

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

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Abstract

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

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

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INTRODUCTION

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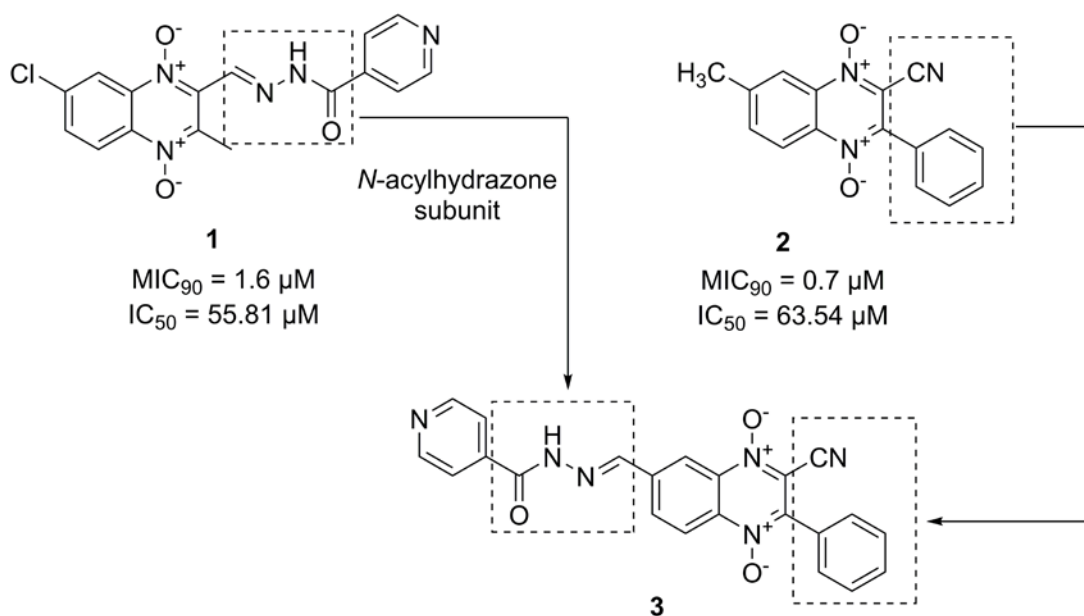
49 Quinoxaline 1,4-di-*N*-oxide (QdNO) represents an important class of *N*-oxide compounds
50 with a wide range of biological activities, such as, antibacterial, antitubercular, antiviral,
51 anticancer, antifungal and anthelmintic [1]. The wide spectrum of biological activities of
52 QdNO derivatives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

57 From a phenotypic-based screening against *Mycobacterium tuberculosis* (MTB)
58 containing more than five thousand compounds of our current library, we have identified
59 the compound 3-cyano-6-methyl-2-phenylquinoxaline 1,4-dioxide **2** (MIC₉₀ = 0.7 μM) in
60 a series of 3-aryl-quinoxaline-2-carbonitrile 1,4-di-*N*-oxide derivatives [10] as a
61 promising scaffold for molecular modifications. Furthermore, we also have reported a
62 series of quinoxaline 1,4-di-*N*-oxide derivatives containing the *N*-acylhydrazone subunit
63 with potent antitubercular activity. The compound (*E*)-6-chloro-3-((2-
64 isonicotinoylhydrazono)methyl)-2-methylquinoxaline 1,4-dioxide **1** showed MIC₉₀ value
65 of 1.6 μM against *Mycobacterium tuberculosis* H₃₇Rv strain and IC₅₀ value of 55 μM
66 against VERO cell lines [11]. *N*-acylhydrazone (NAH) subunit also represents an
67 important scaffold in the medicinal chemistry due to its wide spectrum of biological
68 activities [12–15]. Several NAH derivatives has been described with potent antitubercular
69 activity against MTB H₃₇Rv and multi-drug resistant strains [16,17,14]. Thus, we have

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

72

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



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

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RESULTS AND DISCUSSION

91 We have evaluated the use of four different benzofuroxan derivatives using conventional
92 and microwave-assisted synthesis in order to outline a comparative between these two
93 synthetic methodologies and the best benzofuroxan derivative to obtain the desired
94 hybrid quinoxaline. The aldehyde-benzofuroxan derivative **6** (Synthetic Route B) was
95 obtained from 4-chloro-3-nitrobenzaldehyde **4** as previously described [24]. Next, we
96 obtained the benzofuroxan-*N*-acylhydrazone derivative **8** from compound **6** (Synthetic
97 Route A) as already reported [15] and the dioxolan-benzofuroxan **9** (Synthetic Route D)
98 through an aldehyde protection reaction. The 6-methylbenzo[*c*][1,2,5]oxadiazole 1-oxide
99 **7** (Synthetic Route C) was purchased commercially (Scheme 2). Moreover, we have used
100 different catalysts and solvents in both methodologies (conventional and MW),
101 considering the different reaction conditions that microwave-assisted synthesis requires
102 [25,26].

