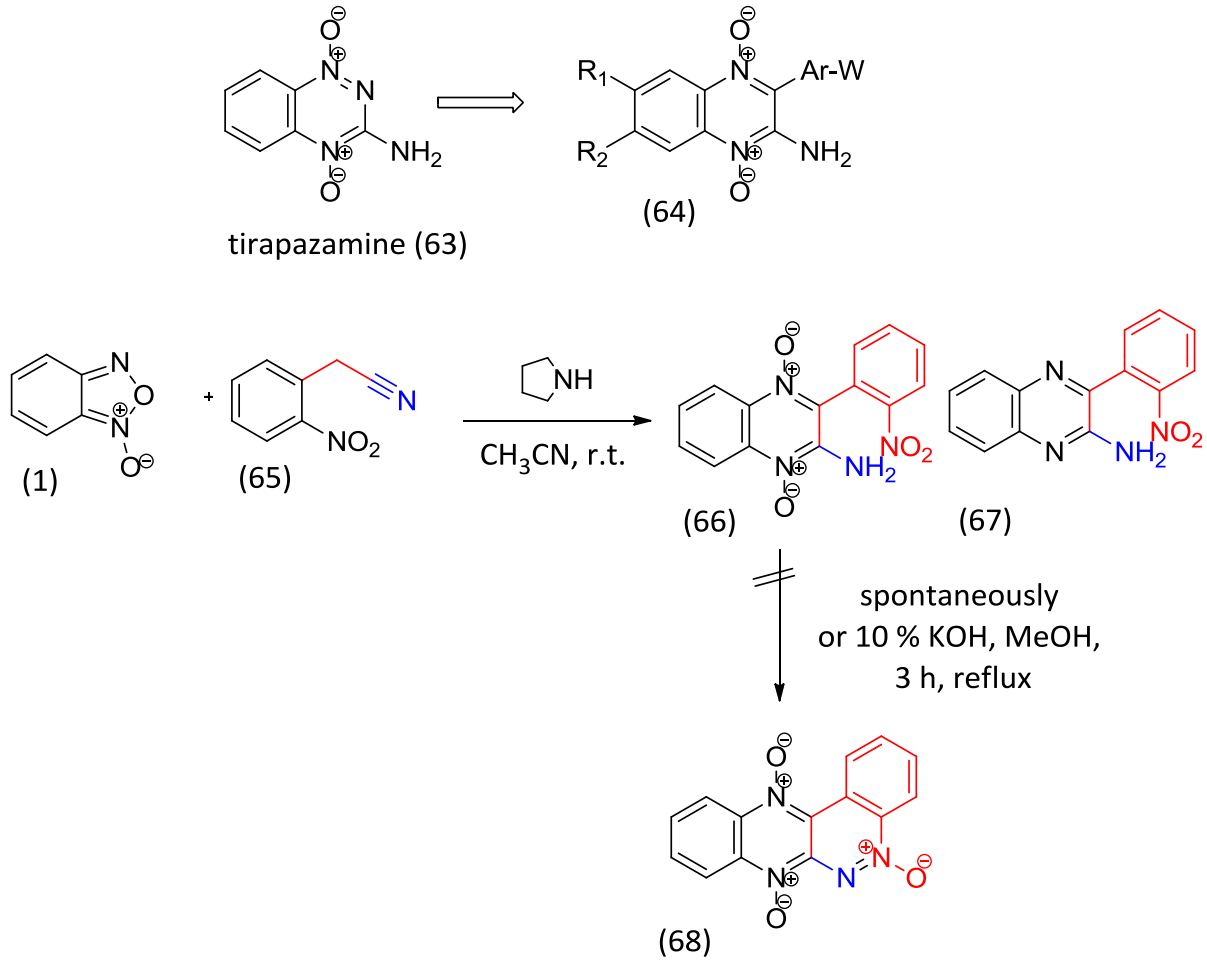
Considering the ability of quinoxaline-*N,N'*-dioxide derivatives act by bioreductive alkylation and cleavage of DNA under hypoxia conditions and aiming to improve selectivity against tumor cells, El-Gogary and coworkers explored the photodynamic therapy concept in the design of quinoxaline-*N,N'*-dioxide derivatives able to absorb radiation at longer wavelengths (λ_{max} ≥350 nM). For these purposes, arylidine and enamine quinoxaline-*N,N'*-dioxide derivatives were synthesized (Scheme 21) and the enamine **62** (AVQD) used as a vehicle to carry iodine-125 to tumor cells. The incorporation of ^{125}I-AVQD in solid tumor sites facilitated tumor imaging, being an interesting model for photodynamic cancer therapy.[38]



