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## Original article

Synthesis of symmetrically substituted 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones, as novel quinoline derivatives with antibacterial activityMatías D. Ferretti<sup>a</sup>, Alexandre T. Neto<sup>b</sup>, Ademir F. Morel<sup>b,\*</sup>, Teodoro S. Kaufman<sup>a</sup>, Enrique L. Larghi<sup>a,\*</sup><sup>a</sup> Instituto de Química Rosario (CONICET-UNR), Suipacha 531, Rosario S2002LRK, Argentina<sup>b</sup> Núcleo de Pesquisas em Produtos Naturais, Prédio 18, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

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## ABSTRACT

A novel series of symmetrically substituted 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones was synthesized and tested as antimicrobials. The minimum inhibitory concentration (MIC) values of the most active heterocycles were slightly higher than those exhibited by levofloxacin, employed as comparator. Structural factors affecting the activity were explored along three diversification points, including the substituents of the aromatic rings of the 3-benzyl moieties, as well as the functionalization of both, the homocyclic ring of the heterocycle and the quinolonic nitrogen atom. 6-Chloro and 3,3-bis(4'-chlorobenzyl) derivatives showed the lower MIC values. Optimally substituted heterocycles were synthesized, which exhibited enhanced activity.

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## 1. Introduction

Bacterial infection is one of the most complex global health issues of this century. The rise of resistant microorganisms is perceived as a serious threat, which aggravates the problem. As a result, increasing efforts have been placed during recent years towards the search for new antimicrobials [1] in order to neutralize this hazard. If left unsolved, this can evolve into a potentially dangerous public health crisis of worldwide dimensions [2].

Quinolines are privileged heterocycles which have been developed into important therapeutic agents, embodying a wide variety of biological activities [3]. Antimicrobial activity has been found among 4-oxo-1,4-dihydro-quinoline-3-carboxylic acids (collectively known as 'quinolones'), 1*H*-quinolin-2-ones (carbostyrils) [4] and 1*H*-quinoline-2,4-diones (4-hydroxy-2-quinolones) [5].

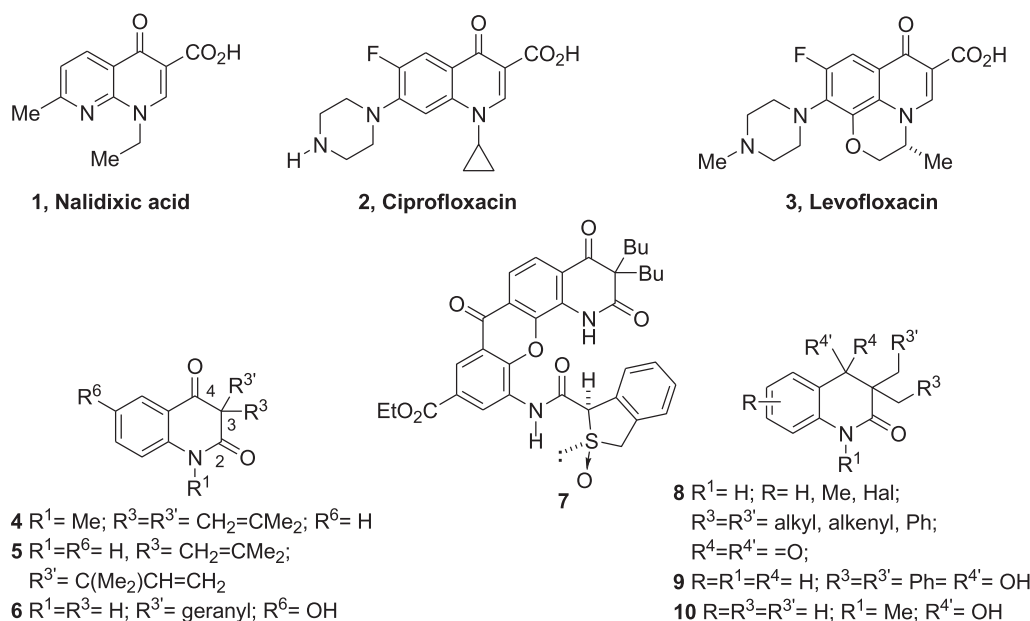
The quinolones are one of the most useful and versatile families of antibacterial agents [6]. The first generation of quinolones was developed after observations regarding nalidixic acid (1, Fig. 1), a

side product found during the synthesis of the antimalarial agent chloroquine. Continued research resulted in the discovery of more suitable analogs, successively leading to second and third generations of these antibacterials, with representatives such as ciprofloxacin (2) and levofloxacin (3), respectively, characterized by improvements in their potency, spectrum and bioavailability. Thus, nalidixic acid and its congeners were active only against Gram-negative microorganisms exhibiting poor pharmacokinetics, while ciprofloxacin increased both the spectrum and activity against aerobic Gram-negatives and levofloxacin broadened the spectrum to include some aerobic Gram-positive bacteria. Fourth generation quinolones, like moxifloxacin, are also currently available [6a]. These methoxy-quinolones are even more active against Gram-positives and anaerobes bacteria.

Functionalization of the related carbostyryl skeleton is also a fertile source of biologically important molecules that exert a plethora of bio- and pharmacological activities. These include enzyme inhibitors [7] and receptor antagonists [8], as well as antithrombotic [9], antiviral (against hepatitis B [10] and HIV [11]), antihelminthic [12], antioxidant [13], antiproliferative [14], anti-tumor [15], and cytotoxic against human tumor cell lines [16] agents. However, relatively few 1*H*-quinolin-2-one derivatives have been reported as antibacterials [17]; among them, the 4-

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**Fig. 1.** Representative examples of the quinolone antimicrobials (**1–3**) and examples of natural (**4–6**) and synthetic (**7, 8**) 3,3-disubstituted-1H-quinoline-2,4-diones and related compounds (**9, 10**).

substituted analogs rationally designed by the group of Jayashree, based on docking experiments with bacterial DNA gyrase [18].

With regards to the antibacterial activity of 1H-quinoline-2,4-diones [19,20], O'Donnell et al. [5] recently performed a detailed study on the inhibition of bacterial growth by a series of sixty synthetic and natural products, carrying different substitution patterns on their homo and heterocyclic rings.

Symmetrically and unsymmetrically 3,3-disubstituted 1H-quinoline-2-one derivatives are structurally related compounds that have been found in nature. They are represented by compound **4** [21], the HIV-1RT inhibitor buchapsine (**5**, buchapsine,  $\text{IC}_{50} = 8 \mu\text{M}$ ) [22], the derivatives of which exhibited antitumor and antimycobacterial activity [23] and the cytotoxic and pro-apoptotic agent severibuxine (**6**) [24]. Analogous compounds have also been synthesized for structural analysis and method development purposes [25], as selective 5-HT<sub>6</sub> receptor antagonists [26] and as intermediates towards antithrombotic agents [27]. In addition, the quinoline-2,4-dione derivative **7** has been used as a cleft-type receptor in chiral recognition [28].

In a previous study, we have observed through bioautographic techniques that 3,3-disubstituted-1H-quinoline-2,4-diones, including the 3,3-dimethyl, 3,3-diallyl and 3,3-dibenzyl analogs (**8**), exhibited antibacterial activity. In this preliminary work, we have explored the activity of the related 3,3-dibenzyl-4-hydroxy derivative **9**, which displayed intriguing bacterial growth inhibition [29]. On the other hand, Daruwala et al. proved that related 3,3-dialkyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-one **10** was relatively non-

toxic ( $\text{LD}_{50} > 150 \text{ mg/kg}$ ) and a mild sedative, in the method involving sodium pentobarbital sleeping time potentiation ( $\text{ED}_{50} = 85 \text{ mg/kg}$ ) [30].

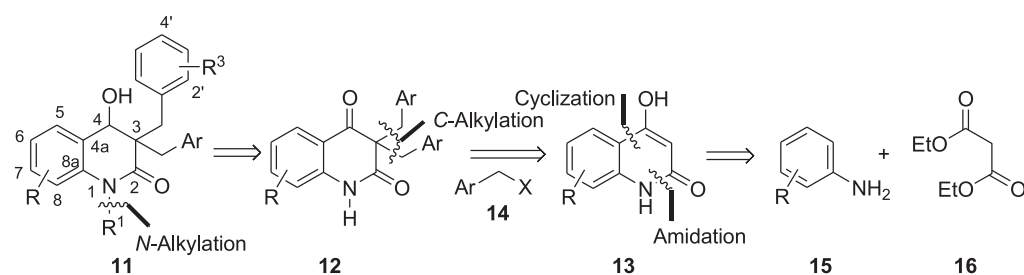
Compounds **9** and **10** belong to a class of heterocycles, the antimicrobial activity of which has not been yet systematically explored. Therefore, we decided to perform a more detailed study, by executing the synthesis of a series of 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-ones, carrying out structural modifications at three main points and evaluating their antimicrobial activity against a panel of microorganisms. Centres subjected to structural variation were the isocyclic ring, the C-3 pendant benzyl moieties and position 1 of the quinolinic core.

## 2. Results and discussion

### 2.1. Chemistry

A library of symmetrically substituted 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-one derivatives (**11**) was prepared, employing a modular approach based on the retrosynthetic analysis shown in Scheme 1, which hinged upon the intermediacy of 4-hydroxy-1H-quinoline-2-one intermediates (**13**) and uncovered anilines (**15**) and diethyl malonate (**16**) as suitable starting materials.

In this way, substituents with different H-bond donor/acceptor characteristics and steric/electronic properties were introduced during the syntheses through the use of properly substituted



**Scheme 1.** Retrosynthetic analysis of the target 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-ones **11**.

aromatic precursors **14** and **15** leading to **12**. *N*-alkylation of the latter provided a third point of structural diversification.

Accordingly, six substituted anilines were condensed at high temperature with diethyl malonate (**16**) under solventless condition, affording the expected bis-malonamide intermediates (**17–22**) in 44–91% yield (Scheme 2). In turn, these dianilides were cyclized under promotion of polyphosphoric acid, as eco-friendly Lewis acid, at 120 °C during a standard time of 6 h [31], to give the desired 4-hydroxy-carbostyryls (**23–28**) in 17–98% yield.

With these synthetic intermediates in hand, and in order to introduce the second point of diversification, the regioselective C-3 dibenzilation of heterocycles **23–28** was next undertaken, employing an adaptation of previous procedures, with benzyl halides as alkylating agents and K<sub>2</sub>CO<sub>3</sub> as base [32]. Under these conditions, the desired 3,3-dibenzyl-1*H*-quinoline-2,4-diones **29–38** were obtained in 34–69% yield after 24–36 h.

With the purpose of including the third point of structural variation, the regioselective *N*-benzylation of 2,4-dione **29** was initially explored in anhydrous DMF, employing NaH as base [33]; however, only decomposition products were detected. Better results were obtained when the K<sub>3</sub>PO<sub>4</sub>/*N,N*-dimethylacetamide (DMA) reagent system was used [34] which afforded exclusively the desired *N*-benzylated product (**41**), albeit in moderate yield (36%).

The selective *N*-alkylation was carried out employing K<sub>2</sub>CO<sub>3</sub> in refluxing MeCN [35]. This procedure accepted a diversity of electrophiles and allowed access to *N*-alkylated products **39–44** in good to very good yields (60–89%).

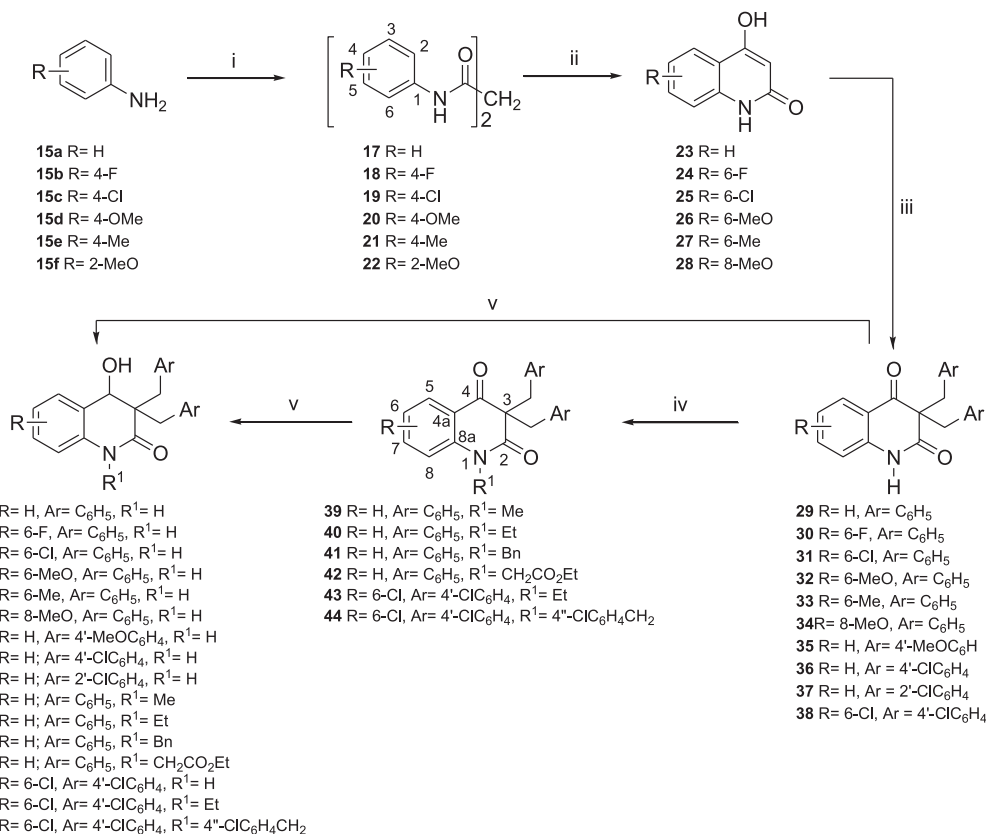
Finally, the ketonic carbonyl moiety of the quinoline-2,4-diones **29–44** was selectively reduced with NaBH<sub>4</sub> in a EtOAc-EtOH mixture, uneventfully rendering the expected carbinols (**45–60**) in 37–99% yield.

## 2.2. Biological activity

Initially, the 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones **44–53** were tested as antimicrobials employing the broth micro dilution technique, with levofloxacin (**3**) as comparator. The results (Table 1) revealed that among the 3,3-dibenzyl congeners **45–50**, the derivatives on the homocyclic ring exhibited the lowest MIC. The 6-chloro derivative **47** was the most potent compound, followed by the 6-fluoro analog **46**. These values were 2–4 times lower than those observed for **45** (unsubstituted on the aromatic ring of the heterocyclic scaffold) and the 6-methyl (**49**), 6-methoxy (**48**) and 8-methoxy substituted derivatives (**50**).

