A Comparative Study of Conventional and Microwave-Assisted Synthesis of Quinoxaline 1,4-di-*N*-oxide *N*-acylhydrazones Derivatives Designed as Antitubercular Drug Candidates

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Abstract

Quinoxaline 1,4-di-*N*-oxide (QdNO) and *N*-acylhydrazone subunit are considered privileged scaffolds in medicinal chemistry due to its wide spectrum of biological activities, such as antibacterial, antitubercular, antiviral, anticancer and antifungal. Beirut's reaction is the mostly commonly employed synthetic method to obtain QdNO; however, extended time, low yields and byproducts formation are commonly features observed during the synthesis. Microwave-assisted organic synthesis (MW) has gained popularity as an effective way to speed up chemical reactions, increasing yields and selectivity of a variety of reactions. Therefore, in an effort to synthesize compounds with potential to tuberculosis treatment, we reported herein the use of MW as a tool to obtain new QdNO derivatives containing the *N*-acylhydrazone subunit. Four different synthetic routes were evaluated by using different benzofuroxan derivatives in the Beirut's reaction. The synthetic route D, which employed a dioxolan-benzofuroxan derivative, has showed to be the best condition to obtain the desired hybrid quinoxaline. MW drastically reduces the reaction time to obtain all compounds compared to conventional heating. For compound **13**, for example, the use of MW instead of conventional heating was able to reduce the reaction time in 192-fold. In conclusion, the use of a benzofuroxan derivative without additional electrophilic sites besides *N*-oxide nitrogen and the employment of the microwave-assisted synthesis have proved to be the optimum condition to obtain quinoxaline 1,4-di-*N*-oxide *N*-acylhydrazone derivatives.

Keywords: quinoxaline 1,4-di-*N*-oxide, *N*-acylhydrazone, Beirut reaction, microwave-assisted synthesis, tuberculosis.

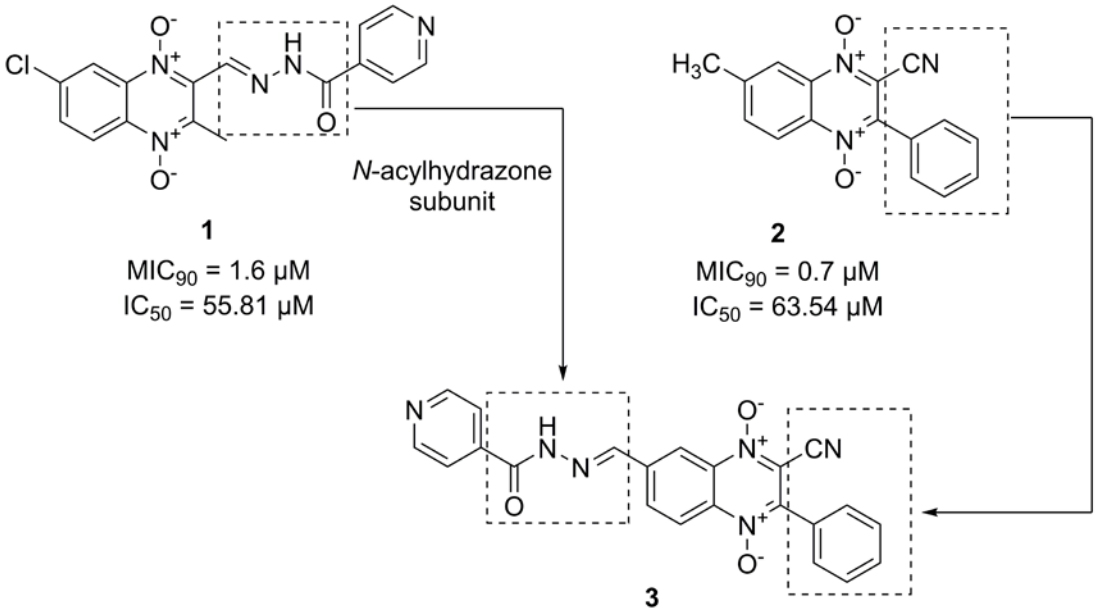
INTRODUCTION

Quinoxaline 1,4-di-*N*-oxide (QdNO) represents an important class of *N*-oxide compounds with a wide range of biological activities, such as, antibacterial, antitubercular, antiviral, anticancer, antifungal and anthelmintic [1]. The wide spectrum of biological activities of QdNO derivatives has been associated to its ability to generate reactive oxygen species (ROS) after biotransformation under hypoxic conditions, leading to DNA damage [2–4]. The antitubercular activity of QdNO derivatives have been described in several papers published by our research group [5–9], reinforcing the potential of this scaffold to be used during the design of new antitubercular compounds.