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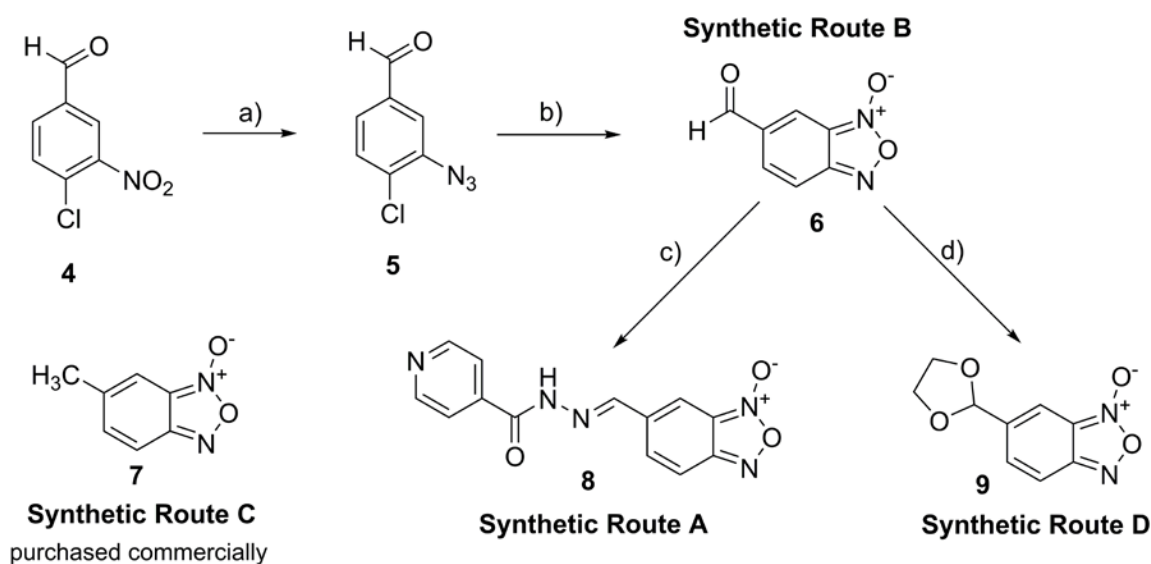
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110 **Scheme 2.** Synthetic methodologies to obtain the different benzofuroxan derivatives.

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

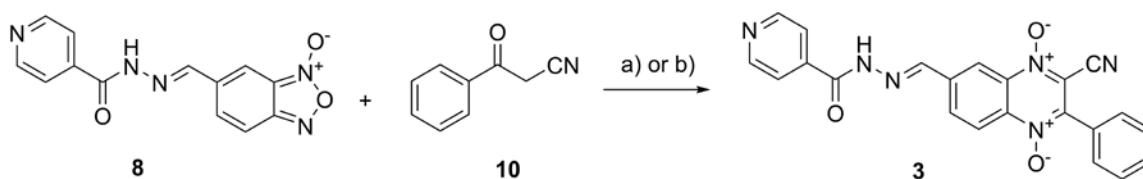
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113 **Synthetic Route A**

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

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Scheme 3. General procedure for synthetic route A.

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

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Synthetic Route B

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After the failure of synthetic route A, a second alternative was planned. In the synthetic

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route B, the Beirut reaction was carried out between a benzofuroxan derivative **6**

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containing an aldehyde function at position 6 and benzoylacetonitrile **10**, which would

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lead the formation of intermediate quinoxaline **11**. Next, compound **11** would be reacted

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with isonicotinohydrazide in order to obtain the hybrid quinoxaline **3** (Scheme 4). Once

133

again, the Beirut reaction generated an even more complex black-oil mixture than the

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previous synthetic route. At this point, we realized that a more selective synthetic route

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and the use of a benzofuroxan derivative with less electrophilic sites would be necessary.

