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Synthesis of Quinoline Derivatives with Potential Antibacterial Activity

Abstract of a Thesis for the Educational and Scientific Degree "Doctor"

Field of higher education – 4. Natural sciences, mathematics and informatics; Professional direction – 4.2. Chemical sciences

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CONTENTS

I. INTRODUCTION

The quinoline ring system (Fig. 1) is present in many bioactive compounds of natural[1,2] and synthetic[3,4] origin. Along with the broad spectrum of biological activities, the quinoline derivatives exhibit remarkable structural diversity, determined by the possibility of full or partial saturation of the aromatic ring system, as well as by the possible substitutions in positions 1 – 8. Among the quinoline derivatives, of particular interest are those with carbonyl functional group in positions 2 or 4 – quinolin-2(1*H*)-ones (Fig. 2, B) and quinolin-4(1*H*)-ones (Fig. 2, A), respecively. For brevity, these ring systems are usually referred to as 4-quinolones and 2-quinolones – a terminology adopted in this doctoral thesis.

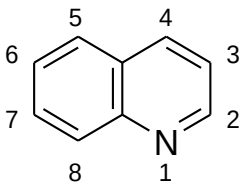


Figure 1. The quinoline ring system, with position numbering.

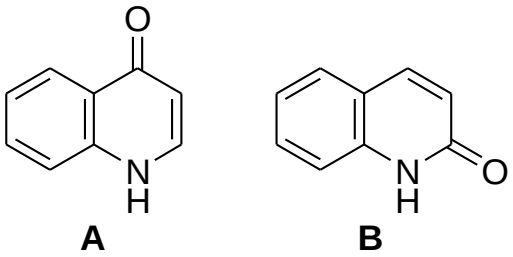


Figure 2. Quinoline derivatives with carbonyl functional group in the heterocyclic ring – quinolin-4(1*H*)-ones (**A**) and quinolin-2(1*H*)-ones (**B**).

The interest towards synthetic methods for construction of 2- and 4-quinolone rings stems from their presence in many alakaloids of microbial or plant origin, as well as in some synthetic pharmaceuticals. The development of novel synthetic methods expands the space of available structural analogs, aids the investigations on structure-activity relationship, and is of foremost importance in the development of novel drugs from natural lead compounds. In view of that, the objective of this doctoral thesis is development of novel methods for **synthesis of quinoline derivativesis**, and specifically derivatives of 2- and 4-quinolone type. With regard to the possible biological activitiy of the synthesized compounds, our efforts were

[1] *Med. Res. Rev.* **2018**, *38*, 775–828. DOI: 10.1002/med.21466
[2] *Med. Res. Rev.* **2018**, *38*, 1614–1660. DOI: 10.1002/med.21492
[3] *Eur. J. Med. Chem.* **2019**, *164*, 121–170. DOI: 10.1016/j.ejmech.2018.11.026
[4] *Org. Biomol. Chem.* **2020**, *18*, 9775–9790. DOI: 10.1039/d0ob02000a

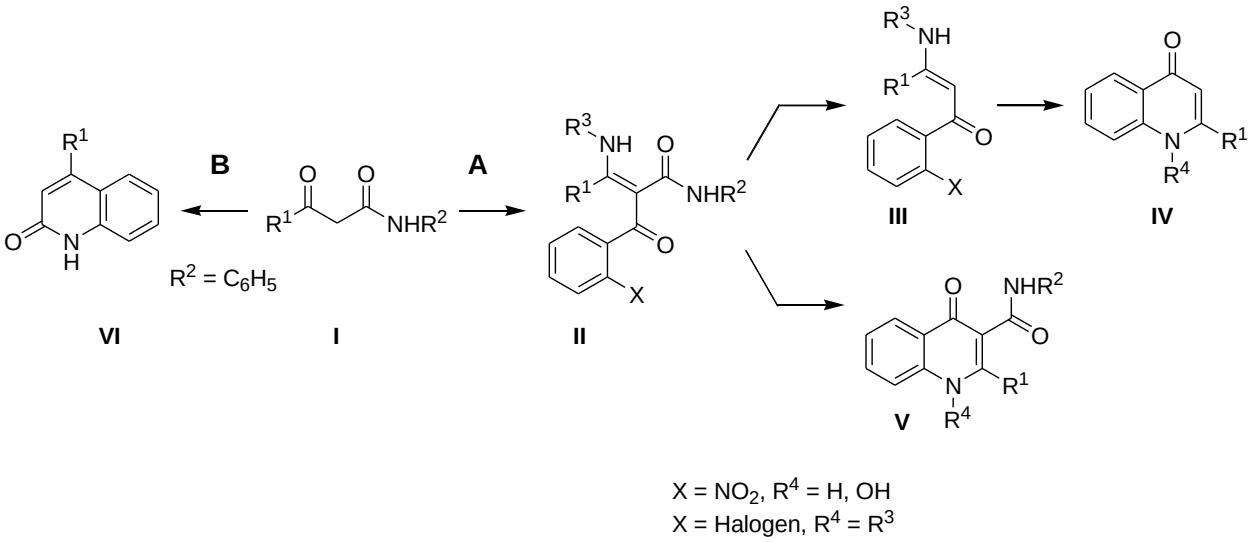
aimed mainly at quinolones **with potential antibacterial activity**, structurally similar to known synthetic antibacterials and natural bacterial metabolites. This objective is motivated by the current problem with the dramatic increase of bacterial resistance towards the established antibacterial drugs. In 2014 the World Health Organization points out the imminent danger of the so called "post-antibiotic era" in which the known antibiotics will lose their efficiency against many pathogens and consequently infections, previously considered trivial, will again become untreatable and life-threatening.[5] The same problem is presented in a detailed analysis of the British Chief Medical Officer from 2011.[6] Both documents underscore that after the introduction of the fluoroquinolone antibacterials in the 1980s, there have been no new discoveries of principally novel antibacterial medicines for the last 30 years. One approach to this problem is to intensify the research on natutal antimicrobial compounds and their synthetic analogs.

The present thesis aims mainly at the development of novel synthetic approach towards 4-quinolones, including known metabolites of the opportunistic pathogen *Pseudomonas aeruginosa* and new synthetic analogs with possible antibacterial activity. To a lesser extent is investigated the synthesis of 2-quinolones and some problems in the application of functionalized ketoamides in the classic Knorr synthesis. A common starting ground towards both target strucutres are compounds of β-keto amide type, which are available through a methodology, previously developed at the Department of Organic Chemistry at the University of Plovdiv.

[5] http://www.who.int/drugresistance/documents/surveillancereport/en/
[6] https://www.gov.uk/government/publications/chief-medical-officer-annual-report-volume-2

II. RESULTS AND DISCUSSION

To accomplish the objective defined in the introduction (synthesis of quinoline derivatives with potential antibacterial activity) we investigated two approaches, which we expected to allow us to synthesize quinoline derivatives of 4-quinolone and 2-quinolone type, respectively. As illustrated in Scheme 1, a feature common to both approaches is the application of β-keto amides as starting compounds. Approach **A** is a multistage synthesis, based on original methodology conceived by us, while approach **B** is basically a single-stage implementation of the classic Knorr synthesis, which attracted our attention with possible competing reactions and subsequent modifications of the 2-quinolone products, obtainable from β-keto amides carrying functionalized R^1 residue.



Scheme 1. *General plan of the investigations on synthetic sequences and expected intermediates, leading to derivatives of 4-quinolone (approach **A**) and 2-quinolone (approach **B**) type.*

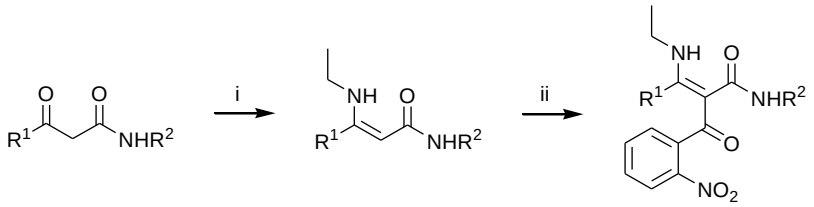
1. Synthetic approach to 2-alkyl-4-quinolones and 2-alkyl-4-quinolone-3-carboxamides based on common β-keto amide precursors

Despite the variety of synthetic approaches to the construction and functionalization of the 4-quinolone ring system, most of the recent studies related to microbial 2-alkyl-4-quinolones rely on variations of the age-old Conrad-Limpach and Camps methods for the construction of the heterocyclic quinolone core. These methods usually give poor overall yield of the target quinolone products and require rather harsh conditions during the ring-forming step – prolonged heating in Ph_2O (270 ^oC) or in dioxane/NaOH (110 ^oC), respectively. This, along with the importance of the C-3 substitution in analogues of microbial behavioural modulators, prompted us to investigate a new synthetic approach that could provide a straightforward access to both 2-alkyl-4-quinolones and 2-alkyl-4-quinolone-3-carboxamides.