From a phenotypic-based screening against *Mycobacterium tuberculosis* (MTB) containing more than five thousand compounds of our current library, we have identified the compound 3-cyano-6-methyl-2-phenylquinoxaline 1,4-dioxide **2** (MIC_{90} = 0.7 μM) in a series of 3-aryl-quinoxaline-2-carbonitrile 1,4-di-*N*-oxide derivatives [10] as a promising scaffold for molecular modifications. Furthermore, we also have reported a series of quinoxaline 1,4-di-*N*-oxide derivatives containing the *N*-acylhydrazone subunit with potent antitubercular activity. The compound (*E*)-6-chloro-3-((2-isonicotinoylhydrazono)methyl)-2-methylquinoxaline 1,4-dioxide **1** showed MIC_{90} value of 1.6 μM against *Mycobacterium tuberculosis* $H_{37}Rv$ strain and IC_{50} value of 55 μM against VERO cell lines [11]. *N*-acylhydrazone (NAH) subunit also represents an important scaffold in the medicinal chemistry due to its wide spectrum of biological activities [12–15]. Several NAH derivatives has been described with potent antitubercular activity against MTB $H_{37}Rv$ and multi-drug resistant strains [16,17,14]. Thus, we have

selected these two compounds (**1** and **2**) to design a novel quinoxaline hybrid derivative **3** (Scheme 1).

Scheme 1. Design of the hybrid quinoxaline *N*-acylhydrazone derivative.



The most commonly employed method in the synthesis of QdNO is through the Beirut reaction, which involves the cycloaddition between benzofuroxan with enamines, α,β-unsaturated ketones, 1,3-dinitriles or enolates leading to formation of quinoxaline-1,4-di-*N*-oxide [18–21]. Nonetheless, low yields, extended time and non-reaction are commonly features observed during the synthesis of several QdNO derivatives [8,22].

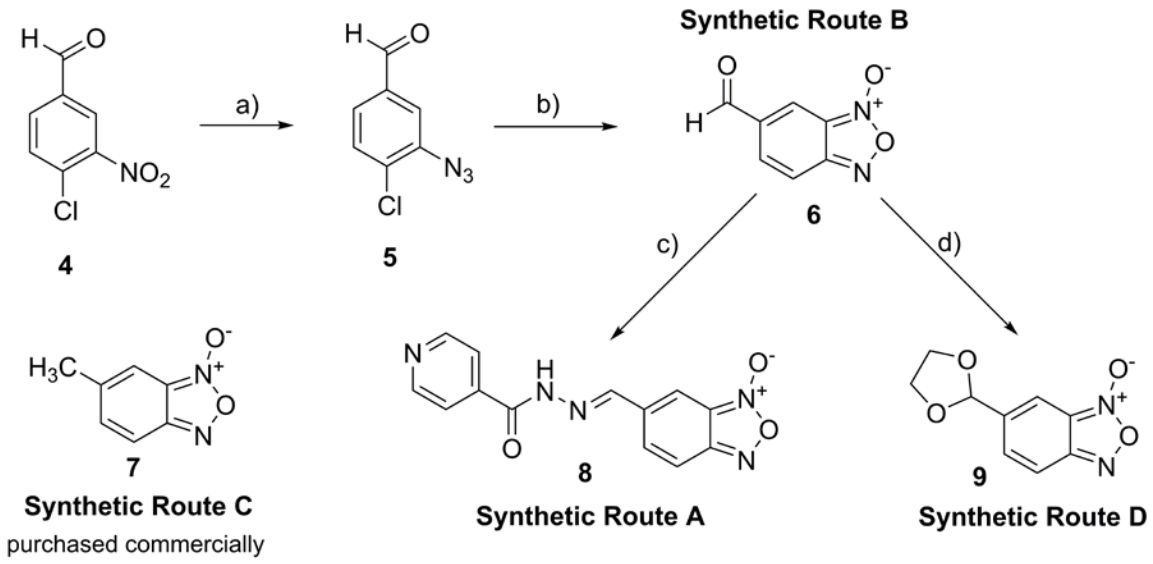
Microwave-assisted organic synthesis (MW) has gained notoriety as an effective way to speed up chemical reactions, increase yields and selectivity in a wide range of reactions [23]. Therefore, in a continuing effort to develop new drug candidates to treat tuberculosis infection, we report herein the synthesis of a quinoxaline 1,4-di-*N*-oxide *N*-acylhydrazone derivative **3** (Scheme 1) exploiting the conventional and the microwave-

assisted synthesis. Furthermore, we also described a comparative study using different benzofuroxan derivatives in order to optimize the synthetic conditions for obtaining these hybrid compounds.

RESULTS AND DISCUSSION

We have evaluated the use of four different benzofuroxan derivatives using conventional and microwave-assisted synthesis in order to outline a comparative between these two synthetic methodologies and the best benzofuroxan derivative to obtain the desired hybrid quinoxaline. The aldehyde-benzofuroxan derivative **6** (Synthetic Route B) was obtained from 4-chloro-3-nitrobenzaldehyde **4** as previously described [24]. Next, we obtained the benzofuroxan-*N*-acylhydrazone derivative **8** from compound **6** (Synthetic Route A) as already reported [15] and the dioxolan-benzofuroxan **9** (Synthetic Route D) through an aldehyde protection reaction. The 6-methylbenzo[c][1,2,5]oxadiazole 1-oxide **7** (Synthetic Route C) was purchased commercially (Scheme 2). Moreover, we have used different catalysts and solvents in both methodologies (conventional and MW), considering the different reaction conditions that microwave-assisted synthesis requires [25,26].