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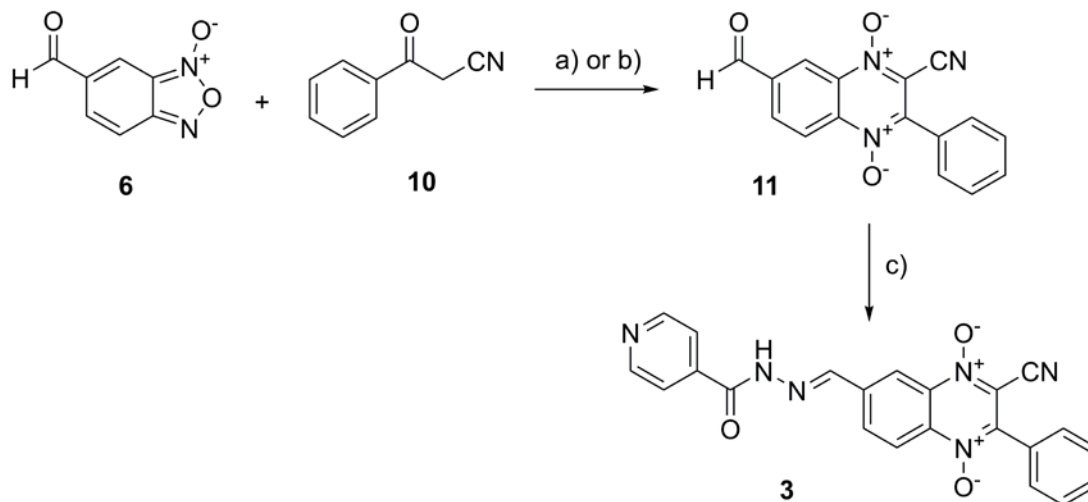
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Scheme 4. General procedure for synthetic route B.

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

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146 **Synthetic Route C**

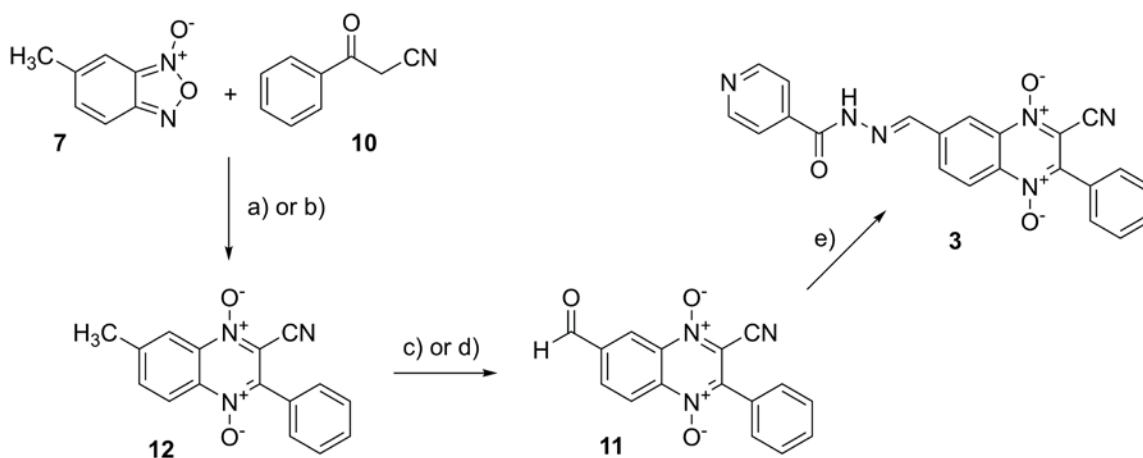
147 Therefore, the synthetic route C was designed in order to improve the selectivity of
 148 Beirut reaction. A benzofuroxan derivative **7** containing a methyl group at position 6
 149 (Scheme 5) was selected for the Beirut reaction with benzoylacetonitrile **10** leading the
 150 formation of the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