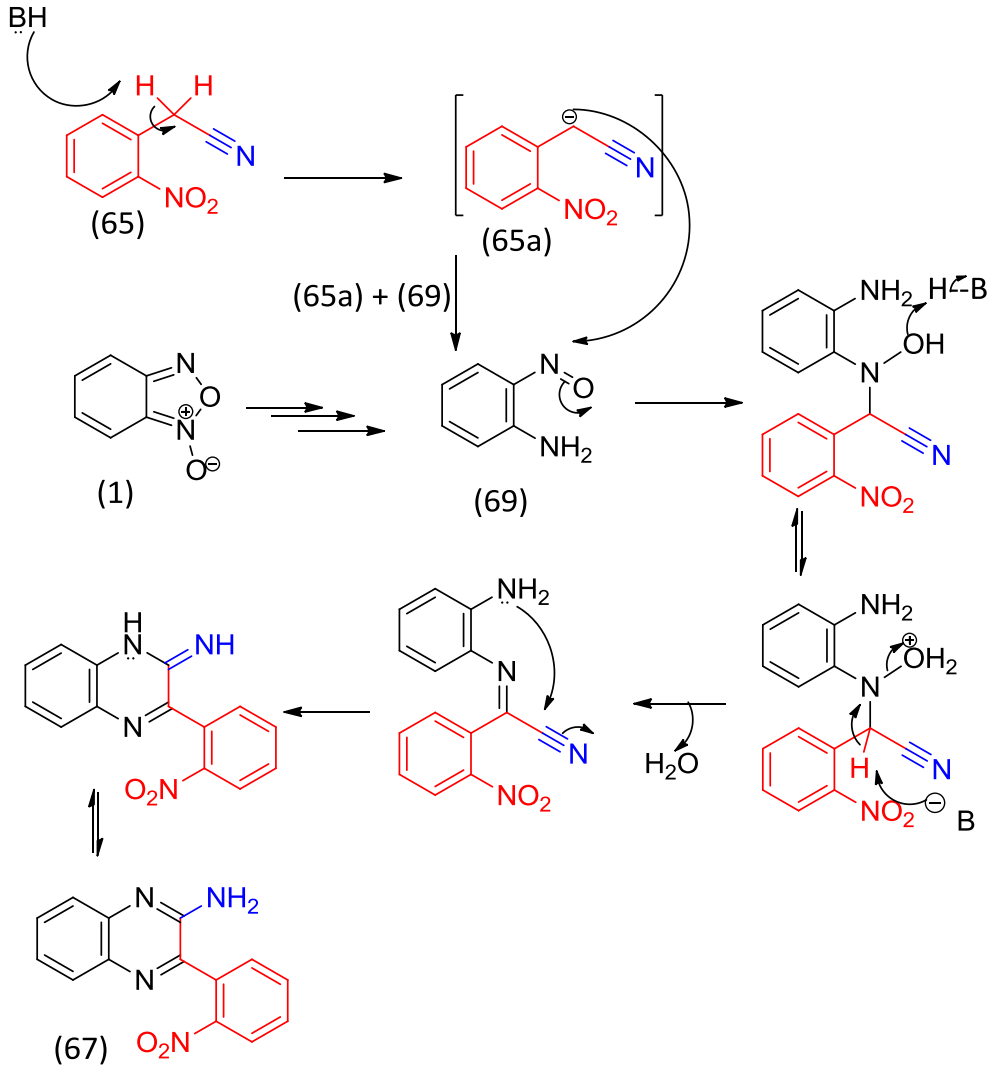
Scheme 21. Examples of arylidine and enamine quinoxaline-*N,N'*-dioxide derivatives