Scheme 2. Synthetic methodologies to obtain the different benzofuroxan derivatives.

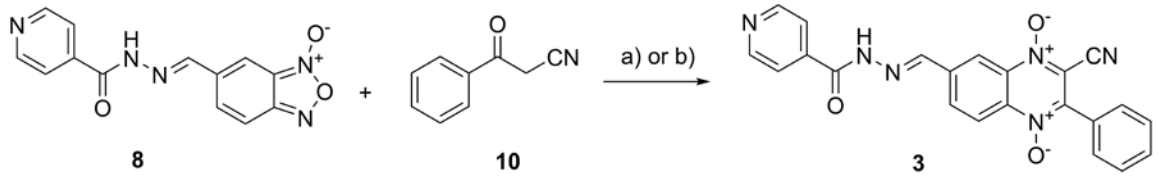


a) NaN_3, DMSO, 75 ºC, 1h; **b)** toluene, reflux, 2h; **c)** isonicotinohydrazide, ethanol, acetic acid, 24h; **d)** toluene, ethylene glycol, *p*-toluenesulfonic acid, reflux, 12h.

Synthetic Route A

A benzofuroxan derivative **8** already containing the *N*-acylhydrazone subunit was used in this synthetic route. In the synthetic design, we selected this benzofuroxan derivative due to the few steps involved in this route (Scheme 3). The only synthetic step was the Beirut reaction between compound **8** and benzoylacetonitrile **10**, which would lead to formation of the hybrid quinoxaline **3**. However, when we tried to perform this reaction, we did not get the desired product using both methodologies (conventional and MW). A complex black-oil mixture was obtained with several byproducts and overlapped retention factors (R_f) in the thin layer chromatography (TLC), becoming it difficult to identification and separation the desired compound.

Scheme 3. General procedure for synthetic route A.

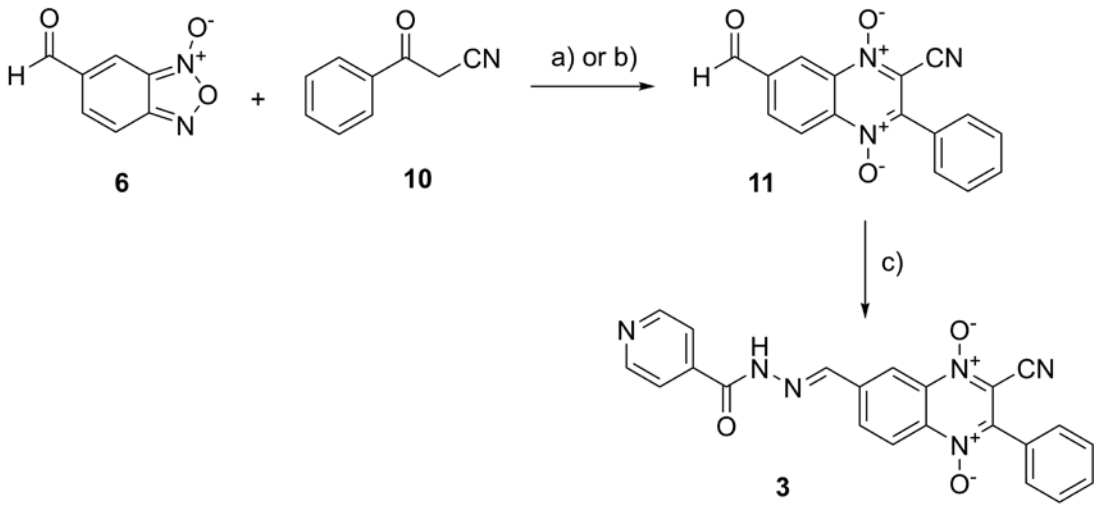


a) dichloromethane, K_2CO_3, 40 ºC; 48h; **b)** toluene, triethylamine, MW, 70 W, 40 ºC, 30 min.

Synthetic Route B

After the failure of synthetic route A, a second alternative was planned. In the synthetic route B, the Beirut reaction was carried out between a benzofuroxan derivative **6** containing an aldehyde function at position 6 and benzoylacetonitrile **10**, which would lead the formation of intermediate quinoxaline **11**. Next, compound **11** would be reacted with isonicotinohydrazide in order to obtain the hybrid quinoxaline **3** (Scheme 4). Once again, the Beirut reaction generated an even more complex black-oil mixture than the previous synthetic route. At this point, we realized that a more selective synthetic route and the use of a benzofuroxan derivative with less electrophilic sites would be necessary.

Scheme 4. General procedure for synthetic route B.



a) dichloromethane, K_2CO_3, 40 ºC; 48h; **b)** toluene, triethylamine, MW, 70 W, 40 ºC, 30 min; **c)** isonicotinohydrazide, ethanol, acetic acid, 12h.