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

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167 Synthetic Route D

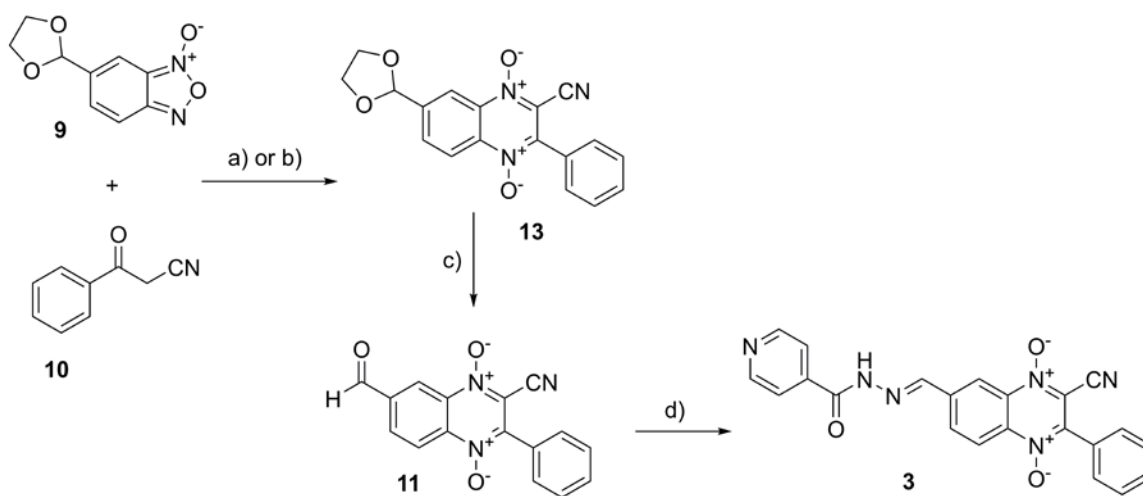
168 Considering the issues involved in synthetic route A - C, a benzofuroxan derivative
 169 without additional electrophilic sites besides the *N*-oxide nitrogen was selected for
 170 synthetic route D. The dioxolan-benzofuroxan derivative **9** was exploited because the
 171 aldehyde group remains 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**

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

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Scheme 6. General procedure for synthetic route D.



183

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

184

185 Synthetic Conditions

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

191 (KF/Al₂O₃) 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 K₂CO₃ 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

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

218 We also evaluated the increase of temperature in all synthetic routes. Temperature above
219 40 °C led to an increase in formation of byproducts in both synthetic methods. Regarding
220 synthetic route A and B, we also carried out both methods at room temperature in order
221 to evaluate whether the byproducts formation would be reduced, however, the same
222 complex black-oil mixture was observed.

223

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

Synthetic Method	Synthetic Route	Catalyst	Solvent	Temperature (°C)	Time	MW Potency (W)	MW Pressure (psi)	Yield (%)
Conventional	A	K ₂ CO ₃	CHCl ₃	40	48 h	-	-	Not obtained
	B	K ₂ CO ₃	CHCl ₃	40	48 h	-	-	Not obtained
	C	K ₂ CO ₃	CHCl ₃	40	48 h	-	-	12
	D	K ₂ CO 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

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CONCLUSIONS

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

244

245

EXPERIMENTAL

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

254 solvent. Chemical shifts were expressed in parts per million (ppm) relative to
255 tetramethylsilane and coupling constants (*J*) values are given in Hertz (Hz). Signal
256 multiplicities are represented as singlet (s), doublet (d), doublet of doublet (dd), and
257 multiplet (m). The reaction progress of all compounds was monitored by thin-layer
258 chromatography (TLC), which was performed on 2.0- by 6.0-cm² aluminum sheets
259 precoated with silica gel 60 (HF-254; Merck) to a thickness of 0.25 mm and revealed
260 under UV light (265 nm). Purification procedures were performed on a chromatography
261 column with silica gel (60 Å pore size, 35-75-µm particle size) and the following solvents
262 were used as mobile phase: methanol, ethyl acetate, dichloromethane and hexane.

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

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

273

274 **Synthetic route A**

275 **General procedure for preparation of compound 3**

276 **Conventional synthesis.** Compound **8** (0.3 g; 1.06 mmol) was dissolved in
277 dichloromethane (15 mL) and then cooled by placing it on ice batch. Next,
278 benzoylacetonitrile (0.15 g; 1.06 mmol) was added in small portions and K₂CO₃ (0.18g;
279 1.32 mmol) was added as base. The reaction mixture was stirred at 40 °C for 48 hours,
280 depending on the benzofuroxan derivative used. After the reaction time, the solvent was
281 evaporated under reduced pressure and the obtained oil or solid was dissolved in 50 mL
282 of ethyl acetate and washed with water. The organic phase was dried with anhydrous
283 Na₂SO₄ and the solvent was evaporated under reduced pressure giving a complex black
284 oil mixture. The desired compound was not obtained.