3. Miscellaneous

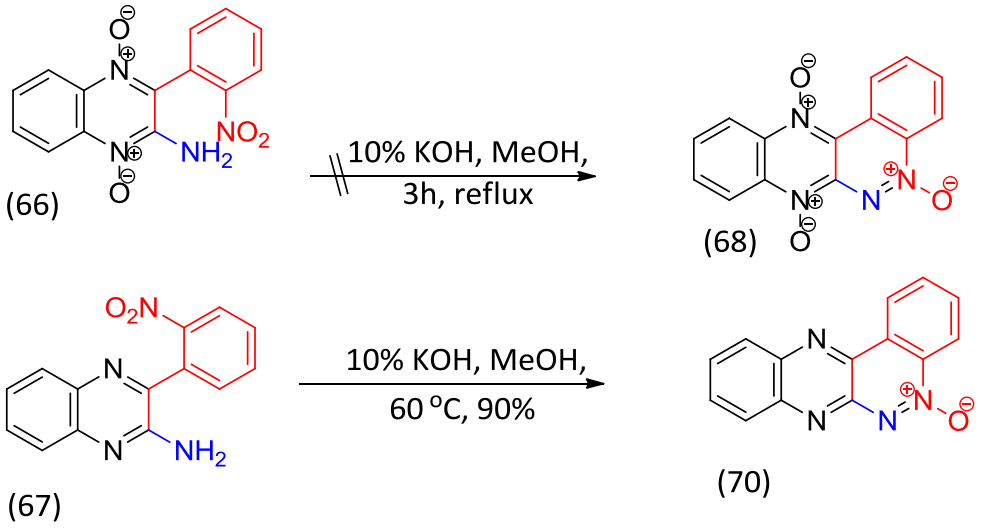
With focus on the anticancer activity of tirapazamine (**63**) and in the possibility of quinoxaline-*N,N'*-dioxide bearing an amino group at position 2 (e.g. **64**) resemble the prototype **63**, Haddadin and coworkers (2011) described the synthesis of a series of 2-amino-3-(2-nitrophenyl)quinoxaline 1,4-dioxide (e.g. **66**) and the attempts for subsequent cyclization to quinoxaline[2,3-c]cinnoline 5,7,12-tri-*N*-oxide (e.g. **68**) (Scheme 22). The Beirut reaction of benzofuroxan (**1**) and 2-nitrobenzylcyanide (**65**) with pyrrolidine as catalyst in acetonitrile resulted in a mixture of almost equivalent amount of compounds **66** and **67** (Scheme 22). This unusual course of Beirut reaction was speculated to be caused by the generation of 2-nitrosoaniline (**69**) which, in turn, reacted with 2-nitrobenzylcyanide (**65**) to give the 2-amino-3-(2-nitrophenyl)quinoxaline (**67**) (Scheme 23). This compound (**67**) could be easy converted to quinoxalino[2,3-*c*]cinnoline 5-*N*-oxide (**70**), showing reactivity profile different from quinoxaline-*N,N'*-dioxide **66** (Scheme 24).[39]



Scheme 22. Attempt to synthetize quinoxalino[2,3-c]cinnoline 5,7,12-tri-*N*-oxide (**68**) and the unexpected quinoxaline (**67**) obtained by unusual Beirut reaction



