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# Antitrypanosomatid Pharmacomodulation at Position 3 of the 8-Nitroquinolin-2(1*H*)-one Scaffold Using Palladium-Catalysed Cross-Coupling Reactions

Julien Pedron<sup>+, [a]</sup>, Clotilde Boudot<sup>+, [b]</sup>, Sandra Bourgeade-Delmas,<sup>[c]</sup> Alix Sournia-Saquet,<sup>[a]</sup> Lucie Paloque,<sup>[a]</sup> Maryam Rastegari,<sup>[a]</sup> Mansour Abdoulaye,<sup>[a]</sup> Hussein El-Kashef,<sup>[d]</sup> Colin Bonduelle,<sup>[a]</sup> Geneviève Pratviel,<sup>[a]</sup> Susan Wyllie,<sup>[e]</sup> Alan H. Fairlamb,<sup>[e]</sup> Bertrand Courtioux,<sup>[b]</sup> Pierre Verhaeghe,<sup>\*, [a]</sup> and Alexis Valentin<sup>[c]</sup>

An antikinoplastid pharmacomodulation study at position 3 of the recently described hit molecule 3-bromo-8-nitroquinolin-2(1*H*)-one was conducted. Twenty-four derivatives were synthesised using the Suzuki–Miyaura cross-coupling reaction and evaluated in vitro on both *Leishmania infantum* axenic amastigotes and *Trypanosoma brucei brucei* trypomastigotes. Introduction of a *para*-carboxyphenyl group at position 3 of the scaffold led to the selective antitrypanosomal hit molecule 3-(4-carboxyphenyl)-8-nitroquinolin-2(1*H*)-one (**21**) with a lower

reduction potential (−0.56 V) than the initial hit (−0.45 V). Compound **21** displays micromolar antitrypanosomal activity (IC<sub>50</sub> = 1.5 μM) and low cytotoxicity on the human HepG2 cell line (CC<sub>50</sub> = 120 μM), having a higher selectivity index (SI = 80) than the reference drug eflornithine. Contrary to results previously obtained in this series, hit compound **21** is inactive toward *L. infantum* and is not efficiently bioactivated by *T. brucei brucei* type I nitroreductase, which suggests the existence of an alternative mechanism of action.

## Introduction

Kinetoplastids are flagellated protozoan parasites responsible for lethal neglected tropical diseases such as human African trypanosomiasis (HAT) and visceral leishmaniasis (VL). They are characterised by the presence of a circular piece of DNA, called a kinetoplast, adjacent to the flagellar basal body. *Trypanosoma* parasites are transmitted by the bite of an infected sandfly and are the causative agents of HAT, also known as sleeping sickness. There are many species of *Trypanosoma*, but only *T. brucei gambiense* and *T. brucei rhodesiense* are responsible for HAT.<sup>[1]</sup> During the process of infection, metacyclic trypomasti-

gotes enter the blood circulation from the bite of the tsetse fly and spread throughout the whole organism, where they differentiate into bloodstream trypomastigotes; this is the hemolymphatic stage. After several weeks or months of symptoms such as headaches, anaemia and hepatosplenomegaly, trypomastigotes cross the blood–brain barrier and cause damage to the central nervous system, leading to sleeping disorders, behavioural disorders, seizures, coma and finally death: this is the meningoencephalic stage.<sup>[2]</sup> *Leishmania*, which is responsible for leishmaniasis, is another protozoan parasite and is transmitted by the bite of an infected sandfly. *L. donovani* and *L. infantum* are the two major species causing the most severe form of the disease: VL.<sup>[3]</sup> In brief, metacyclic promastigotes penetrate the skin during the blood meal of an infected sandfly. They are internalised by mononuclear phagocytic cells such as macrophages, in which they differentiate into amastigotes. The parasites continue to multiply until the destruction of these cells and disseminate into many organs, such as liver and spleen, leading to death.<sup>[4]</sup>

It is estimated that more than 1 billion people are at risk of contracting HAT and VL and that each year there are 55 000 new cases with more than 25 000 deaths from these diseases.<sup>[5,6]</sup> These numbers are likely underestimated because of the difficulty in accessing rural areas and the unspecific symptoms in the early stages of the diseases. Currently, there are few efficient and safe drugs available on the market against these neglected tropical diseases. Pentamidine and suramin are used for the treatment of the first stage of HAT but these drugs are highly toxic and require the patient to be hospitalised for i.v.

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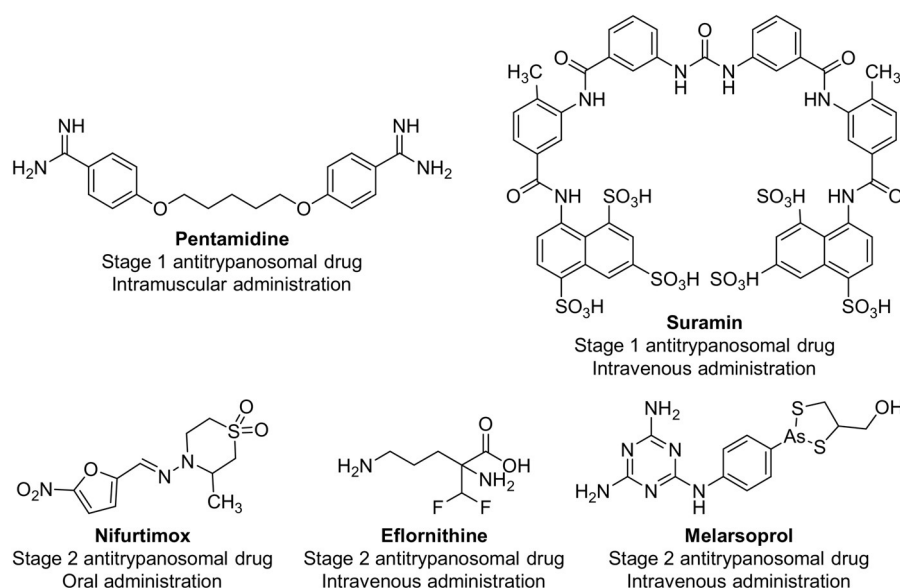


Figure 1. Structure, indication and route of administration of antitrypanosomal drugs on the market.

administration.<sup>[7]</sup> Melarsoprol, an arsenic-containing drug, and a combination of eflornithine and nifurtimox are suitable for the second stage of HAT (Figure 1).<sup>[8]</sup> The same is true of VL, for which only amphotericin B, miltefosine, antimonial derivatives, pentamidine and paromomycin are available. Among these drugs, miltefosine is the only orally available drug. These drugs are either expensive (e.g., liposomal amphotericin B), present severe side effects (e.g., the nephrotoxicity of amphotericin B, the teratogenicity of miltefosine) or show an increasing lack of efficacy due to the emergence of resistant parasites (antimony derivatives and miltefosine).<sup>[9]</sup> This global issue calls for the discovery of new antikinoplastid molecules.

Unfortunately, today, there are only two new chemical entities in clinical trials against HAT and none against VL (Figure 2).<sup>[10]</sup> Acoziborole is an orally active benzoxaborole in phase IIb/III of clinical trials; it is active against both stages of HAT.<sup>[11]</sup> Fexinidazole, a 5-nitroimidazole, was recently in phase II of clinical trials against VL but showed a lack of efficacy, whereas it progressed to a phase IIIb study against HAT.<sup>[12,13]</sup> This molecule is rapidly metabolised in vivo into two metabolites (a

sulfoxide and a sulfone derivative) which are active against the *Trypanosoma* parasites. Fexinidazole is selectively bioactivated by type I parasitic nitroreductases (NTRs) leading, through a successive two-electron reduction, to cytotoxic electrophilic metabolites such as nitroso and hydroxylamine derivatives.<sup>[14]</sup> Two NTRs have been identified in *Leishmania* (NTR1 and NTR2)<sup>[15,16]</sup> and only one in *Trypanosoma*.<sup>[17]</sup> These nitroreductases are absent from mammalian cells. Therefore, substrates of these enzymes could be selective antikinoplastid candidates. Unfortunately, no X-ray structure of these parasitic NTRs is available, which restricts the use of most classical rational medicinal chemistry approaches, such as docking, for the design of new substrates of these enzymes.

Our research team has been working on the synthesis of new antikinoplastid molecules for several years. Starting from a chemical study on 2-substituted nitroquinoline derivatives with antiparasitic potential,<sup>[18]</sup> we identified a new antileishmanial hit: 8-nitroquinolin-2(1*H*)-one.<sup>[19]</sup> Pharmacomodulation studies at position 4 of this scaffold were then realised.<sup>[20,21]</sup> Recently, we described a comprehensive electrochemistry-guided study of this pharmacophore and the development of a computational model able to predict the redox potentials of each molecule in the series.<sup>[22]</sup> Thus, a new antikinoplastid hit molecule was identified (Figure 3). This molecule was not genotoxic in a comet assay and was selectively bioactivated by type 1 NTRs of *L. donovani* and *T. brucei brucei*.<sup>[22]</sup>

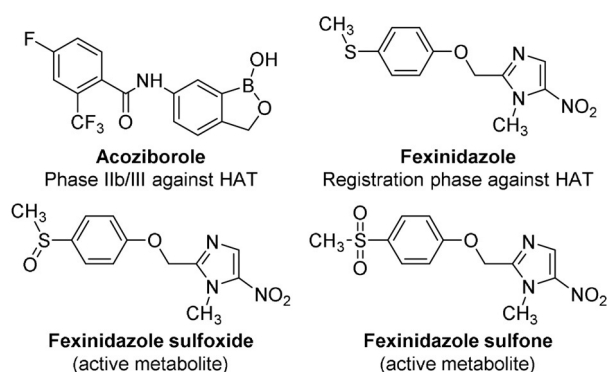


Figure 2. Molecular structure of drug candidates acoziborole and fexinidazole (with its active metabolites).

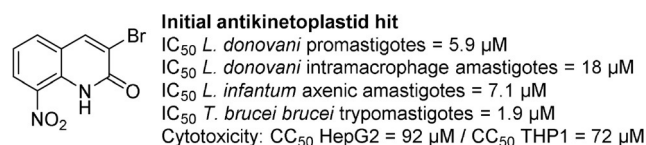


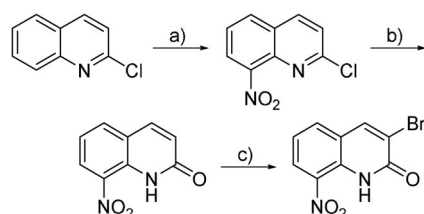
Figure 3. Structure and biological profile of the previously identified antikinoplastid hit 3-bromo-8-nitroquinolin-2(1*H*)-one.

Here, we present a pharmacomodulation study at position 3 of the scaffold, using Suzuki–Miyaura or Sonogashira cross-coupling reactions. To explore structure–activity relationships, 26 molecules were synthesised and evaluated on both *L. infantum* axenic amastigotes and *T. brucei brucei* trypomastigotes. These molecules were also assessed for their cytotoxicity on the HepG2 human cell line.

## Results and Discussion

### Chemistry

3-Bromo-8-nitroquinolin-2(1*H*)-one was prepared in three steps, as presented in Scheme 1.<sup>[22]</sup> Nitration of 2-chloroquinoline mainly led to the intermediate 2-chloro-8-nitroquinoline, which was transformed in a second step into the corresponding lactam, according to a previously reported procedure.<sup>[23]</sup> 8-Nitroquinolin-2(1*H*)-one was finally selectively halogenated at



**Scheme 1.** Synthesis of the initial antikinetoplastid hit. a)  $\text{H}_2\text{SO}_4$ ,  $\text{HNO}_3$ , RT, 2 h, 50%; b)  $\text{CH}_3\text{CN}$ ,  $\text{HClO}_4$ , 100 °C, 72 h, 96%; c)  $\text{NaBrO}_3$ ,  $\text{HBr}$  (48% in  $\text{H}_2\text{O}$ ), 100 °C, 5 h, 68%.

position 3 by heating in  $\text{HBr}$  (48% aqueous solution) at reflux in the presence of sodium bromate, as reported by O'Brien and co-workers.<sup>[24]</sup>

Then, the microwave-assisted Suzuki–Miyaura cross-coupling reaction between 3-bromo-8-nitroquinolin-2(1*H*)-one and *p*-methoxyphenylboronic acid was studied to find optimal conditions (Table 1). Several parameters were investigated such as the nature of the solvent, base, Pd catalyst and the reaction temperature.