285 **Microwave-assisted synthesis.** Compound **8** (0.5 g; 1.7 mmol) was dissolved in toluene
286 (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice batch. Next,
287 benzoylacetonitrile (0.25 g; 1.7 mmol) was added in small portions and triethylamine
288 (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at
289 room temperature for 15 minutes, and after that, the reaction mixture was inserted in a
290 microwave synthesizer and then subject to an optimized method: microwave irradiation
291 at 70 W for 30 minutes, keeping the temperature at 40 °C. Once the reaction time
292 finished, the solvent was eliminated under reduced pressure. A black oil was obtained
293 and it was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase
294 was dried with anhydrous Na₂SO₄ and the solvent was evaporated under reduced pressure
295 giving a black oil. The desired compound was not obtained.

296

297 **Synthetic route B**

298 **General procedure for preparation of compound 11**

299 **Conventional synthesis.** Compound **6** (0.3 g; 1.06 mmol) was dissolved in
300 dichloromethane (15 mL) and then cooled by placing it on ice batch. Next,
301 benzoylacetone (0.15 g; 1.06 mmol) was added in small portions and K_2CO_3 (0.18g;
302 1.32 mmol) was added as base. The reaction mixture was stirred at 40 °C for 48 hours,
303 depending on the benzofuroxan derivative used. After the reaction time, the solvent was
304 evaporated under reduced pressure and the obtained oil or solid was dissolved in 50 mL
305 of ethyl acetate and washed with water. The organic phase was dried with anhydrous
306 Na_2SO_4 and the solvent was evaporated under reduced pressure giving a complex black
307 oil mixture. The desired compound was not obtained.

308 **Microwave-assisted synthesis.** Compound **6** (0.5 g; 1.7 mmol) was dissolved in toluene
309 (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice batch. Next,
310 benzoylacetone (0.25 g; 1.7 mmol) was added in small portions and triethylamine
311 (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at
312 room temperature for 15 minutes, and after that, the reaction mixture was inserted in a
313 microwave synthesizer and then subject to an optimized method: microwave irradiation
314 at 70 W for 30 minutes, keeping the temperature at 40 °C. Once the reaction time
315 finished, the solvent was eliminated under reduced pressure. A black oil was obtained
316 and it was dissolved in 50 mL of ethyl acetate and washed with water. The organic phase
317 was dried with anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure
318 giving a black oil. The desired compound was not obtained.

319

320 **Synthetic route C**

321 **General procedure for preparation of compound 12**

322 **Conventional synthesis.** Compound **7** (0.3 g; 1.06 mmol) was dissolved in
323 dichloromethane (15 mL) and then cooled by placing it on ice bath. Next,
324 benzoylacetonitrile (0.15 g; 1.06 mmol) was added in small portions and K₂CO₃ (0.18g;
325 1.32 mmol) was added as base. The reaction mixture was stirred at 40 °C for 48 hours,
326 depending on the benzofuroxan derivative used. After the reaction time, the solvent was
327 evaporated under reduced pressure and the obtained solid was dissolved in 50 mL of
328 ethyl acetate and washed with water. The organic phase was dried with anhydrous
329 Na₂SO₄ and the solvent was evaporated under reduced pressure giving a yellow powder.
330 The obtained yellow solid was purified by silica gel column chromatography using
331 hexane and ethyl acetate (70:30 v/v) as eluent to give the appropriate compound **12** as a
332 yellow powder.