Functionalization of the 3-benzyl substituents strongly affected the antibacterial activity. Thus, while the 4'-MeO substituted analog **51** was as active as the non-substituted precursor **45**, the 3,3-bis(4'-chlorobenzyl) derivative **52** showed MIC values 8–60 times lower than **45** indicating a higher level of antibacterial activity. In addition, the position of the chlorine atom within the aromatic ring of the benzyl group of the analogs also proved to be relevant, as the MIC value of 4'-chloro derivative **52** was up to eight times lower than that of its 2'-chloro substituted congener **53**. These observations suggest that steric and also electronic factors may have some impact on the potency of these compounds.

Compounds **54–57** served to understand the effect of *N*-substitution. When compared to **45**, it was observed that the introduction of small alkyl groups such as methyl (**54**) and ethyl (**55**) afforded minor improvements on the bioactivity and only against *Bacillus subtilis* and *Escherichia coli*, while the presence of the bulkier benzyl group (**56**) afforded a product which displayed essentially the same profile of the parent heterocycle **45** and installation of an alkyl acetate moiety (**57**) resulted in a substance devoid of activity.



**Scheme 2.** Synthesis of 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones **45–60**. Reagents and conditions: i) H<sub>2</sub>C(CO<sub>2</sub>Et)<sub>2</sub> (**16**), neat, 210–220 °C, 1–2 h. ii) PPA, 130–140 °C, 6 h. iii) ArCH<sub>2</sub>X (X = Br, Cl), K<sub>2</sub>CO<sub>3</sub>, PhMe/H<sub>2</sub>O (1:1, v/v), 110 °C, 24–48 h. iv) (R<sup>1</sup>O)<sub>2</sub>SO<sub>2</sub> or R<sup>1</sup>Br, K<sub>2</sub>CO<sub>3</sub>, MeCN, 80 °C, 24–40 h. v) NaBH<sub>4</sub>, EtOAc-EtOH (1:1, v/v), r.t., 2–5 h.

**Table 1**MIC and MLC ( $\mu\text{g/mL}$ ) results obtained for the 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-ones **45–60** against a panel of five microorganisms.<sup>a</sup>

Compound	Microorganisms									
	Gram-positive						Gram-negative			
	<i>B. subtilis</i>		<i>S. aureus</i>		<i>S. sonnei</i>		<i>P. aeruginosa</i>		<i>E. coli</i>	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
<b>45</b>	100	>200	50	>200	50	>200	100	>200	100	>200
<b>46</b>	25	200	50	>200	25	>200	50	>200	25	>200
<b>47</b>	12.5	200	25	>200	12.5	>200	50	>200	12.5	200
<b>48</b>	50	>200	100	>200	100	>200	100	>200	100	>200
<b>49</b>	50	>200	50	>200	50	>200	100	>200	25	>200
<b>50</b>	100	>200	100	>200	100	>200	100	>200	100	>200
<b>51</b>	100	>200	100	200	100	>200	100	>200	100	>200
<b>52</b>	1.56	25	6.25	100	6.25	100	100	100	1.52	25
<b>53</b>	12.5	200	12.5	200	12.5	200	100	>200	12.5	200
<b>54</b>	50	>200	100	>200	100	>200	100	>200	50	>200
<b>55</b>	12.5	>200	100	>200	200	>200	100	>200	100	>200
<b>56</b>	100	>200	100	>200	200	>200	>200	—	100	>200
<b>57</b>	>200	—	>200	—	>200	—	>200	—	100	>200
<b>58</b>	3.12	50	3.12	12.5	3.12	12.5	50	100	3.12	50
<b>59</b>	3.12	25	6.25	50	50	100	50	50	50	100
<b>60</b>	>100	>200	50	100	50	100	50	50	50	>100
<b>Levofloxacin</b>	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12	3.12

<sup>a</sup> Mean of 4 determinations.

From these results it may be inferred that functionalization of the nitrogen atom affects the potency of the heterocycles and that the nature of the substituent has some influence on it, presumable through steric (**54** and **55** vs. **56** and **57**) as well as electronic (**55** and **56** vs. **57**) effects.

Based on the structures of the heterocycles that showed lower MIC values, a new compound was designed to embody modifications at two diversification points; therefore compound **58** carrying a 6-chloro substituent and two 4'-chlorobenzyl groups on C-3, was synthesized through the intermediacy of **38**. The resulting trichloro derivative retained the activity, exhibiting approximately the same spectrum and about one half the MIC of the parent compound **52**, demonstrating that the effects of the substituents were non-additive.

In a further modification, and taking into account the improved performance of **55** with regards to **45**, compound **59** (the *N*-ethyl derivative of **58**) was synthesized by *N*-alkylation of **38**, followed by reduction of the resulting quinoline-2,4-dione **43**. Interestingly, this compound displayed a narrower spectrum, remaining a similar MIC value than **58** against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, while being essentially inactive against the Gram-negative test strains (Table 1). Not unexpectedly, however, in light of the profile of **56**, no improvements were observed when the bulkier and more lipophilic 4'-chlorobenzyl moiety was attached to the nitrogen atom (**60**).

Interestingly, the tested compounds exhibited comparatively higher MIC values when exposed to *Pseudomonas aeruginosa*. This

observation reflects some degree of selectivity on the side of the assayed compounds. *P. aeruginosa* has demonstrated to exhibit differential sensitivity against different quinolone antibacterials [36].

Compounds **58** and **59** were also examined against an additional set of five clinically relevant bacteria. The results (Table 2) revealed only subtle differences between their profiles.

Therefore, in order to get some insights on the mode of action of these heterocycles, the behavior responses of **58** and **59** against *Enterococcus* spp. were studied, at concentration levels in the range 0.5–8 times the MIC, and the responses were assessed against those of ampicillin and levofloxacin (**3**), as comparators. The modes of action of these antibacterials (interaction with PBPs and type II bacterial topoisomerases, respectively), trigger a series of events which may lead (on a species specific basis) to filamentation or lysis, thus modifying the turbidity of the medium. The determination of the optical densities at 620 nm ( $\text{OD}_{620}$ ) was employed as a measure of the turbidity of the medium [37], and in both cases controls devoid of the antibacterials were used, which displayed a continuous increase of the  $\text{OD}_{620}$  due to normal bacterial growth. The results are displayed in Figs. 2 and 3.

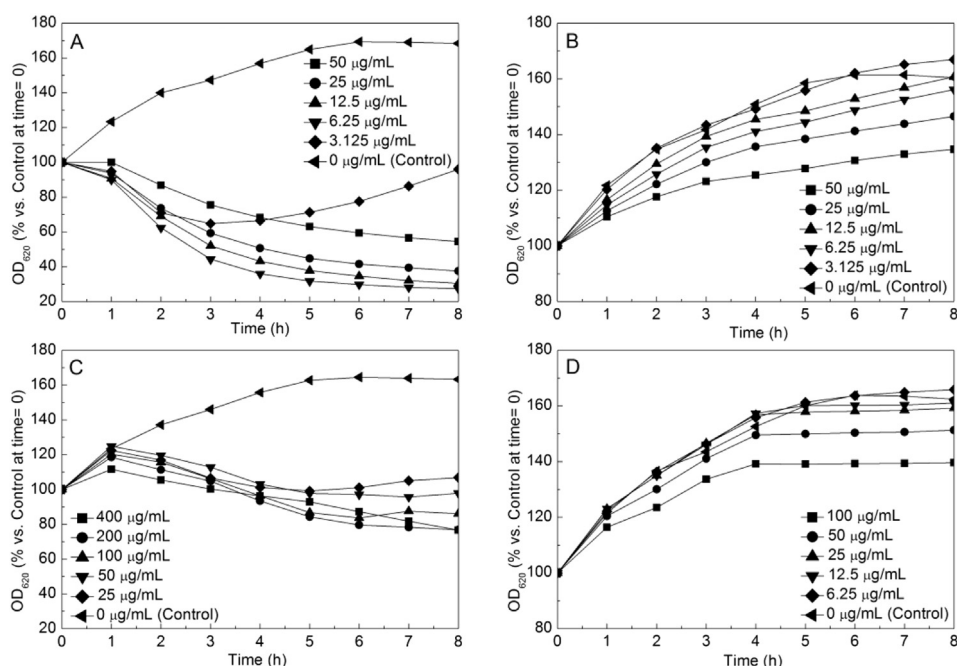
The experiments revealed that the turbidity diminished rapidly after treatment with **58** (Fig. 2A) at concentrations as low as the MIC (6.25  $\mu\text{g/mL}$ ). In the presence of **58** the media exhibited a typical decrease in the  $\text{OD}_{620}$  after a short initial growth period, resembling the response of ampicillin (Fig. 2C); however, the effect of **58** took place at lower concentration and the variation in

**Table 2**MIC and MLC ( $\mu\text{g/mL}$ ) results for **58** and **59** against selected microorganisms.<sup>a</sup>

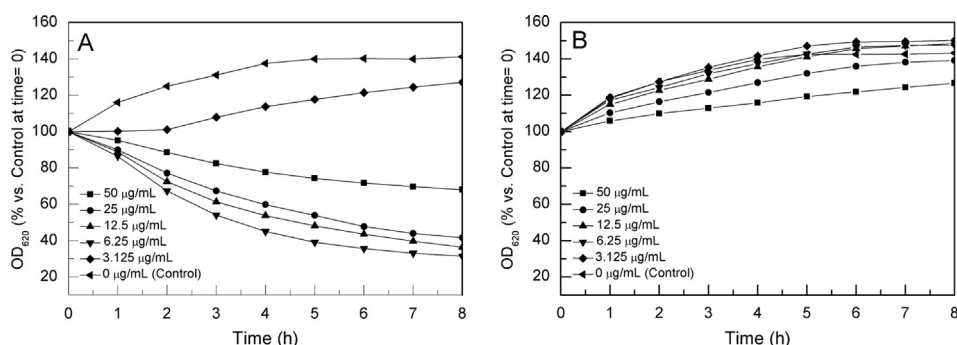
Compound	Microorganisms										
	Gram-positive					Gram-negative					
	<i>Enterococcus</i> spp.		<i>Enterobacter aerogenes</i>			<i>Salmonella typhimurium</i>		<i>Burkholderia cepacia</i>		<i>Morganella morganii</i>	
	MIC	MLC	MIC	MLC		MIC	MLC	MIC	MLC	MIC	MLC
<b>58</b>	6.25	50	50	>100		50	100	6.25	6.25	1.56	3.12
<b>59</b>	6.25	25	50	>100		50	100	6.25	12.5	3.12	12.5
<b>Levofloxacin</b>	0.78	0.78	0.78	0.78		0.78	0.78	0.78	0.78	0.78	1.56

<sup>a</sup> Mean of 4 determinations.





**Fig. 2.** Growth inhibition effect of compounds **58** (A) and **59** (B) against *Enterococcus* spp. For the sake of comparison, the responses of ampicillin (C) and levofloxacin (D) are also shown. Cells were incubated in casein soy broth medium, and each compound was added at time zero. OD<sub>620</sub> = Optical density at 620 nm. Average of readings from quadruplicate experiments; with error < 5%.



**Fig. 3.** Growth inhibition effect of compounds **58** (A) and **59** (B) against *Morganella morganii*. Cells were incubated in casein soy broth medium, and each compound was added at time zero. OD<sub>620</sub> = Optical density at 620 nm. Average of readings from quadruplicate experiments; error < 5%.

turbidity levels was more pronounced. Interestingly, at 3.12 µg/mL, the medium containing **53** displayed an initial decay in the OD<sub>620</sub>, followed by bacterial regrowth after 3 h.

A closer inspection of the curves of *Enterococcus* spp. treated with **58** (Fig. 2A) revealed an apparent paradox. The decrease of OD<sub>620</sub> was lower at higher concentrations of the tested heterocycle. This is reminiscent to the behavior of nalidixic acid (**1**) and related quinolones, as well as other antibacterials (Eagle effect) [38]. Nalidixic acid was found to be most bactericidal at 50–200 µg/mL. However, concentrations of **1** above this range reduced rather than increased its bactericidal effect. At higher levels, around 400 µg/mL, the drug was relatively bacteriostatic. When the mode of action of nalidixic acid was investigated, it was found that at the most bactericidal concentration the drug inhibited production of DNA, but not RNA or protein synthesis. However, at higher concentrations, where the drug was least bactericidal, RNA and protein synthesis were both found to be inhibited [39].

On the other side, compound **59** was also capable of causing growth retardation in a concentration-dependent manner,

exhibiting response curves (Fig. 2B) similar to those of levofloxacin (Fig. 2D). These curves were characterized by a relatively rapid increase of the OD<sub>620</sub> during the first 3 h, followed by increments at a much slower pace after the fourth hour. Additionally, compound **58** was found to be a more effective antibacterial than ampicillin (Fig. 2A and C), reaching higher inhibition levels in a shorter time.

A similar behavior was noted when cultures of *Morganella morganii* were exposed to the heterocycles **58** and **59** at concentrations between 3.12 and 50 µg/mL (Fig. 3). Compound **58** caused a decrease in OD<sub>620</sub> at the MIC (6.25 µg/mL) and above (Fig. 3A); furthermore, the curves exhibited characteristics analogous to those observed when the heterocycle was made to interact with *Enterococcus* spp. (Fig. 2A). On the contrary, the *N*-ethyl derivative **59** displayed a different behavior (Fig. 3B); its effects were not significant up to 12.5 µg/mL (2 × MIC), causing a noticeable concentration-dependent growth retardation above 25 µg/mL.

The mechanism of action of the tested 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones is still unclear. Nevertheless, these compounds lack the 3-carboxy-4-oxo motif, characteristic of the

quinolone antibacterials, which is the minimal structural requirement for chelation with  $Mg^{++}$  at the DNA-gyrase site [40]. Thus, **58** and **59** would in principle be operating under a different mechanism from that of the traditional quinolones, however further experimentation is needed in order to solve this question.

### 3. Conclusions

The synthesis and biological activity of a library of sixteen 3,4-dihydro-1*H*-quinolin-2-one derivatives, embodying a 3,3-dibenzyl-4-hydroxy pattern and three main sites of structural diversification, were reported. The design of the library allowed gaining preliminary insights into the structural requirements for the activity of the heterocycles and helped to improve their antibacterial profile.

In some cases, the antibacterial activity of the heterocycles against a panel of five different microorganisms, including Gram-positive and Gram-negative strains reached levels comparable to those of levofloxacin. However, unlike the latter, these novel compounds are of simple preparation, which can be performed from easily available starting materials and low cost reagents.

Further experimentation is under way in order to acquire knowledge on the influence of the configuration of C-4 hydroxyl group of the 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones on their activity or potency. This will shed light on the mechanism of action of these heterocycles, and help to establish a predictive SAR model. The results will be published in due time.