The first Suzuki–Miyaura reaction conditions (Table 1, entry 1) were inspired from a previously described protocol for introducing an aryl moiety at position 3 of the quinolinone ring.<sup>[25]</sup> This reaction was achieved in DMF with microwave (MW) heating, using 3 equivalents of  $\text{Na}_2\text{CO}_3$  as a base, 0.1 equivalents of  $\text{Pd}(\text{OAc})_2$  as a catalyst and 1.2 equivalents of *p*-methoxyphenylboronic acid in a sealed tube, to afford the desired compound in 43% yield. The first parameter studied was the solvent (Table 1, entries 1–7). By using toluene, dioxane, THF, 1,2-dimethoxyethane (DME) or DMSO, partial conversion or degradation of the substrate was observed, whereas the use of a DMF/ $\text{H}_2\text{O}$  mixture led to a 46% yield. Then, two other bases were studied (Table 1, entries 8–9) but in each case led to the degradation of the substrate within 1 h. In DMF, the replacement of  $\text{Pd}(\text{OAc})_2$  with  $\text{Pd}(\text{PPh}_3)_4$  decreased the yield of the reaction from 43% to 26% (Table 1, entry 10). Then, adapting another previously described protocol using DME and  $\text{Pd}(\text{PPh}_3)_4$  (Table 1, entry 11), a slightly better yield was obtained.<sup>[26]</sup> This result was then improved by replacing  $\text{Na}_2\text{CO}_3$  with  $\text{K}_2\text{CO}_3$ , which afforded a yield of 84%. The nature of the

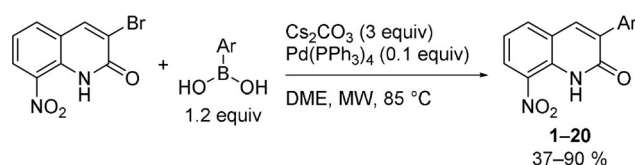
**Table 1.** Optimisation of the Suzuki–Miyaura cross-coupling reaction between 3-bromo-8-nitroquinolin-2(1*H*)-one and *p*-methoxyphenylboronic acid.

| Entry | Solvent                         | Base, 3 equiv            | Catalyst                                           | Temperature [°C] | Time [h] | Yield [%] <sup>[a]</sup> |
|-------|---------------------------------|--------------------------|----------------------------------------------------|------------------|----------|--------------------------|
| 1     | DMF                             | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 150              | 1        | 43                       |
| 2     | DMSO                            | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 150              | 1        | — <sup>[b]</sup>         |
| 3     | Toluene                         | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 110              | 8        | — <sup>[c]</sup>         |
| 4     | Dioxane                         | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 100              | 8        | — <sup>[c]</sup>         |
| 5     | THF                             | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 66               | 8        | — <sup>[c]</sup>         |
| 6     | DME                             | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 85               | 8        | — <sup>[c]</sup>         |
| 7     | DMF/ $\text{H}_2\text{O}$ (8:2) | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 100              | 1        | 46                       |
| 8     | DMF/ $\text{H}_2\text{O}$ (8:2) | $\text{K}_2\text{CO}_3$  | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 100              | 1        | — <sup>[b]</sup>         |
| 9     | DMF/ $\text{H}_2\text{O}$ (8:2) | $\text{Cs}_2\text{CO}_3$ | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 100              | 1        | — <sup>[b]</sup>         |
| 10    | DMF                             | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{PPh}_3)_4$ , 0.1 equiv            | 150              | 1        | 26                       |
| 11    | DME                             | $\text{Na}_2\text{CO}_3$ | $\text{Pd}(\text{PPh}_3)_4$ , 0.1 equiv            | 85               | 3        | 52                       |
| 12    | DME                             | $\text{K}_2\text{CO}_3$  | $\text{Pd}(\text{PPh}_3)_4$ , 0.1 equiv            | 85               | 4        | 84                       |
| 13    | DME                             | $\text{K}_2\text{CO}_3$  | $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ , 0.1 equiv | 85               | 8        | — <sup>[c]</sup>         |
| 14    | DME                             | $\text{K}_2\text{CO}_3$  | $\text{Pd}(\text{dppf})\text{Cl}_2$ , 0.1 equiv    | 85               | 8        | — <sup>[c]</sup>         |
| 15    | DME                             | $\text{K}_2\text{CO}_3$  | $\text{Pd}(\text{OAc})_2$ , 0.1 equiv              | 85               | 8        | — <sup>[c]</sup>         |
| 16    | DME                             | $\text{Cs}_2\text{CO}_3$ | $\text{Pd}(\text{PPh}_3)_4$ , 0.1 equiv            | 85               | 2        | 88                       |
| 17    | DME                             | $\text{CsF}$             | $\text{Pd}(\text{PPh}_3)_4$ , 0.1 equiv            | 85               | 2        | 88                       |
| 18    | DME                             | $\text{Cs}_2\text{CO}_3$ | $\text{Pd}(\text{PPh}_3)_4$ , 0.05 equiv           | 85               | 4        | 56                       |

[a] The yield was calculated after purification by chromatography on silica gel. [b] Degradation of the substrate was observed by TLC. [c] Only partial conversion of the substrate was observed by TLC.

catalyst was then studied (Table 1, entries 13–15), but none of these reactions led to an improvement in the yield, and only a few percent conversion was observed. Finally, the best results were obtained by using Cs<sub>2</sub>CO<sub>3</sub> or CsF as a base (Table 1, entries 16 and 17), which afforded efficient Suzuki–Miyaura cross-coupling reactions of this substrate. Decreasing of the amount of catalyst to 0.05 equivalents led to a lower reaction yield (56% Table 1, entry 18) in comparison with the reaction conditions of entry 16, which were chosen as the most suitable.

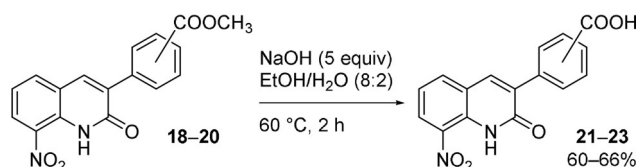
Next, the procedure was extended to 19 other arylboronic acids, to afford new derivatives bearing phenyl, thiophene, furan or pyridine moieties at position 3 of the scaffold (Scheme 2). The reactions yields were generally greater than 65% (for 14 derivatives) but were lower for 4-hydroxyphenylboronic acid and 4-aminophenylboronic acid with 37% and 41% yields, respectively.



**Scheme 2.** General procedure for the Suzuki–Miyaura cross-coupling reaction between 3-bromo-8-nitroquinolin-2(1*H*)-one and various arylboronic acids.

Three additional compounds (**21–23**) were synthesised by the saponification of compounds **18–20** into the carboxylic acid derivatives, using an excess of sodium hydroxide in an ethanol/water mixture (Scheme 3).

In parallel, with the aim of broadening the chemical diversity at position 3 of the scaffold and introduce alkynyl moieties, a Sonogashira cross-coupling reaction was devised. The initial conditions were adapted from a previously described protocol.<sup>[27]</sup> As presented in Table 2, after brief optimisation of the reaction between 3-bromo-8-nitroquinolinone and phenylacetylene, DME was chosen as the solvent, and the reaction was carried out at 15 °C (Table 2, entry 6). Interestingly, it was noted



**Scheme 3.** Preparation of compounds **21–23**.

that temperature had a strong influence on the reaction. At 0 °C in DME, the reaction did not take place, whereas it proceeded at 15 and 25 °C, affording compound **24**. Surprisingly, the unexpected 8-nitro-2-phenylfuro[2,3-*b*]quinoline (**25**) formed with heating at 40 °C in DME or DMF. Compound **25** was also obtained upon attempting to recrystallise **24** from acetonitrile, underlining the instability of this product. Such a consecutive Sonogashira coupling and cyclisation reaction, leading to furo[2,3-*b*]pyridine derivatives, has been reported in refs. [28] and [29].

The optimisation of both Suzuki–Miyaura and Sonogashira cross-coupling reactions led to the synthesis of 25 new molecules with either an aryl or an alkynyl group at position 3 of the scaffold. With the exception of compound **24**, which was considered too unstable, all these molecules were evaluated *in vitro* to determine their antikinoplastid potential. Compound **25** could not be evaluated because of low aqueous solubility.

### Compound evaluation

Initially, the cytotoxicity of these molecules was assessed *in vitro* on the HepG2 human cell line, and the corresponding CC<sub>50</sub> values were compared with that of the reference drug doxorubicin (Table 3). The biological results showed that compounds **1–11** were not soluble enough in aqueous medium to be tested. The water solubility was improved with compounds **10**, **12–17** and **21–23** bearing either a pyridin-3-yl or a phenyl moiety at position 3 of the scaffold; the phenyl group being substituted by a hydrophilic group such as hydroxymethyl or aldehyde or an ionised carboxyl group. These compounds dis-

**Table 2.** Optimisation of the Sonogashira cross-coupling reactions of 3-bromo-8-nitroquinolin-2(1*H*)-one.

| Entry | Solvent           | Temperature [°C] | Yield [%] <sup>[a]</sup>           |                                    | Time [h] |
|-------|-------------------|------------------|------------------------------------|------------------------------------|----------|
|       |                   |                  | Yield <b>24</b> [%] <sup>[a]</sup> | Yield <b>25</b> [%] <sup>[a]</sup> |          |
| 1     | DMF               | 25               | – <sup>[b]</sup>                   | – <sup>[b]</sup>                   | 48       |
| 2     | THF               | 25               | – <sup>[b]</sup>                   | – <sup>[b]</sup>                   | 48       |
| 3     | Et <sub>3</sub> N | 25               | – <sup>[b]</sup>                   | – <sup>[b]</sup>                   | 48       |
| 4     | DME               | 25               | <b>56</b>                          | 0                                  | 0.25     |
| 5     | DME               | 0                | – <sup>[b]</sup>                   | – <sup>[b]</sup>                   | 48       |
| 6     | DME               | 15               | <b>72</b>                          | 0                                  | 1.5      |
| 7     | DME               | 40               | 0                                  | <b>51</b>                          | 0.5      |
| 8     | DMF               | 40               | 0                                  | <b>56</b>                          | 36       |

[a] The yield was calculated after purification by chromatography on silica gel. [b] Only partial conversion of the substrate was observed by TLC.

**Table 3.** In vitro antileishmanial, antitrypanosomal and cytotoxic activities of compounds 1–25 and reference standards.

| Compound                           | R (% yield)                       | IC <sub>50</sub> [μM]                 |                                         | HepG2 CC <sub>50</sub> [μM] | Antitrypanosomal selectivity index <sup>[g]</sup> |
|------------------------------------|-----------------------------------|---------------------------------------|-----------------------------------------|-----------------------------|---------------------------------------------------|
|                                    |                                   | <i>L. infantum</i> axenic amastigotes | <i>T. brucei brucei</i> trypomastigotes |                             |                                                   |
| 1                                  | Phenyl (90)                       | > 12 <sup>[a]</sup>                   | 4.7 ± 2.7                               | > 12 <sup>[a]</sup>         | > 2                                               |
| 2                                  | 4-OCH <sub>3</sub> -phenyl (88)   | > 6 <sup>[a]</sup>                    | –                                       | > 6 <sup>[a]</sup>          | –                                                 |
| 3                                  | 4-OH-phenyl (37)                  | > 3 <sup>[a]</sup>                    | –                                       | > 3 <sup>[a]</sup>          | –                                                 |
| 4                                  | 4-NH <sub>2</sub> -phenyl (41)    | > 3 <sup>[a]</sup>                    | –                                       | > 3 <sup>[a]</sup>          | –                                                 |
| 5                                  | 4-Cl-phenyl (73)                  | > 3 <sup>[a]</sup>                    | –                                       | > 3 <sup>[a]</sup>          | –                                                 |
| 6                                  | 4-F-phenyl (70)                   | > 6 <sup>[a]</sup>                    | –                                       | > 6 <sup>[a]</sup>          | –                                                 |
| 7                                  | 4-CF <sub>3</sub> -phenyl (60)    | > 6 <sup>[a]</sup>                    | –                                       | > 6 <sup>[a]</sup>          | –                                                 |
| 8                                  | 3-thienyl (92)                    | > 6 <sup>[a]</sup>                    | –                                       | > 6 <sup>[a]</sup>          | –                                                 |
| 9                                  | 2-furyl (65)                      | > 3 <sup>[a]</sup>                    | –                                       | > 3 <sup>[a]</sup>          | –                                                 |
| 10                                 | 3-pyridyl (62)                    | > 50 <sup>[a]</sup>                   | 2.8 ± 0.8                               | > 25 <sup>[a]</sup>         | > 9                                               |
| 11                                 | 4-pyridyl (71)                    | NS <sup>[b]</sup>                     | –                                       | NS <sup>[b]</sup>           | –                                                 |
| 12                                 | 4-CH <sub>2</sub> OH-phenyl (77)  | > 25 <sup>[a]</sup>                   | 1.9 ± 0.3                               | > 25 <sup>[a]</sup>         | > 13                                              |
| 13                                 | 3-CH <sub>2</sub> OH-phenyl (87)  | 29.3 ± 4.2                            | 1.5 ± 0.3                               | > 25 <sup>[a]</sup>         | > 17                                              |
| 14                                 | 2-CH <sub>2</sub> OH-phenyl (72)  | 22 ± 2.0                              | 7.2 ± 0.6                               | > 100 <sup>[c]</sup>        | > 14                                              |
| 15                                 | 4-CHO-phenyl (51)                 | 35 ± 1.7                              | 0.5 ± 0.1                               | 30 ± 3.7                    | 60                                                |
| 16                                 | 3-CHO-phenyl (55)                 | 10.2 ± 0.6                            | 5.6 ± 0.4                               | > 100 <sup>[c]</sup>        | > 18                                              |
| 17                                 | 2-CHO-phenyl (65)                 | 9.8 ± 1.2                             | 7.5 ± 0.4                               | > 50 <sup>[a]</sup>         | > 7                                               |
| 18                                 | 4-COOCH <sub>3</sub> -phenyl (72) | > 12.5 <sup>[a]</sup>                 | –                                       | > 12.5 <sup>[a]</sup>       | –                                                 |
| 19                                 | 3-COOCH <sub>3</sub> -phenyl (71) | NS <sup>[b]</sup>                     | –                                       | NS <sup>[b]</sup>           | –                                                 |
| 20                                 | 2-COOCH <sub>3</sub> -phenyl (71) | NS <sup>[b]</sup>                     | –                                       | NS <sup>[b]</sup>           | –                                                 |
| 21                                 | <b>4-COOH-phenyl (65)</b>         | > 100 <sup>[c]</sup>                  | <b>1.5 ± 0.2</b>                        | <b>120 ± 7</b>              | <b>80</b>                                         |
| 22                                 | 3-COOH-phenyl (60)                | > 100 <sup>[c]</sup>                  | 7.5 ± 1.0                               | > 100 <sup>[c]</sup>        | > 13                                              |
| 23                                 | 2-COOH-phenyl (66)                | > 100 <sup>[c]</sup>                  | > 50 <sup>[a]</sup>                     | > 100 <sup>[c]</sup>        | –                                                 |
| Initial hit <sup>[22]</sup>        | Br                                | 7.1 ± 1.5                             | 1.9 ± 0.44                              | 92 ± 13.0                   | 48                                                |
| 8-Nitroquinolinone <sup>[22]</sup> | H                                 | 15.5 ± 0.5                            | 23.4 ± 5.7                              | 164 ± 28                    | 7                                                 |
| Doxorubicin <sup>[d]</sup>         | –                                 | –                                     | –                                       | 0.2 ± 0.02                  | –                                                 |
| Amphotericin B <sup>[e]</sup>      | –                                 | 0.06 ± 0.001                          | –                                       | 7.0 ± 0.25                  | –                                                 |
| Miltefosine <sup>[e]</sup>         | –                                 | 0.8 ± 0.2                             | –                                       | 84.5 ± 8.8                  | –                                                 |
| Fexinidazole <sup>[e,f]</sup>      | –                                 | 3.3 ± 0.7                             | 0.4 ± 0.18                              | > 100 <sup>[c]</sup>        | > 250                                             |
| Suramin <sup>[f]</sup>             | –                                 | –                                     | 0.03 ± 0.009                            | > 100 <sup>[c]</sup>        | > 3333                                            |
| Eflornithine <sup>[f]</sup>        | –                                 | –                                     | 15.8 ± 2.1                              | > 100 <sup>[c]</sup>        | > 6                                               |