333 **Microwave-assisted synthesis.** Compound **7** (0.5 g; 1.7 mmol) was dissolved in toluene
334 (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice bath. Next,
335 benzoylacetonitrile (0.25 g; 1.7 mmol) was added in small portions and triethylamine
336 (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at
337 room temperature for 15 minutes, and after that, the reaction mixture was inserted in a
338 microwave synthesizer and then subject to an optimized method: microwave irradiation
339 at 70 W for 30 minutes, keeping the temperature at 40 °C. Once the reaction time
340 finished, the solvent was eliminated under reduced pressure. A black oil or a solid was
341 obtained and it was dissolved in 50 mL of ethyl acetate and washed with water. The
342 organic phase was dried with anhydrous Na₂SO₄ and the solvent was evaporated under
343 reduced pressure giving a yellow solid. The obtained yellow solid was purified by silica

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

346

347 **3-cyano-6-methyl-2-phenylquinoxaline 1,4-di-N-oxide (12)**. Yellow powder; yield,
348 26%; mp, 188 to 190 °C. IR ν_{\max} (cm⁻¹; KBr pellets): 3065 (C-H aromatic), 2241 (CN
349 nitrile), 1347 (N-O). ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm) δ : 8.58 (1H, m, Ar-H), 8.40
350 (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),
351 2.67 (3H, s, 13-CH₃) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆, δ ppm) δ : 144.9, 136.6, 132.0,
352 130.4, 129.3, 127.0, 126.9, 121.3 (2C), 120.8, 120.5, 120.0 (2C), 110.5, 22.2 ppm. *Anal.*
353 *Calcd.* (%) for C₁₆H₁₁N₃O₂: C: 69.31; H: 4.00; N: 15.15. Found: C: 69.54; H: 4.10; N:
354 15.41.

355

356 **General procedure for preparation of compound 11**

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

360 **Microwave-assisted synthesis.** Compound **12** (0.35 g; 1.26 mmol) and selenium dioxide
361 (0.14 g; 1.26 mmol) were dissolved in ethyl acetate (10 mL) in a microwave vessel (35
362 mL). The mixture reaction was stirred at room temperature for 15 minutes and then
363 placed in a microwave reactor. The mixture was then subjected to microwave irradiation
364 at 200 W for 1 hour at 70 °C. No changes in TLC were observed after the reaction time.

365

366 **Synthetic route D**

367 **General procedure for preparation of compound 9.** A mixture of **6** (0.8 g; 4.8 mmol),
368 ethylene glycol (2 mL; 35 mmol), *p*-toluenesulfonic acid (0.14 g; 2%) and toluene (15
369 mL) was stirred under reflux for 12 hours. After the reaction time, the solvent was
370 eliminated under reduced pressure. The obtained oil was dissolved in 50 mL of
371 dichloromethane and washed with saturated aqueous NaHCO₃, water and brine. The
372 organic phase was dried with anhydrous Na₂SO₄ and the solvent was evaporated under
373 reduced pressure giving a brown solid. The obtained solid was purified by silica gel
374 column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give
375 compound **9** as a yellow powder.

376

377 **6-(1,3-dioxolan-2-yl)benzo[*c*][1,2,5]oxadiazole 1-oxide (9).** Yellow powder; yield, 85%;
378 mp, 62 to 63 °C. IR ν_{\max} (cm⁻¹; KBr pellets): 3065 (C-H aromatic), 1359 (N-O), 1078 (C-
379 O ether). ¹H NMR (400 MHz, CDCl₃, δ ppm) δ : 7.47 (3H, m, Ar-H), 5.80 (1H, s, 11-
380 CH), 4.09 (4H, m, 14-CH, 15-CH) ppm. ¹³C NMR (75 MHz, CDCl₃, δ ppm) δ : 102.4,
381 65.9 (2C) ppm. *Anal. Calcd.* (%) for C₉H₈N₂O₄: C: 51.93; H: 3.87; N: 13.46. Found: C:
382 52.30; H: 3.91; N: 13.54.

383

384 **General procedure for preparation of compound 13**

385 **Conventional synthesis.** Compound **9** (0.3 g; 1.06 mmol) was dissolved in
386 dichloromethane (15 mL) and then cooled by placing it on ice bath. Next,
387 benzoylacetone (0.15 g; 1.06 mmol) was added in small portions and K₂CO₃ (0.18g;
388 1.32 mmol) was added as base. The reaction mixture was stirred at 40 °C for 96 hours,
389 depending on the benzofuroxan derivative used. After the reaction time, the solvent was

390 evaporated under reduced pressure and the obtained solid was dissolved in 50 mL of
391 ethyl acetate and washed with water. The organic phase was dried with anhydrous
392 Na₂SO₄ and the solvent was evaporated under reduced pressure giving a yellow powder.
393 The obtained yellow solid was purified by silica gel column chromatography using
394 hexane and ethyl acetate (70:30 v/v) as eluent to give the appropriate compound **13** as a
395 yellow powder.