As the starting point of our synthetic experiments we used a set of β-ketoamides **1**. One of these compounds (**1g**) was acquired from a commercial supplier, others (**1h** and **1i**) were prepared by acetoacetylation of the corresponding amine, and the rest (**1a-f**) were prepared according to our previously published method.[7] The intermediate β-enamino amides **2** are easily available by condensation of the corresponding β-keto amide **1** and an amine (Scheme 1, i). As the amine here plays only an auxiliary role, for the purpose of this research we opted for inexpensive ethylamine. Compounds **2** were obtained by simply stirring dichloromethane solution of the corresponding ketoamide **1** with slight excess of 70% aqueous ethylamine over Na_2SO_4 and were used directly in the next step, without purification. These compounds are highly reactive at their α-position towards acylating reagents and this provides an opportunity to prepare the key *o*-nitrobenzoyl intermediates **3** in a reaction with *o*-nitrobenzoyl chloride (Scheme 1, ii). The acylation of **2** to **3** proceeded with variable yields, depending on the substituents R^1 and R^2. Derivatives **2** with primary carboxamide group (R^2 = H) gave generally lower and poorly reproducible yields of the desired products **3**. On the other hand, when R^2 was aryl or benzyl the yields of **3** over two steps were very good, in the range of 75–92% (Table 1). The R^1 substituent influenced the yield of **3** to a lower extent, with unfavorable effect of

[7] Angelov, P. *Synlett* **2010**, 1273–1275. https://doi.org/10.1055/s-0029-1219836

sterically bulkier substituents. Any α-substitution in R^1 drove the yields of **3** below 50% and for this reason isolation and further elaboration of such products was considered impractical.



1 **2** **3**

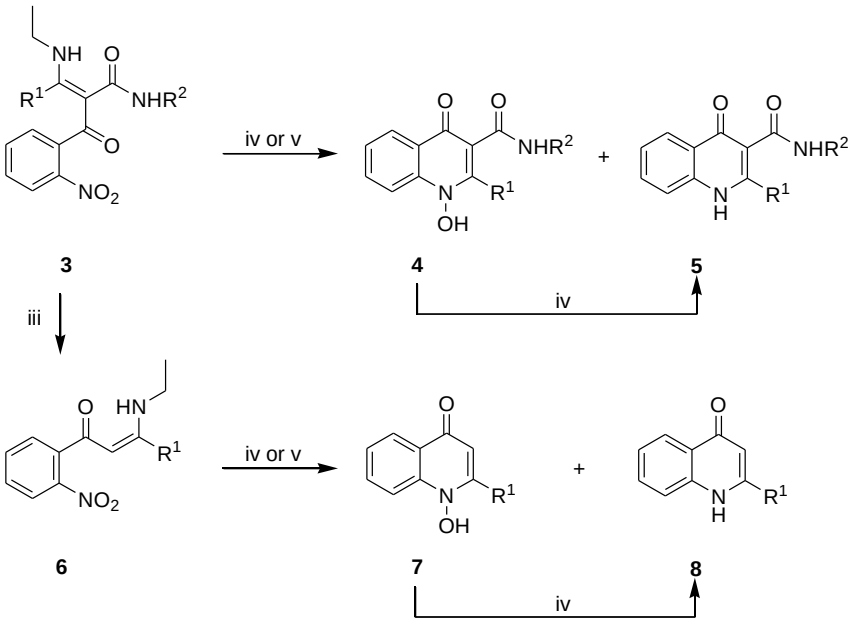
Scheme 1. *Preparation of α-(o-nitrobenzoyl)-β-enaminoamides **3**, reagents and conditions: i) $EtNH_2$ (70% aq., 1.05 – 1.15 equiv.), CH_2Cl_2, Na_2SO_4, 24 h r.t.; ii) NMM (1 equiv.), DMAP (0.2 equiv), o-nitrobenzoyl chloride (1 equiv.), CH_2Cl_2, 2h, r.t.*

Table 1. *Yields of α-(o-nitrobenzoyl)-β-enaminoamides **3** prepared according to Scheme 1:*

3	**R^1**	**R^2**	**Yield* % (3)**
a	*n*-C_3H_7	C_6H_5	90
b	*i*-C_4H_9	C_6H_5	75
c	*n*-C_5H_{11}	C_6H_5	89
d	*n*-C_7H_{15}	C_6H_5	88
e	*n*-C_7H_{15}	*p*-$C_6H_4OCH_3$	86
f	*n*-C_7H_{15}	*p*-C_6H_4Cl	91
g	CH_3	*p*-C_6H_4Cl	90
h	CH_3	*p*-$C_6H_4OCH_3$	92
i	CH_3	$CH_2C_6H_5$	90

* Over two steps, without purification of intermediate **2**.

Once prepared, the key intermediates **3** could be transformed either directly to 2-alkyl-4-quinolone-3-carboxamides **5** or to 2-alkyl-4-quinolones **8**, after an additional decarbamoylative step (Scheme 2). The decarbamoylation of **3a-d** was carried out by heating at 60 °C in neat H_3PO_4 for 90 minutes and gave the corresponding β-enaminoketones **6a-d** in good yields (Table 2). The NMR spectra of compounds **6** in DMSO-d_6 in all cases indicated a mixture of *Z/E* isomers in approximately 85:15 ratio. The same spectra in $CDCl_3$ showed broad coalescent signals for the characteristic vinyl CH protons, which is indicative of dynamic equilibrium between the isomers.



Scheme 2. *Alternative manipulations of intermediates **3**, leading to either 2-alkyl-4-quinolones **8** (via enaminoketones **6**) or 2-alkyl-4-quinolone-3-carboxamides **5** (by direct reduction/cyclocondensation). Reagents and conditions: iii) H_3PO_4, 60 °C, 90 min.; iv) Zn/AcOH/CH_2Cl_2, r.t., overnight; v) $HCOONH_4$, Pd/C, CH_3OH, r.t. See main text for details.*

Table 2. *Yields of β-enaminoketones **6** prepared by decarbamoylation of intermediates **3**, according to Scheme 2:*

6	**R^1**	**Yield (6) %**
a	*n*-C_3H_7	90
b	*i*-C_4H_9	91
c	*n*-C_5H_{11}	91
d	*n*-C_7H_{15}	93

For both types of nitro-intermediates (**3** and **6**) the final ring-forming step required reduction of the nitro group with subsequent cyclisation of the reduced intermediate (Scheme 2, iv). We tried to carry out these reactions either with Zn in acetic acid/dichloromethane or by transfer hydrogenation with ammonium formate in the presence of Pd on charcoal. Both types of reductive conditions presented a challenge with regard to the chemoselectivity of the desired transformation, as they initially gave mixtures of 4-quinolones (**5** or **8**, respectively) and their corresponding *N*-hydroxy derivatives (**4** or **7**, respectively). Such a result is not surprising, considering that the reduction of the aromatic nitro-derivatives **3** and **6** proceeds through the corresponding hydroxylamines, capable of intramolecular cyclisation to products **4** or **7**.

Fortunately, under Zn/AcOH reductive conditions this was resolved by extending the duration of the reaction to 18 – 24h, providing enough time for compounds **4/7** to get reduced to quinolones **5/8**, which were isolated in good yields (Table 3 and Table 4).