Synthetic Route C

Therefore, the synthetic route C was designed in order to improve the selectivity of Beirut reaction. A benzofuroxan derivative **7** containing a methyl group at position 6 (Scheme 5) was selected for the Beirut reaction with benzoylacetonitrile **10** leading the formation of the intermediate methyl-quinoxaline derivative **12**, which would be subsequently oxidized to an aldehyde-quinoxaline **11** [11]. The last step would involve the condensation reaction with isonicotinohydrazide leading the formation of the hybrid quinoxaline **3**. The first step was successfully achieved and the methyl-quinoxaline was obtained with moderate yields using conventional and microwave-assisted methodologies, 28% and 35%, respectively. Following the synthetic methodology, the next step would be performed through an oxidation reaction of the methyl group to aldehyde using selenium dioxide [27]. However, despite the selenium dioxide be one of the most used oxidizing agents in the oxidation of methyl groups to aldehydes, this

159 reaction did not occurred. Even after a extend reaction time in both methods (MW and
160 conventional), the TLC only showed the starting reactants and no change was observed in
161 the reaction medium. The failure of this reaction forced us to plan a new synthetic route
162 to obtain the desired quinoxaline.

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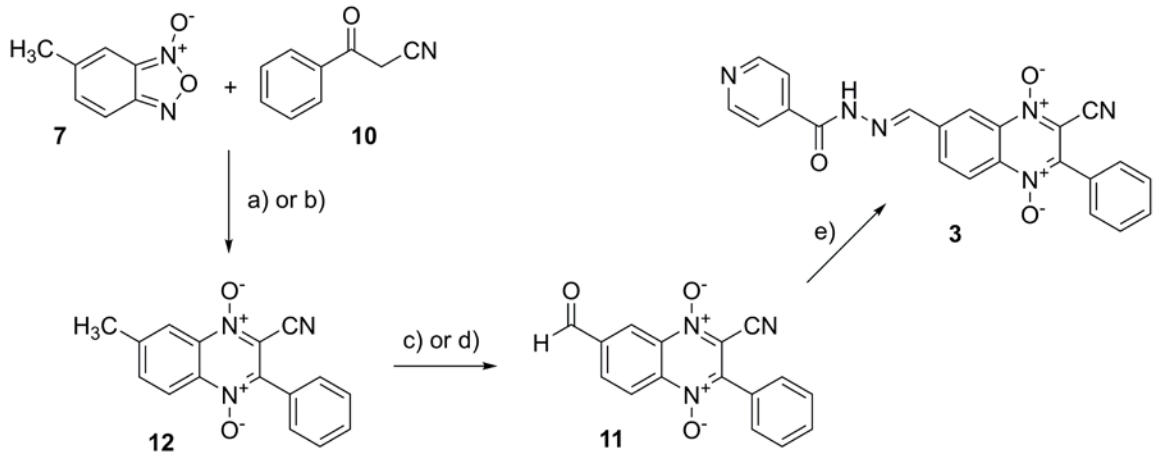
164 **Scheme 5.** General procedure for synthetic route C.



a) dichloromethane, K_2CO_3, 40 ºC; 48h; **b)** toluene, triethylamine, MW, 70 W, 40 ºC, 10 min; **c)** ethyl acetate, selenium dioxide, MW, 200W, 70 ºC, 1h; **d)** ethyl acetate, selenium dioxide, reflux, 24h; **e)** isonicotinohydrazide, ethanol, acetic acid, 12h.

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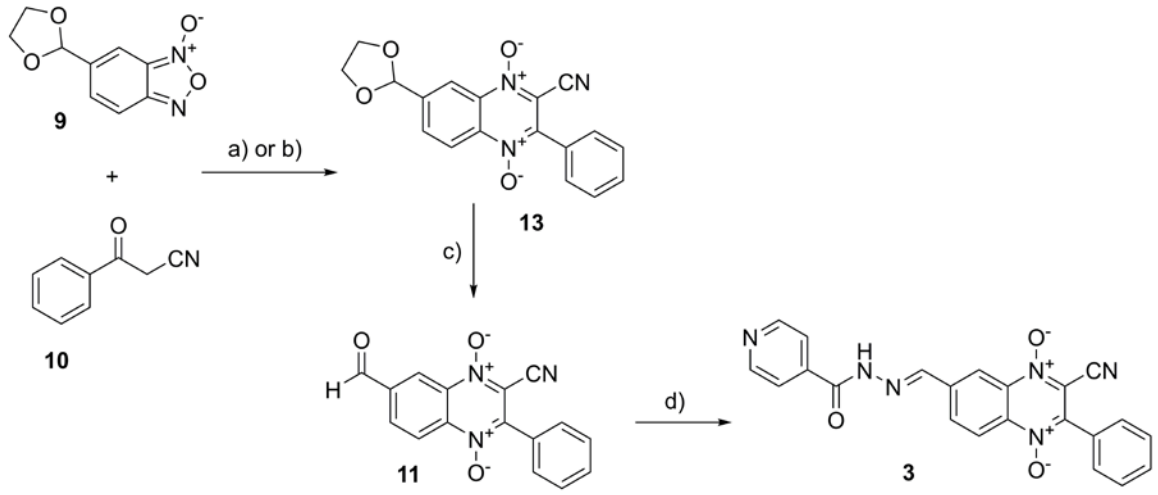
167 **Synthetic Route D**