[a] The product could not be tested at higher concentrations due to low solubility in aqueous media. [b] The product was not soluble at any test concentration. [c] The IC<sub>50</sub> or CC<sub>50</sub> value was not reached at the highest tested concentration. [d] Doxorubicin was used as a cytotoxic reference drug. [e] Amphotericin B, miltefosine and fexinidazole were used as antileishmanial reference drugs or drug candidates. [f] Fexinidazole, suramin and eflornithine were used as antitrypanosomal reference drugs or drug candidates. [g] The antitrypanosomal selectivity index was calculated according to the following formula: SI = CC<sub>50</sub> HepG2/IC<sub>50</sub> *T. brucei brucei*.

played low cytotoxicity on the HepG2 human cell line with CC<sub>50</sub> values > 25 μM. The compound bearing an aldehyde group in the *para* position of the phenyl ring (**15**) was the most cytotoxic of this series with a CC<sub>50</sub> value of 30 μM.

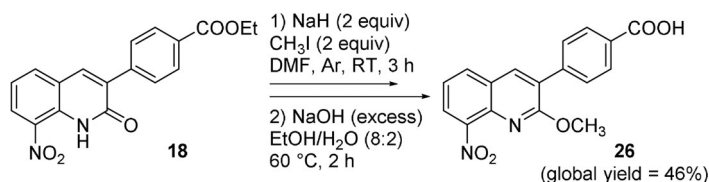
Then, all synthesised compounds were tested in vitro against *L. infantum* axenic amastigotes. Their IC<sub>50</sub> values were determined and compared with those of two antileishmanial reference drugs (amphotericin B and miltefosine) and to the drug candidate fexinidazole.

With respect to antileishmanial activity, apart for aldehyde-containing compounds **16** and **17**, the tested series appeared either weakly active (IC<sub>50</sub> = 22–35 μM) or even inactive (IC<sub>50</sub> > 100 μM for carboxylic acid derivatives) toward *L. infantum* in comparison with the reference drugs. Thus, introducing an aryl moiety at position 3 of the pharmacophore did not seem to favour antileishmanial activity.

In a second assay, only the compounds with appropriate aqueous solubility were tested in vitro against *T. brucei brucei* trypomastigotes and compared with reference antitrypanosomal drugs (suramin and eflornithine) and the drug candidate fexinidazole. All tested molecules displayed good antitrypanosomal activity (0.5 μM ≤ IC<sub>50</sub> ≤ 7.5 μM), better than that of eflornithine (IC<sub>50</sub> = 15.8 μM) and similar to that of fexinidazole (IC<sub>50</sub> = 0.4 μM). Interestingly, compounds substituted at the *ortho* position of the phenyl ring (**14**, **17**, **23**) displayed higher IC<sub>50</sub> values than their *meta*- and *para*-substituted analogues. Compounds with a *para*-substituted phenyl ring generally showed lower IC<sub>50</sub> values than their *meta*-substituted analogues, for example, **15** being 11 times more potent than **16**, and **21** five times more potent than **22**. Compounds **15** and **21** appeared to be the most promising antitrypanosomal molecules in this series with respective IC<sub>50</sub> values of 0.5 and

1.5  $\mu\text{M}$ . Compound **21** emerged as a new antitrypanosomal hit, with an activity against *T. brucei brucei* close to that of the parent compound ( $\text{IC}_{50}$  = 1.9  $\mu\text{M}$ ) but with a better cytotoxicity profile, leading to a better selectivity index ( $\text{SI}$  = 80 versus 48 for the initial hit). By comparison with reference drugs, **21** appears less active than suramin but more active than eflornithine. With respect to fexinidazole, another nitroheterocycle, compound **21** presents the same cytotoxic profile with  $\text{CC}_{50}$  = 120  $\mu\text{M}$ , and is three times less active than the drug candidate. Indeed, unlike fexinidazole and 3-bromo-8-nitroquinolin-2(1*H*)-one, compound **21** displayed a selective antitrypanosomal activity, being inactive ( $\text{IC}_{50}$  > 100  $\mu\text{M}$ ) against *L. infantum*. This is a first indicator that molecule **21** shows a specific antiparasitic profile in the studied series.

Finally, the *O*-methylated analogue of hit compound **21** (**26**) was synthesised in two steps: *O*-methylation of **18**, using methyl iodide in DMF under an inert atmosphere, followed by saponification by the same procedure as described for compounds **21**–**23** (Scheme 4). Compound **26** was then tested in vitro against both *L. infantum* and *T. brucei brucei* (Figure 4).



Scheme 4. Preparation of compound **26**.

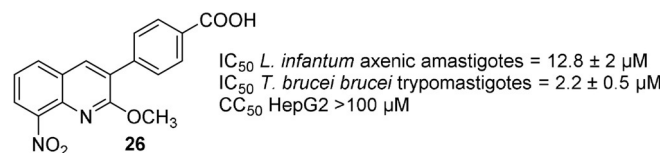


Figure 4. In vitro antiparasitic activities and cytotoxicity of compound **26**.

It was as active against *T. brucei brucei* as **21** ( $\text{IC}_{50}$  = 2.2  $\mu\text{M}$ ). Compound **26** was also active against *L. infantum* ( $\text{IC}_{50}$  = 12.8  $\mu\text{M}$ ), whereas **21** was not ( $\text{IC}_{50}$  > 100  $\mu\text{M}$ ). These results are surprising, considering that the hydrogen bond between the lactam function and the nitro group appeared mandatory for conferring antileishmanial activity on the 8-nitroquinolin-2(1*H*)-one series by increasing the reduction potential.<sup>[22]</sup> This is a second indicator that the introduction of an aryl group at position 3 of the 8-nitroquinolin-2(1*H*)-one scaffold might lead to a new antiketoplastid mechanism of action, different from that of 3-bromo-8-nitroquinolin-2(1*H*)-one.

To assess if the antitrypanosomal nitroheterocycle **21** was bioactivated by the NTR of *T. brucei brucei*, its  $\text{IC}_{50}$  value was measured on both a wild-type *T. brucei brucei* trypomastigote strain and a NTR-overexpressing strain (Table 4). The results were compared with those obtained for the initial hit.<sup>[22]</sup> The latter is clearly bioactivated by the trypanosomal NTR, being 4.5-fold more active against the strain overexpressing the NTR

Table 4. Study of the bioactivation of **21** by trypanosomal nitroreductase.

| Compound    | <i>T. brucei brucei</i> trypomastigotes $\text{IC}_{50}$ [ $\mu\text{M}$ ] |                           | Fold change |
|-------------|----------------------------------------------------------------------------|---------------------------|-------------|
|             | Wild-type strain                                                           | NTR-overexpressing strain |             |
| <b>21</b>   | 5.4 ± 0.12                                                                 | 4.2 ± 0.2                 | 1.3         |
| Initial hit | 17.7 ± 1.0                                                                 | 3.9 ± 0.1                 | 4.5         |
| Nifurtimox  | 1.9 ± 0.05                                                                 | 0.6 ± 0.05                | 3.1         |

than the wild type, whereas **21** is only 1.3 times more active on the strain overexpressing the NTR. This result is a third piece of evidence that suggests that **21**, which is less intensively bioactivated by the trypanosomal NTR than the initial hit, could act on another parasite target. This assay also showed that compound **21** presents the same level of activity toward *T. brucei brucei* than the drug nifurtimox, which was used as a bioactivation control.

In parallel, an electrochemistry study was carried out by measuring in DMSO the reduction potentials of five 8-nitroquinolin-2(1*H*)-one derivatives bearing an aryl group at position 3, using cyclic voltammetry (Table 5). For all compounds, a reversible single-electron reduction was observed (formation of an anion radical). The redox potentials of the new compounds bearing an aryl group at position 3 ranged between −0.53 and −0.59 V, higher than for the initial hit (−0.45 V) but similar to that of 8-nitroquinolin-2(1*H*)-one (−0.54 V). As previously noted in the studied series,<sup>[22]</sup> the *O*-methylation of compound **21** to give **24**, is responsible for an important decrease in the redox potential value from −0.56 to −0.93 V. This shift is mainly due to the removal of the intramolecular hydrogen bond between the lactam function and the nitro group. It can be concluded that the introduction of a phenyl ring at the position 3 of the scaffold has no significant impact on the redox potential of the studied series but that it allows access to novel antitrypanosomal molecules that display lower reduction potential than the initial hit with the same level of efficacy.

Thus, to understand the selective antitrypanosomal activity of **21**, other parasitic targets should be investigated. Among the parasitic targets that were recently reported in literature concerning the antitrypanosomal activity of new diverse nitroheterocycles, *S*-adenosylmethionine decarboxylase was identified by a high-throughput mass spectrometry-based assay conducted on 400 000 molecules,<sup>[30]</sup> and should be considered.

## Conclusion

An optimised Suzuki–Miyaura reaction at position 3 of 3-bromo-8-nitroquinolin-2(1*H*)-one led to the synthesis of 24 new derivatives. These molecules were screened in vitro against *L. infantum* and *T. brucei brucei* to evaluate their antiketoplastid potential. Among these molecules, a new selective antitrypanosomal hit **21**, bearing a *para*-carboxyphenyl group, was identified. Compound **21** was not cytotoxic on the HepG2 human cell line ( $\text{CC}_{50}$  = 120  $\mu\text{M}$ ), displayed good antitrypanoso-

**Table 5.** Effects of substitution of the phenyl ring on reduction potentials  $E^\circ$  in the Suzuki–Miyaura series.

| Compound                  | Structure | $E^\circ$ [V] <sup>[a]</sup> |
|---------------------------|-----------|------------------------------|
| 1                         |           | -0.59                        |
| 7                         |           | -0.53                        |
| 13                        |           | -0.53                        |
| 21                        |           | -0.56                        |
| 26                        |           | -0.93                        |
| Initial hit               |           | -0.45                        |
| 8-Nitroquinolin-2(1H)-one |           | -0.54                        |

[a] Cyclic voltammetry conditions: DMSO/TBAPF<sub>6</sub>, SCE/GC; one-electron reversible reduction; values are expressed in V versus NHE.

mal activity ( $IC_{50} = 1.5 \mu\text{M}$ ,  $SI = 80$ ), which is better than that of the drug eflornithine and similar to that of fexinidazole, a 5-nitroimidazole in phase III of clinical trials against HAT. Interestingly, **21** was inactive against *L. infantum*, showing a parasitic selectivity among kinetoplastids. Unlike fexinidazole and the previously identified hit molecule in the series, this molecule was not efficiently metabolised by the type I trypanosomal NTR, suggesting a probable alternative mechanism of action in this series.

## Experimental Section

### Chemistry

All reagents and solvents were obtained from commercial sources (Fluorochem, Sigma–Aldrich or Alfa Aesar) and used as received. The progress of the reactions was monitored on ALUGRAM SIL G/UV<sub>254</sub> (Macherey–Nagel) pre-coated thin layer chromatography (TLC) sheets and were visualised under ultraviolet light at 254 nm.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on Bruker UltraShield 300 MHz, Bruker IconNMR 400 MHz or Bruker Avance NEO 600 MHz instruments at the Laboratoire de Chimie de Coordination, and data are presented as follows: chemical shift  $\delta$  in parts per million (ppm) using tetramethylsilane (TMS) as reference, coupling constant  $J$  in Hertz (Hz), and multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublets of doublets; m, multiplet; brs, broad singlet. Melting points were measured on a Stuart Melting Point SMP3 instrument. High-resolution mass measurements were recorded on a GCT Premier Spectrometer (DCI,  $\text{CH}_4$ ) or Xevo G2 QTOF (Waters, ESI+) instrument at the Université Paul Sabatier, Toulouse (France). Microwave reactions were performed in a CEM Discover microwave reactor.

3-Bromo-8-nitroquinolin-2(1H)-one was prepared according to a previously reported procedure.<sup>[22]</sup>

### General procedure for the preparation of compounds 1–20 and 25

3-Bromo-8-nitroquinolin-2(1H)-one (300 mg, 1.1 mmol, 1 equiv), cesium carbonate (1.1 g, 3.3 mmol, 3 equiv), Pd(PPh<sub>3</sub>)<sub>4</sub> (127 mg, 0.12 mmol, 0.1 equiv) and the appropriate phenylboronic acid (1.2 equiv) were added in a sealed 25 mL flask. Under an argon atmosphere, anhydrous DME (10 mL) was then added. The reaction mixture was heated at 85 °C in a microwave reactor for 2 h. The reaction mixture was poured into water and extracted with dichloromethane (3 × 100 mL). The organic layers were combined, washed with water, dried over anhydrous MgSO<sub>4</sub> and evaporated in vacuo. The crude residue was purified by chromatography on silica gel and recrystallised if necessary to give compounds 1–20 or 25.