Table 3. *Yields of 2-alkyl-4-quinolone-3-carboxamides **5**, prepared according to Scheme 2:*

5	**R^1**	**R^2**	**Yield % (5)**
a	*n*-C_3H_7	C_6H_5	90
b	*i*-C_4H_9	C_6H_5	56
c	*n*-C_5H_{11}	C_6H_5	63
d	*n*-C_7H_{15}	C_6H_5	90
e	*n*-C_7H_{15}	*p*-$C_6H_4OCH_3$	72
f	*n*-C_7H_{15}	*p*-C_6H_4Cl	83
g	CH_3	*p*-C_6H_4Cl	92
h	CH_3	*p*-$C_6H_4OCH_3$	92
i	CH_3	$CH_2C_6H_5$	79

Table 4. *Yields of 2-alkyl-4-quinolones **8**, prepared according to Scheme 2:*

8	**R^1**	**Yield % (8)**
a	*n*-C_3H_7	72
b	*i*-C_4H_9	74
c	*n*-C_5H_{11}	90
d	*n*-C_7H_{15}	90

In the case of Pd-catalysed transfer hydrogenation of intermediates **3** the yields of products **5** in most cases were lower than those obtained with Zn/AcOH, regardless of the reaction duration. On the other hand, limiting the reaction time to 60 – 90 min under these conditions allowed some of the *N*-hydroxy derivatives **4** to be isolated in good yield (Table 5), even though it did not entirely prevent the formation of products **5**. Pd-catalysis was not appropriate for the hydrogenation of compounds **3f** and **3g**, because of concomitant reduction at the C-Cl bond.

Table 5. *Yields of 1-hydroxy-2-alkyl-4-quinolone-3-carboxamides **4**, prepared according to Scheme 2:*

4	**R^1**	**R^2**	**Yield % (4)**
a	*n*-C_3H_7	C_6H_5	57
b	*i*-C_4H_9	C_6H_5	75
c	*n*-C_5H_{11}	C_6H_5	60
d	*n*-C_7H_{15}	C_6H_5	70
e	*n*-C_7H_{15}	*p*-$C_6H_4OCH_3$	64

Intermediates **6**, similarly to **3**, gave mixture of products **7/8** under Pd-catalysed transfer hydrogenation conditions. In contrast to **3**, however, limiting the reaction time here did not help to develop preparatively useful procedure for preferential isolation of *N*-hydroxy derivatves **7**. Further experiments for Pd-catalysed hydrogenation with H_2 at atmospheric pressure did not show any advantage over the transfer hydrogenation conditions.

Overall, the described synthetic approach (Scheme 1 and Scheme 2) allowed us to prepare in an operationally simple manner 2-alkyl-4-quinolones **8a-d**, all of which are known from the literature [25, 36, 61, 67, 68] and two of them are natural products of microbial origin (**8c** [69] and **8d** [71]). More importantly, the utility of the approach was demonstrated with the synthesis of novel 2-alkyl-4-quinolone-3-carboxamides **5a-i** and some of their *N*-hydroxy derivatives **4a-e**. Compounds of this type with C-2 substitution other than methyl [71] have not been previously described.

All of the obtained products were screened for antimicrobial activity at concentration of 1 mg/mL against *S. aureus* and *E. coli*, using the hole-plate method in Mueller–Hinton agar, with 100 µg loading of each compound in 100 µL DMSO (Table 6). Interestingly, at this concentration most of the compounds showed weak to moderate activity against *E. coli*, while *S. aureus* was inhibited only by C_5 and C_7 analogs. Among the novel compounds, only **4d** and **4e** gave inhibition zones of more than 20 mm and were further analysed to determine their minimum inhibitory concentrations (MIC) by serial broth dilutions [72]. The MICs measured for **4d** and **4e** were ≤ 6.25 µg/mL and ≤ 3.12 µg/mL respectively, with MIC ≤ 0.78 µg/mL for Levofloxacin as the positive control.

Table 6. *Antibacterial activity of the synthesized quinolone derivatives **4**, **5** and **8**.*

Compound*	Sterile zone diameter (mm)**	
	S. aureus ATCC 25923	*E. coli* ATCC 25922
4b	-	17
4d	27	16
4e	22	-
5a	-	16
5b	-	15
5c	-	14
5d	19	14
5e	15	16
5f	15	15
8a	-	16
8b	-	15
8c	18	15
8d	21	13

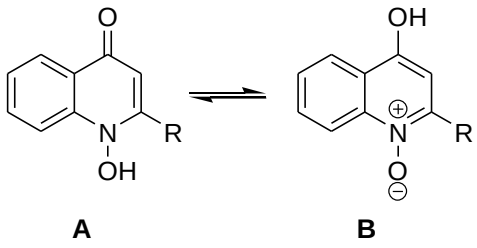
* Compounds giving sterile zones of less than 10 mm are not listed.
** Including well diameter of 8 mm.

In conclusion, we have demonstrated that β-keto amides and 2-nitrobenzoyl chloride can be used as convenient precursors to a variety of 4-quinolone derivatives. The described approach is realised in a small number of steps, under mild conditions, and allows easy installation of long-chain substituents at the C-2 position of the quinolone core. These characteristics of the synthetic method could be particularly attractive in the search of novel mimics of the *Pseudomonas* quorum-sensing signal molecules. The high activity of compounds **4d** and **4e** against *S. aureus* provides a good lead for further structural optimisations.

2. Synthesis of 4-Quinolone N-Oxides *via* Controlled Partial Hydrogenation of 2-Nitrobenzoyl Enamines

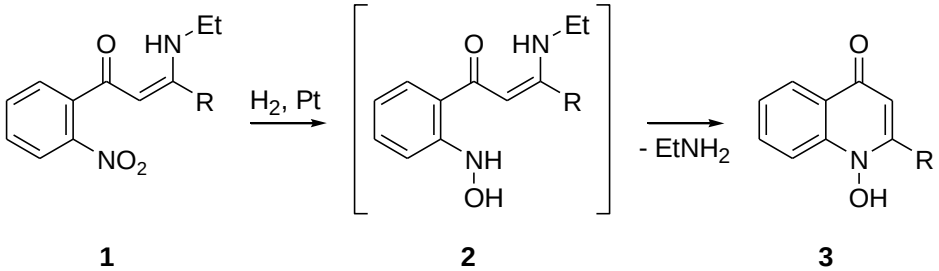
Various bacterial species of genera, such as *Pseudomonas*, *Burkholderia*, *Arthrobacter*, *Stigmatella*, and *Rhodococcus,* produce 2-alkyl-4-quinolone *N*-oxides (AQNOs)with antibiotic activities. The widely accepted model of their antibiotic effect is based on the disruption of the respiratory chain of the competing microorganisms. The interesting biological profile of these compounds has motivated the development of synthetic methods for preparation of natural AQNOs and structural analogs as potential antibacterials. AQNOs can exist in two

tautomeric forms (Scheme 1) and for this reason they are also referred to as 4-hydroxyquinolines and 4-hydroxyquinoline *N*-oxides, respectively. Although the oxidized 4-quinolone tautomer is actually an *N*-hydroxy derivative (Scheme 1, A), the term "4-quinolone *N*-oxide" has gained widespread acceptance as a universal reference to this type of compound, disregarding the precise tautomeric form.



Scheme 1. Tautomeric forms of AQNOs: *N*-hydroxy-4-quinolone (**A**) and 4-hydroxyquinoline *N*-oxide (**B**).

The conditions for partial reduction of 2-nitrobenzoylated β-enamino amides under Pd-catalyzed transfer hydrogenation conditions, described in the previous section, did not provide complete control on the degree of hydrogenation and were not compatible with chlorinated substrates. Further, the structurally simpler natural AQNOs were completely out of the scope of this method, because of the poor control on the degree of hydrogenation. Considering this drawback, and taking into account the general interest in convenient preparative methods for AQNOs, we continued our efforts in this direction by screening a variety of conditions for chemoselective partial reduction of the nitro group in aromatic substrates. We were particularly interested in developing a procedure for the partial reduction of 2-nitrobenzoyl enamines **1** (Scheme 2), available through acylation/decarbamoylation of β-enamino amides. The required procedure was expected to stop the reduction of the target substrates at the hydroxylamine level (**2**) and was also expected not to reduce the subsequently formed products **3** (Scheme 2).



3	R	Yield %
a	*n*-C_3H_7	70
b	iBu	58
c	*n*-C_5H_{11}	70
d	*n*-C_7H_{15}	68

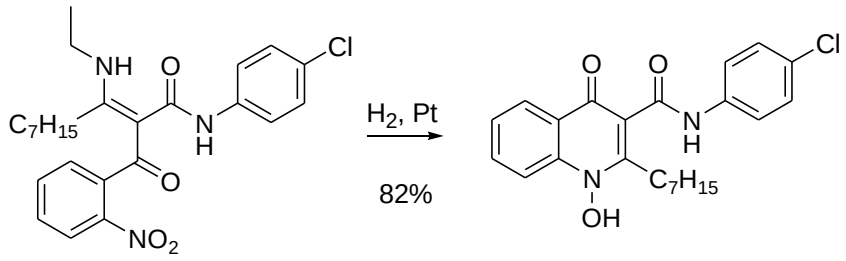
Scheme 2. Synthesis of AQNOs. Reagents and conditions: H_2 (balloon), 5 wt% Pt/Al_2O_3, n-$BuNH_2$, DMSO, isopropanol, 24 h, r.t.