### 4. Experimental section

#### 4.1. General conditions

Melting points were taken on an Ernst Leitz Wetzlar model 350 hot-stage microscope and are reported uncorrected. The  $^1H$ ,  $^{13}C$  and  $^{19}F$  NMR spectra were usually acquired in  $CDCl_3$  (unless otherwise stated) in a Bruker Avance spectrometer (300.13, 75.48 and 282.23 MHz for  $^1H$ ,  $^{13}C$  and  $^{19}F$ , respectively) with tetramethylsilane (TMS) as internal standard. The chemical shifts ( $\delta$ ) are reported in ppm downfield from TMS and the coupling constants ( $J$ ) are expressed in Hertz. The DEPT 135 experiment was used to aid the interpretation of the fully decoupled  $^{13}C$  NMR spectra. In special cases, 2D NMR experiments (COSY, HMBC and HSQC) were also employed. Low resolution mass spectra (EI = 70 eV) were obtained with a Shimadzu QP-2010*plus* instrument by direct injection with a probe temperature program ( $T_{init}$  = 40 °C,  $T_{end}$  = 350 °C,  $\Delta t$  = 20 °C/min). High resolution mass spectra were obtained with a Bruker MicroTOF-Q II instrument (Bruker Daltonics, Billerica, MA). Detection of the ions was performed in electrospray ionization, positive ion mode.

The reactions requiring inert atmospheres were carried out under dry Argon, employing oven-dried glassware. The reagents were used as received; dry DMF was prepared by distillation from anhydrous BaO; absolute EtOH was prepared by distillation after refluxing the reagent for 24 h over  $I_2$ -activated Mg turnings; dry MeCN was prepared by a 4 h reflux over  $P_2O_5$  followed by distillation; anhydrous solvents were stored in dry Young ampoules. In the conventional purification procedure, the residue was submitted to flash column chromatography with silica gel 60H (particle size = 63–200  $\mu m$ ). The elution was carried out with hexane-EtOAc mixtures, under  $N_2$  positive pressure and employing gradient of solvent polarity techniques.

All new compounds gave single spots on TLC plates of Kieselgel 60 GF<sub>254</sub>, run in different hexane-EtOAc and  $CH_2Cl_2$ -PhMe solvent systems. The chromatographic spots were detected by exposure of the plates to UV light (254 nm), followed by spraying with ethanolic

ninhydrin (amines) or with ethanolic *p*-anisaldehyde/sulfuric acid reagent and careful heating for 2 min.

The effect of the compounds on bacterial growth was tested in 96-well microplates placed in a Spectra Max M2 microplate reader (Molecular Devices Corp.) programmed to obtain measurements at 23 °C in order to prevent the evaporation of samples [33]. The optical densities of the cell cultures were recorded at 620 nm ( $OD_{620}$ ) at 15 min intervals with shaking (30 s) between measurements. Bacterial concentrations of the cell cultures were adjusted employing a SP-22 spectrophotometer (Biospectro, Brazil).

#### 4.2. General procedure for the synthesis of dianilides **17–22**

The aniline (**15a–f**, 2.0 equiv.) and diethyl malonate (**16**, 1.0 equiv.) were mixed in a round bottom flask, the mixture was degassed under Argon and placed in a pre-heated bath at 210–220 °C, allowing to escape the EtOH formed. Once the evolution of EtOH had stopped (1–2 h), the reaction was cooled to room temperature and the thus formed solid was washed several times with EtOAc.

##### 4.2.1. $N^1,N^3$ -diphenylmalonamide (**17**)

Yield: 11.6 g (91%). White solid; m.p.: 230–232 °C (Lit. 228–230 °C [41]). IR (KBr,  $\nu$ ): 3273 (N–H), 3149 (C–H), 1668 (C=O), 1647, 1597, 1535, 1498, 1444, 1413, 1357, 1249, 752 and 688  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.49 (s, 2H,  $CH_2$ ), 7.06 (t,  $J$  = 7.8, 2H, H-4), 7.32 (t,  $J$  = 7.8, 4H, H-3), 7.62 (d,  $J$  = 7.8, 4H, H-2) and 10.17 (s, 2H, NH).  $^{13}C$  NMR (DMSO- $d_6$ ):  $\delta$  45.9 ( $CH_2$ ), 119.0 ( $4 \times C-2$ ), 123.4 ( $2 \times C-4$ ), 128.7 ( $4 \times C-3$ ), 138.9 ( $2 \times C-1$ ) and 165.3 ( $2 \times C=O$ ). EI-MS ( $m/z$ , %): 254 ( $M^+$ , 41), 135 (55), 120 (8), 93 (100), 91 (13) and 77 (22).

##### 4.2.2. $N^1,N^3$ -bis(4-fluorophenyl)malonamide (**18**)

Yield: 0.28 g (44%). White solid; m.p.: 211–213 °C (Lit. 214–216 °C [42]). IR (KBr,  $\nu$ ): 3286 (N–H), 3088 (C–H), 1672, 1645 (C=O), 1618, 1558, 1512, 1409, 1222 and 752  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.42 (s, 2H,  $CH_2$ ), 7.13 (t,  $J$  = 9.1, 4H, H-2), 7.58 (dd,  $J$  = 5.0 and 9.1, 4H, H-3) and 10.20 (s, 2H, NH).  $^{13}C$  NMR (DMSO- $d_6$ ):  $\delta$  46.1 ( $CH_2$ ), 115.8 (d,  $^2J_{CF}$  = 22.5,  $4 \times C-3$ ), 121.3 (d,  $^3J_{CF}$  = 8.2,  $4 \times C-2$ ), 135.7 (d,  $^4J_{CF}$  = 2.2,  $2 \times C-1$ ), 158.5 (d,  $^1J_{CF}$  = 238.7,  $2 \times C-4$ ) and 165.7 ( $2 \times C=O$ ).  $^{19}F$  NMR (DMSO- $d_6$ ):  $\delta$  –119.1 (m, Ar–F). EI-MS ( $m/z$ , %): 290 ( $M^+$ , 56), 153 (42), 137 (8), 111 (100), 95 (10), 83 (21) and 75 (7).

##### 4.2.3. $N^1,N^3$ -bis(4-chlorophenyl)malonamide (**19**)

Yield: 1.4 g (49%). White solid; m.p.: 250–252 °C (Lit. 256–257 °C [41]). IR (KBr,  $\nu$ ): 3253 (N–H), 3045 (C–H), 1643 (C=O), 1533, 1012, 977 and 501  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.48 (s, 2H,  $CH_2$ ), 7.37 (d,  $J$  = 8.8, 4H, H-2), 7.63 (d,  $J$  = 8.8, 4H, H-3) and 10.33 (s, 2H, NH).  $^{13}C$  NMR (DMSO- $d_6$ ):  $\delta$  45.8 ( $CH_2$ ), 120.6 ( $4 \times C-2$ ), 126.9 ( $2 \times C-4$ ), 128.6 ( $4 \times C-3$ ), 137.8 ( $2 \times C-1$ ) and 165.4 ( $2 \times C=O$ ). EI-MS ( $m/z$ , %): 322 ( $M^+$ , 51), 169 (39), 153 (12), 127 (100), 99 (14), 91 (34) and 75 (13).

##### 4.2.4. $N^1,N^3$ -bis(4-methoxyphenyl)malonamide (**20**)

White solid. Yield: 0.4 g (63%). White solid; m.p.: 233–235 °C (Lit. 232–234 °C [41]). IR (KBr,  $\nu$ ): 3269 (N–H), 2954 (C–H), 1645 (C=O), 1616, 1533, 1508, 1411, 1244, 1035 and 821  $cm^{-1}$ .  $^1H$  NMR (DMSO- $d_6$ ):  $\delta$  3.39 (s, 2H,  $CH_2$ ), 3.72 (s, 6H,  $ArOCH_3$ ), 6.88 (d,  $J$  = 9.0, 4H, H-3), 7.51 (d,  $J$  = 9.0, 4H, H-2) and 10.00 (s, 2H, NH).  $^{13}C$  NMR (DMSO- $d_6$ ):  $\delta$  45.5 ( $CH_2$ ), 55.7 ( $2 \times ArOCH_3$ ), 113.8 ( $4 \times C-3$ ), 120.6 ( $4 \times C-2$ ), 132.1 ( $2 \times C-1$ ), 155.3 ( $2 \times C-4$ ) and 164.9 ( $2 \times C=O$ ). EI-MS ( $m/z$ , %): 314 ( $M^+$ , 100), 165 (41), 149 (7), 121 (73), 108 (37) and 80 (7).



#### 4.2.5. *N*<sup>1</sup>,*N*<sup>3</sup>-di(*p*-tolyl)malonamide (**21**)

Yield: 0.47 g (71%). White solid; m.p.: 246–248 °C (Lit. 251–253 °C [43]). IR (KBr,  $\nu$ ): 3269 (N–H), 3034 (C–H), 1649 (C=O), 1614, 1531, 1514, 1404 and 1357 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.25 (s, 6H, ArCH<sub>3</sub>), 3.42 (s, 2H, CH<sub>2</sub>), 7.11 (d, *J* = 8.3, 4H, H-3), 7.48 (d, *J* = 8.5, 4H, H-2) and 10.05 (s, 2H, NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  20.3 (2 × ArCH<sub>3</sub>), 45.7 (CH<sub>2</sub>), 119.0 (4 × C-3), 129.1 (4 × C-2), 132.2 (2 × C-1), 136.4 (2 × C-4) and 165.2 (2 × C=O). EI-MS (*m/z*, %): 282 (M<sup>+</sup>, 100), 149 (48), 133 (7), 106 (90), 91 (23) and 77 (18).

#### 4.2.6. *N*<sup>1</sup>,*N*<sup>3</sup>-bis(2-methoxyphenyl)malonamide (**22**)

Yield: 0.63 g (49%). White solid; m.p.: 160–162 °C (Lit. 163 °C [41]). IR (KBr,  $\nu$ ): 3367 (N–H), 3292 (N–H), 3005 (C–H), 1674 (C=O), 1597, 1529, 1489, 1463, 1255, 1228, 1118 and 1024 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  3.68 (s, 2H, CH<sub>2</sub>), 3.82 (s, 6H, ArOCH<sub>3</sub>), 6.86–7.11 (m, 8H, 2 × ArH), 8.00 (d, *J* = 7.6, 2H, H-6) and 9.67 (s, 2H, NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  45.5 (CH<sub>2</sub>), 55.7 (2 × ArOCH<sub>3</sub>), 110.1 (2 × C-3), 120.2 (2 × Ar), 120.7 (2 × Ar), 124.2 (2 × Ar), 127.1 (2 × C-1), 148.4 (2 × C-2) and 164.7 (2 × C=O). EI-MS (*m/z*, %): 314 (M<sup>+</sup>, 100), 165 (33), 134 (11), 123 (68), 108 (50) and 80 (9).

### 4.3. General procedure for the synthesis of 4-hydroxy-1*H*-quinolin-2-ones **23–28**

The dianilide (1.0 equiv.) was placed in a round bottom flask and mixed with 10 times (by weight) of polyphosphoric acid. The syrupy mixture was heated at 130–140 °C during 6 h, when it was poured onto crushed ice and made alkaline with 4 N NaOH. The solids were filtered off and the remaining liquids were transferred to a separation funnel and washed with Et<sub>2</sub>O (3 × 5 mL). The aqueous phase was transferred to a beaker placed in an ice bath and acidified with 6 N HCl to pH = 1, causing precipitation of the 4-hydroxy-3,4-dihydro-1*H*-quinolin-2-one product. The solid was filtered in a Büchner funnel, washed several times with H<sub>2</sub>O and dried in a desiccator over P<sub>2</sub>O<sub>5</sub>, under vacuum during 24 h. This product was used without further purification.

#### 4.3.1. 4-Hydroxy-1*H*-quinolin-2-one (**23**) [14c]

Yield: 1.26 g (67%). Pale pinkish solid; m.p.: 350–352 °C (Lit. > 300 °C [44]). IR (KBr,  $\nu$ ): 3400 (O–H, N–H), 3093 (C–H), 2951 (C–H), 2908, 2821, 2762, 1635 (C=O), 1593, 1506, 1419, 1379, 1332, 1234 and 866 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  5.74 (s, 1H, H-3), 7.13 (t, *J* = 7.7, 1H, H-6), 7.25 (d, *J* = 8.3, 1H, H-8), 7.44–7.52 (m, 1H, H-7), 7.78 (d, *J* = 7.8, 1H, H-5), 11.17 (s, 2H, OH and NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  98.1 (C-3), 115.0 (C-4a and C-8), 120.9 (C-5), 122.6 (C-6), 130.7 (C-7), 139.1 (C-8a), 162.5 (C-4)\* and 163.5 (C-2)\*. EI-MS (*m/z*, %): 161 (M<sup>+</sup>, 100), 133 (16), 119 (83), 105 (19), 92 (68) and 77 (11).

#### 4.3.2. 6-Fluoro-4-hydroxy-1*H*-quinolin-2-one (**24**) [14c]

Yield: 0.10 g (52%). White solid; m.p.: 356 °C, dec. (Lit. 345 °C [42]). IR (KBr,  $\nu$ ): 3446 (O–H), 3097 (N–H), 2949 (C–H), 2899, 1647 (C=O), 1600, 1517, 1456, 1319, 1251, 1240 and 1197 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  5.94 (s, 1H, H-3), 7.27–7.55 (m, 3H, H-5, H-7 and H-8) and 11.31 (bs, 2H, OH and NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  99.6 (C-3), 108.0 (d, <sup>2</sup>*J*<sub>CF</sub> = 23.7, C-5), 116.3 (d, <sup>3</sup>*J*<sub>CF</sub> = 8.1, C-4a), 117.5 (d, <sup>3</sup>*J*<sub>CF</sub> = 8.1, C-8), 119.2 (d, <sup>2</sup>*J*<sub>CF</sub> = 24.2, C-7), 136.3 (C-8a), 157.1 (d, <sup>1</sup>*J*<sub>CF</sub> = 237.5, C-6), 162.1 (d, <sup>4</sup>*J*<sub>CF</sub> = 2.8, C-4) and 163.8 (C-2). <sup>19</sup>F NMR (DMSO-*d*<sub>6</sub>):  $\delta$  −121.5 (m, Ar–F). EI-MS (*m/z*, %): 179 (M<sup>+</sup>, 100), 151 (10), 137 (83), 123 (19), 110 (53), 95 (16), 82 (29) and 75 (19).

#### 4.3.3. 6-Chloro-4-hydroxy-1*H*-quinolin-2-one (**25**) [45]

Yield: 0.81 g (98%). White solid; m.p.: 308 °C, dec. (Lit. 350 °C [42]). IR (KBr,  $\nu$ ): 3124 (N–H), 2972 (C–H), 2837, 1670 (C=O), 1610, 1302, 1231 and 998 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  5.78 (s, 1H, H-3), 7.28 (d, *J* = 8.7, 1H, H-8), 7.54 (d, *J* = 7.6, 1H, H-7), 7.73 (s, 1H, H-5)

and 11.39 (bs, 2H, OH and NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  99.0 (C-3), 116.2 (C-4a), 117.1 (C-5), 121.7 (C-8), 125.1 (C-6), 130.7 (C-7), 137.8 (C-8a), 161.3 (C-4) and 163.3 (C-2). EI-MS (*m/z*, %): 195 (M<sup>+</sup>, 100), 167 (12), 153 (92), 139 (15), 126 (37), 99 (13), 90 (23) and 75 (29).