**8-Nitro-3-phenylquinolin-2(1H)-one (1)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (97:3) as eluent, and recrystallised from acetonitrile to yield a yellow solid (264 mg, 0.99 mmol, 90%).  $T_{\text{dec}} = 177^\circ\text{C}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.31\text{--}7.35$  (m, 1H; H6), 7.41–7.50 (m, 3H; H3', H4' and H5'), 7.75–7.77 (m, 2H; H2' and H6'), 7.91 (s, 1H; H4), 7.94 (dd,  $J = 7.6$ , 1.4 Hz, 1H; H5), 8.49 (dd,  $J = 8.4$ , 1.4 Hz, 1H; H7), 11.40 ppm (brs, 1H; NH);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 121.4$  (CH), 122.7 (C), 127.3 (CH), 128.5 (2 × CH), 128.7 (2 × CH), 129.1 (CH), 132.7 (C), 133.1 (C), 134.5 (C), 134.6 (C), 135.6 (CH), 136.7 (CH), 161.0 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{11}\text{N}_2\text{O}_3$ : 267.0770  $[M + \text{H}]^+$ ; found: 267.0762.

**3-(4-Methoxyphenyl)-8-nitroquinolin-2(1H)-one (2)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (95:5) as eluent, and recrystallised from acetonitrile to yield an orange solid (287 mg, 0.97 mmol, 88%); mp: 229 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 3.87$  (s, 3H; CH<sub>3</sub>), 6.99–7.02 (m, 2H; H3' and H5'), 7.29–7.33 (m, 1H; H6), 7.73–7.77 (m, 2H; H2' and H6'), 7.87 (s, 1H; H4), 7.91 (dd,  $J = 7.6$ , 1.4 Hz, 1H; H5), 8.47 (dd,  $J = 8.3$ , 1.4 Hz, 1H; H7), 11.38 ppm (brs, 1H; NH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta = 55.4$  (CH<sub>3</sub>), 114.0 (2 × CH), 121.3 (CH), 122.9 (C), 125.6 (C), 126.9 (CH), 130.0 (2 × CH), 132.7 (C), 132.9 (C), 134.0 (C), 135.3 (CH), 135.4 (CH), 160.3 (C), 161.2 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{13}\text{N}_2\text{O}_4$ : 297.0875  $[M + \text{H}]^+$ ; found: 297.0864.

**3-(4-Hydroxyphenyl)-8-nitroquinolin-2(1H)-one (3)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (75:25) as eluent to yield an orange solid (115 mg, 0.41 mmol, 37%); mp: 266 °C;  $^1\text{H}$  NMR ( $[\text{D}_2\text{O}]$  DMSO, 400 MHz):  $\delta = 6.84\text{--}6.88$  (m, 2H; H3' and H5'), 7.40–7.44 (m, 1H; H6), 7.67–7.71 (m, 2H; H2' and H6'), 8.20 (dd,  $J = 7.7$ , 1.4 Hz, 1H; H5), 8.26 (s, 1H; H4), 8.40 (dd,  $J = 8.3$ , 1.4 Hz, 1H; H7), 9.74 (s, 1H; OH), 11.12 ppm (brs, 1H; NH);



$^{13}\text{C}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 75 MHz):  $\delta = 115.4$  (2 $\times$ CH), 122.0 (CH), 122.8 (C), 125.8 (C), 127.1 (CH), 130.4 (2 $\times$ CH), 132.3 (C), 132.5 (C), 133.5 (C), 136.0 (CH), 136.1 (CH), 158.5 (C), 160.8 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{11}\text{N}_2\text{O}_4$ : 283.0719  $[\text{M} + \text{H}]^+$ ; found: 283.0706.

**3-(4-Aminophenyl)-8-nitroquinolin-2(1H)-one (4)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (95:5) as eluent to yield an orange solid (128 mg, 0.45 mmol, 41%); mp: 280 °C;  $^1\text{H}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 400 MHz):  $\delta = 5.44$  (s, 2H;  $\text{NH}_2$ ), 6.61–6.65 (m, 2H;  $\text{H}_3'$  and  $\text{H}_5'$ ), 7.37–7.41 (m, 1H;  $\text{H}_6$ ), 7.58–7.62 (m, 2H;  $\text{H}_2'$  and  $\text{H}_6'$ ), 8.15–8.18 (m, 2H;  $\text{H}_4$  and  $\text{H}_5$ ), 8.36 (dd,  $J = 8.3$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 11.07 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 100 MHz):  $\delta = 113.6$  (2 $\times$ CH), 121.9 (CH), 122.1 (C), 123.1 (C), 126.5 (C), 129.9 (2 $\times$ CH), 131.9 (C), 132.9 (C), 133.4 (C), 134.1 (CH), 135.8 (CH), 150.0 (CH), 161.0 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{12}\text{N}_3\text{O}_3$ : 282.0879  $[\text{M} + \text{H}]^+$ ; found: 282.0874.

**3-(4-Chlorophenyl)-8-nitroquinolin-2(1H)-one (5)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (97:3) as eluent to yield a yellow solid (241 mg, 0.80 mmol, 73%); mp: 222 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.33$ –7.37 (m, 1H;  $\text{H}_6$ ), 7.40–7.42 (m, 2H;  $\text{H}_3'$  and  $\text{H}_5'$ ), 7.66–7.75 (m, 2H;  $\text{H}_2'$  and  $\text{H}_6'$ ), 7.92 (s, 1H;  $\text{H}_4$ ), 7.95 (dd,  $J = 7.6$ , 1.5 Hz, 1H;  $\text{H}_5$ ), 8.51 (dd,  $J = 8.4$ , 1.5 Hz, 1H;  $\text{H}_7$ ), 11.42 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 121.6$  (CH), 122.4 (C), 126.9 (CH), 127.7 (CH), 128.7 (CH), 129.1 (CH), 129.7 (CH), 132.8 (C), 133.1 (C), 133.2 (C), 134.4 (C), 135.7 (CH), 136.2 (C), 137.3 (CH), 160.6 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{10}\text{ClN}_2\text{O}_3$ : 301.0380  $[\text{M} + \text{H}]^+$ ; found: 301.0373.

**3-(4-Fluorophenyl)-8-nitroquinolin-2(1H)-one (6)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (98:2) as eluent to yield a yellow solid (219 mg, 0.77 mmol, 70%); mp: 217 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.12$ –7.20 (m, 2H;  $\text{H}_2'$  and  $\text{H}_6'$ ), 7.31–7.36 (m, 1H;  $\text{H}_6$ ), 7.73–7.80 (m, 2H;  $\text{H}_3'$  and  $\text{H}_5'$ ), 7.89 (s, 1H;  $\text{H}_4$ ), 7.94 (dd,  $J = 7.6$ , 1.5 Hz, 1H;  $\text{H}_5$ ), 8.50 (dd,  $J = 8.4$ , 1.5 Hz, 1H;  $\text{H}_7$ ), 11.41 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 115.5$  (d,  $J = 21.6$  Hz; 2 $\times$ CH), 121.5 (CH), 122.6 (C), 124.4 (C), 127.4 (CH), 130.6 (d,  $J = 8.3$  Hz; 2 $\times$ CH), 132.7 (C), 133.1 (C), 133.5 (C), 135.5 (CH), 136.5 (CH), 160.9 (C), 163.2 ppm (d,  $J = 249.2$  Hz, C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{15}\text{H}_{10}\text{FN}_2\text{O}_3$ : 285.0675  $[\text{M} + \text{H}]^+$ ; found: 285.0676.

**8-Nitro-3-(4-trifluoromethylphenyl)quinolin-2(1H)-one (7)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (98:2) as eluent to yield a yellow solid (220 mg, 0.66 mmol, 60%); mp: 168 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.34$ –7.38 (m, 1H;  $\text{H}_6$ ), 7.72–7.75 (m, 2H;  $\text{H}_2'$  and  $\text{H}_6'$ ), 7.88 (m, 2H;  $\text{H}_3'$  and  $\text{H}_5'$ ), 7.95–7.98 (m, 2H;  $\text{H}_4$  and  $\text{H}_5$ ), 8.53 (dd,  $J = 8.4$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 11.45 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 150 MHz):  $\delta = 121.7$  (CH), 122.4 (C), 124.0 (q,  $J = 272.3$  Hz, C), 125.4 (q,  $J = 3.7$  Hz, 2 $\times$ CH), 127.9 (CH), 129.1 (2 $\times$ CH), 130.9 (q,  $J = 32.5$  Hz, C), 132.8 (C), 133.2 (C), 133.4 (C), 135.8 (CH), 137.6 (CH), 138.0 (C), 160.5 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{10}\text{F}_3\text{N}_2\text{O}_3$ : 335.0644  $[\text{M} + \text{H}]^+$ ; found: 335.0629.

**8-Nitro-3-(thiophen-3-yl)quinolin-2(1H)-one (8)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate (98:2) as eluent to yield a yellow solid (275 mg, 1.01 mmol, 92%); mp: 199 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.31$ –7.35 (m, 1H;  $\text{H}_6$ ), 7.41–7.43 (m, 1H;  $\text{H}_4'$ ), 7.59 (dd,  $J = 5.1$ , 1.3 Hz, 1H;  $\text{H}_5'$ ), 7.94 (dd,  $J = 7.7$ , 1.4 Hz, 1H;  $\text{H}_5$ ), 8.05 (s, 1H;  $\text{H}_4$ ), 8.39 (dd,  $J = 3.0$ , 1.3 Hz, 1H;  $\text{H}_2'$ ), 8.48 (dd,  $J = 8.4$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 11.42 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 75 MHz):  $\delta = 121.4$  (CH), 122.6 (C), 123.6 (C), 125.6 (CH), 126.2 (CH), 127.0 (CH), 127.1 (CH), 128.6 (C), 132.4 (C), 134.1 (CH), 134.3 (C), 135.4 (CH), 160.6 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{13}\text{H}_9\text{N}_2\text{O}_3\text{S}$ : 273.0334  $[\text{M} + \text{H}]^+$ ; found: 273.0330.

**3-(2-Furanyl)-8-nitroquinolin-2(1H)-one (9)** was purified by chromatography on silica gel using dichloromethane as eluent to yield a dark red solid (183 mg, 0.71 mmol, 65%); mp: 257 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz):  $\delta = 6.58$  (dd,  $J = 3.3$  Hz, 1.9 Hz, 1H;  $\text{H}_4'$ ), 7.30–7.35 (m, 1H;  $\text{H}_6$ ), 7.55–7.56 (m, 2H;  $\text{H}_3'$  and  $\text{H}_5'$ ), 7.96 (dd,  $J = 7.6$ , 1.4 Hz, 1H;  $\text{H}_5$ ), 8.23 (s, 1H;  $\text{H}_4$ ), 8.46 (dd,  $J = 8.3$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 11.42 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 112.6$  (CH), 114.2 (CH), 121.6 (CH), 122.5 (C), 123.6 (C), 126.9 (CH), 130.7 (CH), 132.0 (C), 132.8 (C), 135.5 (CH), 143.3 (CH), 147.7 (C), 158.7 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{13}\text{H}_9\text{N}_2\text{O}_4$ : 257.0562  $[\text{M} + \text{H}]^+$ ; found: 257.0558.

**8-Nitro-3-(pyridin-3-yl)quinolin-2(1H)-one (10)** was purified by chromatography on silica gel using dichloromethane/ethyl acetate as eluent to yield a yellow solid (182 mg, 0.68 mmol, 62%); mp: 237 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.35$ –7.39 (m, 1H;  $\text{H}_6$ ), 7.40–7.43 (m, 1H;  $\text{H}_5'$ ), 7.97–7.99 (m, 2H;  $\text{H}_4$  and  $\text{H}_5$ ), 8.20–8.23 (m, 1H;  $\text{H}_6'$ ), 8.53 (dd,  $J = 8.4$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 8.66 (dd,  $J = 4.9$ , 1.7 Hz; 1H;  $\text{H}_4'$ ), 8.90 (dd,  $J = 2.3$ , 0.9 Hz, 1H;  $\text{H}_2'$ ), 11.45 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta = 121.7$  (CH), 122.3 (C), 123.1 (CH), 127.9 (CH), 130.5 (C), 131.4 (C), 132.8 (C), 133.3 (C), 135.8 (CH), 136.4 (CH), 137.2 (CH), 149.0 (CH), 150.0 (CH), 160.6 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{14}\text{H}_{10}\text{N}_3\text{O}_3$ : 268.0722  $[\text{M} + \text{H}]^+$ ; found: 268.0712.

**8-Nitro-3-(pyridin-4-yl)quinolin-2(1H)-one (11)** was purified by chromatography on silica gel using dichloromethane/acetone (80:20) as eluent to yield a yellow solid (209 mg, 0.78 mmol, 71%); mp: 297 °C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta = 7.36$ –7.40 (m, 1H;  $\text{H}_6$ ), 7.70–7.72 (m, 2H;  $\text{H}_2'$  and  $\text{H}_6'$ ), 7.98 (dd,  $J = 7.9$ , 1.4 Hz, 1H;  $\text{H}_5$ ), 8.03 (s, 1H;  $\text{H}_4$ ), 8.55 (dd,  $J = 8.4$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 8.73–8.74 (m, 2H;  $\text{H}_3'$  and  $\text{H}_5'$ ), 11.46 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $\text{CF}_3\text{CO}_2\text{D}$ , 100 MHz):  $\delta = 121.6$  (C), 123.7 (CH), 126.1 (C), 126.7 (2 $\times$ CH), 130.7 (CH), 132.4 (C), 133.4 (C), 137.5 (CH), 140.6 (2 $\times$ CH), 144.1 (CH), 153.5 (C), 161.1 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{14}\text{H}_{10}\text{N}_3\text{O}_3$ : 268.0722  $[\text{M} + \text{H}]^+$ ; found: 268.0713.