In the event, we found that the conditions reported by Takenaka and colleagues for the preparation of aromatic hydroxylamines[8] gave excellent results, in the context of AQNO preparation from 2-nitrobenzoyl enamines **1**. This partial hydrogenation procedure is carried out with H_2 at atmospheric pressure and uses a combination of DMSO and *n*-butylamine to fine-tune the activity of the alumina-supported Pt catalyst. When the 2-nitrobenzoyl enamines **1** were subjected to these conditions, the reaction led directly to the desired *N*-oxides **3** in very good yields and purity. The intermediate hydroxylamines **2** (Scheme 2) cyclized spontaneously and could not be isolated. The products **3** were easily isolated after filtration of the catalyst, evaporation of the solvent under reduced pressure and a simple extractive workup, removing the residual DMSO and *n*-butylamine.

The NMR spectra of the obtained AQNO products **3** were measured at 25 °C in DMSO-d_6 and were indicative of the 4-hydroxyquinoline-*N*-oxide tautomeric form (Scheme 1, **B**), with the only exception of the poorly soluble **3b**, which had to be measured at 70 °C and appeared as the *N*-hydroxy-4-quinolone form (Scheme 1, **A**). This is in contrast to their reduced AQ analogs, which show preference for the 4(1*H*)-quinolone form in the same solvent at 25 °C.[14] The ^{1}H-NMR signals that are most indicative of the tautomeric form are those of the C3-H protons, appearing around 6 ppm for the keto tautomers and at 7.02 – 7.15 for the aromatized 4-hydroxy *N*-oxide tautomers. In the ^{13}C-NMR spectra, the most significant difference between the tautomers is observed in the chemical shifts of the C4 signals (174 – 177 ppm for a carbonyl C4, and 166 – 167 ppm for an aromatic C4-OH). Interestingly, all previously reported spectra of the known products **3c** and **3d** have been measured in deuterated methanol and indicate a preference for the *N*-hydroxy-4-quinolone form in this solvent, with the C4-carbonyl ^{13}C signal visible only in HMBC.

After the successful preparation of the *N*-oxides **3**, we tried the same hydrogenation conditions with the chlorinated carboxamide substrate **4**. In this case the procedure also worked excellently and gave the expected product **5** in 82% yield, without any concomitant reduction at the C–Cl bond (Scheme 3).

[8] *Green Chem.,* **11**, 1385 (2009). https://doi.org/10.1039/B904672K



4 **5**

Scheme 3. Synthesis of a halogenated carboxamide analog (**5**) of the P. aeruginosa metabolite HQNO.

The antibacterial activity of product **5** was of particular interest to us, as we had already observed high activity in similar compounds with different substituents in the aromatic carboxamide residue. The activity of compound **5** was initially assessed by the agar diffusion method against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *C. albicans* (NBIMCC 74). At a concentration of 1 mg/mL the compound was inactive against *E. coli* and *C. albicans*, but significantly inhibited the growth of *S. aureus*. Its minimal inhibitory concentration (MIC) against this strain was further determined by serial broth dilutions to be ≤ 3.125 µg/mL, compared to MIC ≤ 0.781 µg/mL for Levofloxacin as the positive control in a parallel measurement.

In this way, we accomplished a chemoselective and operationally simple synthesis of 4-hydroxyquinoline *N*-oxides from 2-nitrobenzoyl enamines. One of the obtained products (**3d** or HQNO) is a known bacterial toxin, produced by *P. aeruginosa*. The synthetic procedure is also compatible with chlorinated substrates and extends the range of accessible analogs for biological studies.

3. Synthesis of Pseudane IX, Its N-Oxide, and Novel Carboxamide Analogs with Antibacterial Activity

The natural product pseudane IX, or 2-nonyl-4-quinolone, was isolated for the first time by Hays et al. from cultures of *Pseudomonas aeruginosa* and its structure was determined later by Wells, who accomplished the first synthesis of this compound.[9] Since then, pseudane IX has been isolated from various *Pseudomonas* species and, also, from plants such as *Vepris ampody*

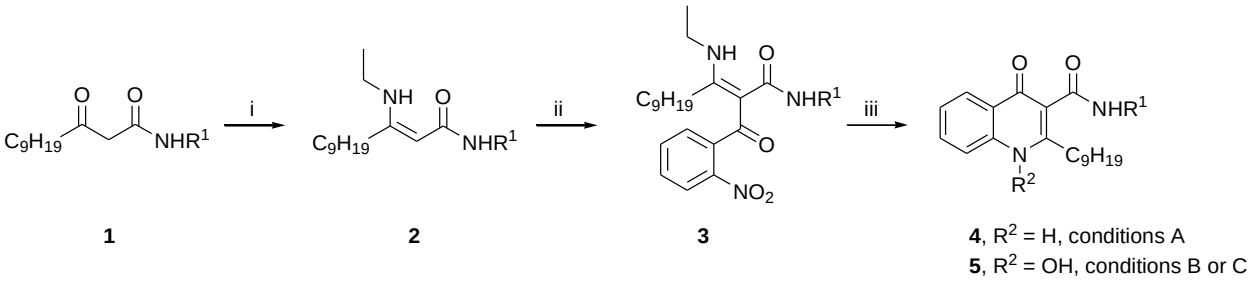
[9] *J. Biol. Chem.* **1952**, *196*, 331–340. https://doi.org/10.1016/S0021-9258(18)55737-9

(Rutaceae) and *Ruta angustifolia*. An interesting spectrum of biological activities has been reported for pseudane IX, including swarming motility inhibition in *Bacillus atrophaeus*, activity against *Plasmodium falciparum* and other protozoa, and inhibition of *Candida albicans* biofilm formation. Notably, pseudane IX has shown remarkable antiviral activity against the hepatitis C virus, exceeding that of the standard drug ribavirin.[10] The *N*-oxide of pseudane IX (NQNO) is also a known bacterial metabolite with antibiotic activity. The natural sources of pseudane IX do not offer convenient isolation and sufficiently large amounts of this compound. For this reason, most biological studies rely on synthetic pseudane IX. The only method used for this purpose until now is based on the classic Conrad–Limpach reaction, in which an enamino ester undergoes ring closure at 270 °C in refluxing diphenyl ether. The overall yield of this approach is low, and the harsh conditions impose certain limitations on the preparation of functionalized analogs. The potential of pseudane IX as a lead compound in the search for novel antimicrobials has motivated us to attempt a new synthesis of this natural product.

In order to synthesize a few structural analogs of pseudane IX along with the targeted natural product, we first prepared three different amides of 3-oxododecanoic acid (**1a**–**c**). This was performed by acylation/deacetylation of the corresponding commercially available acetoacetamides, following our published method. The β-keto amides **1** were then condensed with ethylamine, to provide enamines **2**, which were directly subjected to acylation with 2-nitrobenzoyl chloride (Scheme 1). This way, good yields of the intermediates **3** were obtained (80–85%). Next, a reduction of the nitro group in intermediates **3** with *Zn* in CH_2Cl_2/*HOAc* was carried out. This was accompanied by spontaneous cyclization of the reduced intermediates to the corresponding 2-nonyl-4-quinolone-3-carboxamide derivatives **4** (R^2 = H). Compared with our previous experiments on similar substrates with shorter alkyl chains, the full conversion of **3** to **4** here was slightly slower and required a larger excess of the reducing agent. Alternatively, controlled partial reduction of the nitro group under *Pd*- or *Pt*-catalyzed hydrogenation conditions led to the *N*-hydroxy derivatives **5** (R^2 = OH). Compounds **5a**,**b** were obtained by *Pd*-catalyzed transfer hydrogenation of **3a**,**b** with ammonium formate, while compound **3c** was hydrogenated with H_2 over DMSO-inhibited Pt/Al_2O_3 to give **5c** without

[10] *Fitoterapia* **2014**, *99*, 276–283. https://doi.org/10.1016/j.fitote.2014.10.011

concomitant reduction at the *C–Cl* bond. This way, a set of six new analogs of the natural product were prepared (Scheme 1, Table 1).