168 Considering the issues involved in synthetic route A - C, a benzofuroxan derivative
169 without additional electrophilic sites besides the *N*-oxide nitrogen was selected for
170 synthetic route D. The dioxolan-benzofuroxan derivative **9** was exploited because the
171 aldehyde group remains protected by a cyclic acetal during the Beirut reaction (Scheme
172 6). The first reaction step was the protection of the aldehyde group using ethylene glycol
173 and acid catalysis leading the formation of the dioxolan-benzofuroxan **9** with good yield
174 (85%) [28]. Next, the dioxolan-benzofuroxan **9** was reacted with benzoylacetonitrile **10**

through the Beirut reaction in order to generate the dioxolan-quinoxaline **13**. This step was successfully in achieve the desired compound with moderate yields (30%) in both methods (MW and conventional). Finally, the deprotection reaction was carried out using acid catalysis and the condensation reaction with isonicotinohydrazide was performed *in situ* without further purification of the aldehyde, leading the formation of the hybrid quinoxaline 1,4-di-*N*-oxide-*N*-acylhydrazone derivative **3** with good yield (66%).

Scheme 6. General procedure for synthetic route D.



a) dichloromethane, K_2CO_3, 40 ºC, 96h; **b)** toluene, triethylamine, MW, 70 W, 40 ºC, 30 min; **c)** acetone, HCl, r.t., 48h; **d)** isonicotinohydrazide, ethanol, acetic acid, 12h.

Synthetic Conditions

Our research group previously evaluated the use of several bases as catalysts and solvents in Beirut reaction, therefore, wide ranges of synthetic conditions were described for quinoxaline 1,4-di-*N*-oxide synthesis. For instance, the preparation of 2-(carboethoxy)-3-phenyl- quinoxaline 1,4-dioxide was achieved with good yields and short reaction time by using potassium carbonate (K_2CO_3) in acetone or potassium fluoride on alumina

191 (KF/Al_2O_3) in the absence of an organic solvent [29]. Moreno and coworkers also
192 demonstrated the synthesis of a series of 1,4-di-*N*-oxide-quinoxaline-2-carboxylic acid
193 aryl amide derivatives using ethanolamine as catalyst and methanol as solvent in a
194 reaction time ranging from 1 to 48 h based on the benzofuroxan used [8]. On the other
195 hand, some reaction conditions have led to quinoxaline derivatives with low yields [7,22]
196 and others have required longer reaction times [30]. We also have reported the potential
197 of the microwave-assisted synthesis to obtain QdNO derivatives in very short reaction
198 times. For instance, a series of 2-(4-fluorobenzoyl)-3-(trifluoromethyl)quinoxaline 1,4-
199 dioxide was obtained in 15 minutes using microwave irradiation [31].
200 Therefore, it was necessary the use of different conditions due to previous trials in order
201 to define the optimum solvent and basic catalysis conditions for each method of synthesis
202 (conventional and MW). Thus, the conventional heating synthesis was carried out using
203 dichloromethane as solvent [32] and K_2CO_3 as catalyst [29] whereas triethylamine and
204 toluene were used in the microwave-assisted synthesis methodology [11,32].
205 Although the synthetic route C has not been able to lead the formation of the hybrid
206 quinoxaline-*N*-acylhydrazone, this route was capable to generate an intermediate
207 quinoxaline in both synthetic methods. By the other hand, the synthetic route D was
208 successfully to obtain the final desired quinoxaline. It is noteworthy that the microwave-
209 assisted synthesis was outstanding useful to reduce the reaction time and formation of
210 byproducts when compared to conventional method. For instance, in the synthetic route
211 C, the reaction time in conventional methodology was carried out during 48 hours with
212 12% of yield for compound **12**, which was obtained in 10 minutes with 26% of yield
213 through the microwave-assisted methodology (Table 1). A similar result was observed in

the synthetic route D, in which compound **13** was obtained in 30 minutes with 30% of yield using microwave-assisted synthesis. Nevertheless, this compound **13** was achieved with an extremely extended reaction time of 96 hours and several byproducts in the conventional methodology; however, the yield remained in about 30% (Table 1).

We also evaluated the increase of temperature in all synthetic routes. Temperature above 40 ºC led to an increase in formation of byproducts in both synthetic methods. Regarding synthetic route A and B, we also carried out both methods at room temperature in order to evaluate whether the byproducts formation would be reduced, however, the same complex black-oil mixture was observed.

Table 1 Different conditions for the synthesis of 3-aryl-quinoxaline-2-carbonitrile 1,4-di-*N*-oxide derivative **3** using conventional and microwave-assisted synthesis.