#### 4.3.4. 6-Methoxy-4-hydroxy-1*H*-quinolin-2-one (**26**)

Yield: 0.45 g (19%). White solid; m.p.: 310–312 °C (Lit. > 300 °C [44]). IR (KBr,  $\nu$ ): 3344 (O–H), 3143 (N–H), 2993 (C–H), 2839, 1670 (C=O), 1614, 1037, 898 and 821 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  3.75 (s, 3H, Ar–OCH<sub>3</sub>), 5.71 (s, 1H, H-3), 7.07–7.22 (m, 3H), 11.06 (s, 1H, NH) and 11.26 (s, 1H, OH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  55.5 (Ar–OCH<sub>3</sub>), 98.6 (C-3), 103.9 (C-5), 115.5 (C-4a), 116.7 (CH), 120.1 (CH), 133.7 (C-8a), 153.8 (C-6), 162.1 (C-4) and 163.3 (C-2). EI-MS (*m/z*, %): 191 (M<sup>+</sup>, 100), 176 (56), 149 (22), 134 (65), 121 (25), 106 (84) and 80 (20).

#### 4.3.5. 6-Methyl-4-hydroxy-1*H*-quinolin-2-one (**27**)

Yield: 0.46 g (17%). White solid; m.p.: 259 °C, dec. [44]. IR (KBr,  $\nu$ ): 3419 (O–H), 3288 (N–H), 2916 (C–H), 1670 (C=O), 1610, 1244, 1091 and 1041 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  2.33 (s, 3H, ArCH<sub>3</sub>), 5.73 (s, 1H, H-3), 7.16 (d, *J* = 8.2, 1H, H-8), 7.30 (dd, *J* = 1.7 and 6.4, 1H, H-7), 7.56 (s, 1H, H-5) and 11.10 (s, 1H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  21.0 (ArCH<sub>3</sub>), 97.7 (C-3), 115.6 (C-4a), 116.0 (C-8), 122.6 (C-5), 131.2 (C-6), 132.9 (C-7), 137.3 (C-8a), 163.7 (C-4)\* and 163.8 (C-2)\*. EI-MS (*m/z*, %): 175 (M<sup>+</sup>, 100), 147 (8), 133 (95), 119 (20), 106 (42), 104 (58), 91 (15) and 77 (42).

#### 4.3.6. 8-Methoxy-4-hydroxy-1*H*-quinolin-2-one (**28**)

Yield: 0.60 g (36%). White solid; m.p.: 246–248 °C (Lit. 248 °C [46]). IR (KBr,  $\nu$ ): 3149 (N–H), 2960 (C–H), 2835, 1663 (C=O), 1591, 1504, 1317, 1242 and 1053 cm<sup>−1</sup>. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>):  $\delta$  3.88 (s, 3H, ArOCH<sub>3</sub>), 5.75 (s, 1H, H-3), 7.03–7.19 (m, 2H, H-5,8), 7.37 (dd, *J* = 1.6, 7.6, 1H, H-7), 10.09 (s, 1H, NH) and 11.31 (s, 1H, OH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>):  $\delta$  56.4 (ArOCH<sub>3</sub>), 99.1 (C-3), 111.7 (C-7), 114.7 (C-5), 116.0 (C-5a), 121.4 (C-6), 129.7 (C-8a), 146.1 (C-8), 163.0 (C-4)\* and 163.3 (C-3)\*. EI-MS (*m/z*, %): 191 (M<sup>+</sup>, 100), 190 (55), 162 (45), 148 (29), 134 (41), 121 (31), 106 (52), 91 (28) and 77 (18).

### 4.4. General procedure for the C,C-dibenzylation of the 4-hydroxy-1*H*-quinolin-2-ones **29–38**

The 4-hydroxy-3,4-dihydro-1*H*-quinolin-2-one (1.0 equiv.), K<sub>2</sub>CO<sub>3</sub> (8.0 equiv.) and H<sub>2</sub>O (9.0 mL/mmol), were placed in a round bottom flask and the mixture was stirred until complete dissolution of the heterocycle (approximately 15 min). Then, a solution of the benzyl halide (6.0 equiv., 9.0 mL/mmol) in toluene was added. The reaction was refluxed during 48 h, cooled to room temperature, diluted with distilled H<sub>2</sub>O and extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with brine (2 × 10 mL), dried over MgSO<sub>4</sub>, concentrated under reduced pressure and the residue was purified by column chromatography.

#### 4.4.1. 3,3-Dibenzyl-1*H*-quinoline-2,4-dione (**29**)

Yield: 0.097 g (46%). White solid; m.p.: 187–189 °C (Lit. 206–208 °C [32c]). IR (KBr,  $\nu$ ): 3286 (N–H), 3066 (C–H), 1695 (C=O, ketone), 1684, 1661 (C=O, amide), 1645, 1610, 1598, 1485, 1358, 1247, 1211, 763, 705 and 503 cm<sup>−1</sup>. <sup>1</sup>H NMR:  $\delta$  3.43 (d, *J* = 12.8, 2H, ArCH<sub>2</sub>), 3.51 (d, *J* = 12.8, 2H, ArCH<sub>2</sub>), 6.45 (d, *J* = 8.2, 1H, H-8), 6.93 (t, *J* = 7.5, 1H, H-7), 6.99–7.24 (m, 11H, 2 × ArH and H-6), 7.77 (dd, *J* = 1.0 and 7.9, 1H, H-5) and 8.44 (s, 1H, NH). <sup>13</sup>C NMR:  $\delta$  45.2 (2 × ArCH<sub>2</sub>), 64.6 (C-3), 115.3 (C-8), 119.8 (C-8a), 123.0 (C-6), 126.8 (2 × *p*-C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub> and C-5), 127.9 (4 × *m*-C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>), 129.7 (4 × *o*-C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>), 135.5 (2 × *ipso*-C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>), 135.7 (C-7), 139.8 (C-4a), 173.0 (C-2) and 197.0 (C-4). EI-MS (*m/z*, %): 340 (M<sup>+</sup>, 32), 250 (60), 153 (8), 141 (9), 127 (14), 111 (25), 99 (33), 91 (46), 85 (68) and 71 (100).

**4.4.2. 3,3-Dibenzyl-6-fluoro-1H-quinoline-2,4-dione (30)**

Yield: 0.11 g (61%). White solid; m.p.: 176–178 °C. IR (KBr,  $\nu$ ): 3230 (N–H), 3034 (C–H), 1685 (C=O, ketone), 1653 (C=O, amide), 1624, 1500, 1411, 1363, 1249 and 1166  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.43 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.49 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 6.49 (dd,  $J = 4.0$  and 8.8, 1H, H-8), 6.94–7.12 (m, 11H,  $2 \times \text{ArH}$  and H-7), 7.44 (dd,  $J = 3.0$ , 8.2, 1H, H-5) and 8.72 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  45.3 ( $2 \times \text{ArCH}_2$ ), 64.4 (C-3), 112.4 (d,  $^2J_{\text{CF}} = 23.1$ , C-7), 117.2 (d,  $^3J_{\text{CF}} = 7.1$ , C-8), 120.7 (d,  $^3J_{\text{CF}} = 6.6$ , C-4a), 123.3 (d,  $^2J_{\text{CF}} = 24.2$ , C-5), 126.9 ( $2 \times p\text{-C}_6\text{H}_5\text{CH}_2$ ), 128.1 ( $4 \times m\text{-C}_6\text{H}_5\text{CH}_2$ ), 129.7 ( $4 \times o\text{-C}_6\text{H}_5\text{CH}_2$ ), 135.4 ( $2 \times ipso\text{-C}_6\text{H}_5\text{CH}_2$ ), 136.4 (C-8a), 158.3 (d,  $^1J_{\text{CF}} = 244.2$ , C-6), 173.2 (C-2) and 196.5 (C-4).  $^{19}\text{F}$  NMR:  $\delta$  –118.5 (m, Ar–F). EI-MS ( $m/z$ , %): 359 ( $\text{M}^+$ , 2.4), 268 (100), 250 (3), 138 (4), 131 (4), 103 (7), 91 (72) and 77 (5). HRMS (ESI–TOF),  $m/z$  found: 382.1208 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{18}\text{FNNaO}_2$  requires 382.1214.

**4.4.3. 3,3-Dibenzyl-6-chloro-1H-quinoline-2,4-dione (31)**

Yield: 0.13 g (69%). White solid; m.p.: 201–203 °C. IR (KBr,  $\nu$ ): 3325 (N–H), 2929 (C–H), 1697 (C=O, ketone), 1654 (C=O, amide), 1606, 1489, 1280, 1247 and 1192  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.42 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.50 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 6.46 (dd,  $J = 2.2$  and 8.6, 1H, H-8), 6.96–7.13 (m, 10H,  $2 \times \text{ArH}$ ), 7.21 (dd,  $J = 2.4$  and 8.6, 1H, H-7), 7.74 (d,  $J = 2.4$ , 1H, H-5) and 8.75 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  45.3 ( $2 \times \text{ArCH}_2$ ), 64.7 (C-3), 117.1 (C-8), 120.7 (C-4a), 126.4 (C-5), 127.0 ( $2 \times p\text{-C}_6\text{H}_5\text{CH}_2$ ), 128.1 ( $4 \times m\text{-C}_6\text{H}_5\text{CH}_2$ ), 128.7 (C-8a), 129.7 ( $4 \times o\text{-C}_6\text{H}_5\text{CH}_2$ ), 135.3 ( $2 \times ipso\text{-C}_6\text{H}_5\text{CH}_2$ ), 135.6 (C-7), 138.5 (C-6), 173.3 (C-2) and 196.3 (C-4). EI-MS ( $m/z$ , %): 375 ( $\text{M}^+$ , 2.4), 284 (100), 266 (3), 154 (3), 131 (5), 115 (3), 103 (7), 91 (69) and 77 (4). HRMS (ESI–TOF),  $m/z$  found: 398.0919 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{18}\text{ClNNaO}_2$  requires 398.0918.

**4.4.4. 3,3-Dibenzyl-6-methoxy-1H-quinoline-2,4-dione (32)**

Yield: 0.023 g (40%). White solid; m.p.: 180–182 °C. IR (KBr,  $\nu$ ): 3175 (N–H), 3057 (C–H), 2902 (C–H), 1685 (C=O, ketone), 1651 (C=O, amide), 1508, 1490, 1419 and 1350  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.42 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.49 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.76 (s, 3H, Ar–OCH<sub>3</sub>), 6.51 (d,  $J = 8.8$ , 1H, H-8), 6.88 (dd,  $J = 3.0$ , 8.8, 1H, H-7), 6.97–7.13 (m, 10H,  $2 \times \text{ArH}$ ), 7.23 (d,  $J = 3.0$ , 1H, H-5) and 9.37 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  45.2 ( $2 \times \text{ArCH}_2$ ), 55.7 (ArOCH<sub>3</sub>), 64.1 (C-3), 107.9 (C-8), 117.3 (C-5), 120.3 (C-8a), 124.5 (C-7), 126.8 ( $2 \times p\text{-C}_6\text{H}_5\text{CH}_2$ ), 128.0 ( $4 \times m\text{-C}_6\text{H}_5\text{CH}_2$ ), 129.7 ( $4 \times o\text{-C}_6\text{H}_5\text{CH}_2$ ), 134.5 (C-4a), 135.6 ( $2 \times ipso\text{-C}_6\text{H}_5\text{CH}_2$ ), 155.5 (C-6), 173.6 (C-2) and 197.1 (C-4). EI-MS ( $m/z$ , %): 371 ( $\text{M}^+$ , 9), 280 (100), 150 (3), 131 (6), 106 (3) and 91 (45). HRMS (ESI–TOF),  $m/z$  found: 394.1404 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{24}\text{H}_{21}\text{NNaO}_3$  requires 394.1414.

**4.4.5. 3,3-Dibenzyl-6-methyl-1H-quinoline-2,4-dione (33)**

Yield: 0.035 g (38%). White solid; m.p.: 200–202 °C. IR (KBr,  $\nu$ ): 3311 (N–H), 3084 (C–H), 2929 (C–H), 1689 (C=O, ketone), 1651 (C=O, amide), 1618, 1508, 1419 and 704  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  2.23 (s, 3H, Ar–CH<sub>3</sub>), 3.41 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.49 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 6.34 (d,  $J = 8.0$ , 1H, H-8), 7.00–7.13 (m, 11H,  $2 \times \text{ArH}$  and H-7), 7.55–7.59 (m, 1H, H-5) and 7.92 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  20.5 (ArCH<sub>3</sub>), 45.2 ( $2 \times \text{ArCH}_2$ ), 64.5 (C-3), 115.5 (C–H), 119.7 (C-8a), 126.6 (C–H), 126.8 ( $2 \times p\text{-C}_6\text{H}_5\text{CH}_2$ ), 128.0 ( $4 \times m\text{-C}_6\text{H}_5\text{CH}_2$ ), 129.8 ( $4 \times o\text{-C}_6\text{H}_5\text{CH}_2$ ), 132.8 (C-6), 135.7 ( $2 \times ipso\text{-C}_6\text{H}_5\text{CH}_2$ ), 136.8 (C–H), 138.0 (C-4a), 173.4 (C-2) and 197.2 (C-4). EI-MS ( $m/z$ , %): 355 ( $\text{M}^+$ , 1.6), 264 (100), 246 (2), 131 (5), 103 (6), 91 (35) and 77 (6). HRMS (ESI–TOF),  $m/z$  found: 356.1635 [ $\text{M} + \text{H}$ ] $^+$ ,  $\text{C}_{24}\text{H}_{22}\text{NNaO}_2$  requires 356.1645.

**4.4.6. 3,3-Dibenzyl-8-methoxy-1H-quinoline-2,4-dione (34)**

Yield: 0.14 g (62%). White solid; m.p.: 196–198 °C. IR (KBr,  $\nu$ ): 3184 (N–H), 3064 (C–H), 2929 (C–H), 1693 (C=O, ketone), 1654 (C=O, amide), 1506, 1382 and 1267  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.42 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.48 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.70 (s, 3H,

ArOCH<sub>3</sub>), 6.77 (dd,  $J = 1.4$  and 8.0, 1H, H-8), 6.84 (t,  $J = 7.8$ , 1H, H-7), 6.99–7.13 (m, 10H,  $2 \times \text{ArH}$ ), 7.34 (dd,  $J = 1.0$  and 7.8, 1H, H-5) and 7.83 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  45.4 ( $2 \times \text{ArCH}_2$ ), 60.0 (ArOCH<sub>3</sub>), 64.7 (C-3), 115.8 (C-7), 118.0 (C-5), 120.1 (C-4a), 122.2 (C-6), 126.8 ( $2 \times p\text{-C}_6\text{H}_5\text{CH}_2$ ), 128.0 ( $4 \times m\text{-C}_6\text{H}_5\text{CH}_2$ ), 129.7 ( $4 \times o\text{-C}_6\text{H}_5\text{CH}_2$ ), 130.6 (C-8a), 135.9 ( $2 \times ipso\text{-C}_6\text{H}_5\text{CH}_2$ ), 145.2 (C-8), 172.0 (C-2) and 197.3 (C-4). EI-MS ( $m/z$ , %): 371 ( $\text{M}^+$ , 10), 370 (26), 280 (100), 264 (6), 131 (6), 91 (67) and 71 (22). HRMS (ESI–TOF),  $m/z$  found: 394.1415 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{24}\text{H}_{21}\text{NNaO}_3$  requires 394.1414.