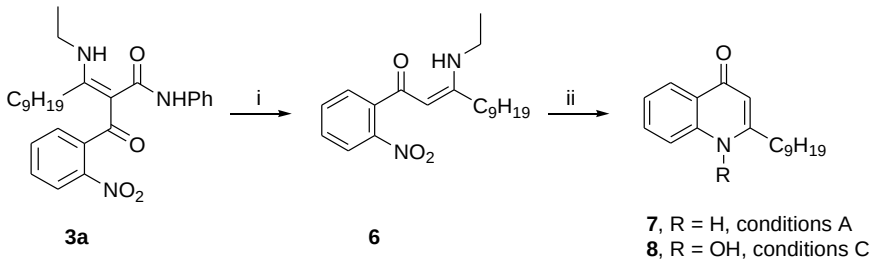
Scheme 1. Reagents and conditions: (i) $EtNH_2$ (70% aq, 1.05–1.15 equiv), CH_2Cl_2, Na_2SO_4, 24 h, rt; (ii) NMM (1 equiv), DMAP (0.2 equiv), 2-nitrobenzoyl chloride (1 equiv), CH_2Cl_2, 2 h, rt; (iii) either A: $Zn/HOAc/CH_2Cl_2$, rt, overnight, B: $HCOONH_4$, Pd/C, CH_3OH, rt, or C: H_2 (balloon), 5 wt% Pt/Al_2O_3, *n*-$BuNH_2$, DMSO, isopropanol, 24 h, rt.

Table 1. Isolated yield of products **4** and **5**, obtained according to Scheme 1.

Product	R^1	R^2	Conditions	Yield (%)
4a	C_6H_5	H	A	84
4b	*p*-$MeOC_6H_4$	H	A	80
4c	*p*-ClC_6H_4	H	A	72
5a	C_6H_5	OH	B	70
5b	*p*-$MeOC_6H_4$	OH	B	70
5c	*p*-ClC_6H_4	OH	C	82

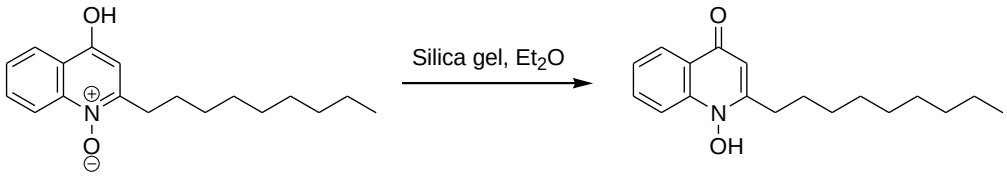
The synthesis of pseudane IX required one additional decarbamoylation step after the acylation stage (Scheme 2). In theory, any of the nitrobenzoylated intermediates **3** should be susceptible to such decarbamoylation and would give the same β-enamino ketone **6** upon heating in neat H_3PO_4. However, we only used intermediate **3a** for this purpose as it offered the best atom economy and the lowest overall price of the synthesis. This reaction was carried out by stirring compound **3a** in neat H_3PO_4 for 2 h at 60 °C. It should be noted that the time needed for the completion of the reaction was longer than previously observed for similar substrates with shorter alkyl chains. The β-enamino ketone **6** was obtained in an 86% yield and its ^1H NMR spectrum in DMSO-d_6 indicated a mixture of *Z*/*E* isomers in a ratio of 85/15. In the last step, the β-enamino ketone **6** successfully underwent a reduction of the nitro group with *Zn* in CH_2Cl_2/*HOAc* and the ensuing spontaneous cyclization completed the synthesis of pseudane IX in 72% yield (**7**, Scheme 2). To stop the reduction at the *N*-oxide level, we carried

out atmospheric pressure hydrogenation of the β-enamino ketone **6** with H_2 over DMSO-inhibited Pt/Al_2O_3. Under these conditions, the *N*-oxide of pseudane IX (**8**) was cleanly obtained in 70% yield.



Scheme 2. Synthesis of pseudane IX (**7**) and its *N*-oxide (**8**) from intermediate **3a**. Reagents and conditions: (i) H_3PO_4, 60 °C, 2 h; (ii) either A: $Zn/HOAc/CH_2Cl_2$, rt, overnight, or C: H_2 (balloon), 5 wt% Pt/Al_2O_3, $n\text{-}BuNH_2$, DMSO, isopropanol, 24 h, rt.

Depending on the method of isolation, compound **8** was obtained in two distinctly different forms. When the crude product was only triturated with diethyl ether, it solidified as a white powder with good solubility in DMSO and a mp of 103–104 °C. The NMR spectrum of this material was taken in DMSO-d_6 at 25 °C and was indicative of a 4-quinolinol-*N*-oxide tautomeric form (Scheme 3), with the C3-H signal appearing at 7.08 ppm. On the other hand, when compound **8** passed through a silica gel column with diethyl ether as the eluent, it crystallized as colorless needles with a mp of 146–147 °C and a very poor DMSO solubility at 25 °C. Because of the poor solubility, the NMR spectrum in DMSO-d_6 had to be run at 70 °C, and this time it clearly indicated an *N*-hydroxy-4-quinolone tautomeric form, with the C3-H signal appearing at 5.97 ppm. A similar change was not observed in compound **7**, which was registered only as the 4-quinolone tautomer, regardless of the isolation method.



Scheme 3. Tautomeric change of compound **8** (NQNO) during chromatography on silica gel.

The antibacterial activity of the novel analogs **4** and **5** was tested at a concentration of 100 µg/mL against a set of six bacterial strains, using the agar diffusion method. The known natural compounds pseudane IX (**7**) and its *N*-oxide (**8**) were also included in the assay for comparison. The *N*-oxide **8** is known for its antibiotic activity and served as the positive control. The

observed inhibition zones (Table 2) indicated higher activity of the *N*-hydroxy derivatives **5** compared with that of their reduced counterparts **4**, with *S. aureus* ATCC 25923 being the most susceptible among the studied bacteria. Notably, there was a sharp contrast in the susceptibility of the other assayed *S. aureus* strain (ATCC 6538), which was moderately inhibited by only one of the novel compounds (**5c**). Compound **5c** was also the one with the broadest activity spectrum, inhibiting all of the studied bacteria and approaching the activity of the natural antibiotic **8**. Against the resilient *S. aureus* strain (ATCC 6538), **5c** even outperformed **8** with a slightly larger inhibition zone.

Table 2. Antibacterial assay of the synthesized compounds at 100 µg/mL in DMSO, with 60 µL loading in 6 mm agar wells.

	Sterile Zone Diameter (mm) [1]					
	E. coli **ATCC 25922**	***E. coli*** **ATCC 8739**	***S. aureus*** **ATCC 25923**	***S. aureus*** **ATCC 6538**	***Enterococcus faecalis*** **ATCC 29212**	***B. subtilis*** **NBIMCC 1208**
4a	-	-	19	-	-	-
4b	-	-	11	-	-	-
4c	-	-	9	-	-	9
5a	-	15	25	-	-	15
5b	-	-	20	-	15	15
5c	15	15	19	16	15	15
7	-	-	14	-	-	9
8	14	15	27	14	14	20

[1] Including 6 mm well diameter.

In conclusion, we have accomplished a convenient synthesis of two natural products with interesting biological profiles—pseudane IX and its *N*-oxide. The overall yield over four steps, starting from the phenylamide of 3-oxododecanoic acid and involving only one chromatographic purification, is 52% and 50% respectively. We have also demonstrated that the synthetic method used for this purpose allows easy access to novel carboxamide analogs of these compounds. A broad antibacterial spectrum was observed in one of the newly obtained analogs, providing a lead for further structural optimization.

4. Attempted Synthesis of the Pseudomonas aeruginosa Metabolite 2-Benzyl-4(1H)-quinolone and Formation of 3-Methylamino-2-(2-nitrobenzoyl)-4H-naphthalen-1-one as an Unexpected Product

We considered the *P. aeruginosa* metabolite 2-benzyl-4(1*H*)-quinolone (Figure 1) to be an interesting target and a good testing ground for our synthetic methodology (Scheme 1). At the core of this methodology are intermediates **III**, which are readily prepared from the corresponding β-keto amides **I**. Compounds **III** are susceptible to decarbamoylation upon heating in H_3PO_4. Finally, the reduction of the nitro group in **IV** is followed by spontaneous cyclization to the corresponding 2-alkyl-4(1*H*)-quinolone **V**, with the elimination of an amine (R^3-NH_2). The type of the R^3 substituent is not of decisive importance to this method, so it is most convenient to use an inexpensive lower aliphatic amine in this auxiliary role.