Synthetic Method	Synthetic Route	Catalyst	Solvent	Temperature (ºC)	Time	MW Potency (W)	MW Pressure (psi)	Yield (%)
Conventional	A	K_2CO_3	$CHCl_3$	40	48 h	-	-	Not obtained
	B	K_2CO_3	$CHCl_3$	40	48 h	-	-	Not obtained
	C	K_2CO_3	$CHCl_3$	40	48 h	-	-	12
	D	K_2CO_3	$CHCl_3$	40	96 h	-	-	30
Microwave	A	triethylamine	toluene	40	30 min	70	20	Not obtained
	B	triethylamine	toluene	40	30 min	70	20	Not obtained
	C	triethylamine	toluene	40	10 min	70	20	26
	D	triethylamine	toluene	40	30 min	70	20	30

CONCLUSIONS

In conclusion, we highlighted the microwave-assisted synthesis as a tool that can speed up the synthesis of quinoxaline 1,4-di-*N*-oxide derivatives through drastic reduction in reaction time, fewer byproducts formation due to increased selectivity and higher yields. Specifically for the synthesis of QdNO-*N*-acylhydrazones derivatives, the use of benzofuroxan derivatives without electrophilic sites besides the *N*-oxide nitrogen has an important role in the Beirut reaction in order to avoid byproducts formation. Four synthetic methods were tested and different benzofuroxan derivatives were employed. The reaction, which was performed using benzofuroxan derivatives with an aldehyde or an *N*-acylhydrazone subunit, had not successful in obtaining the final compound. By the other hand, the reaction that was carried out using a dioxolan-benzofuroxan derivative and microwave-assisted synthesis resulted in the formation of the desired hybrid compound with good yield (66%) and short reaction time (30 min).

EXPERIMENTAL

Microwave-organic synthesis was carried out in a Microwave synthesizer Discover SP (CEM Corporation®). Melting points (mp) were measured using a Mettler FP82+FP80 apparatus (Greifense, Switzerland). Infrared spectroscopy (KBr disc) were performed on a Nicolet Nexu FTIR Thermo® spectrometer, and the frequencies are expressed in cm^{-1}. Elemental analyses (C, H and N) were performed on a Perkin-Elmer model 2400 analyzer and the data were within ± 0.4% of the theoretical values. The NMR for ^{1}H and ^{13}C of all compounds were recorded on a Bruker 400 Ultrashield^{TM} $^{13}C/^{1}H$ (400-MHz) NMR spectrometer using deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide (DMSO-d_6) as

solvent. Chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane and coupling constants (*J*) values are given in Hertz (Hz). Signal multiplicities are represented as singlet (s), doublet (d), doublet of doublet (dd), and multiplet (m). The reaction progress of all compounds was monitored by thin-layer chromatography (TLC), which was performed on 2.0- by 6.0-cm^2 aluminum sheets precoated with silica gel 60 (HF-254; Merck) to a thickness of 0.25 mm and revealed under UV light (265 nm). Purification procedures were performed on a chromatography column with silica gel (60 Å pore size, 35-75-µm particle size) and the following solvents were used as mobile phase: methanol, ethyl acetate, dichloromethane and hexane.

All compounds were analyzed by HPLC, and their purity was confirmed to be greater than 98.5%. HPLC conditions: Shimadzu HPLC model CBM 20-A (Shimadzu®) equipped with UV-VIS detector (model SPD-20A), quaternary pumping system mobile phase (model LC-20AT), solvent degasser (model DGU-20As) and a Agilent® Eclipse XDB C-18 column (250mm x 27 4,6mm; 5µm). For HPLC method it was used an isocratic flow [methanol:water (75:25)].

Reagents and solvents were purchased from commercial suppliers and used as received. Isonicotinohydrazide, benzoylacetonitrile **10** and 6-methylbenzofuroxan **7** were purchased commercially. Compounds **6** and **8** were prepared according to a previously described methodology [24,15].

Synthetic route A

General procedure for preparation of compound 3

Conventional synthesis. Compound **8** (0.3 g; 1.06 mmol) was dissolved in dichloromethane (15 mL) and then cooled by placing it on ice batch. Next, benzoylacetonitrile (0.15 g; 1.06 mmol) was added in small portions and K_2CO_3 (0.18g; 1.32 mmol) was added as base. The reaction mixture was stirred at 40 ºC for 48 hours, depending on the benzofuroxan derivative used. After the reaction time, the solvent was evaporated under reduced pressure and the obtained oil or solid was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a complex black oil mixture. The desired compound was not obtained.

Microwave-assisted synthesis. Compound **8** (0.5 g; 1.7 mmol) was dissolved in toluene (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice batch. Next, benzoylacetonitrile (0.25 g; 1.7 mmol) was added in small portions and triethylamine (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at room temperature for 15 minutes, and after that, the reaction mixture was inserted in a microwave synthesizer and then subject to an optimized method: microwave irradiation at 70 W for 30 minutes, keeping the temperature at 40 ºC. Once the reaction time finished, the solvent was eliminated under reduced pressure. A black oil was obtained and it was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a black oil. The desired compound was not obtained.