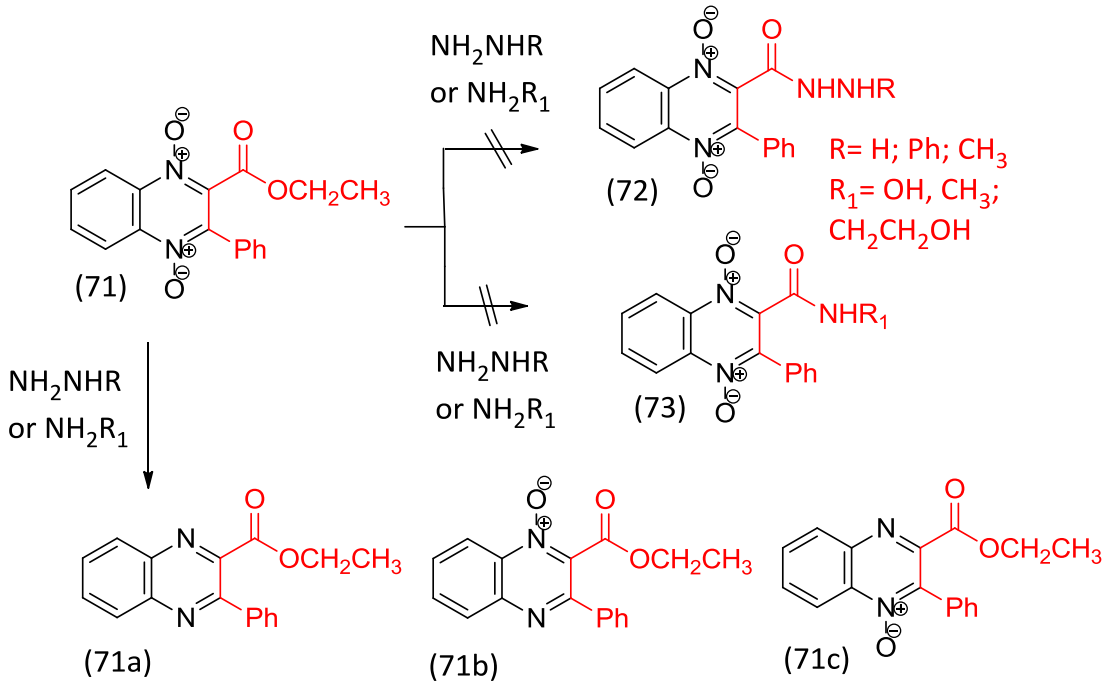
Scheme 23. Proposed mechanism for the synthesis of the 2-amino-3-(2-nitrophenyl)quinoxaline (**67**) (adapted from Haddadin and coworkers, 2011)[39]



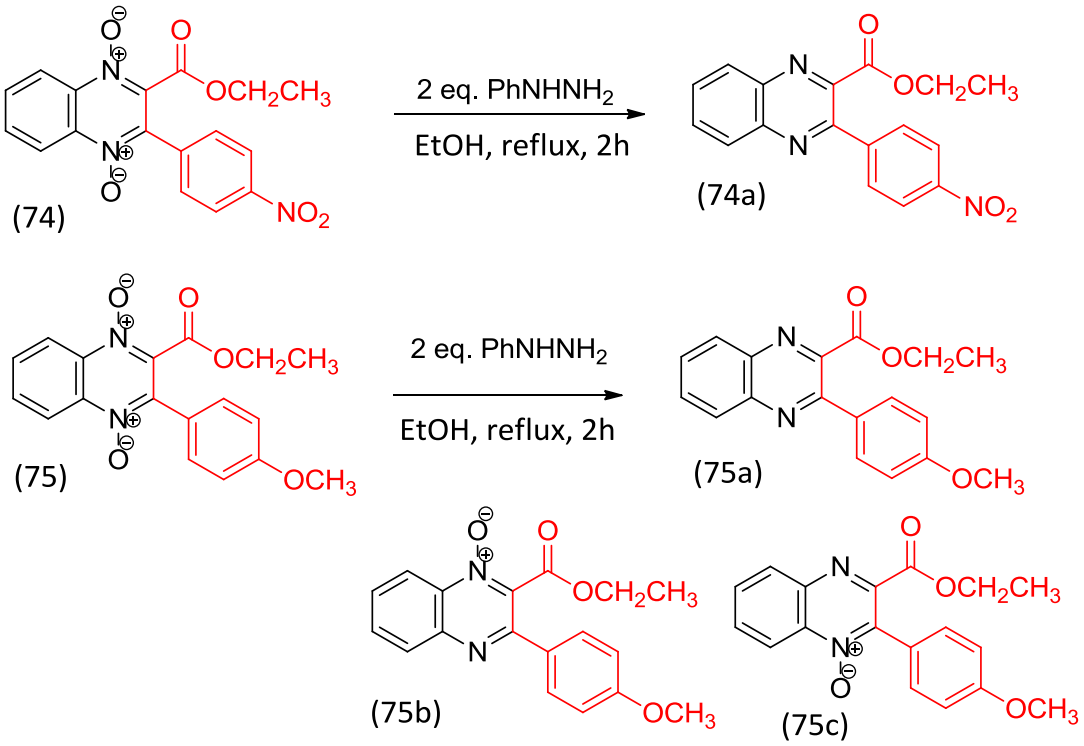
Scheme 24. Synthesis of quinoxalino[2,3-c]cinnoline 5-*N*-oxide (**70**)