**4.4.7. 3,3-Bis(4'-Methoxybenzyl)-1H-quinoline-2,4-dione (35)**

Yield: 0.064 g (52%). White solid; m.p.: 179–181 °C. IR (KBr,  $\nu$ ): 3266 (N–H), 2930 (C–H), 2837, 1682 (C=O, ketone), 1658 (C=O, amide), 1640, 1599, 1510, 1490, 1438, 1379, 1253, 1180 and 824  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.34 (d,  $J = 13.1$ , 2H,  $\text{ArCH}_2$ ), 3.42 (d,  $J = 13.1$ , 2H,  $\text{ArCH}_2$ ), 3.61 (s, 6H, ArOCH<sub>3</sub>), 6.46–7.32 (m, 11H,  $2 \times \text{ArH}$ , H-6, H-7 and H-8), 7.79 (dd,  $J = 1.0$  and 7.8, 1H, H-5) and 8.52 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  44.2 ( $2 \times \text{ArCH}_2$ ), 54.9 ( $2 \times \text{ArOCH}_3$ ), 65.0 (C-3), 113.3 ( $4 \times \text{C-3'}$ ), 115.6 (C-8), 119.8 (C-8a), 123.0 (C-6), 126.8 (C-5), 127.7 ( $4 \times \text{C-2'}$ ), 130.7 ( $2 \times \text{C-1'}$ ), 135.7 (C-7), 140.1 (C-4a), 158.2 ( $2 \times \text{C-4'}$ ), 173.5 (C-2) and 197.3 (C-4). EI-MS ( $m/z$ , %): 401 ( $\text{M}^+$ , 1.6), 280 (69), 161 (2), 121 (100), 91 (6) and 77 (10). HRMS (ESI–TOF),  $m/z$  found: 424.1510 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{25}\text{H}_{23}\text{NNaO}_4$  requires 424.1519.

**4.4.8. 3,3-Bis(4'-Chlorobenzyl)-1H-quinoline-2,4-dione (36)**

Yield: 0.040 g (40%). White solid; m.p.: 232–234 °C. IR (KBr,  $\nu$ ): 3178 (N–H), 3055 (C–H), 2993 (C–H), 2931, 1689 (C=O, ketone), 1653 (C=O, amide), 1595, 1487 and 1388  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.36 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.44 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 6.51 (d,  $J = 6.0$ , 1H, H-8), 6.95–7.07 (m, 9H,  $2 \times \text{ArH}$  and H-7), 7.33 (dt,  $J = 1.4$  and 7.6, 1H, H-6), 7.79 (d,  $J = 6.9$ , 1H, H-5) and 8.25 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  44.5 ( $2 \times \text{ArCH}_2$ ), 64.7 (C-3), 115.5 (C-5), 119.8 (C-8a), 123.6 (C-6), 127.1 (C-5), 128.3 ( $4 \times \text{C-3'}$ ), 131.2 ( $4 \times \text{C-2'}$ ), 132.9 ( $2 \times \text{C-4'}$ ), 133.9 ( $2 \times \text{C-1'}$ ), 136.3 (C-7), 139.8 (C-4a), 172.3 (C-2) and 196.8 (C-4). EI-MS ( $m/z$ , %): 409 ( $\text{M}^+$ , 0.9), 284 (100), 248 (9), 165 (3), 125 (40), 120 (6), 89 (11) and 71 (3). HRMS (ESI–TOF),  $m/z$  found: 432.0510 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{17}\text{Cl}_2\text{NNaO}_2$  requires 432.0529.

**4.4.9. 3,3-Bis(2'-Chlorobenzyl)-1H-quinoline-2,4-dione (37)**

Yield: 0.048 g (38%). White solid; m.p.: 172–174 °C. IR (KBr,  $\nu$ ): 3243 (N–H), 1691 (C=O, ketone), 1660 (C=O, amide), 1613, 1596, 1485, 1474, 1436, 1373, 1227, 755 and 740  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.61 (d,  $J = 14.5$ , 2H,  $\text{ArCH}_2$ ), 3.70 (d,  $J = 14.5$ , 2H,  $\text{ArCH}_2$ ), 6.66 (d,  $J = 8.1$ , 1H, H-8), 6.86–7.24 (m, 9H,  $o\text{-ClC}_6\text{H}_4\text{CH}_2$  and H-7), 7.39 (t,  $J = 7.7$ , 1H, H-6), 7.88 (d,  $J = 6.9$ , 1H, H-5) and 9.65 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  40.3 ( $2 \times \text{ArCH}_2$ ), 61.2 (C-3), 116.2 (C-5), 119.7 (C-8a), 123.5 (C-6), 126.4 ( $2 \times \text{Ar}$ ), 127.6 (C-5), 128.0 ( $2 \times \text{Ar}$ ), 129.5 ( $2 \times \text{Ar}$ ), 130.2 ( $2 \times \text{Ar}$ ), 133.7 ( $2 \times \text{Ar}$ ), 134.9 ( $2 \times \text{Ar}$ ), 136.0 (C-7), 140.2 (C-4a), 173.6 (C-2) and 195.2 (C-4). EI-MS ( $m/z$ , %): 410 ( $\text{M}^+$ , 0.05), 374 (45), 284 (7), 248 (100), 125 (20) and 89 (5). HRMS (ESI–TOF),  $m/z$  found: 410.0704 [ $\text{M} + \text{H}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{18}\text{Cl}_2\text{NO}_2$  requires 410.0709.

**4.4.10. 3,3-Bis(4'-Chlorobenzyl)-6-chloro-1H-quinoline-2,4-dione (38)**

Yield: 0.039 g (34%). White solid; m.p.: 204–206 °C. IR (KBr,  $\nu$ ): 3259 (N–H), 3069 (C–H), 2958 (C–H), 1685 (C=O, ketone), 1662 (C=O, amide), 1489, 1408, 1363, 1095, 837 and 815  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  3.36 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 3.43 (d,  $J = 12.8$ , 2H,  $\text{ArCH}_2$ ), 6.51 (d,  $J = 8.4$ , 1H, H-8), 6.97–7.07 (m, 8H,  $2 \times \text{ArH}$ ), 7.39 (dd,  $J = 2.2$  and 8.4, 1H, H-7), 7.75 (d,  $J = 2.2$ , 1H, H-5) and 8.40 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  44.6 ( $2 \times \text{ArCH}_2$ ), 64.7 (C-3), 117.1 (C-8), 120.4 (C-4a), 126.5 (C-5), 128.4 ( $4 \times \text{Ar}$ ), 129.2 (C-8a), 131.2 ( $4 \times \text{Ar}$ ), 133.1 ( $2 \times \text{Ar}$ ), 133.7 ( $2 \times \text{Ar}$ ), 136.2 (C-7), 138.1 (C-6), 172.3 (C-2) and 196.0 (C-4). EI-MS ( $m/z$ , %): 443 ( $\text{M}^+$ , 1), 318 (65), 282 (9), 165 (3), 125 (100), 99 (3) and

89 (9). HRMS (ESI–TOF),  $m/z$  found: 466.0119 [ $M + Na$ ]<sup>+</sup>, C<sub>23</sub>H<sub>16</sub>Cl<sub>3</sub>NNaO<sub>2</sub> requires 466.0139.

#### 4.5. General procedures for the *N*-alkylation of the 3,3-dibenzyl-1*H*-quinoline-2,4-diones **39–44**

##### 4.5.1. Procedure A

The 1*H*-quinoline-2,4-dione (1.0 equiv.), K<sub>2</sub>CO<sub>3</sub> (3.0 equiv.) and dry acetonitrile (30 mL/mmol) were placed in a round bottom flask equipped with a Liebig condenser. After stirring for 5 min, the alkylating agent (1.2 equiv.) was added dropwise and the system was refluxed under Argon atmosphere until complete consumption of the starting material (24–48 h). Then, the reaction was cooled and the product was concentrated under reduced pressure. The residue was column chromatographed, to afford the *N*-alkylated product.

##### 4.5.2. Procedure B

The 1*H*-quinoline-2,4-dione (1.0 equiv.) was dissolved in anhydrous DMA (13 mL/mmol) and anhydrous K<sub>3</sub>PO<sub>4</sub> (6.0 equiv.) was immediately added. The system was placed under an Argon atmosphere, and treated dropwise with the alkylating agent (1.2 equiv.), while the suspension was vigorously stirred at 120 °C. Once verified the complete consumption of the starting material, the volatiles were distilled off under vacuum, and the remaining oily material was purified by column chromatography to afford the *N*-alkylated product.

##### 4.5.3. 3,3-Dibenzyl-1-methyl-1*H*-quinoline-2,4-dione (**39**)

Synthesized after Procedure A. Yield: 0.012 g (60%). White solid; m.p.: 126–128 °C (Lit. 126–128 °C [29a]). IR (KBr, ν): 2926 (C–H), 1685 (C=O, ketone), 1651 (C=O, amide), 1598, 1490, 1473, 1456, 1367, 1082, 804, 761 and 704 cm<sup>−1</sup>. <sup>1</sup>H NMR: δ 3.16 (s, 3H, CH<sub>3</sub>N), 3.47 (s, 4H, ArCH<sub>2</sub>), 6.62 (d,  $J = 8.2$ , 1H, H-8), 6.90–7.08 (m, 11H, 2 × ArH and H-6), 7.26–7.34 (m, 1H, H-7) and 7.84 (dd,  $J = 1.6$  and 7.8, 1H, H-5). <sup>13</sup>C NMR: δ 29.1 (CH<sub>3</sub>N), 45.9 (2 × ArCH<sub>2</sub>), 64.6 (C-3), 114.2 (C-8), 121.3 (C-8a), 122.5 (C-6), 126.6 (2 × Ar), 127.1 (C-5), 127.8 (4 × Ar), 129.7 (4 × Ar), 135.8 (2 × Ar), 135.9 (C-7), 142.6 (C-4a), 172.0 (C-2) and 197.1 (C-4). EI-MS ( $m/z$ , %): 355 ( $M^+$ , 1.1), 264 (100), 134 (15), 103 (8), 91 (64) and 77 (15).

##### 4.5.4. 3,3-Dibenzyl-1-ethyl-1*H*-quinoline-2,4-dione (**40**)

Synthesized after Procedure A. Yield: 0.018 g (77%). White solid; m.p.: 145–147 °C. IR (KBr, ν): 3064 (C–H), 2933 (C–H), 1685 (C=O, ketone), 1647 (C=O, amide), 1598, 1471, 1377 and 1303 cm<sup>−1</sup>. <sup>1</sup>H NMR: δ 1.01 (t,  $J = 7.1$ , 3H, CH<sub>3</sub>CH<sub>2</sub>N), 3.48 (s, 4H, ArCH<sub>2</sub>), 3.81 (q,  $J = 7.1$ , 2H, CH<sub>3</sub>CH<sub>2</sub>N), 6.65 (d,  $J = 8.4$ , 1H, H-8), 6.92 (t,  $J = 7.5$ , 1H, H-6), 6.97–7.15 (m, 10H, 2 × ArH), 7.26–7.37 (m, 1H, H-7) and 7.85 (dd,  $J = 1.6$  and 7.8, 1H, H-5). <sup>13</sup>C NMR: δ 11.8 (CH<sub>3</sub>CH<sub>2</sub>N), 37.0 (CH<sub>3</sub>CH<sub>2</sub>N), 46.1 (2 × ArCH<sub>2</sub>), 64.1 (C-3), 114.1 (C-8), 121.5 (C-8a), 122.4 (C-6), 126.6 (2 × Ar), 127.4 (C-5), 127.8 (4 × Ar), 129.8 (4 × Ar), 135.8 (2 × Ar), 141.6 (C-4a), 171.7 (C-2) and 197.5 (C-4). EI-MS ( $m/z$ , %): 369 ( $M^+$ , 1.3), 278 (100), 148 (5), 103 (4), 91 (29) and 77 (6). HRMS (ESI–TOF),  $m/z$  found: 370.1806 [ $M + H$ ]<sup>+</sup>, C<sub>25</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> requires 370.1802.

##### 4.5.5. 1,3,3-Tribenzyl-1*H*-quinoline-2,4-dione (**41**)

Synthesized after Procedure B. Yield: 0.014 g (36%). White solid; m.p.: 149–151 °C. IR (KBr, ν): 3030 (C–H), 2926 (C–H), 1683 (C=O, ketone), 1651 (C=O, amide), 1597, 1492, 1467, 1454, 1436, 1377 and 1298 cm<sup>−1</sup>. <sup>1</sup>H NMR: δ 3.48 (d,  $J = 12.6$ , 2H, ArCH<sub>2</sub>), 3.62 (d,  $J = 12.6$ , 2H, ArCH<sub>2</sub>), 4.99 (s, 2H, ArCH<sub>2</sub>N), 6.46 (d,  $J = 8.2$ , 1H, H-8), 6.54–6.62 (m, 2H, ArH), 6.91 (t,  $J = 7.2$ , 1H, H-6), 7.01–7.19 (m, 14H, 3 × ArH and H-7) and 7.91 (dd,  $J = 1.8$  and 7.8, 1H, H-5). <sup>13</sup>C NMR: δ 45.4 (2 × ArCH<sub>2</sub>), 46.3 (ArCH<sub>2</sub>N), 64.8 (C-3), 115.4 (C-8), 121.5 (C-

4a), 122.7 (C-6), 125.9 (Ar), 126.8 (2 × Ar), 126.9 (2 × Ar), 127.2 (C-5), 128.0 (4 × Ar), 128.6 (2 × Ar), 129.9 (4 × Ar), 135.2 (Ar), 135.8 (2 × Ar), 135.9 (C-7), 141.8 (C-8a), 172.3 (C-2) and 197.6 (C-4). EI-MS ( $m/z$ , %): 431 ( $M^+$ , 1.2), 340 (82), 234 (4), 208 (12), 91 (100) and 77 (4). HRMS (ESI–TOF),  $m/z$  found: 454.1799 [ $M + Na$ ]<sup>+</sup>, C<sub>30</sub>H<sub>25</sub>NNaO<sub>2</sub> requires 454.1783.