**3-(4-Hydroxymethylphenyl)-8-nitroquinolin-2(1H)-one (12)** was purified by chromatography on silica gel using dichloromethane/acetone (75:25) as eluent to yield a yellow solid (251 mg, 0.85 mmol, 77%); mp: 219 °C;  $^1\text{H}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 400 MHz):  $\delta = 4.56$  (d,  $J = 5.6$  Hz, 2H;  $\text{CH}_2$ ), 5.26 (t,  $J = 5.6$  Hz, 1H;  $\text{OH}$ ), 7.41–7.46 (m, 3H;  $\text{H}_6$ ,  $\text{H}_3'$  and  $\text{H}_5'$ ), 7.76–7.79 (m, 2H;  $\text{H}_2'$  and  $\text{H}_6'$ ), 8.23 (dd,  $J = 7.6$ , 1.4 Hz, 1H;  $\text{H}_5$ ), 8.36 (s, 1H;  $\text{H}_4$ ), 8.43 (dd,  $J = 8.3$ , 1.4 Hz, 1H;  $\text{H}_7$ ), 11.17 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 100 MHz):  $\delta = 63.1$  ( $\text{CH}_2$ ), 122.1 (CH), 122.6 (C), 126.6 (2 $\times$ CH), 127.6 (CH), 128.8 (2 $\times$ CH), 132.6 (C), 132.7 (C), 133.6 (C), 134.0 (C), 136.4 (CH), 137.6 (CH), 143.6 (C), 160.8 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{13}\text{N}_2\text{O}_4$ : 297.0875  $[\text{M} + \text{H}]^+$ ; found: 297.0878.

**3-(3-Hydroxymethylphenyl)-8-nitroquinolin-2(1H)-one (13)** was purified by chromatography on silica gel using dichloromethane/acetone (75:25) as eluent to yield a yellow solid (267 mg, 0.90 mmol, 82%); mp: 155 °C;  $^1\text{H}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 300 MHz):  $\delta = 4.58$  (d,  $J = 5.6$  Hz, 2H;  $\text{CH}_2$ ), 5.27 (t,  $J = 5.6$  Hz, 1H;  $\text{OH}$ ), 7.37–7.47 (m, 3H;  $\text{H}_6$ ,  $\text{H}_4'$  and  $\text{H}_5'$ ), 7.66–7.68 (m, 1H;  $\text{H}_6'$ ), 7.73–7.75 (m, 1H;  $\text{H}_2'$ ), 8.25 (dd,  $J = 7.8$ , 1.4 Hz, 1H;  $\text{H}_5$ ), 8.35 (s, 1H;  $\text{H}_4$ ), 8.44 (dd,  $J = 8.3$  and 1.4 Hz, 1H;  $\text{H}_7$ ), 11.17 ppm (brs, 1H;  $\text{NH}$ );  $^{13}\text{C}$  NMR ( $[\text{D}_6]\text{DMSO}$ , 75 MHz):  $\delta = 63.3$  ( $\text{CH}_2$ ), 122.1 (CH), 122.6 (C), 127.1 (CH), 127.2 (CH), 127.5 (CH), 127.7 (CH), 128.4 (CH), 132.7 (C), 133.0 (C), 133.7 (C), 135.1 (C), 136.5 (CH), 138.0 (CH), 142.9 (C), 160.6 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{13}\text{N}_2\text{O}_4$ : 297.0875  $[\text{M} + \text{H}]^+$ ; found: 297.0861.

**3-(2-Hydroxymethylphenyl)-8-nitroquinolin-2(1H)-one (14)** was purified by chromatography on silica gel using dichloromethane/

ethyl acetate (50:50) as eluent to yield a yellow solid (235 mg, 0.79 mmol, 72%); mp: 208 °C; <sup>1</sup>H NMR ([D<sub>6</sub>]DMSO, 400 MHz): δ = 4.44 (d, *J* = 5.5 Hz, 2H; CH<sub>2</sub>), 5.09 (s, 1H; OH), 7.28 (dd, *J* = 7.5, 1.4 Hz, 1H; H3'), 7.33–7.37 (m, 1H; H6), 7.42–7.46 (m, 2H; H4' and H5'), 7.57 (dd, *J* = 7.5, 1.4 Hz, 1H; H6'), 8.12 (s, 1H; H4), 8.19 (dd, *J* = 7.6, 1.4 Hz, 1H; H5), 8.45 (dd, *J* = 8.3, 1.4 Hz, 1H; H7), 11.19 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 63.7 (CH<sub>2</sub>), 121.9 (CH), 122.5 (C), 127.8 (CH), 128.4 (CH), 129.8 (CH), 130.1 (CH), 130.6 (CH), 132.7 (C), 133.1 (C), 133.9 (C), 135.7 (CH), 135.8 (C), 139.2 (CH), 139.9 (C), 162.2 ppm (C); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>16</sub>H<sub>13</sub>N<sub>2</sub>O<sub>4</sub>: 297.0875 [*M* + H]<sup>+</sup>; found: 297.0869.

**3-(4-Formylphenyl)-8-nitroquinolin-2(1H)-one (15)** was purified by chromatography on silica gel using dichloromethane/cyclohexane (80:20) as eluent, and recrystallised from acetonitrile to yield a yellow solid (165 mg, 0.56 mmol, 51%); mp: 243 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ = 7.34–7.39 (m, 1H; H6), 7.93–8.00 (m, 6H; H4, H5, H2', H3', H5' and H6'), 8.53 (dd, *J* = 8.5, 1.4 Hz, 1H; H7), 10.08 (s, 1H; CHO), 11.45 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz): δ = 121.7 (CH), 122.3 (C), 128.0 (CH), 129.4 (2×CH), 129.8 (2×CH), 132.8 (C), 133.2 (C), 133.4 (C), 135.9 (CH), 136.4 (C), 137.9 (CH), 140.5 (C), 160.5 (C), 191.7 ppm (CH); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>: 295.0719 [*M* + H]<sup>+</sup>; found: 295.0710.

**3-(3-Formylphenyl)-8-nitroquinolin-2(1H)-one (16)** was purified by chromatography on silica gel using dichloromethane/acetone (75:25) as eluent to yield a yellow solid (104 mg, 0.35 mmol, 32%); mp: 209 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ = 7.34–7.39 (m, 1H; H6), 7.63–7.69 (m, 1H; H5'), 7.94–7.99 (m, 2H; H5 and H6'), 8.01 (s, 1H; H4), 8.10–8.13 (m, 1H; H4'), 8.25–8.26 (m, 1H; H2'), 8.53 (dd, *J* = 8.4, 1.4 Hz, 1H; H7), 10.10 (s, 1H; CHO), 11.5 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 121.7 (CH), 122.4 (C), 127.8 (CH), 129.2 (CH), 129.7 (CH), 130.2 (CH), 132.8 (C), 133.1 (C), 133.3 (C), 134.7 (CH), 135.6 (C), 135.8 (CH), 136.6 (C), 137.5 (CH), 160.7 (C), 192.0 ppm (CH); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>: 295.0719 [*M* + H]<sup>+</sup>; found: 295.0713.

**3-(2-Formylphenyl)-8-nitroquinolin-2(1H)-one (17)** was purified by chromatography on silica gel using cyclohexane/acetone (60:40) as eluent to yield a yellow solid (210 mg, 0.71 mmol, 65%); mp: 230–231 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 7.33–7.37 (m, 1H; H6), 7.44 (dd, *J* = 7.5, 1.3 Hz, 1H; H6'), 7.61–7.65 (m, 1H; H4'), 7.68–7.72 (m, 1H; H5'), 7.82 (s, 1H; H4), 7.93 (dd, *J* = 7.6, 1.4 Hz, 1H; H3'), 7.99 (dd, *J* = 7.8, 1.4 Hz, 1H; H5), 8.53 (dd, *J* = 8.4, 1.4 Hz, 1H; H7), 10.03 (s, 1H; CHO), 11.42 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 121.6 (CH), 122.3 (C), 127.8 (CH), 129.5 (CH), 130.8 (CH), 131.0 (CH), 133.0 (C), 133.7 (C), 133.9 (CH), 134.5 (C), 135.0 (C), 135.7 (CH), 136.1 (C), 138.0 (CH), 160.8 (C), 191.2 ppm (CH); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>: 295.0719 [*M* + H]<sup>+</sup>; found: 295.0718.

**3-(4-Methoxycarbonylphenyl)-8-nitroquinolin-2(1H)-one (18)** was purified by chromatography on silica gel using dichloromethane as eluent, and recrystallised from acetonitrile to yield a yellow solid (257 mg, 0.79 mmol, 72%); mp: 245 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): δ = 3.95 (s, 3H; CH<sub>3</sub>), 7.33–7.38 (m, 1H; H6), 7.84–7.87 (m, 2H; H2' and H6'), 7.95–7.97 (m, 2H; H4 and H5), 8.12–8.15 (m, 2H; H3' and H5'), 8.52 (dd, *J* = 8.3, 1.4 Hz, 1H; H7), 11.43 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 52.3 (CH<sub>3</sub>), 121.6 (CH), 122.4 (C), 127.8 (CH), 128.7 (2×CH), 129.7 (2×CH), 130.4 (C), 132.8 (C), 133.3 (C), 133.5 (C), 135.8 (CH), 137.6 (CH), 139.0 (C), 160.6 (C), 166.7 ppm (C); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>17</sub>H<sub>13</sub>N<sub>2</sub>O<sub>5</sub>: 325.0824 [*M* + H]<sup>+</sup>; found: 325.0818.

**3-(3-Methoxycarbonylphenyl)-8-nitroquinolin-2(1H)-one (19)** was purified by chromatography on silica gel using dichloromethane/

cyclohexane (90:10) as eluent to yield a yellow solid (253 mg, 0.78 mmol, 71%); mp: 212 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 3.95 (s, 3H; CH<sub>3</sub>); 7.33–7.37 (m, 1H; H6), 7.53–7.58 (m, 1H; H5'), 7.96 (dd, *J* = 7.7 Hz, 1.4 Hz, 1H; H5), 7.98 (s, 1H; H4), 8.05–8.11 (m, 2H; H4' and H6'), 8.36–8.37 (m, 1H; H2'), 8.51 (dd, *J* = 8.4, 1.4 Hz, 1H; H7), 11.42 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 52.3 (CH<sub>3</sub>), 121.6 (CH), 122.5 (C), 127.7 (CH), 128.6 (CH), 129.6 (CH), 130.0 (CH), 130.5 (C), 132.8 (C), 133.2 (C), 133.4 (CH), 133.5 (C), 134.8 (C), 135.7 (CH), 137.3 (CH), 160.7 (C), 166.7 ppm (C); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>17</sub>H<sub>13</sub>N<sub>2</sub>O<sub>5</sub>: 325.0824 [*M* + H]<sup>+</sup>; found: 325.0809.

**3-(2-Methoxycarbonylphenyl)-8-nitroquinolin-2(1H)-one (20)** was purified by chromatography on silica gel using cyclohexane/acetone (70:30) as eluent to yield a yellow solid (257 mg, 0.79 mmol, 72%); mp: 212 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 3.82 (s, 3H; CH<sub>3</sub>), 7.29–7.33 (m, 1H; H6), 7.37–7.39 (m, 1H; H6'), 7.49–7.53 (m, 1H; H4'), 7.60–7.64 (m, 1H; H5'), 7.77 (s, 1H; H4), 7.91 (d, *J* = 7.65 Hz, 1H; H3'), 8.02 (d, *J* = 7.8 Hz, 1H; H5), 8.48 (d, *J* = 8.4 Hz, 1H; H7), 11.34 ppm (brs, 1H; NH); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz): δ = 52.3 (CH<sub>3</sub>), 121.4 (CH), 122.7 (C), 127.2 (CH), 129.0 (CH), 130.1 (CH), 130.6 (CH), 131.0 (C), 132.4 (CH), 132.9 (C), 133.4 (C), 135.5 (CH), 135.6 (CH), 135.8 (C), 137.0 (C), 160.9 (C), 167.5 ppm (C); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>17</sub>H<sub>13</sub>N<sub>2</sub>O<sub>5</sub>: 325.0824 [*M* + H]<sup>+</sup>; found: 325.0819.

#### General procedure for the preparation of compounds 21–23

A mixture of H<sub>2</sub>O/ethanol (2:8, 40 mL) was added to 1 equiv (200 mg) of the 3-(methoxycarbonylphenyl)-8-nitroquinolin-2(1H)-one derivative (18–20). Then, NaOH (5 equiv) was added and the reaction mixture was stirred at 80 °C for 3 h. The reaction mixture was then poured into water, acidified to pH 1 with 37% HCl, and extracted with dichloromethane (2×50 mL) and ethyl acetate (2×50 mL). The combined organic layers were washed with water, dried over anhydrous MgSO<sub>4</sub> and evaporated in vacuo. The crude residue was purified by chromatography on silica gel and recrystallised if necessary to give compound 21, 22 or 23.