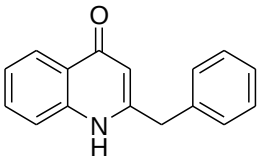
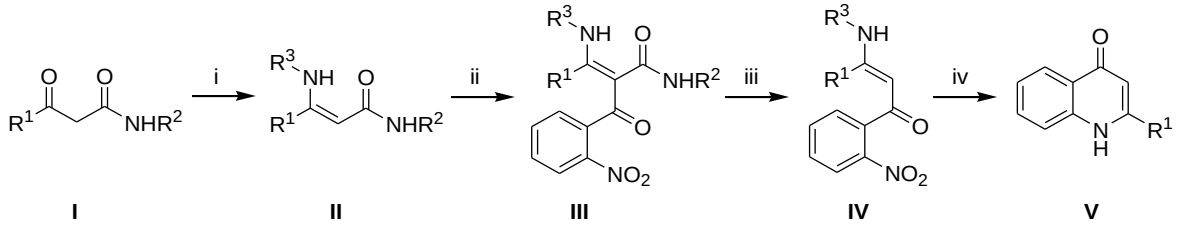
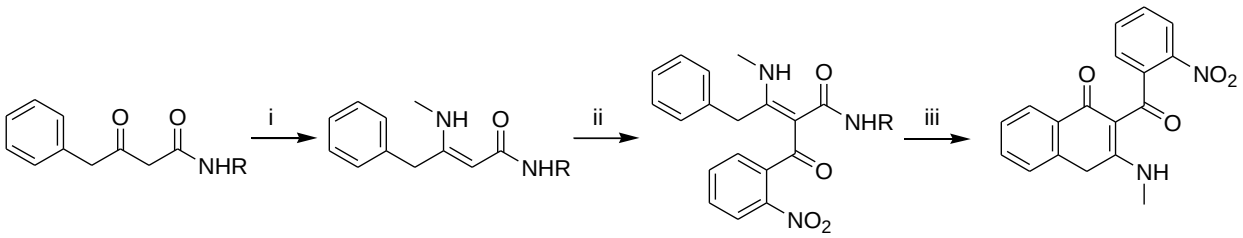


Figure 1. Structure of 2-benzyl-4(1*H*)-quinolone.



Scheme 1. General method for the synthesis of 2-alkyl-4(1*H*)-quinolones (**V**): (**i**) R^3NH_2, CH_2Cl_2, Na_2SO_4, 24 h r.t.; (**ii**) NMM (1 equiv.), DMAP (0.2 equiv), 2-nitrobenzoyl chloride (1 equiv.), CH_2Cl_2, 2h, r.t.; (**iii**) H_3PO_4, 60 °C, 1–2 h; (**iv**) Zn/AcOH or H_2, Pd/C.

With this in mind, three different γ-phenyl β-keto amides **1a**–**c** were prepared and condensed with methylamine to provide the corresponding β-enamino amides **2a**–**c** in quantitative yields (Scheme 2). The acylation of **2a**–**c** with 2-nitrobenzoyl chloride proceeded smoothly and resulted in the corresponding intermediates **3a**–**c** with 65–74% yield.

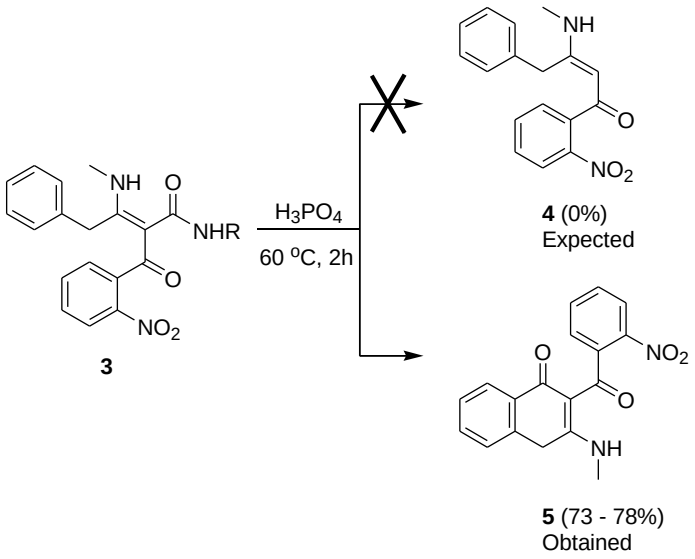


1 **2** **3** **5** (73 - 78%)

	R	Yield **3** (%)
a	C_6H_5	67
b	*p*-$C_6H_4OCH_3$	74
c	*p*-C_6H_4Cl	65

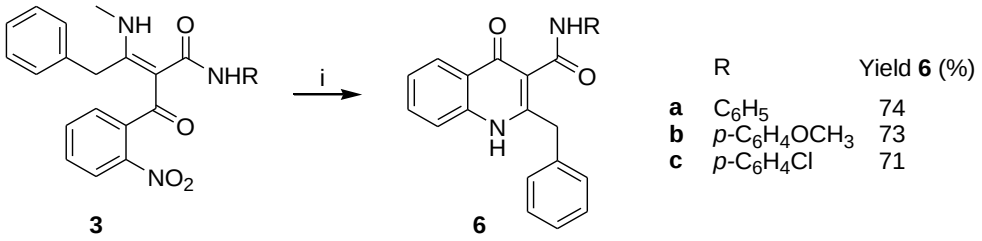
Scheme 2. Reagents and conditions: (**i**) CH_3NH_2 (40% aq., 1.05–1.15 equiv.), CH_2Cl_2, Na_2SO_4, 24 h r.t.; (**ii**) NMM (1 equiv.), DMAP (0.2 equiv), 2-nitrobenzoyl chloride (1 equiv.), CH_2Cl_2, 2h, r.t.; (**iii**) H_3PO_4, 60 °C, 2h.

In the next stage, however, the attempted decarbamoylation of **3** in neat H_3PO_4 at 60 °C did not give the expected result with any of the intermediates **3a**–**c**. In all three cases, cyclization to the same product **5** was observed, with the elimination of the corresponding amine (RNH_2) and no trace of the expected product **4** (Scheme 3). The yields of the unexpected product 3-methylamino-2-(2-nitrobenzoyl)-4*H*-naphthalen-1-one **5** were consistently found to be within the range of 73–78%, regardless of the R substituent. The structure of **5** was elucidated on the basis of NMR and MS data (see Supplementary Materials information) and could be a result of either an intramolecular Friedel–Crafts reaction or a 6π–electrocyclic process, occurring in a tautomer of **3**, possessing the prerequisite conjugated π system. Interestingly, the protonated molecular ion of **5** was observed as the base peak in the mass spectra of intermediates **3a** and **3c**, with abundance exceeding that of the actual $[M+H]^+$ ions of **3**. The base peak for intermediate **3b** corresponded to its own $[M+H]^+$, but the protonated molecular ion of **5** was still present with high abundancy (50%), indicating that the cyclization depicted in Scheme 3 is also a favored process under the conditions of positive electrospray ionization.



Scheme 3. Expected and observed reactions of intermediates **3** in H_3PO_4.

Even though this unexpected reactivity of the benzyl-substituted intermediates **3** did not allow us to accomplish the planned synthesis of 2-benzyl-4(1*H*)-quinolone, we were still in the position to prepare derivatives of this natural product, carrying a carboxamide moiety at the C3 position of the quinolone core. This was easily achieved by the reduction of the nitro group in intermediates **3** with Zn in $AcOH/CH_2Cl_2$, which was followed by spontaneous cyclization to carboxamide derivatives **6** with 71–74% yield (Scheme 4).