##### 4.5.6. Ethyl 2-(3,3-dibenzyl-2,4-dioxo-3,4-dihydroquinolin-1*H*-yl) acetate (**42**)

Synthesized after Procedure A. Yield: 0.022 g (89%); colorless oil. IR (NaCl, ν): 3062 (C–H), 2933 (C–H), 1748 (C=O, ester) 1693 (C=O, ketone), 1658 (C=O, amide), 1600, 1373, 1197, 437 and 410 cm<sup>−1</sup>. <sup>1</sup>H NMR: δ 1.16 (t,  $J = 7.0$ , 3H, CH<sub>3</sub>CH<sub>2</sub>O), 3.47 (s, 4H, ArCH<sub>2</sub>), 4.14 (q,  $J = 7.1$ , 2H, CH<sub>3</sub>CH<sub>2</sub>O), 4.51 (s, 2H, EtO<sub>2</sub>CCH<sub>2</sub>), 6.47 (d,  $J = 8.2$ , 1H, H-8), 6.94–7.10 (m, 11H, Ar–H and H-7), 7.27–7.36 (m, 1H, H-6) and 7.86 (dd,  $J = 1.8$  and 7.8, 1H, H-5). <sup>13</sup>C NMR: δ 14.0 (CH<sub>3</sub>CH<sub>2</sub>O), 43.9 (EtO<sub>2</sub>CCH<sub>2</sub>), 45.5 (2 × ArCH<sub>2</sub>), 61.5 (CH<sub>3</sub>CH<sub>2</sub>O), 64.4 (C-3), 113.8 (C-8), 121.2 (C-8a), 123.0 (C-6), 126.7 (2 × Ar), 127.6 (C-5), 127.9 (4 × Ar), 129.8 (4 × Ar), 135.6 (C-7), 135.9 (2 × Ar), 141.6 (C-4a), 167.7 (EtO<sub>2</sub>CCH<sub>2</sub>), 172.3 (C-2) and 196.4 (C-4). EI-MS ( $m/z$ , %): 427 ( $M^+$ , 2), 336 (100), 290 (34), 262 (50), 234 (3), 132 (23), 91 (41) and 77 (8). HRMS (ESI–TOF),  $m/z$  found: 428.1839 [ $M + Na$ ]<sup>+</sup>, C<sub>27</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub> requires 428.1856.

##### 4.5.7. 6-Chloro-3,3-bis-(4'-chlorobenzyl)-1-ethyl-1*H*-quinoline-2,4-dione (**43**)

Synthesized after Procedure A. Yield: 0.019 g (80%). White solid; m.p.: 151–153 °C. IR (KBr, ν): 2936 (C–H), 1686 (C=O, ketone), 1653 (C=O, amide), 1597, 1489, 1435, 1359, 1273, 1016 and 817 cm<sup>−1</sup>. <sup>1</sup>H NMR: δ 1.02 (t,  $J = 7.1$ , 3H, CH<sub>3</sub>CH<sub>2</sub>N), 3.41 (s, 4H, ArCH<sub>2</sub>), 3.81 (q,  $J = 7.1$ , 2H, CH<sub>3</sub>CH<sub>2</sub>N), 6.67 (d,  $J = 8.8$ , 1H, H-8), 6.95 (d,  $J = 8.3$ , 4H, ArH), 7.02 (d,  $J = 8.3$ , 4H, ArH), 7.32 (dd,  $J = 8.8$ , 2.5, 1H, H-7) and 7.81 (d,  $J = 2.5$ , 1H, H-5). <sup>13</sup>C NMR: δ 11.9 (CH<sub>3</sub>CH<sub>2</sub>N), 37.3 (CH<sub>3</sub>CH<sub>2</sub>N), 45.3 (2 × ArCH<sub>2</sub>), 64.1 (C-3), 116.1 (C-8), 122.1 (C-4a), 126.8 (C-5), 128.2 (4 × Ar), 128.6 (C-8a), 131.2 (4 × Ar), 132.9 (2 × Ar), 133.9 (2 × Ar), 136.1 (C-7), 140.1 (C-6), 171.2 (C-2) and 196.3 (C-4). EI-MS ( $m/z$ , %): 471 ( $M^+$ , 2.5), 346 (100), 182 (7), 125 (60) and 89 (10). HRMS (ESI–TOF),  $m/z$  found: 494.0446 [ $M + Na$ ]<sup>+</sup>, C<sub>25</sub>H<sub>20</sub>Cl<sub>3</sub>NNaO<sub>2</sub> requires 494.0452.

##### 4.5.8. 1,3,3-Tris(4'-Chlorobenzyl)-6-chloro-1*H*-quinoline-2,4-dione (**44**)

Synthesized after Procedure B. Yield: 0.062 g (57%). Glassy solid; m.p.: 144–146 °C. IR (KBr, ν): 3053 (C–H), 2940 (C–H), 1695 (C=O, ketone), 1660 (C=O, amide), 1595, 1485, 1436, 1409, 1360, 1252, 1096, 1015 and 822 cm<sup>−1</sup>. <sup>1</sup>H NMR: δ 3.40 (d,  $J = 12.7$ , 2H, ArCH<sub>2</sub>), 3.57 (d,  $J = 12.7$ , 2H, ArCH<sub>2</sub>), 4.95 (bs, 2H, ArCH<sub>2</sub>N), 6.43 (m, 3H, ArH and H-8), 6.97 (d,  $J = 8.4$ , 4H, ArH), 7.07 (d,  $J = 8.4$ , 4H, ArH), 7.09–7.19 (m, 3H, ArH and H-7) and 7.88 (d,  $J = 2.6$ , 1H, H-5). <sup>13</sup>C NMR: δ 44.9 (ArCH<sub>2</sub>N), 45.6 (2 × ArCH<sub>2</sub>), 64.7 (C-3), 117.2 (C-8), 122.1 (C-4a), 126.8 (C-5), 127.2 (2 × Ar), 128.5 (4 × Ar), 129.0 (2 × Ar), 129.2 (C-8a), 131.2 (4 × Ar), 133.0 (Ar), 133.1 (2 × Ar), 133.3 (Ar), 134.0 (2 × Ar), 136.1 (C-7), 140.0 (C-6), 171.7 (C-2) and 196.4 (C-4). EI-MS ( $m/z$ , %): 569 ( $M^+$ , 2), 442 (22), 276 (4), 127 (30), 125 (100) and 89 (12). HRMS (ESI–TOF),  $m/z$  found: 590.0195 [ $M + Na$ ]<sup>+</sup>, C<sub>30</sub>H<sub>21</sub>Cl<sub>4</sub>NNaO<sub>2</sub> requires 590.0219.

#### 4.6. General procedure for the synthesis of the 3,3-dibenzyl-4-hydroxy-3,4-dihydro-1*H*-quinolin-2-ones **45–60**

The 3,3-dibenzyl-1*H*-quinoline-2,4-dione (1.0 equiv.) was dissolved in a 1:1 mixture of EtOAc/EtOH (10 mL/mmol) contained in a round bottom flask. The solution was cooled in an ice bath, treated with NaBH<sub>4</sub> (1 mol equiv.), and allowed to react at room temperature until complete consumption of the starting material was

observed. Then, the reaction was cooled at 0 °C and saturated NH<sub>4</sub>Cl solution was cautiously added; after stirring for 15 min, the reaction products were extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with brine, dried over MgSO<sub>4</sub> and concentrated under reduced pressure, leaving a residue which upon column chromatography furnished the desired carbinol.

#### 4.6.1. 3,3-Dibenzyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (**45**)

Yield: 0.025 g (99%). White solid; m.p.: 171–173 °C. IR (KBr, ν): 3403 (O–H), 3245 (N–H), 1666 (C=O, amide), 1599, 1491, 1377, 1256, 1048, 763, 743 and 702 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.00 (s, 1H, OH), 2.77 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.01 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.09 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.38 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 4.69 (s, 1H, H-4), 6.56 (d, *J* = 7.6, 1H, H-8), 7.02 (t, *J* = 7.4, 1H, H-7), 7.10–7.24 (m, 9H, 2 × ArH and H-6), 7.26–7.38 (m, 3H, ArH and H-5) and 8.16 (s, 1H, NH). <sup>13</sup>C NMR: δ 36.9 (ArCH<sub>2</sub>), 37.9 (ArCH<sub>2</sub>), 53.0 (C-3), 69.7 (C-4), 114.5 (C-8), 123.3 (C-7), 125.9 (C-5), 126.1 (C-8a), 126.6 (2 × Ar), 127.9 (2 × Ar), 128.2 (2 × Ar), 128.6 (C-6), 130.3 (2 × Ar), 130.8 (2 × Ar), 134.3 (C-4a), 136.5 (Ar), 137.3 (Ar) and 172.5 (C-2). EI-MS (*m/z*, %): 343 (M<sup>+</sup>, 0.3), 252 (100), 234 (12), 216 (4), 174 (21) and 91 (41). HRMS (ESI–TOF), *m/z* found: 366.1464 [M + Na]<sup>+</sup>, C<sub>23</sub>H<sub>21</sub>NNaO<sub>2</sub> requires 366.1465.

#### 4.6.2. 3,3-Dibenzyl-6-fluoro-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (**46**)

Yield: 0.046 g (91%). White solid; m.p.: 187–189 °C. IR (KBr, ν): 3384 (O–H), 3242 (N–H), 3030 (C–H), 2922 (C–H), 1666 (C=O, amide), 1505, 1253, 1237, 1144, 819 and 701 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.80 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 2.98 (d, *J* = 13.9, 1H, ArCH<sub>2</sub>), 3.03 (d, *J* = 13.9, 1H, ArCH<sub>2</sub>), 3.51 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 4.69 (d, *J* = 4.8, 1H, H-4), 6.49 (dd, *J* = 4.6 and 8.6, 1H, H-8), 6.79 (dt, *J* = 2.8 and 8.6, 1H), 7.07–7.25 (m, 10H, 2 × ArH), 7.29–7.35 (m, 1H) and 8.77 (s, 1H, NH). <sup>13</sup>C NMR: δ 37.3 (ArCH<sub>2</sub>), 38.1 (ArCH<sub>2</sub>), 52.9 (C-3), 69.1 (C-4), 112.8 (d, <sup>2</sup>*J*<sub>CF</sub> = 24.2, Ar), 114.8 (d, <sup>2</sup>*J*<sub>CF</sub> = 23.1, Ar), 115.8 (d, <sup>3</sup>*J*<sub>CF</sub> = 7.7, Ar), 126.7 (Ar), 126.8 (Ar), 127.9 (2 × Ar), 128.4 (2 × Ar), 128.5 (C), 130.2 (2 × Ar and C), 130.6 (2 × Ar), 136.2 (Ar), 137.4 (Ar), 159.2 (d, <sup>1</sup>*J*<sub>CF</sub> = 242.0, C-6) and 172.4 (C-2). <sup>19</sup>F NMR: δ –119.2 (m, Ar–F). EI-MS (*m/z*, %): 361 (M<sup>+</sup>, 0.9), 270 (100), 252 (12), 234 (7), 192 (19) and 91 (42). HRMS (ESI–TOF), *m/z* found: 384.1367 [M + Na]<sup>+</sup>, C<sub>23</sub>H<sub>20</sub>FNNaO<sub>2</sub> requires 384.1370.

#### 4.6.3. 3,3-Dibenzyl-6-chloro-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (**47**)

Yield: 0.032 g (65%). White solid; m.p.: 194–196 °C. IR (KBr, ν): 3419 (O–H), 3240 (N–H), 3061 (C–H), 2922 (C–H), 1668 (C=O, amide), 1595, 1492, 1375, 1298, 1049 and 821 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.79 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 2.99 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.04 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.48 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 4.68 (d, *J* = 6.2, 1H, H-4), 6.43 (d, *J* = 8.2, 1H, H-8), 7.05 (dd, *J* = 2.4 and 8.4, 1H, H-7), 7.13–7.23 (m, 6H, 2 × ArH), 7.27–7.40 (m, 4H, ArH) and 7.95 (s, 1H, NH). <sup>13</sup>C NMR: δ 37.3 (ArCH<sub>2</sub>), 38.2 (ArCH<sub>2</sub>), 53.0 (C-3), 69.0 (C-4), 115.5 (C–H), 125.9 (C–H), 126.7 (Ar), 126.8 (Ar), 127.9 (2 × Ar), 128.1 (C), 128.2 (C–H), 128.4 (2 × Ar), 128.5 (C), 130.2 (2 × Ar), 130.7 (2 × Ar), 132.6 (C-6), 136.1 (Ar), 137.3 (Ar) and 172.0 (C-2). EI-MS (*m/z*, %): 377 (M<sup>+</sup>, 1.2), 286 (100), 268 (12), 250 (5), 208 (14), 115 (4) and 91 (52). HRMS (ESI–TOF), *m/z* found: 400.1063 [M + Na]<sup>+</sup>, C<sub>23</sub>H<sub>20</sub>ClNNaO<sub>2</sub> requires 400.1075.

#### 4.6.4. 3,3-Dibenzyl-4-hydroxy-6-methoxy-3,4-dihydro-1H-quinolin-2-one (**48**)

Yield: 0.018 g (92%). White solid; m.p.: 150–152 °C. IR (KBr, ν): 3207 (O–H, N–H), 3061 (C–H), 2929 (C–H), 1683 (C=O, amide), 1500, 1373, 1249 and 1159 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.22 (s, 1H, OH), 2.98 (d, *J* = 13.6, 1H, ArCH<sub>2</sub>), 3.05 (d, *J* = 13.8, 1H, ArCH<sub>2</sub>), 3.39 (d, *J* = 13.8, 1H, ArCH<sub>2</sub>), 3.39 (d, *J* = 13.6, 1H, ArCH<sub>2</sub>), 3.77 (s, 3H, ArOCH<sub>3</sub>), 4.66

(s, 1H, H-4), 6.53 (d, *J* = 8.5, 1H, H-8), 6.67 (dd, *J* = 2.4 and 8.5, 1H, H-7), 6.94 (d, *J* = 2.4, 1H, H-5), 7.10–7.24 (m, 8H, 2 × ArH), 7.31 (d, *J* = 6.6, 2H, ArH) and 8.92 (s, 1H, NH). <sup>13</sup>C NMR: δ 36.8 (ArCH<sub>2</sub>), 37.6 (ArCH<sub>2</sub>), 52.8 (C-3), 55.6 (2 × ArOCH<sub>3</sub>), 69.8 (C-4), 111.6 (C–H), 113.6 (C–H), 115.9 (C–H), 126.6 (Ar), 127.6 (C), 127.7 (C), 127.9 (2 × Ar), 128.2 (2 × Ar), 130.3 (2 × Ar), 130.8 (2 × Ar), 136.6 (Ar), 137.5 (Ar), 156.1 (C-6) and 172.7 (C-2). EI-MS (*m/z*, %): 373 (M<sup>+</sup>, 26), 282 (100), 264 (19), 204 (6), 151 (6), 123 (5) and 91 (34). HRMS (ESI–TOF), *m/z* found: 396.1572 [M + Na]<sup>+</sup>, C<sub>24</sub>H<sub>23</sub>NNaO<sub>3</sub> requires 396.1570.