**3-(4-Carboxyphenyl)-8-nitroquinolin-2(1H)-one (21)** was washed with dilute aqueous hydrochloric acid and recrystallised from acetonitrile to yield a yellow solid (124 mg, 0.40 mmol, 65%); mp: > 310 °C; <sup>1</sup>H NMR ([D<sub>6</sub>]DMSO, 400 MHz): δ = 7.45–7.49 (m, 1H; H6), 7.93–7.96 (m, 2H; H2' and H6'), 8.03–8.06 (m, 2H; H3' and H5'), 8.26 (dd, *J* = 7.9, 1.4 Hz, 1H; H5), 8.46 (dd, *J* = 8.3, 1.4 Hz, 1H; H7), 8.49 (s, 1H; H4), 11.23 (brs, 1H; NH), 13.07 ppm (brs, 1H; COOH); <sup>13</sup>C NMR ([D<sub>6</sub>]DMSO, 100 MHz): δ = 122.3 (CH), 122.35 (C), 128.1 (CH), 129.2 (2×CH), 129.6 (2×CH), 131.0 (C), 131.7 (C), 132.8 (C), 133.8 (C), 136.7 (CH), 139.1 (CH), 139.5 (C), 160.4 (C), 167.5 ppm (C); HRMS (ESI<sup>+</sup>): *m/z* calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>5</sub>: 311.0668 [*M* + H]<sup>+</sup>; found: 311.0669.

**3-(3-Carboxyphenyl)-8-nitroquinolin-2(1H)-one (22)** was washed with dilute aqueous hydrochloric acid and recrystallised from acetonitrile to yield a brown solid (88 mg, 0.28 mmol, 42%). *T*<sub>dec</sub> = 305–306 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ = 7.44–7.48 (m, 1H; H6), 7.60–7.64 (m, 1H; H5'), 8.00–8.02 (m, 1H; H6'), 8.03–8.06 (m, 1H; H4'), 8.27 (dd, *J* = 7.8, 1.4 Hz, 1H; H5), 8.39–8.40 (m, 1H; H2'), 8.46 (dd, *J* = 8.3, 1.4 Hz, 1H; H7), 8.47 (s, 1H; H4), 11.22 (brs, 1H; NH), 13.05 ppm (brs, 1H; COOH); <sup>13</sup>C NMR ([D<sub>6</sub>]DMSO, 100 MHz): δ = 122.2 (CH), 122.5 (C), 127.9 (CH), 129.0 (CH), 129.8 (CH), 129.9 (CH), 131.3 (C), 131.8 (C), 132.8 (C), 133.4 (CH), 133.7 (C), 135.6 (C), 136.7 (CH), 138.6 (CH), 160.5 (C), 167.6 ppm (C); HRMS (DCl, CH<sub>4</sub>): *m/z* calcd for C<sub>16</sub>H<sub>11</sub>N<sub>2</sub>O<sub>4</sub>: 311.0668 [*M* + H]<sup>+</sup>; found: 311.0672.

**3-(2-Carboxyphenyl)-8-nitroquinolin-2(1H)-one (23)** was purified by chromatography on silica gel using dichloromethane/ethyl ace-

tate (50:50) as eluent to yield a yellow solid (109 mg, 0.35 mmol, 57%).  $T_{\text{dec}} = 282\text{--}285\text{ }^{\circ}\text{C}$ ;  $^1\text{H NMR}$  ( $[\text{D}_6]$ DMSO, 400 MHz):  $\delta = 7.43\text{--}7.48$  (m, 2H; H6 and H6'), 7.54–7.58 (m, 1H; H4'), 7.66–7.70 (m, 1H; H5'), 7.91 (dd,  $J = 7.7$ , 1.4 Hz, 1H; H5), 8.13 (s, 1H; H4), 8.20 (dd,  $J = 7.8$ , 1.4 Hz, 1H; H3'), 8.42 (dd,  $J = 8.3$ , 1.4 Hz, 1H; H7); 11.08 (brs, 1H; NH), 12.72 ppm (brs, 1H; COOH);  $^{13}\text{C NMR}$  ( $[\text{D}_6]$ DMSO, 100 MHz):  $\delta = 122.1$  (CH), 122.6 (C), 127.4 (CH), 129.1 (CH), 129.8 (CH), 131.3 (CH), 132.4 (CH), 132.5 (C), 132.7 (C), 133.9 (C), 136.1 (CH), 136.2 (C), 136.3 (CH), 136.4 (C), 160.7 (C), 168.4 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{16}\text{H}_{11}\text{N}_2\text{O}_5$ : 311.0668  $[\text{M} + \text{H}]^+$ ; found: 311.0673.

#### Preparation of 8-nitro-3-phenylethynylquinolin-2(1H)-one (24)

3-Bromo-8-nitroquinolin-2(1H)-one (100 mg, 0.37 mmol, 1 equiv) previously synthesised in our team, CuI (7 mg, 0.037 mmol, 0.1 equiv) and Pd(PPh<sub>3</sub>)<sub>4</sub> (43 mg, 0.037 mmol, 0.1 equiv) were added to a sealed 10 mL flask. Under an argon atmosphere, anhydrous dimethoxyethane (5 mL), Et<sub>3</sub>N (155  $\mu\text{L}$ , 1.11 mmol, 3 equiv) and phenylacetylene (61  $\mu\text{L}$ , 0.56 mmol, 1.5 equiv) were successively added. The reaction mixture was cooled at 15  $^{\circ}\text{C}$  for 1.5 h. The reaction mixture was poured into water and extracted three times with dichloromethane. The organic layers were combined, washed with water, dried over anhydrous MgSO<sub>4</sub> and evaporated in vacuo. The crude residue was purified by chromatography on silica gel using dichloromethane as an eluent to yield **24** as a yellow solid (78 mg, 0.27 mmol, 72%); mp: 186–187  $^{\circ}\text{C}$ ;  $^1\text{H NMR}$  (CDCl<sub>3</sub>, 400 MHz):  $\delta = 7.31\text{--}7.35$  (m, 1H; H6), 7.37–7.38 (m, 2H; H3', H4' and H5'), 7.59–7.63 (m, 2H; H2' and H6'), 7.87 (dd,  $J = 7.6$ , 1.5 Hz, 1H; H5), 8.06 (s, 1H; H4), 8.49 (dd,  $J = 8.4$ , 1.4 Hz, 1H; H7), 11.40 ppm (brs, 1H; NH);  $^{13}\text{C NMR}$  (CDCl<sub>3</sub>, 100 MHz):  $\delta = 83.4$  (C), 97.3 (C), 119.6 (C), 121.8 (CH), 122.0 (C), 122.1 (C), 128.0 (CH), 128.4 (2 $\times$ CH), 129.2 (CH), 132.1 (2 $\times$ CH), 133.0 (C), 133.0 (C), 135.3 (CH), 141.6 (CH), 159.9 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{17}\text{H}_{13}\text{N}_2\text{O}_5$ : 291.0770  $[\text{M} + \text{H}]^+$ ; found: 291.0776.

#### Preparation of 8-nitro-2-phenyl-furo[2,3-b]quinoline (25)

3-Bromo-8-nitroquinolin-2(1H)-one (100 mg, 0.37 mmol, 1 equiv) previously synthesised in our team, CuI (7 mg, 0.037 mmol, 0.1 equiv) and Pd(PPh<sub>3</sub>)<sub>4</sub> (43 mg, 0.037 mmol, 0.1 equiv) were added to a sealed 10 mL flask. Under an argon atmosphere, anhydrous dimethoxyethane (5 mL), Et<sub>3</sub>N (155  $\mu\text{L}$ , 1.11 mmol, 3 equiv) and phenylacetylene (61  $\mu\text{L}$ , 0.56 mmol, 1.5 equiv) were successively added. The reaction mixture was heated at 40  $^{\circ}\text{C}$  for 30 min. The reaction mixture was then poured into water and extracted three times with dichloromethane. The organic layer was washed with water, dried over anhydrous MgSO<sub>4</sub> and evaporated in vacuo. The crude residue was purified by chromatography on silica gel using dichloromethane as an eluent and isolated to yield **25** as a grey solid (55 mg, 0.19 mmol, 51%); mp: 226  $^{\circ}\text{C}$ ;  $^1\text{H NMR}$  (CDCl<sub>3</sub>, 400 MHz):  $\delta = 7.16$  (s, 1H; H3), 7.45–7.55 (m, 3H; H3', H4' and H5'), 7.56–7.59 (m, 1H; H6), 7.99–8.02 (m, 2H; H2' and H6'), 8.08 (dd,  $J = 7.5$ , 1.4 Hz, 1H; H5), 8.14 (dd,  $J = 8.3$ , 1.4 Hz, 1H; H7), 8.41 ppm (s, 1H; H4);  $^{13}\text{C NMR}$  (CDCl<sub>3</sub>, 100 MHz):  $\delta = 99.2$  (CH), 123.3 (CH), 123.4 (CH), 124.0 (C), 125.9 (2 $\times$ CH), 127.7 (C), 128.2 (CH), 128.6 (C), 129.1 (2 $\times$ CH), 130.5 (CH), 132.0 (CH), 136.0 (C), 147.7 (C), 159.6 (C), 162.4 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{17}\text{H}_{11}\text{N}_2\text{O}_3$ : 291.0770  $[\text{M} + \text{H}]^+$ ; found: 291.0768.

#### Preparation of 3-(4-carboxyphenyl)-2-methoxy-8-nitroquinoline (26)

Under an argon atmosphere, 3-(4-methoxycarbonylphenyl)-8-nitroquinolin-2(1H)-one (260 mg, 0.80 mmol, 1 equiv) was dissolved in anhydrous DMF (5 mL). This solution was then added to 60% sodium hydride (64 mg, 1.6 mmol, 2 equiv) in DMF (5 mL). After 10 min of stirring at RT, methyl iodide (100  $\mu\text{L}$ , 1.6 mmol, 2 equiv) was added dropwise. The reaction mixture was stirred at RT overnight, before being poured over ice. A precipitate formed and was filtered off and washed with water. The precipitate was dissolved in dichloromethane and this solution was dried over anhydrous MgSO<sub>4</sub> and evaporated in vacuo. The crude residue was purified by chromatography on silica gel using dichloromethane as eluent. The resulting white solid intermediate was dissolved in a mixture of H<sub>2</sub>O/ethanol (2:8, 40 mL). NaOH (excess, solid) was then added, and the reaction mixture was stirred at 80  $^{\circ}\text{C}$  for 2 h. The reaction mixture was then poured into water, acidified to pH 1 with 37% HCl, and extracted with dichloromethane (2 $\times$ 50 mL) and ethyl acetate (2 $\times$ 50 mL). The combined organic layers were washed with water, dried over anhydrous MgSO<sub>4</sub> and evaporated in vacuo. The residue was washed with alkaline water (pH 8) to yield **26** as a white solid (87 mg, 0.27 mmol, 61%).  $T_{\text{dec}} = 270\text{--}274\text{ }^{\circ}\text{C}$ ;  $^1\text{H NMR}$  ( $[\text{D}_6]$ DMSO, 400 MHz):  $\delta = 4.00$  (s, 3H; CH<sub>3</sub>), 7.61–7.65 (m, 1H; H6), 7.80–7.82 (m, 2H; H2' and H6'), 8.05–8.07 (m, 2H; H3' and H5'), 8.23 (dd,  $J = 7.8$ , 1.4 Hz, 1H; H5), 8.27 (dd,  $J = 8.1$ , 1.4 Hz, 1H; H7), 8.56 (s, 1H; H4), 13.07 ppm (brs, 1H; NH);  $^{13}\text{C NMR}$  ( $[\text{D}_6]$ DMSO, 100 MHz):  $\delta = 54.5$  (CH<sub>3</sub>), 124.1 (CH), 124.7 (C), 126.6 (CH), 127.0 (C), 129.7 (2 $\times$ CH), 130.0 (2 $\times$ CH), 130.9 (C), 132.7 (CH), 136.7 (C), 139.3 (CH), 140.0 (C), 146.2 (C), 160.8 (C), 167.5 ppm (C); HRMS (DCI,  $\text{CH}_4$ ):  $m/z$  calcd for  $\text{C}_{17}\text{H}_{13}\text{N}_2\text{O}_5$ : 325.0824  $[\text{M} + \text{H}]^+$ ; found: 325.0822.

#### Electrochemistry

Voltammetric measurements were carried out with a potentiostat Autolab PGSTAT100 (ECO Chemie, The Netherlands) controlled by GPES 4.09 software. Experiments were performed at room temperature in a homemade airtight three-electrode cell connected to a vacuum/argon line. The reference electrode consisted of a saturated calomel electrode (SCE) separated from the solution by a bridge compartment. The counter electrode was a platinum wire with an apparent surface area of approximately 1 cm<sup>2</sup>. The working electrode was a GC microdisk (1.0 mm diameter; Bio-logic SAS). The supporting electrolyte [*n*Bu<sub>4</sub>N][PF<sub>6</sub>] (99% puriss electrochemical grade, Fluka) and the solvent DMSO (Sigma–Aldrich puriss p.a., dried, < 0.02% water) were used as received and simply degassed under argon. The solutions used during the electrochemical studies were typically 10<sup>−3</sup> and 0.1 M with respect to test compound and supporting electrolyte, respectively. Before each measurement, the solutions were degassed by bubbling argon, and the working electrode was polished with a polishing machine (Presi P230). Under these experimental conditions, the half-wave potential ( $E_{1/2}$ ) of the ferrocene Fc<sup>+</sup>/Fc couple in DMSO was  $E_{1/2} = 0.45$  V versus SCE. Experimental peak potentials were measured versus SCE and converted to NHE by adding 0.241 V.