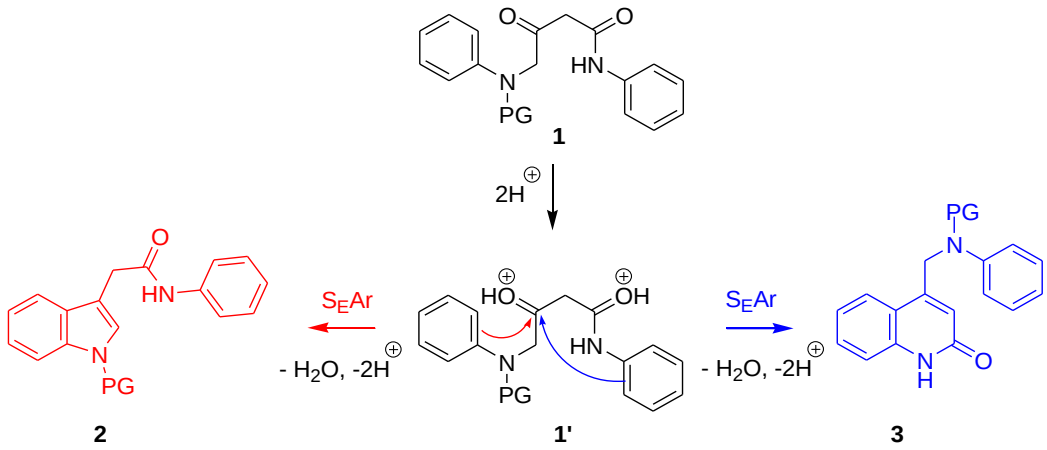


Scheme 4. Preparation of 3-carboxamide derivatives of 2-benzyl-4(1*H*)-quinolone (**6**). Reagents and conditions: (**i**) $Zn/AcOH/CH_2Cl_2$, r.t., 24 h.

The encountered unexpected reactivity of benzyl-substituted intermediates **3** limits the scope of the methodology, but it still allowed 3-carboxamide derivatives of 2-benzyl-4(1*H*)-quinolone to be obtained in high yields. The unexpected product 3-methylamino-2-(2-nitrobenzoyl)-4*H*-naphthalen-1-one (**5**) was also obtained in a good yield, and probably other 3-alkylamino analogs of **5** are within reach by this route.

5. Cyclisation Modes in Anilides of N-Protected 3-Oxo-4-phenylaminobutyric Acid Under Knorr Conditions

The ring closure of oxocarbenium intermediates via intramolecular aromatic electrophilic substitution is an approach applicable to the construction of both quinolin-2-ones and indoles. In the former case, this approach is known as the Knorr synthesis, and it has found numerous applications in the preparation of various quinolin-2-one derivatives from β-ketoanilides. The mechanism of this reaction has been studied in detail, and it has been shown to proceed via distonic superelectrophilic dications.[11] Indoles have also been prepared in a similar manner from appropriate α-phenylaminocarbonyl precursors, although the scope of the approach in this case is narrower and it is rarely used in comparison to other methods of indole synthesis. It was of interest to experiment with substrates that are simultaneously capable of both five- and six-membered ring formation modes (Scheme 1).



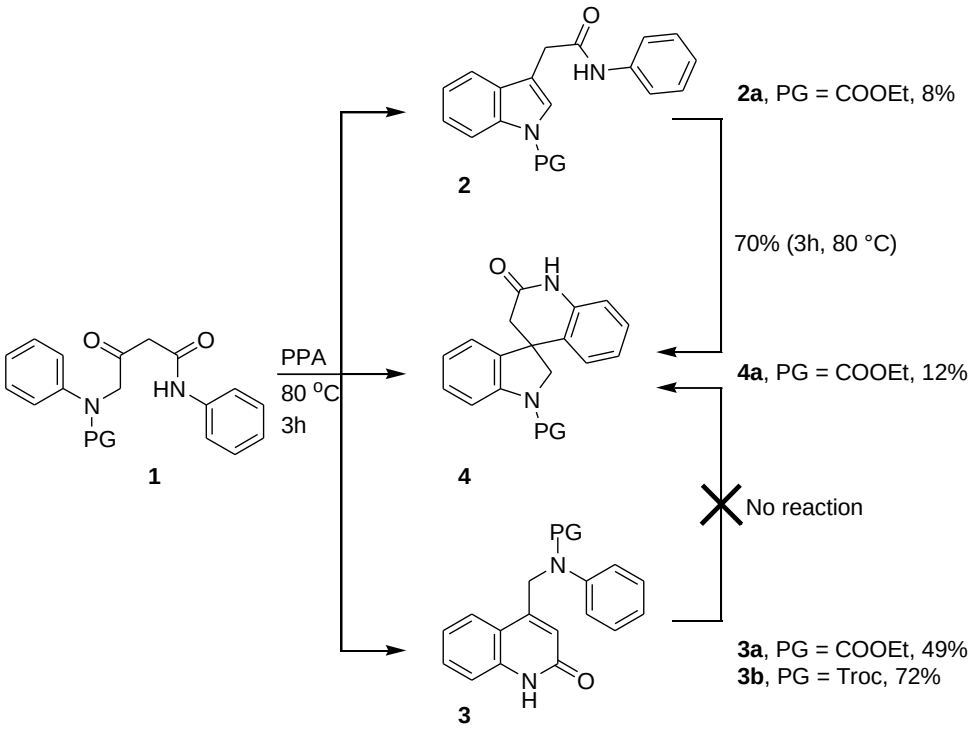
Scheme 1. Possible modes of cyclization in anilides of *N*-protected 3-oxo-4-phenylaminobutyric acid.

To carry out the experiments, we first prepared two anilides of 3-oxo-4-phenylaminobutyric acid, bearing ethoxycarbonyl or 2,2,2-trichloroethyloxycabonyl (Troc) protection at the phenylamine nitrogen (**1**). The substrates **1** were then subjected to Knorr-type conditions via heating in neat polyphosphoric acid (PPA) at 80 °C. The reaction was checked by TLC on 30 min intervals and the heating was maintained until full consumption of the starting compounds **1**, which took 3 h in both cases. Under these conditions, the Troc-protected

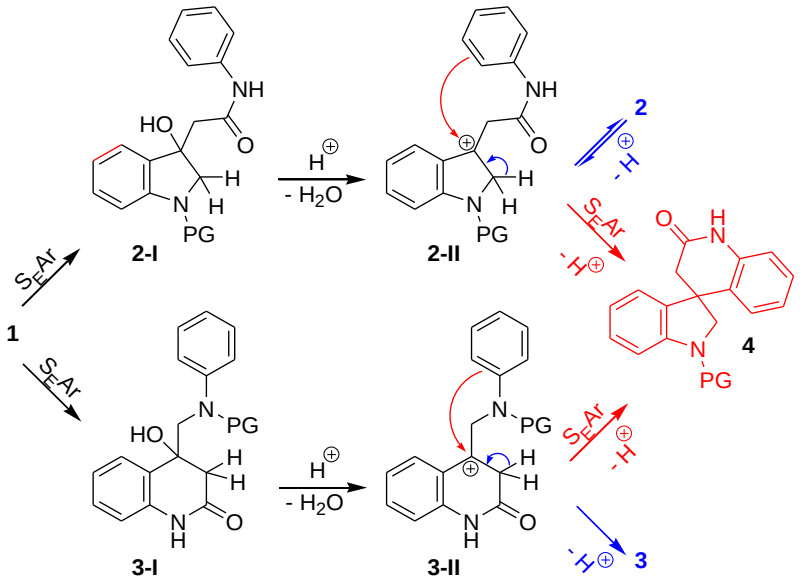
[11] *J. Org. Chem.* **2007**, *72*, 9761–9764. https://doi.org/10.1021/jo7013092

substrate **1b** (PG = Troc) gave only one major product that could be isolated via column chromatography. Its NMR spectra corresponded to the quinolin-2-one **3b,** and the yield was 72%. On the other hand, the ethoxycarbonyl protected substrate **1a** (PG = COOEt) gave a mixture of three products with clear spots on the TLC plate. These products were easily separated via column chromatography on silica gel, and their spectral characteristics corresponded to the indolylacetanilide **2a** (8%), the quinolin-2-one **3a** (49%), and the spirocyclic derivative **4a** (12%) (Scheme 2).