In the attempt to synthetize hydrazide (**72**) and amide (**73**) derivatives from ethyl 3-phenylquinoxaline-2-carboxylate 1,4-dioxide (**71**), exploring hydrazinolysis and aminolysis reactions, respectively, unexpected results were obtained and the quinoxaline **71c** and the mono-oxide derivatives **71a** and **71b** were isolated and characterized (Scheme 25). In order to study the distinct reductive profiles of the selected amines and the chemoselectivity of the process, the experimental conditions were standardized to the use of two equivalents of amine in ethanol under reflux for two hours. Under these conditions, with the exception of hydrazine hydrate, a well-known reducing agent, which converted compound **71** to a 3-phenyl-2-quinoxalinecarbohydrazide derivative, the amines were unable to act as nucleophiles and operated exclusively as reducing agents. Hydroxylamine, methylamine, ethanolamine, methyl hydrazine and phenylhydrazine reduced compound **71** to the mono-oxide derivatives **71a** and **71b** and to quinoxaline **71c**, while aniline and triethylamine, a common amine used as catalyst in Beirut reaction, were unable to reduce compound **71**, with no significant difference noted when compared to the experiment carried out in the absence of amine.[40]

The electronic profile of the substituent linked to quinoxaline-*N,N'*-dioxide ring seems to influence the reduction process (Scheme 26). Quinoxaline-*N,N'*-dioxide ester, bearing an electron-withdrawing group (NO_2), was selectively reduced to quinoxaline **74a** in the presence of phenylhydrazine (Scheme 26), while no chemoselectivity was observed under the treatment with hydrazine hydrate and, consequently, the nitro group was also reduced and the hydrazinolysis product formed (data not shown). By the way, the presence of an electron-donating group, like OCH_3, led to obtain in similar proportions quinoxaline **75a** and the mono-oxide derivatives **75b** and **75c** (Scheme 26).[40]