#### Biology

##### Antileishmanial activity on *L. infantum axenic amastigotes*<sup>[31]</sup>

*L. infantum* promastigotes (MHOM/MA/67/ITMAP-263, CNR Leishmania, Montpellier, France, expressing luciferase activity) in the logarithmic phase, cultivated in RPMI 1640 medium supplemented

with 5% foetal calf serum, L-glutamine (2 mM) and antibiotics (100 U mL<sup>-1</sup> penicillin and 100 µg mL<sup>-1</sup> streptomycin), were centrifuged at 900 *g* for 10 min. The supernatant was removed carefully and was replaced by the same volume of RPMI 1640 complete medium at pH 5.4 and incubated for 24 h at 24 °C. The acidified promastigotes were incubated for 24 h at 37 °C in a ventilated flask. Promastigotes were then transformed into axenic amastigotes. The effects of the test compounds on the growth of *L. infantum* axenic amastigotes were assessed as follows. *L. infantum* amastigotes were incubated at a density of 2 × 10<sup>6</sup> parasites mL<sup>-1</sup> in sterile 96-well plates with various concentrations of compounds dissolved in DMSO (final concentration < 0.5% v/v), in duplicate. The appropriate controls DMSO, amphotericin B, miltefosine and fexinidazole (reference drugs purchased from Sigma–Aldrich) were added to each set of experiments. After a 48 h incubation period at 37 °C, each well was examined by microscope for precipitate formation. To estimate the luciferase activity of axenic amastigotes, 80 µL of each well were transferred to white 96-well plates. Steady Glow reagent (Promega) was added according to the manufacturer's instructions, and plates were incubated for 2 min. The luminescence was measured using a Microbeta luminescence counter (PerkinElmer). IC<sub>50</sub> was defined as the concentration of drug required to inhibit by 50% the metabolic activity of *L. infantum* amastigotes relative to the control. IC<sub>50</sub> values were calculated by nonlinear regression analysis of dose–response curves, using TableCurve 2D V5 software. IC<sub>50</sub> values represent the mean of three independent experiments.

#### Antitrypanosomal activity on *T. brucei brucei* trypomastigotes

Assays were performed on *T. brucei brucei* AnTat 1.9 strain (IMTA, Antwerp, Belgium). Trypanosomes were cultured in MEM containing Earle's salts, supplemented according to the protocol of Baltz et al.<sup>[32]</sup> with the following additions: mercaptoethanol (0.5 mM, Sigma–Aldrich, France), L-cysteine (1.5 mM, Sigma–Aldrich), bathocuproine disulfonate (0.05 mM, Sigma–Aldrich) and 20% heat-inactivated horse serum (Gibco, France), at 37 °C in an atmosphere containing 5% CO<sub>2</sub>. The parasites were incubated at an average density of 2000 parasites per well in sterile 96-wells plates (Mc2, France) with various concentrations of compounds dissolved in DMSO (Sigma–Aldrich), in duplicate. Reference drugs suramin, eflornithine, and fexinidazole (purchased from Sigma–Aldrich, France and Fluorochem, UK) suspended in 0.9% NaCl or DMSO, were added to each set of experiments. The effects of the test compounds were assessed using the viability marker Alamar Blue assay described by R  z et al.<sup>[33]</sup> After a 69 h incubation period at 37 °C, Alamar Blue (10 µL, Fisher, France) was added to each well, and the plates were incubated for 5 h.<sup>[34]</sup> The plates were read using a PerkinElmer (Germany) ENSPIRE microplate reader using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. IC<sub>50</sub> values were calculated by nonlinear regression analysis of dose–response curves using GraphPad Prism software. IC<sub>50</sub> was defined as the concentration of drug required to inhibit by 50% the viability of *T. brucei brucei* relative to the control. IC<sub>50</sub> values were calculated from three independent experiments in duplicate.

#### Antitrypanosomal activity on *T. brucei* trypomastigotes overexpressing the nitroreductase NTR1

*Trypanosoma brucei* bloodstream-form “single marker” S427 (T7RPOL TETR NEO) and drug-resistant cell lines were cultured at 37 °C in HMI9-T medium<sup>[35]</sup> supplemented with G418 (2.5 µg mL<sup>-1</sup>,

Melford) to maintain expression of T7 RNA polymerase and the tetracycline repressor protein. Bloodstream trypanosomes overexpressing the *T. brucei* nitroreductase (NTR1)<sup>[36]</sup> were grown in medium supplemented with phleomycin (2.5 µg mL<sup>-1</sup>) and expression of NTR was induced by the addition of tetracycline (1 µg mL<sup>-1</sup>). Cultures were initiated with 1 × 10<sup>5</sup> cells per mL and sub-cultured if the cell density approached 1 × 10<sup>6</sup>–2 × 10<sup>6</sup> cells per mL. To examine the effects of inhibitors on the growth of these parasites, triplicate cultures containing the inhibitor were seeded at 1 × 10<sup>5</sup> trypanosomes per mL. Cells overexpressing NTR were induced with tetracycline 48 h prior to EC<sub>50</sub> analysis. Cell densities were determined after culture for 72 h, as previously described.<sup>[37]</sup> EC<sub>50</sub> values were determined using the following two-parameter equation by nonlinear regression using GraFit [Eq. (1)]:

$$y = \frac{100}{1 + \left(\frac{[I]}{EC_{50}}\right)^m} \quad (1)$$

The experimental data were corrected for background cell density and expressed as a percentage of the uninhibited control cell density. In this equation, [I] represents inhibitor concentration and *m* is the slope factor.

#### Cytotoxicity evaluation on HepG2 cells

The evaluation of the cytotoxicity of the test molecules on HepG2 cells (hepatocarcinoma cell line from ECACC purchased from Sigma–Aldrich, ref. 85011430-1VL, certificated without mycoplasma) was performed according to the method of Mosmann with slight modifications.<sup>[38]</sup> In brief, cells (1 × 10<sup>5</sup> cells per mL) in complete medium [100 µL, Alpha MEM Eagle from PAN BIOTECH supplemented with 10% foetal bovine serum, L-glutamine (2 mM) and antibiotics (100 U mL<sup>-1</sup> penicillin and 100 µg mL<sup>-1</sup> streptomycin)] were seeded into each well of 96-well plates and incubated at 37 °C and 5% CO<sub>2</sub>. After a 24 h incubation, medium (100 µL) with various product concentrations and appropriate controls was added and the plates were incubated for 72 h at 37 °C and 5% CO<sub>2</sub>. Each well was then examined by microscope for possible precipitate formation before the medium was aspirated from the wells. MTT solution (100 µL, 0.5 mg mL<sup>-1</sup> in Alpha MEM Eagle) was then added to each well. Cells were incubated for 2 h at 37 °C and 5% CO<sub>2</sub>. After this time, the MTT solution was removed and DMSO (100 µL) was added to dissolve the resulting formazan crystals. Plates were shaken vigorously (300 rpm) for 5 min. The absorbance was measured at 570 nm with a microplate spectrophotometer (Eon BioTek). DMSO was used as a blank and doxorubicin (Sigma–Aldrich) as a positive control. CC<sub>50</sub> values were calculated by nonlinear regression analysis of dose–response curves using TableCurve 2D V5 software. CC<sub>50</sub> values represent the mean value calculated from three independent experiments.

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## Conflict of interest

The authors declare no conflict of interest.

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## Supporting Information

### **Antitrypanosomatid Pharmacomodulation at Position 3 of the 8-Nitroquinolin-2(1H)-one Scaffold Using Palladium-Catalysed Cross-Coupling Reactions**

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# 1. Experimental spectra

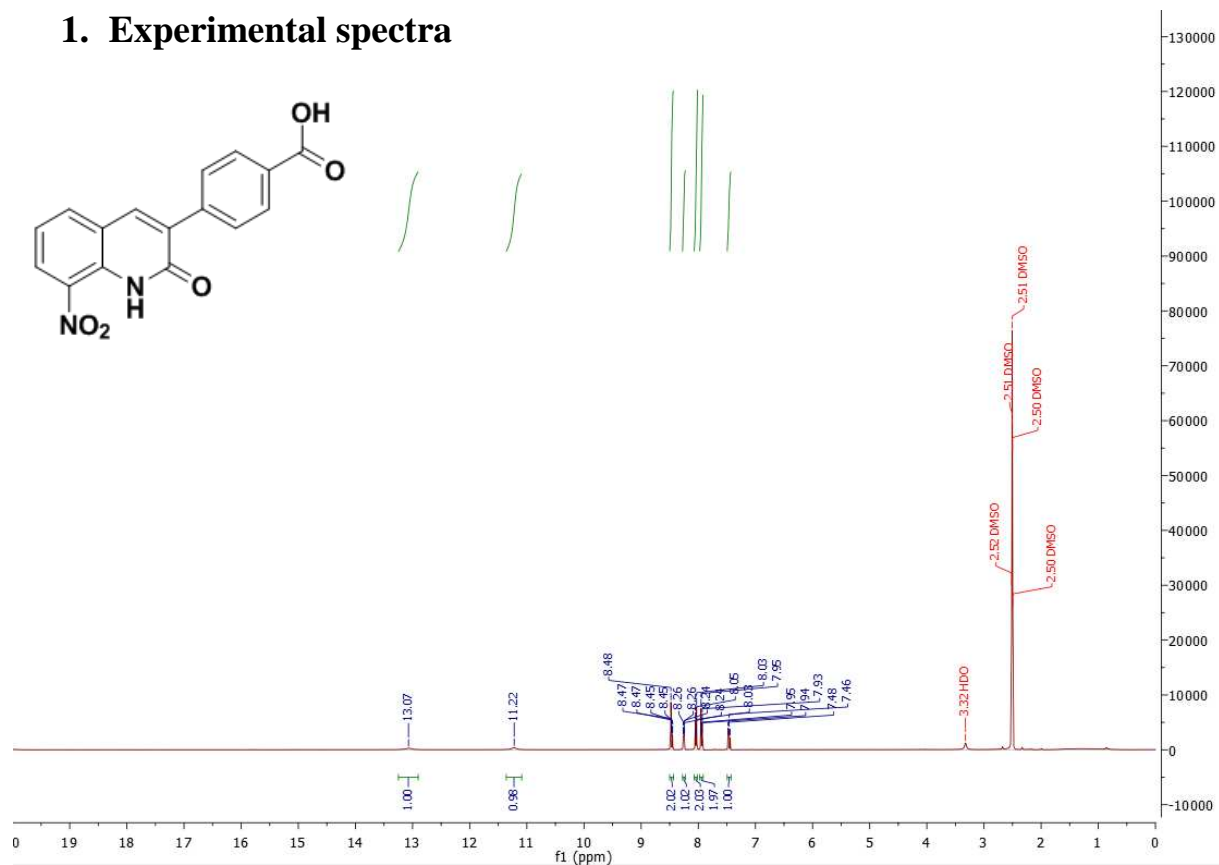


Figure S1:  $^1\text{H}$  NMR of the hit compound **21**.