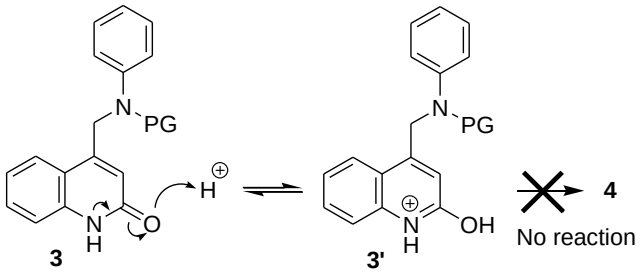
There are two possible mechanistic sequences leading to the formation of the spirocyclic product **4a**. This product could be a result of S_EAr cyclization occurring in either of two different cationic intermediates, formed after the first ring closure (Scheme 3, **2-II** and **3-II**). The cationic intermediates **2-II** and **3-II** could either eliminate a proton to give indole **2** and quinolone **3**, respectively, or undergo a second S_EAr process, leading to the spirocyclic product **4a**. Should the proton elimination in **2-II** and **3-II** be reversible, then the spirocyclic product could also be obtained from any of the competing products **2** and **3**. To clarify this, we carried out additional experiments, starting with pure, chromatographically isolated compounds **2a** and **3a**. Both compounds were heated separately in PPA, but only the indole derivative **2a** underwent a dearomative spirocylization to **4a**. The conversion of **2a** to **4a** took 3h at 80 °C and gave an isolated yield of 70%. Compound **3a** on the other hand did not react even after 5h at 100 °C in either PPA or H_3PO_4. This suggests that the protonation of the quinolone **3** occurs preferentially at the amide oxygen, with formation of a stable aromatic 2-hydroxyquinolinium ion **3'** (Scheme 4). Although a protonation at the amide carbonyl is also likely for the indole derivative **2a**, this apparently does not preclude a dearomative protonation in the five-membered ring of this compound.



Scheme 2. Products obtained from anilides of *N*-protected 3-oxo-4-phenylaminobutyric acid under Knorr conditions.



Scheme 3. Alternative pathways to the spirocyclic product **4a**.



Scheme 4. Protonation of quinolones **3**.

In another experiment, the cyclization of **1a** in PPA was carried out for prolonged period of 5 h, which provided enough time for product **2a** to fully convert to **4a** in the same reaction vessel. In this way, only products **3a** and **4a** were isolated in 50% and 21% yield, correspondingly.

The structures of the obtained products **2**, **3**, and **4** were unequivocally determined on the basis of their NMR spectra. In the ^{1}H NMR spectrum of the spirocyclic product **4**, there are signals for two AB spin systems, arising from the presence of a stereogenic center in the molecule. These signals correspond to the diastereotopic protons in the methylene groups that are adjacent to the stereogenic center. One of the methylene groups gives two doublets with ^{2}J = 16 Hz at δ_A = 2.69 ppm and δ_B = 3.05 ppm. The protons of this AB system are bonded to a carbon with ^{13}C δ = 42.2 ppm according to the observed HSQC correlation. This, along with the HMBC correlation of the same protons to the amide carbonyl (δ = 168.5 ppm), is sufficient to assign that methylene group to the the six-membered ring. The other methylene group appears in the ^{1}H NMR spectrum as two doublets with ^{2}J = 12 Hz at δ_A = 3.89 and δ_B = 4.03 ppm. These doublets correlate to a carbon with ^{13}C δ = 60.7 ppm in the HSQC spectrum and show no correlation to the amide carbonyl in the HMBC spectrum, which assigns the corresponding methylene group to the five-membered indoline ring. Both, the five- and the six-membered-ring methylene groups, show HMBC correlation to the quaternary carbon at the spiro ring juncture, which appears at δ = 46.1 ppm in the ^{13}C NMR spectrum. The ^{1}H signals of the ethoxycarbonyl group in **4a** are broad and unresolved, most likely due to a rotameric exchange. With regard to products **2** and **3**, the most significant difference in their NMR spectra is observed in the chemical shifts of the methylene signals—δ ^{1}H/^{13}C = 3.79/34.7 for **2a**, where the CH_2 is neighbored by an indole ring and an amide carbonyl, compared to δ ^{1}H/^{13}C = 5.15/50.7 for **3a**, where the CH_2 is allylic and next to a nitrogen. In both cases, the ^{1}H

methylene signals appear as narrow doublets due to allylic coupling to a CH in the neighboring ring (^4J = 1.0 and 0.9 Hz respectively). The new sp^2-CH signals in **2a**/**3a** that appear as a result of the cyclization are easily identifiable by COSY and HSQC. In the indole ring of **2a**, this is the aromatic C2-H signal at δ ^{1}H/^{13}C = 7.68/124.4 ppm, while in the quinolone **3a**, this is the C3-H at δ ^{1}H/^{13}C = 6.29/119.4 ppm. Here, the ^{1}H signals are unresolved triplets because of the weak ^4J coupling.

In conclusion, we have shown that the competing reaction pathways of anilides of *N*-protected 3-oxo-4-phenylaminobutyric acids under Knorr-conditions are influenced by the nature of the protecting group in the substrates. While the Troc-protected substrate is preferentially converted to a quinolin-2-one derivative, the COOEt-protected one gives a mixture of quinolone, indole, and spirocyclic products. Although the latter variant is of little preparative value, it revealed an interesting possibility of dearomative spirocyclization in indolylacetanilides, which may be useful in other contexts.

III. SUMMARY

1. Three different approaches to the synthesis of quinolone derivatives have been investigated, starting from various β-keto amides.

2. A new method for the synthesis of 2-alkyl-4-quinolones, 2-alkyl-4-quinolone-3-carboxamides and their *N*-hydroxy derivatives through reductive cyclocondensations of ortho-nitrobenzoylated enamine intermediates has been developed. This has allowed the synthesis of six known natural products, including bacterial metabolites and plant alkaloids, along with a series of novel structural analogs.

3. A new method for the synthesis of 1,2-dialkyl-4-quinlone-3-carboxamides through intramolecular aromatic nucleophilic substitution in ortho-fluorobenzoylated enamine intermediates has been developed.

4. The cyclisation of of anilides of *N*-protected 3-oxo-4-phenylaminobutyric acids under Knorr-conditions has been investigated. It has been shown that the competing reaction pathways are influenced by the nature of the protecting group in the substrates. While the Troc-protected substrate is preferentially converted to a quinolin-2-one derivative, the COOEt-protected one gives a mixture of quinolone, indole, and spirocyclic products. A dearomative spirocyclization of indolylacetanilide has been demonstrated.

5. The novel compounds obtained in this thesis have been screened for antibacterial activity, which has led to the discovery of some highly active compounds against *S. aureus* and one highly active compound against a spectrum of Gram-positive and Gram-negative bacteria.

IV. PUBLICATIONS

Results from this doctoral thesis have been published in the following articles:

1. Mollova-Sapundzhieva, Y.; Angelov, P.; Georgiev, D.; Yanev, P. Synthetic approach to 2-alkyl-4-quinolones and 2-alkyl-4-quinolone-3-carboxamides based on common β-keto amide precursors. *Beilstein journal of organic chemistry* **2023,** *19,* 1804–1810. https://doi.org/10.3762/bjoc.19.132

2. Angelov, P.; Mollova-Sapundzhieva, Y.; Alonso, F.; Goranov, B.; Nedialkov, P.; Bachvarova, D. Concise Synthesis of Pseudane IX, Its N-Oxide, and Novel Carboxamide Analogs with Antibacterial Activity. *Molecules* **2024,** *29,* 3676. https://doi.org/10.3390/molecules29153676

3. Angelov, P.; Mollova-Sapundzhieva, Y.; Nedialkov, P. Attempted Synthesis of the Pseudomonas aeruginosa Metabolite 2-Benzyl-4(1*H*)-quinolone and Formation of 3-Methylamino-2-(2-nitrobenzoyl)-4*H*-naphthalen-1-one as an Unexpected Product. *Molbank* **2024,** *2024,* M1877. https://doi.org/10.3390/m1877

4. Mollova, Y.; Angelov, P.; Yanev, P. 3-Carbamoylmethyl-Indole-1-Carboxylic Acid Ethyl Ester. *Molbank* **2022**, *2022*, M1324. https://doi.org/10.3390/M1324

5. Mollova-Sapundzhieva. Y.; Alonso, F; Angelov, P; Nedialkov P. Synthesis of 4-Quinolone N-Oxides via Controlled Partial Hydrogenation of 2-Nitrobenzoyl Enamines. *ChemRxiv* **2024**, https://doi.org/10.26434/chemrxiv-2024-4w06r

6. Angelov, P.; Mollova-Sapundzhieva, Y. Cyclization Modes in Anilides of *N*-Protected 3-Oxo-4-phenylaminobutyric Acid Under Knorr Conditions. *Molbank* **2024**, *2024*, M1933. https://doi.org/10.3390/M1933

V. ACKNOWLEDGEMENT

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