396 **Microwave-assisted synthesis.** Compound **9** (0.5 g; 1.7 mmol) was dissolved in toluene
397 (10 mL) in a microwave vessel (35 mL). The mixture was cooled with ice batch. Next,
398 benzoylacetonitrile (0.25 g; 1.7 mmol) was added in small portions and triethylamine
399 (0.35 mL; 55 mmol) was added dropwise as base. The reaction mixture was stirred at
400 room temperature for 15 minutes, and after that, the reaction mixture was inserted in a
401 microwave synthesizer and then subject to an optimized method: microwave irradiation
402 at 70 W for 30 minutes, keeping the temperature at 40 °C. Once the reaction time
403 finished, the solvent was eliminated under reduced pressure. A black oil or a solid was
404 obtained and it was dissolved in 50 mL of ethyl acetate and washed with water. The
405 organic phase was dried with anhydrous Na₂SO₄ and the solvent was evaporated under
406 reduced pressure giving a yellow solid. The obtained yellow solid was purified by silica
407 gel column chromatography using hexane and ethyl acetate (70:30 v/v) as eluent to give
408 the appropriate compound **13** as a yellow powder.

409

410 **3-cyano-6-(1,3-dioxolan-2-yl)-2-phenylquinoxaline 1,4-di-N-oxide (13).** Yellow
411 powder; yield, 30%; mp, 158 to 159 °C. IR ν_{\max} (cm⁻¹; KBr pellets): 3089 (C-H
412 aromatic), 2235 (CN nitrile), 1335 (N-O), 1098 (C-O ether). ¹H NMR (400 MHz,

413 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),
414 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-
415 CH) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆, δ ppm) δ : 144.1, 143.0, 139.2, 131.0, 130.6,
416 130.1 (2C), 128.9, 128.5 (2C), 127.7, 126.9, 121.0, 117.1, 101.0, 65.2 (2C) ppm. *Anal.*
417 *Calcd.* (%) for C₁₈H₁₃N₃O₄: C: 64.48; H: 3.91; N: 12.53. Found: C: 64.58; H: 4.11; N:
418 12.82.

419

420 **General procedure for preparation of compound 11.** Compound **13** (0.2 g; 0.6 mmol)
421 was dissolved in acetone (15 mL). Next, hydrochloric acid (0.3 mL) was added dropwise.
422 The reaction mixture was stirred at room temperature for 48 hours. After the reaction
423 time, the solvent was evaporated under reduced pressure and dissolved in 30 mL of
424 dichloromethane and washed with saturated aqueous NaHCO₃, water and brine. Next, the
425 solvent was dried with anhydrous Na₂SO₄ and evaporated under reduced pressure giving
426 an orange solid **11**. The obtained powder was used in the next reaction without further
427 purification.

428

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

435 was purified by silica gel column chromatography using dichloromethane and methanol
436 (95:5 v/v) as eluent to give compound **3** as an orange powder.

437

438 *(E)-3-cyano-6-((2-isonicotinoylhydrazono)methyl)-2-phenylquinoxaline 1,4-di-N-oxide*

439 (**3**). Orange powder; yield, 66%; mp, 243 to 244 °C. IR ν_{\max} (cm⁻¹; KBr pellets): 3284

440 (N-H), 3084 (C-H aromatic), 1702 (C=O amide), 1675 (C=N imine), 1608 (N-N), 1347

441 (N-O), 1314 (C-N aromatic). ¹H NMR (400 MHz, DMSO-*d*₆, δ ppm) δ : 12.52 (1H, s,

442 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-

443 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)

444 ppm. ¹³C NMR (75 MHz, DMSO-*d*₆, δ ppm) δ : 162.1, 150.4 (2C), 146.0, 145.9, 139.4,

445 137.3, 131.1, 130.9, 130.1 (2C), 129.9, 128.6 (2C), 127.6, 125.0, 121.6 (2C), 120.9, 119.1

446 ppm. *Anal. Calcd.* (%) for C₂₂H₁₄N₆O₃: C: 64.39; H: 3.44; N: 20.48. Found: C: 64.58; H:

447 3.62; N: 20.64.

448

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