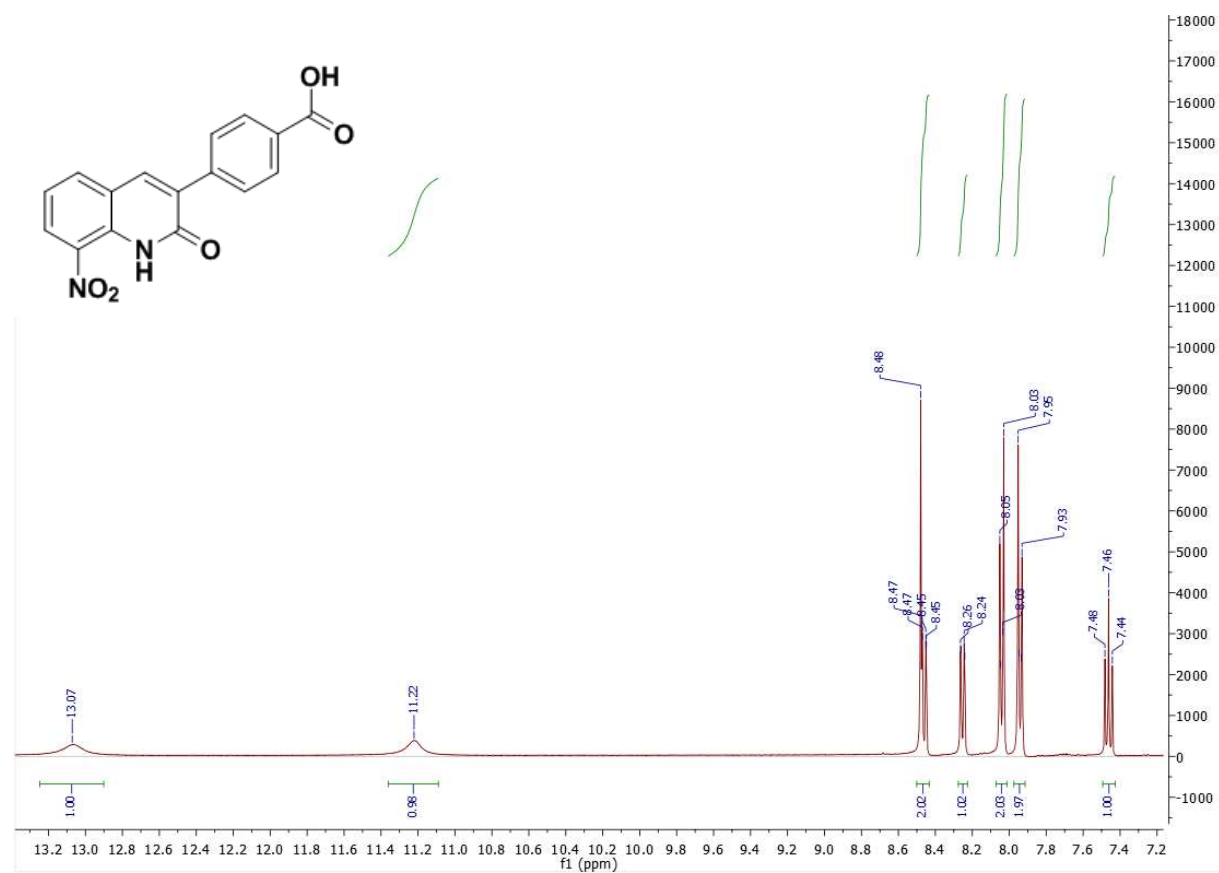
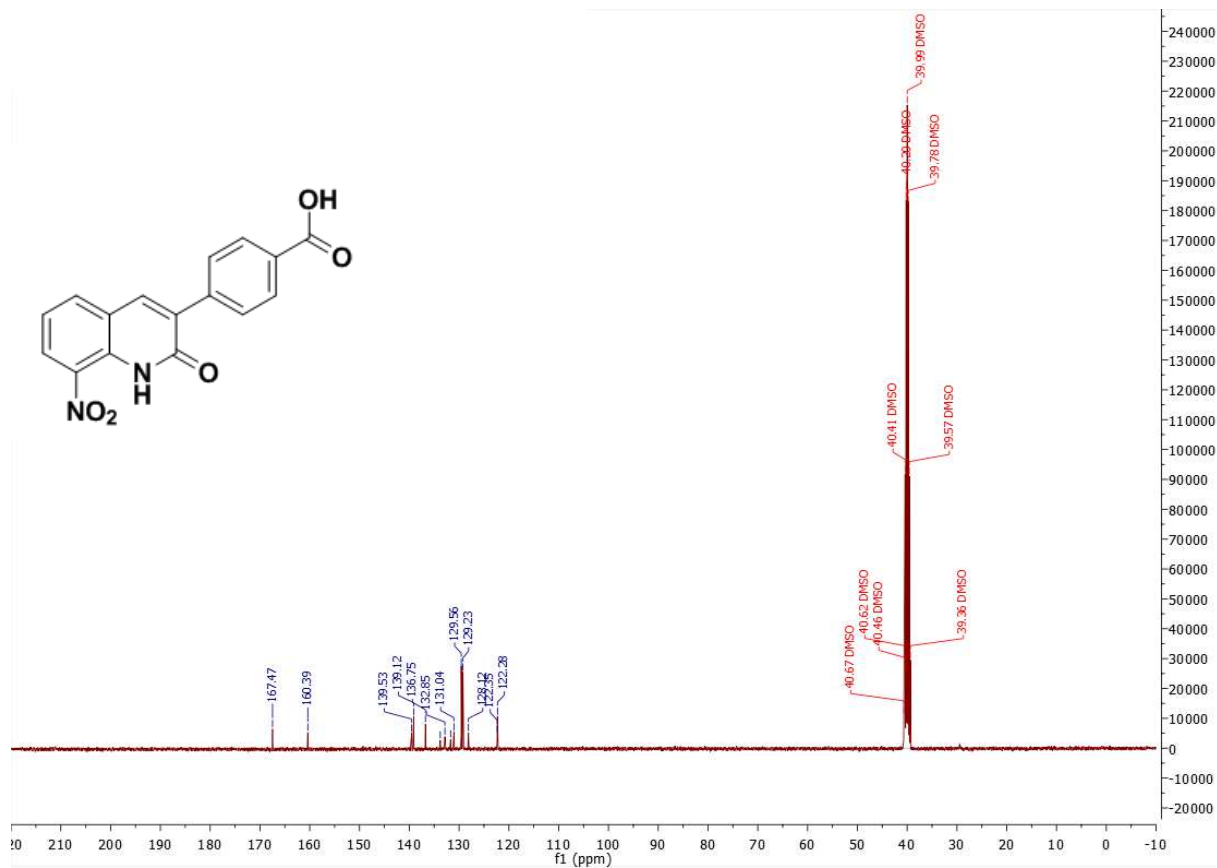
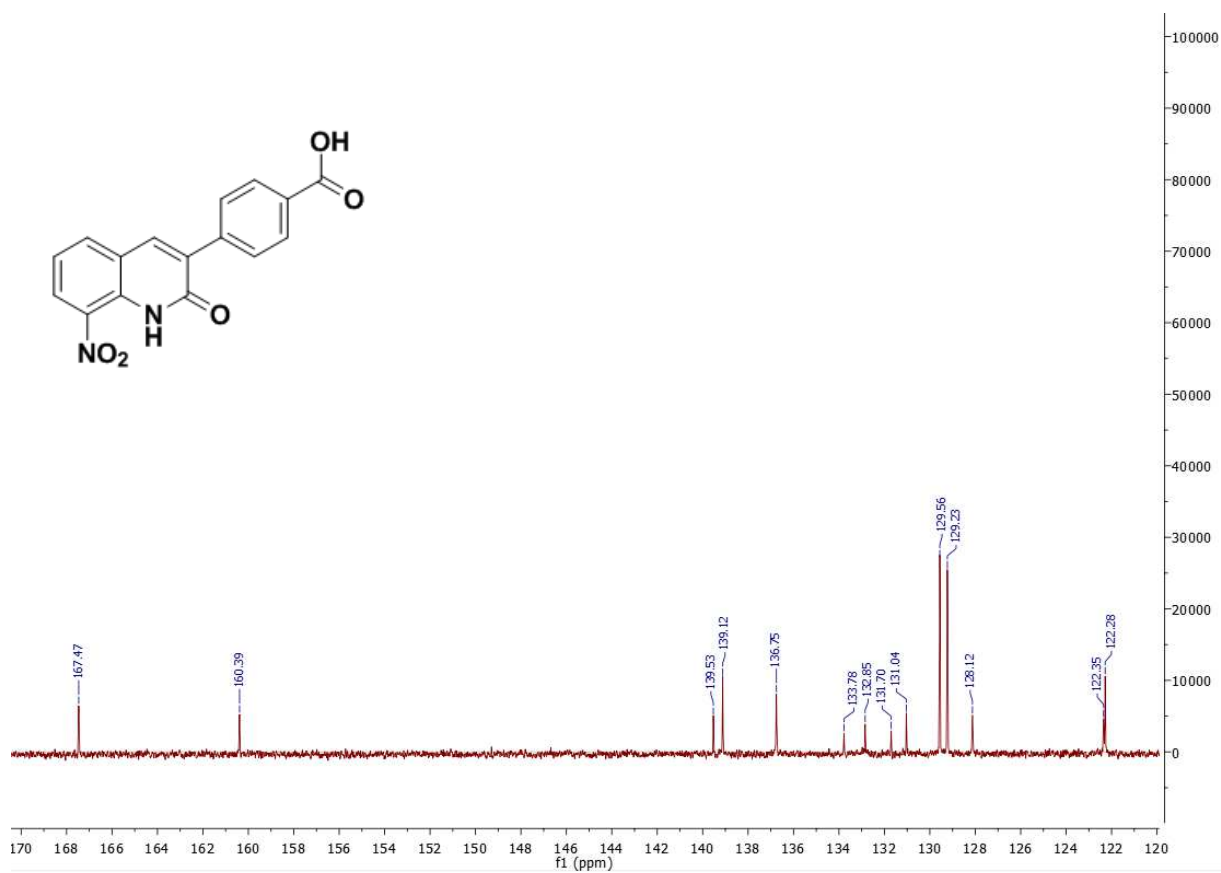


Figure S2:  $^1\text{H}$  NMR of the hit compound **21**, zoom between 7.2 and 13.2 ppm.

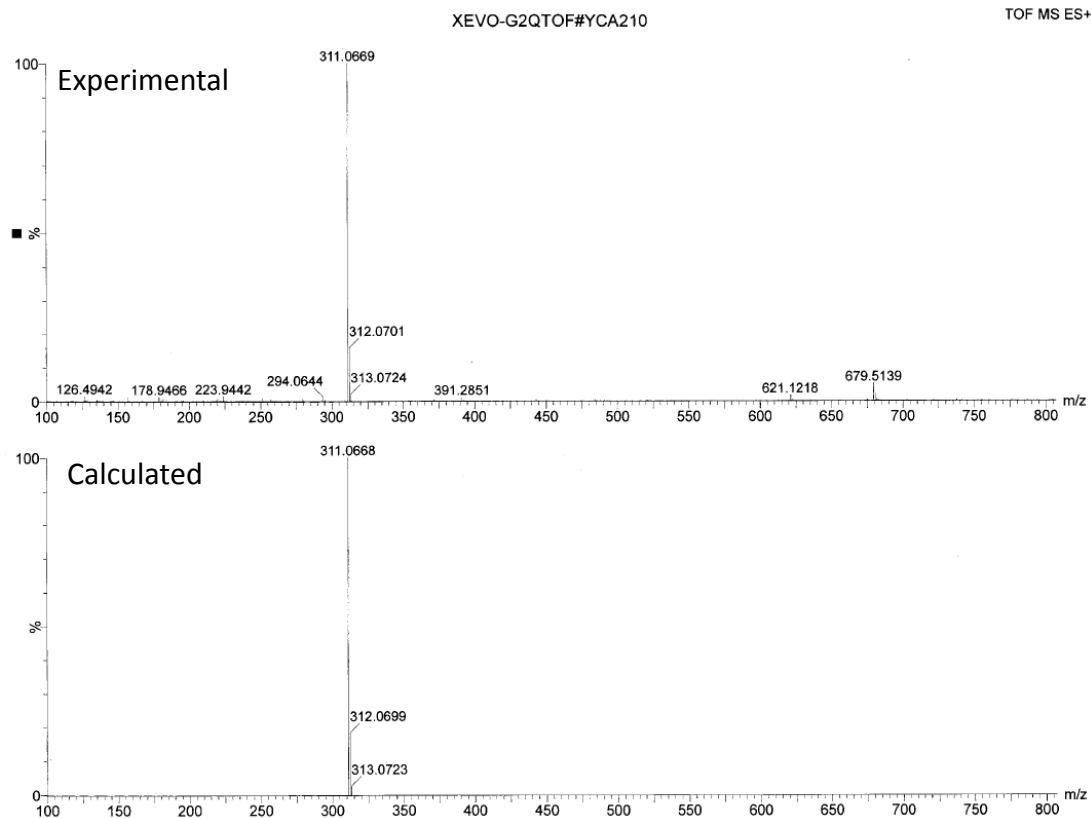


**Figure S3:**  $^{13}\text{C}$  NMR of the hit compound **21**.



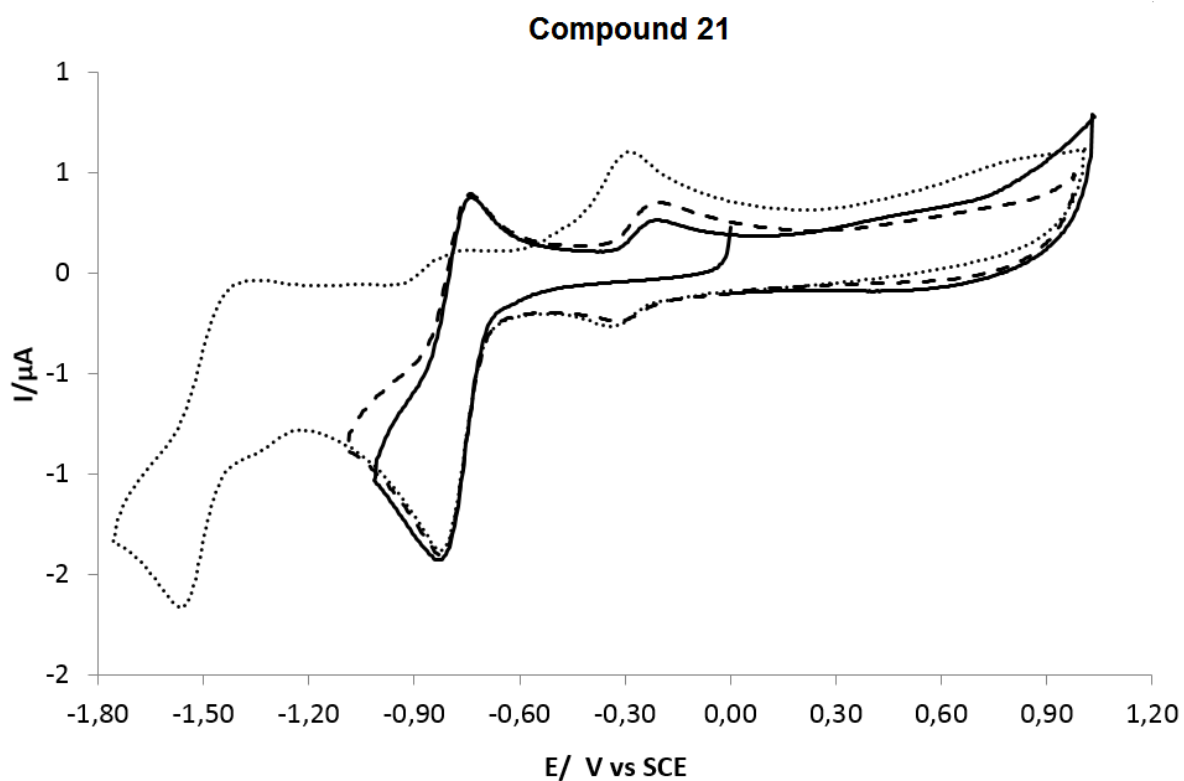
**Figure S4:**  $^{13}\text{C}$  NMR of the hit compound **21**, zoom between 120 and 170 ppm.





**Figure S5:** HRMS of the hit compound **21** between  $m/z = 100$  and  $800$  amu.

## 2. Cyclic voltammogram



**Figure S6:** Cyclic voltammetry of compound **21** ( $10^{-3}$  mol L $^{-1}$ ) in DMSO + 0.1 mol L $^{-1}$  of (n-Bu $_4$ N)[PF $_6$ ] on GC microdisk ( $r = 0.5$ mm) at room temperature. Scan rate: 0.2 V s $^{-1}$ .