Synthetic route B

General procedure for preparation of compound 11

Conventional synthesis. Compound **6** (0.3 g; 1.06 mmol) was dissolved in dichloromethane (15 mL) and then cooled by placing it on ice batch. Next, benzoylacetonitrile (0.15 g; 1.06 mmol) was added in small portions and K_2CO_3 (0.18g; 1.32 mmol) was added as base. The reaction mixture was stirred at 40 ºC for 48 hours, depending on the benzofuroxan derivative used. After the reaction time, the solvent was evaporated under reduced pressure and the obtained oil or solid was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a complex black oil mixture. The desired compound was not obtained.

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Synthetic route C

General procedure for preparation of compound 12

Conventional synthesis. Compound **7** (0.3 g; 1.06 mmol) was dissolved in dichloromethane (15 mL) and then cooled by placing it on ice batch. Next, benzoylacetonitrile (0.15 g; 1.06 mmol) was added in small portions and K_2CO_3 (0.18g; 1.32 mmol) was added as base. The reaction mixture was stirred at 40 ºC for 48 hours, depending on the benzofuroxan derivative used. After the reaction time, the solvent was evaporated under reduced pressure and the obtained solid was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a yellow powder. The obtained yellow solid was purified by silica gel column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give the appropriate compound **12** as a yellow powder.

Microwave-assisted synthesis. Compound **7** (0.5 g; 1.7 mmol) was dissolved in toluene (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice batch. Next, benzoylacetonitrile (0.25 g; 1.7 mmol) was added in small portions and triethylamine (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at room temperature for 15 minutes, and after that, the reaction mixture was inserted in a microwave synthesizer and then subject to an optimized method: microwave irradiation at 70 W for 30 minutes, keeping the temperature at 40 ºC. Once the reaction time finished, the solvent was eliminated under reduced pressure. A black oil or a solid was obtained and it was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a yellow solid. The obtained yellow solid was purified by silica

gel column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give the appropriate compound **12** as a yellow powder.

3-cyano-6-methyl-2-phenylquinoxaline 1,4-di-N-oxide (12). Yellow powder; yield, 26%; mp, 188 to 190 °C. IR V_{max} (cm^{-1}; KBr pellets): 3065 (C-H aromatic), 2241 (CN nitrile), 1347 (N-O). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ: 8.58 (1H, m, Ar-H), 8.40 (1H, s, Ar-H), 7.81 (1H, d, J = 2.3 Hz, Ar-H), 7.74 (2H, m, Ar-H), 7.61 (3H, m, Ar-H), 2.67 (3H, s, 13-CH_3) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm) δ: 144.9, 136.6, 132.0, 130.4, 129.3, 127.0, 126.9, 121.3 (2C), 120.8, 120.5, 120.0 (2C), 110.5, 22.2 ppm. *Anal. Calcd.* (%) for $C_{16}H_{11}N_3O_2$: C: 69.31; H: 4.00; N: 15.15. Found: C: 69.54; H: 4.10; N: 15.41.

General procedure for preparation of compound 11

Conventional synthesis. Compound **12** (0.35 g; 1.26 mmol) and selenium dioxide (0.14 g; 1.26 mmol) were dissolved in ethyl acetate (10 mL). The mixture reaction was stirred under reflux for 24 hours. No changes in TLC were observed after the reaction time.

Microwave-assisted synthesis. Compound **12** (0.35 g; 1.26 mmol) and selenium dioxide (0.14 g; 1.26 mmol) were dissolved in ethyl acetate (10 mL) in a microwave vessel (35 mL). The mixture reaction was stirred at room temperature for 15 minutes and then placed in a microwave reactor. The mixture was then subjected to microwave irradiation at 200 W for 1 hour at 70 ºC. No changes in TLC were observed after the reaction time.

Synthetic route D

General procedure for preparation of compound 9. A mixture of **6** (0.8 g; 4.8 mmol), ethylene glycol (2 mL; 35 mmol), *p*-toluenesulfonic acid (0.14 g; 2%) and toluene (15 mL) was stirred under reflux for 12 hours. After the reaction time, the solvent was eliminated under reduced pressure. The obtained oil was dissolved in 50 mL of dichloromethane and washed with saturated aqueous $NaHCO_3$, water and brine. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a brown solid. The obtained solid was purified by silica gel column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give compound **9** as a yellow powder.

6-(1,3-dioxolan-2-yl)benzo[c][1,2,5]oxadiazole 1-oxide (9). Yellow powder; yield, 85%; mp, 62 to 63 °C. IR V_{max} (cm^{-1}; KBr pellets): 3065 (C-H aromatic), 1359 (N-O), 1078 (C-O ether). ^1H NMR (400 MHz, $CDCl_3$, δ ppm) δ: 7.47 (3H, m, Ar-H), 5.80 (1H, s, 11-CH), 4.09 (4H, m, 14-CH, 15-CH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) δ: 102.4, 65.9 (2C) ppm. *Anal. Calcd.* (%) for $C_9H_8N_2O_4$: C: 51.93; H: 3.87; N: 13.46. Found: C: 52.30; H: 3.91; N: 13.54.