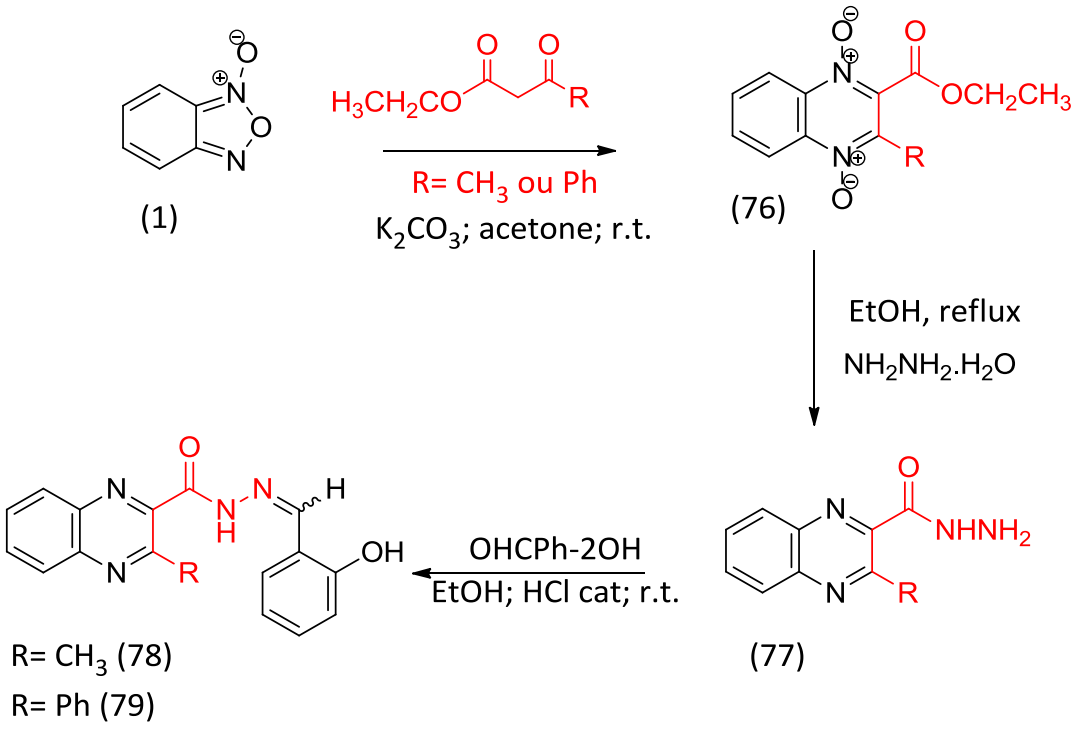
Scheme 25. Unexpected quinoxaline **71c** and the mono-oxide quinoxalines **71a** and **71b** obtained from the attempt to synthetize hydrazide **72** or amide **73** from ester **71**



Scheme 26. Reduction of quinoxaline-*N,N'*-dioxide esters (**74-75**) attached to electron-withdrawing and electron-donating groups using phenylhydrazine

Two new basic conditions for the synthesis of quinoxaline-*N,N'*-dioxide derivatives in moderate to good yields were described by Lima and coworkers. These conditions, exemplified by the use of K_2CO_3 in acetone or KF/Al_2O_3 in the absence of an organic solvent, were reproducible and applicable to the synthesis of 2-(carboethoxy)-3-phenylquinoxaline-*N,N'*-dioxide derivatives substituted in position 4 with electron-donating or electron-withdrawing groups.[41]

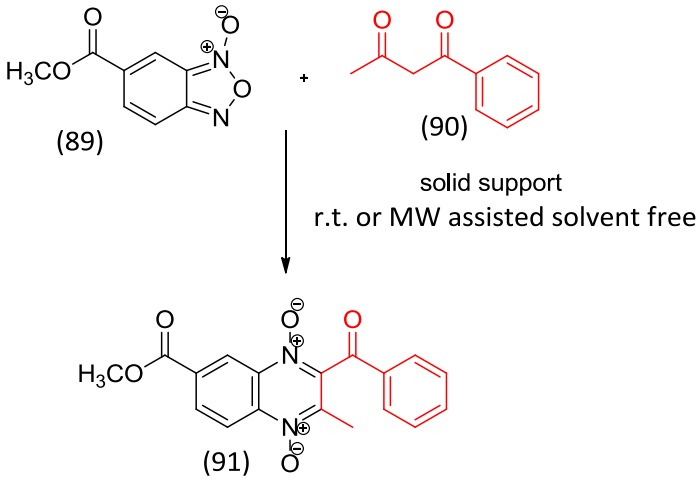
In attempt to obtain in one-step the quinoxaline-2-hydrazide from quinoxaline-*N,N'*-dioxide derivatives, the 2-(ethoxycarbonyl)-3-methyl-(or3-phenyl)-quinoxaline-1,4-dioxide (**76**) was treated with hydrazine monohydrate in ethanol under reflux, affording the title hydrazides (**77**, Scheme 27) in good yields. Considering that salicylaldehyde *N*-acylhydrazones are described as inhibitors of some cysteine proteases like *Trypanosoma cruzi* cruzain, these hydrazides intermediates were condensed with 2-hydroxybenzaldehyde giving compounds **78** and **79** in good yields (Scheme 27). These compounds showed trypanocidal activity with IC_{50} values of the same magnitude order than the standard drug nifurtimox (Nfx) and were non-toxic at the highest assayed concentration showing selectivity indexes [IC_{50}(macrophages)/IC_{50}(Trypanosoma cruzi)] ≥ 20.[42]