#### 4.6.5. 3,3-Dibenzyl-4-hydroxy-6-methyl-3,4-dihydro-1H-quinolin-2-one (**49**)

Yield: 0.011 g (73%). Colorless oil. IR (NaCl, ν): 3444 (O–H), 3392 (N–H), 3055 (C–H), 2929 (C–H), 2873, 1732, 1672 (C=O, amide), 1373, 1265, 1249, 738, 702 and 437 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.29 (s, 3H, ArCH<sub>3</sub>), 2.02 (d, *J* = 4.8, 1H, OH), 2.74 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 2.99 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.08 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.33 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 4.64 (d, *J* = 4.8, 1H, H-4), 6.48 (d, *J* = 7.8, 1H, H-8), 6.94 (d, *J* = 8.0, 1H, H-7), 7.10–7.31 (m, 11H, 2 × ArH and H-5) and 8.26 (s, 1H, NH). <sup>13</sup>C NMR: δ 20.9 (ArCH<sub>3</sub>), 36.6 (ArCH<sub>2</sub>), 37.6 (ArCH<sub>2</sub>), 52.9 (C-3), 69.9 (C-4), 114.6 (C–H), 125.9 (C), 126.6 (2 × Ar and C), 127.9 (2 × Ar), 128.2 (2 × Ar), 129.0 (C–H), 130.3 (2 × Ar), 130.8 (2 × Ar), 131.9 (C), 132.9 (C), 136.8 (Ar), 137.4 (Ar), 172.4 (C-2). EI-MS (*m/z*, %): 357 (M<sup>+</sup>, 2), 266 (100), 248 (15), 188 (12), 135 (6), 107 (8) and 91 (33). HRMS (ESI–TOF), *m/z* found: 380.1628 [M + Na]<sup>+</sup>, C<sub>24</sub>H<sub>23</sub>NNaO<sub>2</sub> requires 380.1621.

#### 4.6.6. 3,3-Dibenzyl-4-hydroxy-8-methoxy-3,4-dihydro-1H-quinolin-2-one (**50**)

Yield: 0.011 g (37%). White solid; m.p.: 182–184 °C. IR (KBr, ν): 3371 (O–H), 2929 (C–H), 1653 (C=O, amide), 1494, 1448, 1417, 1369, 1273, 1247 and 1055 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.08 (d, *J* = 5.6, 1H, OH), 2.75 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.02 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.09 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.36 (d, *J* = 13.7, 1H, ArCH<sub>2</sub>), 3.76 (s, 3H, ArOCH<sub>3</sub>), 4.67 (d, *J* = 4.4, 1H, H-4), 6.64–6.72 (m, 1H, H-7), 6.89–6.97 (m, 2H, H-5 and H-6), 7.08–7.30 (m, 10H, 2 × ArH) and 7.78 (s, 1H, NH). <sup>13</sup>C NMR: δ 37.1 (ArCH<sub>2</sub>), 38.2 (ArCH<sub>2</sub>), 52.9 (C-3), 55.8 (ArOCH<sub>3</sub>), 69.7 (C-4), 110.1 (C–H), 117.7 (C–H), 123.0 (C–H), 123.5 (C), 126.5 (2 × Ar), 126.6 (C), 127.9 (2 × Ar), 128.1 (2 × Ar), 130.3 (2 × Ar), 130.8 (2 × Ar), 136.6 (Ar), 137.4 (Ar), 145.1 (C-8) and 171.4 (C-2). EI-MS (*m/z*, %): 373 (M<sup>+</sup>, 4), 282 (100), 264 (9), 249 (5), 204 (11), 151 (4), 123 (5) and 91 (28). HRMS (ESI–TOF) *m/z* found: 396.1583 [M + Na]<sup>+</sup>, C<sub>24</sub>H<sub>23</sub>NNaO<sub>3</sub> requires 396.1570.

#### 4.6.7. 3,3-Bis(4-Methoxybenzyl)-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (**51**)

Yield: 0.030 g (99%). White solid; m.p.: 155–157 °C. IR (KBr, ν): 3397 (O–H), 3237 (N–H), 2914 (C–H), 1667 (C=O, amide), 1612, 1596, 1511, 1378, 1300, 1251, 1176 and 1032 cm<sup>-1</sup>. <sup>1</sup>H NMR: δ 2.08 (s, 1H, OH), 2.70 (d, *J* = 13.9, 1H, ArCH<sub>2</sub>), 2.94 (d, *J* = 13.9, 1H, ArCH<sub>2</sub>), 3.02 (d, *J* = 13.9, 1H, ArCH<sub>2</sub>), 3.28 (d, *J* = 13.9, 1H, ArCH<sub>2</sub>), 3.69 (s, 3H, OCH<sub>3</sub>), 3.73 (s, 3H, OCH<sub>3</sub>), 4.68 (s, 1H, H-4), 6.59 (dd, *J* = 0.8 and 7.8, 1H, H-8), 6.67–6.80 (m, 4H, *p*-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>), 7.02 (dt, *J* = 1.0, 7.4, 1H, H-7), 7.10–7.23 (m, 5H, *p*-CH<sub>3</sub>OC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub> and H-6), 7.33 (d, *J* = 7.4, 1H, H-5) and 8.51 (s, 1H, NH). <sup>13</sup>C NMR: δ 35.8 (ArCH<sub>2</sub>), 36.8 (ArCH<sub>2</sub>), 52.9 (C-3), 55.1 (2 × ArOCH<sub>3</sub>), 69.8 (C-4), 113.4 (2 × Ar), 113.6 (2 × Ar), 114.7 (C-8), 123.3 (C-7), 126.0 (C-5), 126.3 (C-8a), 128.5 (2 × Ar), 128.6 (C-6), 129.3 (2 × Ar), 131.3 (Ar), 131.7 (Ar), 134.4 (C-4a), 158.2 (Ar), 158.3 (Ar) and 172.9 (C-2). EI-MS (*m/z*, %): 403 (M<sup>+</sup>, 2), 282 (100), 264 (25), 174 (22), 121 (95), 91 (6) and 77 (10). HRMS (ESI–TOF), *m/z* found: 426.1683 [M + Na]<sup>+</sup>, C<sub>25</sub>H<sub>25</sub>NNaO<sub>4</sub> requires 426.1676.

**4.6.8. 3,3-Bis(4-Chlorobenzyl)-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (52)**

Yield: 0.060 g (63%). White solid; m.p.: 95–97 °C. IR (KBr,  $\nu$ ): 3383 (O–H), 3265 (N–H), 2924 (C–H), 1670 (C=O, amide), 1614, 1597, 1489, 1409, 1382, 1091, 1016 and 754  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  2.02 (d,  $J = 5.8$ , 1H, OH), 2.71 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 2.97 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.04 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.28 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 4.64 (d,  $J = 5.6$ , 1H, H-4), 6.54 (dd,  $J = 0.8$ , 7.6, 1H, H-8), 7.04 (dt,  $J = 1.0$ , 7.6, 1H, H-7), 7.08–7.23 (m, 9H,  $p\text{-ClC}_6\text{H}_4\text{CH}_2$ , H-5), 7.32 (d,  $J = 6.8$ , 1H, H-6) and 8.01 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  36.2 ( $\text{ArCH}_2$ ), 37.3 ( $\text{ArCH}_2$ ), 52.8 (C-3), 69.4 (C-4), 114.6 (C-8), 123.6 (C-7), 125.9 (Ar and C-5), 128.0 ( $2 \times \text{Ar}$ ), 128.2 ( $2 \times \text{Ar}$ ), 128.8 (Ar and C-8a), 131.6 ( $2 \times \text{Ar}$ ), 132.0 ( $2 \times \text{Ar}$ ), 132.6 (C-6), 134.1 (C-4a), 134.7 (Ar), 135.5 (Ar) and 171.8 (C-2). EI-MS ( $m/z$ , %): 411 ( $\text{M}^+$ , 0.4), 286 (100), 268 (19), 250 (6), 233 (8), 174 (23), 125 (34) and 93 (9). HRMS (ESI–TOF),  $m/z$  found: 434.0676 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{19}\text{Cl}_2\text{NaNO}_2$  requires 434.0685.

**4.6.9. 3,3-Bis(2-Chlorobenzyl)-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (53)**

Yield: 0.038 g (99%). Colorless oil. IR (NaCl,  $\nu$ ): 3209 (O–H, N–H), 3063 (C–H), 2925 (C–H), 1667 (C=O, amide), 1589, 1475, 1440, 1362, 1264, 1053, 1043, 754 and 735  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  2.83 (s, 1H, OH), 3.22 (d,  $J = 14.2$ , 1H,  $\text{ArCH}_2$ ), 3.37 (d,  $J = 9.7$ , 1H,  $\text{ArCH}_2$ ), 3.42 (d,  $J = 9.7$ , 1H,  $\text{ArCH}_2$ ), 3.55 (d,  $J = 14.2$ , 1H,  $\text{ArCH}_2$ ), 4.80 (s, 1H, H-4), 6.60 (d,  $J = 7.6$ , 1H, H-8), 6.95–7.63 (m, 11H,  $2 \times \text{ArH}$ , H-5, H-6 and H-7) and 9.21 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  33.5 ( $\text{ArCH}_2$ ), 33.6 ( $\text{ArCH}_2$ ), 54.3 (C-3), 69.2 (C-4), 114.4 (C-8), 123.5 (C-7), 125.1 (C-8a), 126.2 (Ar and C-5), 127.2 (Ar), 128.0 (Ar), 128.2 (Ar), 128.5 (C-6), 129.4 ( $2 \times \text{Ar}$ ), 132.2 (Ar), 132.9 (Ar), 134.0 (Ar), 134.4 (Ar), 134.6 (C-4a), 135.4 (Ar), 135.5 (Ar) and 172.7 (C-2). EI-MS ( $m/z$ , %): 411 ( $\text{M}^+$ , 0.07), 376 (100), 286 (20), 250 (15), 234 (15), 174 (9) and 125 (22). HRMS (ESI–TOF),  $m/z$  found: 434.0682 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{19}\text{Cl}_2\text{NaNO}_2$  requires 434.0685.

**4.6.10. 3,3-Dibenzyl-4-hydroxy-1-methyl-3,4-dihydro-1H-quinolin-2-one (54)**

Yield: 0.060 g (52%). White solid; m.p.: 147–149 °C. IR (KBr,  $\nu$ ): 3408 (O–H), 3028 (C–H), 2933 (C–H), 2879, 1653 (C=O, amide), 1602, 1473, 1367, 1124 and 700  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  1.96 (s, 1H, OH), 2.70 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.04 (s, 2H,  $\text{ArCH}_2$ ), 3.24 (s, 3H,  $\text{NCH}_3$ ), 3.38 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 4.60 (s, 1H, H-4), 6.68 (d,  $J = 8.0$ , 1H, H-8), 7.02 (dt,  $J = 0.6$  and 7.4, 1H, H-7), 7.07–7.24 (m, 11H,  $2 \times \text{ArH}$  and H-6) and 7.33 (d,  $J = 7.2$ , 1H, H-5).  $^{13}\text{C}$  NMR:  $\delta$  29.8 ( $\text{N-CH}_3$ ), 37.6 ( $\text{ArCH}_2$ ), 39.1 ( $\text{ArCH}_2$ ), 52.7 (C-3), 69.3 (C-4), 113.7 (C-8), 122.9 (C-7), 125.8 (C-8a), 126.4 (C-5), 127.4 ( $2 \times \text{Ar}$ ), 127.8 ( $2 \times \text{Ar}$ ), 128.0 ( $2 \times \text{Ar}$ ), 128.6 (C-6), 130.2 ( $2 \times \text{Ar}$ ), 130.8 ( $2 \times \text{Ar}$ ), 136.8 (C), 137.5 (C), 137.9 (C) and 171.1 (C-2). EI-MS ( $m/z$ , %): 357 ( $\text{M}^+$ , 1.40), 266 (100), 248 (18), 188 (9), 106 (3) and 91 (28). HRMS (ESI–TOF),  $m/z$  found: 358.1797 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{24}\text{H}_{24}\text{NNaO}_2$  requires 358.1802.

**4.6.11. 3,3-Dibenzyl-1-ethyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (55)**

Yield: 0.090 g (45%). Colorless oil. IR (NaCl,  $\nu$ ): 3441 (O–H), 3053 (C–H), 2981, 1666 (C=O, amide), 1602, 1494, 1462, 1379, 1265, 1134, 1049 and 894  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  1.21 (t,  $J = 7.1$ , 3H,  $\text{CH}_3\text{CH}_2\text{N}$ ), 1.93 (d,  $J = 3.6$ , 1H, OH), 2.70 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 2.99 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.05 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.36 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.79 (sextet,  $J = 7.1$ , 1H,  $\text{CH}_3\text{CH}_2\text{N}$ ), 3.98 (sextet,  $J = 7.1$ , 1H,  $\text{CH}_3\text{CH}_2\text{N}$ ), 4.57 (d,  $J = 3.6$ , 1H, H-4), 6.82 (d,  $J = 8.0$ , 1H, H-8), 7.03 (dt,  $J = 1.0$  and 7.4, 1H, H-7), 7.11–7.24 (m, 11H,  $2 \times \text{ArH}$  and H-6) and 7.35 (d,  $J = 7.4$ , 1H, H-5).  $^{13}\text{C}$  NMR:  $\delta$  12.6 ( $\text{CH}_3\text{CH}_2\text{N}$ ), 37.2 ( $\text{CH}_3\text{CH}_2\text{N}$ ), 38.3 ( $\text{ArCH}_2$ ), 38.6 ( $\text{ArCH}_2$ ), 52.4 (C-3), 69.5 (C-4), 113.8 (C-8), 122.9 (C-7), 126.1 (C-8a), 126.4 (C-5), 126.5 ( $2 \times \text{Ar}$ ), 127.8 ( $2 \times \text{Ar}$ ), 127.9 (Ar), 128.0 (Ar), 128.6 (C-6), 130.3 ( $2 \times \text{Ar}$ ), 130.9

( $2 \times \text{Ar}$ ), 136.8 (C), 137.1 (C), 137.6 (C) and 170.5 (C-2). EI-MS ( $m/z$ , %): 371 ( $\text{M}^+$ , 1.4), 280 (100), 262 (13), 251 (4), 234 (5), 202 (8) and 91 (28). HRMS (ESI–TOF),  $m/z$  found: 372.1947 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{25}\text{H}_{26}\text{NNaO}_2$  requires 372.1958.