General procedure for preparation of compound 13

Conventional synthesis. Compound **9** (0.3 g; 1.06 mmol) was dissolved in dichloromethane (15 mL) and then cooled by placing it on ice batch. Next, benzoylacetonitrile (0.15 g; 1.06 mmol) was added in small portions and K_2CO_3 (0.18g; 1.32 mmol) was added as base. The reaction mixture was stirred at 40 ºC for 96 hours, depending on the benzofuroxan derivative used. After the reaction time, the solvent was

evaporated under reduced pressure and the obtained solid was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a yellow powder. The obtained yellow solid was purified by silica gel column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give the appropriate compound **13** as a yellow powder.

Microwave-assisted synthesis. Compound **9** (0.5 g; 1.7 mmol) was dissolved in toluene (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice batch. Next, benzoylacetonitrile (0.25 g; 1.7 mmol) was added in small portions and triethylamine (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at room temperature for 15 minutes, and after that, the reaction mixture was inserted in a microwave synthesizer and then subject to an optimized method: microwave irradiation at 70 W for 30 minutes, keeping the temperature at 40 ºC. Once the reaction time finished, the solvent was eliminated under reduced pressure. A black oil or a solid was obtained and it was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure giving a yellow solid. The obtained yellow solid was purified by silica gel column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give the appropriate compound **13** as a yellow powder.

3-cyano-6-(1,3-dioxolan-2-yl)-2-phenylquinoxaline 1,4-di-N-oxide (13). Yellow powder; yield, 30%; mp, 158 to 159 °C. IR V_{max} (cm^{-1}; KBr pellets): 3089 (C-H aromatic), 2235 (CN nitrile), 1335 (N-O), 1098 (C-O ether). ^1H NMR (400 MHz,

DMSO-d_6, δ ppm) δ: 8.55 (2H, d, J = 9.1 Hz, Ar-H), 8.11 (1H, dd, J = 28.4 Hz, Ar-H), 7.74 (2H, s, Ar-H), 7.63 (3H, m, Ar-H), 6.10 (1H, s, 11-CH), 4.10 (4H, m, 22-CH, 23-CH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm) δ: 144.1, 143.0, 139.2, 131.0, 130.6, 130.1 (2C), 128.9, 128.5 (2C), 127.7, 126.9, 121.0, 117.1, 101.0, 65.2 (2C) ppm. *Anal. Calcd.* (%) for $C_{18}H_{13}N_3O_4$: C: 64.48; H: 3.91; N: 12.53. Found: C: 64.58; H: 4.11; N: 12.82.

General procedure for preparation of compound 11. Compound **13** (0.2 g; 0.6 mmol) was dissolved in acetone (15 mL). Next, hydrochloric acid (0.3 mL) was added dropwise. The reaction mixture was stirred at room temperature for 48 hours. After the reaction time, the solvent was evaporated under reduced pressure and dissolved in 30 mL of dichloromethane and washed with saturated aqueous $NaHCO_3$, water and brine. Next, the solvent was dried with anhydrous Na_2SO_4 and evaporated under reduced pressure giving an orange solid **11**. The obtained powder was used in the next reaction without further purification.

General procedure for preparation of compound 3. Compound **11** (0.15 g; 0.52 mmol) was dissolved in 20 mL of ethanol and then, acetic acid was added dropwise until the solution reached pH 5. The reaction mixture was stirred for 15 minutes. Next, isonicotinohydrazide (0.077 g; 0.56 mmol) was added. The reaction mixture was stirred at room temperature for 12 hours. After the reaction time, an orange solid was precipitated and it was filtered and washed with cold ethanol. The obtained orange solid

was purified by silica gel column chromatography using dichloromethane and methanol (95:5 v/v) as eluent to give compound **3** as an orange powder.

(E)-3-cyano-6-((2-isonicotinoylhydrazono)methyl)-2-phenylquinoxaline 1,4-di-N-oxide (3). Orange powder; yield, 66%; mp, 243 to 244 °C. IR V_{max} (cm^{-1}; KBr pellets): 3284 (N-H), 3084 (C-H aromatic), 1702 (C=O amide), 1675 (C=N imine), 1608 (N-N), 1347 (N-O), 1314 (C-N aromatic). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) δ: 12.52 (1H, s, NH), 8.81 (3H, d, *J* = 10.2 Hz, Ar-H), 8.73 (1H, s, 11-CH), 8.60 (1H, t, *J* = 8.2 Hz, Ar-H), 8.48 (1H, m, Ar-H), 7.86 (2H, m, Ar-H), 7.75 (2H, m, Ar-H), 7.64 (3H, m, Ar-H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, δ ppm) δ: 162.1, 150.4 (2C), 146.0, 145.9, 139.4, 137.3, 131.1, 130.9, 130.1 (2C), 129.9, 128.6 (2C), 127.6, 125.0, 121.6 (2C), 120.9, 119.1 ppm. *Anal. Calcd.* (%) for $C_{22}H_{14}N_6O_3$: C: 64.39; H: 3.44; N: 20.48. Found: C: 64.58; H: 3.62; N: 20.64.

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