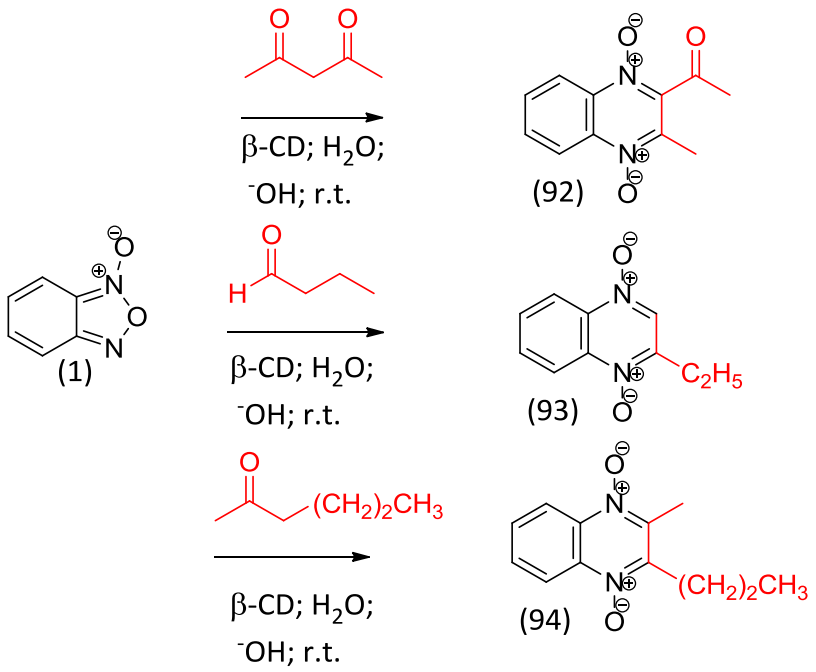
Scheme 27. Synthesis of trypanocidal quinoxaline acylhydrazones (**78** and **79**) starting by Beirut reaction

Systematic studies of base and acid catalyst in the Beirut reaction exploring the condensation of benzofurazan oxide (**1**) with ethyl benzoylacetate (**80**), as well as the comparative use of solvent free KF-Al_2O_3 and K_2CO_3 in acetone were performed by Lima and coworkers. The use of triethylamine, morpholine, piperidine in the presence of polar solvents ($CHCl_3$ or EtOH) resulted in obtaining compound **81** in very low yield. The use of inorganic base as potassium carbonate in the presence of acetone or *N,N*-dimethylformamide were investigated and resulted in improvement of the yield. Considering the versatility of potassium fluoride on alumina (KF/Al_2O_3) it was employed as base and solid support for the aforementioned chemical transformation. In this condition, the condensation step was carried out spontaneously resulting in formation of quinoxaline-*N,N'*-dioxide **81** in 38% yield. No significant differences were observed for the condensation step using KF/Al_2O_3 when the ethyl benzoylacetate was substituted in the *para*-position of phenyl ring by an electron-donating or electron-withdrawing group. However, the Beirut reaction in this condition seemed to depend on the physical state of □-keto-ester and at least one of the reagents must be a liquid in order to chemical conversion to be a success, since no reaction was observed using a solid □-keto-ester, such as 4-nitrobenzoylacetate (Scheme 28).[41]

Another mild, environmentally friendly and highly efficient procedure for Beirut reaction was described by Sun and coworkers, who reported the synthesis of quinoxaline-*N,N'*-dioxide derivatives exploring the condensation between benzofurazan oxide (**1**) and ketones or aldehydes in the presence of β-cyclodextrin in water at room temperature (Scheme 30).[44]



Scheme 29. Beirut reaction using solid support at room temperature or microwave assisted solvent free



Scheme 30. Beirut reaction in the presence of β-cyclodextrin in water at room temperature

Acknowledgments

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