**4.6.12. 1,3,3-Tribenzyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (56)**

Yield: 0.011 g (58%). Colorless oil. IR (NaCl,  $\nu$ ): 3441 (O–H), 2918 (C–H), 1666 (C=O, amide), 1602, 1454, 1373, 1265 and 1045  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  1.91 (d,  $J = 5.6$ , 1H, OH), 2.77 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.07 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.18 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 3.36 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 4.63 (d,  $J = 5.44$ , 1H, H-4), 5.07 (d,  $J = 16.1$ , 1H,  $\text{ArCH}_2\text{N}$ ), 5.18 (d,  $J = 16.1$ , 1H,  $\text{ArCH}_2\text{N}$ ), 6.78 (d,  $J = 7.6$ , 1H, H-8), 7.00 (dt,  $J = 1.0$  and 7.4, 1H, H-7), 7.10 (dt,  $J = 1.2$  and 7.2, 1H, H-6), 7.14–7.30 (m, 15H,  $2 \times \text{ArH}$  and  $\text{C}_6\text{H}_5\text{CH}_2\text{N}$ ) and 7.34 (d,  $J = 7.2$ , 1H, H-5).  $^{13}\text{C}$  NMR:  $\delta$  37.2 ( $\text{ArCH}_2$ ), 38.4 ( $\text{ArCH}_2$ ), 45.8 ( $\text{ArCH}_2\text{N}$ ), 52.6 (C-3), 69.7 (C-4), 114.9 (C-8), 123.1 (C-7), 126.1 (C-5), 126.5 ( $2 \times \text{Ar}$ ), 126.6 ( $2 \times \text{Ar}$ ), 126.9 ( $2 \times \text{Ar}$ ), 127.0 (Ar), 127.8 ( $2 \times \text{Ar}$ ), 128.0 (Ar), 128.1 (Ar), 128.5 (Ar), 128.6 (C-6), 130.3 ( $2 \times \text{Ar}$ ), 130.9 ( $2 \times \text{Ar}$ ), 136.8 (C), 136.9 (C), 137.0 (C), 137.5 (C) and 171.7 (C-2). EI-MS ( $m/z$ , %): 433 ( $\text{M}^+$ , 0.8), 342 (26), 324 (6), 250 (14), 174 (4) and 91 (67). HRMS (ESI–TOF),  $m/z$  found: 434.2116 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{30}\text{H}_{28}\text{NNaO}_2$  requires 434.2115.

**4.6.13. Ethyl 2-(3,3-dibenzyl-4-hydroxy-2-oxo-3,4-dihydro-1H-quinolin-1-yl)acetate (57)**

Yield: 0.013 g (74%). Colorless oil. IR (NaCl,  $\nu$ ): 3447 (O–H), 2981 (C–H), 1732 (C=O, ester), 1681 (C=O, amide), 1604, 1494, 1462, 1371, 1265, 1246, 1193, 1045 and 1031  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  1.26 (t,  $J = 7.1$ , 3H,  $\text{CH}_3\text{CH}_2\text{O}$ ), 2.40 (s, 1H, OH), 2.70 (d,  $J = 13.9$ , 1H,  $\text{ArCH}_2$ ), 2.96 (d,  $J = 13.9$ , 1H,  $\text{ArCH}_2$ ), 3.14 (d,  $J = 13.9$ , 1H,  $\text{ArCH}_2$ ), 3.15 (d,  $J = 13.9$ , 1H,  $\text{ArCH}_2$ ), 4.17–4.29 (m, 2H,  $\text{CH}_3\text{CH}_2\text{O}$ ), 4.32 (d,  $J = 17.5$ , 1H,  $\text{CH}_3\text{CH}_2\text{O}$ ), 4.59 (s, 1H, H-4), 4.96 (d,  $J = 17.5$ , 1H,  $\text{EtO}_2\text{CCH}_2$ ), 6.70 (d,  $J = 7.6$ , 1H, H-8), 7.09 (dt,  $J = 0.8$  and 7.4, 1H, H-7), 7.12–7.32 (m, 11H,  $2 \times \text{ArH}$  and H-6) and 7.38 (d,  $J = 7.4$ , 1H, H-5).  $^{13}\text{C}$  NMR:  $\delta$  14.1 ( $\text{CH}_3\text{CH}_2\text{O}$ ), 36.3 ( $\text{ArCH}_2$ ), 37.6 ( $\text{ArCH}_2$ ), 44.9 ( $\text{EtO}_2\text{CCH}_2$ ), 52.5 (C-3), 61.7 ( $\text{CH}_3\text{CH}_2\text{O}$ ), 69.8 (C-4), 114.1 (C-8), 123.7 (C-7), 126.5 (C-5 and C), 126.6 ( $2 \times \text{Ar}$ ), 128.0 ( $2 \times \text{Ar}$ ), 128.1 ( $2 \times \text{Ar}$ ), 128.8 (C-6), 130.2 ( $2 \times \text{Ar}$ ), 131.1 ( $2 \times \text{Ar}$ ), 136.7 (C), 137.1 (C), 137.4 (C), 169.0 ( $\text{EtO}_2\text{CCH}_2$ ) and 171.9 (C-2). EI-MS ( $m/z$ , %): 429 ( $\text{M}^+$ , 0.3), 338 (100), 320 (61), 292 (63), 264 (8), 246 (10), 218 (13), 134 (23) and 91 (59). HRMS (ESI–TOF),  $m/z$  found: 881.3741 [ $2\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{54}\text{H}_{54}\text{N}_2\text{NaO}_8$  requires 881.3772.

**4.6.14. 3,3-Bis(4-Chlorobenzyl)-6-chloro-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (58)**

Yield: 0.021 g (69%). White solid; m.p.: 186–188 °C. IR (KBr,  $\nu$ ): 3284 (O–H), 3265 (N–H), 1674 (C=O, amide), 1595, 1490, 1409, 1375, 1093, 1014, 839, 819 and 808  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  2.38 (d,  $J = 6.0$ , 1H, OH), 2.73 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 2.93 (d,  $J = 13.9$ , 1H,  $\text{ArCH}_2$ ), 2.98 (d,  $J = 13.9$ , 1H,  $\text{ArCH}_2$ ), 3.36 (d,  $J = 13.7$ , 1H,  $\text{ArCH}_2$ ), 4.63 (d,  $J = 5.8$ , 1H, H-4), 6.48 (d,  $J = 8.2$ , 1H, H-8), 7.03–7.23 (m, 9H,  $2 \times \text{ArH}$  and H-7), 7.34 (d,  $J = 1.6$ , 1H, H-5) and 8.70 (s, 1H, NH).  $^{13}\text{C}$  NMR:  $\delta$  36.5 ( $\text{ArCH}_2$ ), 37.3 ( $\text{ArCH}_2$ ), 52.8 (C-3), 68.7 (C-4), 115.7 (C-8), 125.9 (C-7), 127.9 (Ar), 128.0 ( $2 \times \text{Ar}$ ), 128.4 ( $2 \times \text{Ar}$ ), 128.8 (Ar), 129.1 (Ar), 129.9 (C), 131.6 ( $2 \times \text{Ar}$ ), 131.9 ( $2 \times \text{Ar}$ ), 132.4 (C-4a), 132.8 (C-6), 134.4 (Ar), 135.5 (Ar) and 171.7 (C-2). EI-MS ( $m/z$ , %): 445 ( $\text{M}^+$ , 3), 320 (100), 302 (15), 284 (5), 267 (8), 208 (14), 125 (40) and 89 (9). HRMS (ESI–TOF),  $m/z$  found: 468.0276 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{23}\text{H}_{18}\text{Cl}_3\text{NNaO}_2$  requires 468.0295.

**4.6.15. 3,3-Bis(4-Chlorobenzyl)-6-chloro-1-ethyl-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (59)**

Yield: 0.019 g (99%). Colorless oil. IR (NaCl,  $\nu$ ): 3443 (O–H), 2976 (C–H), 2934 (C–H), 1645 (C=O, amide), 1597, 1489, 1427, 1377,



1263, 1121, 1094, 1016, 841 and 812  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  1.20 (t,  $J$  = 7.1, 3H,  $\text{CH}_3\text{CH}_2\text{N}$ ), 1.61 (bs, 1H, OH), 2.69 (d,  $J$  = 13.6, 1H,  $\text{ArCH}_2$ ), 2.91 (s, 2H,  $\text{ArCH}_2$ ), 3.38 (d,  $J$  = 13.6, 1H,  $\text{ArCH}_2$ ), 3.75 (sextet,  $J$  = 7.0, 1H,  $\text{CH}_3\text{CH}_2\text{N}$ ), 3.94 (sextet,  $J$  = 7.0, 1H,  $\text{CH}_3\text{CH}_2\text{N}$ ), 4.53 (d,  $J$  = 5.7, 1H, H-4), 6.71 (d,  $J$  = 8.8, 1H, H-8), 6.97 (d,  $J$  = 8.1, 2H,  $\text{ArH}$ ), 7.12 (d,  $J$  = 8.1, 2H,  $\text{ArH}$ ), 7.15–7.20 (m, 5H,  $\text{ArH}$  and H-7) and 7.36 (d,  $J$  = 2.1, 1H, H-5).  $^{13}\text{C}$  NMR:  $\delta$  12.6 ( $\text{CH}_3\text{CH}_2\text{N}$ ), 36.8 ( $\text{ArCH}_2$ ), 38.3 ( $\text{ArCH}_2$ ), 38.6 ( $\text{CH}_3\text{CH}_2\text{N}$ ), 52.6 (C-3), 68.4 (C-4), 115.2 (Ar and C-8), 125.8 (C-5 and C), 127.9 ( $2 \times \text{Ar}$ ), 128.3 ( $2 \times \text{Ar}$ ), 128.6 (C), 129.8 (C), 131.7 ( $2 \times \text{Ar}$ ), 132.0 ( $2 \times \text{Ar}$ ), 132.6 (C-6), 132.9 (C), 134.7 (C), 135.2 (C), 135.8 (C) and 169.9 (C-2). EI-MS ( $m/z$ , %): 473 ( $\text{M}^+$ , 10), 348 (100), 302 (5), 236 (15), 125 (175) and 89 (15). HRMS (ESI–TOF),  $m/z$  found: 496.0605 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{25}\text{H}_{22}\text{Cl}_3\text{NNaO}_2$  requires 496.0608.

#### 4.6.16. 1,3,3-Tris(4-chlorobenzyl)-6-chloro-4-hydroxy-3,4-dihydro-1H-quinolin-2-one (60)

Yield: 0.056 g (93%). Colorless oil. IR (NaCl,  $\nu$ ): 3458 (O–H), 3049 (C–H), 2931 (C–H), 1645 (C=O, amide), 1598, 1487, 1410, 1371, 1307, 1265, 1188, 1095, 1016, 984, 843 and 810  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR:  $\delta$  2.18 (bs, 1H, OH), 2.72 (d,  $J$  = 13.8, 1H,  $\text{ArCH}_2$ ), 2.99 (d,  $J$  = 13.8, 1H,  $\text{ArCH}_2$ ), 3.06 (d,  $J$  = 13.8, 1H,  $\text{ArCH}_2$ ), 3.34 (d,  $J$  = 13.8, 1H,  $\text{ArCH}_2$ ), 4.56 (bs, 1H, H-4), 4.98 (d,  $J$  = 16.0, 1H,  $\text{ArCH}_2\text{N}$ ), 5.07 (d,  $J$  = 16.0, 1H,  $\text{ArCH}_2\text{N}$ ), 6.61 (d,  $J$  = 8.6, 1H, H-8), 6.99–7.23 (m, 13H,  $2 \times \text{ArH}$ ,  $\text{ArCH}_2\text{N}$  and H-7) and 7.34 (d,  $J$  = 2.1, 1H, H-5).  $^{13}\text{C}$  NMR:  $\delta$  36.7 ( $\text{ArCH}_2$ ), 38.2 ( $\text{ArCH}_2$ ), 45.3 ( $\text{ArCH}_2\text{N}$ ), 52.6 (C-3), 68.6 (C-4), 116.0 (C-8), 126.2 (C-5), 128.1 ( $2 \times \text{Ar}$ ), 128.3 ( $4 \times \text{Ar}$ ), 128.4 (C-7), 128.9 ( $2 \times \text{Ar}$ ), 129.0 (Ar), 129.8 (Ar), 131.6 ( $2 \times \text{Ar}$ ), 132.1 ( $2 \times \text{Ar}$ ), 132.7 (Ar), 132.8 (Ar), 133.3 (Ar), 134.6 (Ar), 134.7 (Ar), 134.9 (Ar), 135.6 (C-8a) and 171.1 (C-2). EI-MS ( $m/z$ , %): 571 ( $\text{M}^+$ , 4), 444 (25), 302 (10), 127 (30), 125 (100) and 89 (8). HRMS (ESI–TOF),  $m/z$  found: 592.0371 [ $\text{M} + \text{Na}$ ] $^+$ ,  $\text{C}_{30}\text{H}_{23}\text{Cl}_4\text{NNaO}_2$  requires 592.0375.

### 4.7. Biological activity

#### 4.7.1. Determination of MIC and MLC

The compounds were evaluated for their antimicrobial activity against the following bacterial strains: *B. subtilis* (ATCC 6633), *Burkholderia cepacia* (ATCC 17759), *Enterobacter aerogenes* (ATCC 13048), *Escherichia coli* (ATCC 25922), *Enterococcus* spp. (ATCC 6589), *M. morganii* (ATCC 8019), *P. aeruginosa* (ATCC 17759), *S. aureus* (ATCC 6538p), *Shigella sonnei* (ATCC 15305) and *Salmonella typhimurium* (ATCC 14028).

The minimum inhibitory concentration (MIC) of the compounds was determined in 96-well culture plates by the micro dilution method [47] using a microorganism suspension in casein soy broth (17 g of enzymatic digest of casein, 3 g of enzymatic digest of soybean meal, 2.5 g of dextrose, 5 g of sodium chloride, 2.5 g of dipotassium phosphate per liter, pH 7.3) at a density of  $10^5$  CFU/mL, incubated for 24 h at 37 °C. The cultures that did not exhibit bacterial growth were used to inoculate plates of Mueller Hinton Agar in order to determine the minimum lethal concentration (MLC). Transfers were performed employing a calibrated pipette and levofloxacin was used as comparator. Controls were assayed simultaneously and samples were tested in quadruplicate.

#### 4.7.2. Optical density (OD) measurements [33]

The microorganisms were cultivated in casein soy broth medium at 37 °C. The overnight bacterial culture cells were harvested by centrifugation (10 min, 3000 rpm), re-suspended in fresh medium, and adjusted to approximately  $5 \times 10^7$  cells/mL ( $\text{OD}_{620} = 0.2$ ). Aliquots of the adjusted bacterial culture (180  $\mu\text{L}$ ) were added to a 96-well microplate and further cultivated up to their mid-logarithmic phase, when twofold serial dilutions of the test samples or the comparator antibacterials (20  $\mu\text{L}$ ) were added, dissolved in fresh medium. The experiments for each compound

and concentration level were run in quadruplicate. The plate was placed in the microplate reader and the  $\text{OD}_{620}$  values were periodically recorded at 23 °C during 10 h in order to prevent the evaporation of samples [33,43a]. Data analyses were carried out using the SPSS v. 18.0 statistical software (SPSS Inc., Chicago, IL). The results are presented as % variation of  $\text{OD}_{620}$  relative to the control group at the time of the addition of the test compound.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2014.05.024>.

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