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An amidation/cyclization approach to the synthesis of *N*-hydroxyquinolinones and their biological evaluation as potential anti-plasmodial, anti-bacterial, and iron(II)-chelating agents

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ABSTRACT

A 26-member library of novel *N*-hydroxyquinolinone derivatives was synthesized by a one-pot Buchwald-type palladium catalyzed amidation and condensation sequence. The design of these rare scaffolds was inspired from *N*-hydroxypyridones and 2-quinolinones classes of compounds which have been shown to have rich biological activities. The synthesized compounds were evaluated for their anti-plasmodial and anti-bacterial properties. In addition, these compounds were screened for their iron(II)-chelation properties. Notably, four of these compounds exhibited anti-plasmodial activities comparable to that of the natural product cordypyridone B.

Molecular hybridization, a strategy of combining pharmacophoric elements of two or more biologically active small molecules, results in hybrid compounds that may retain the biological characteristics of the parent compounds or lead to new or enhanced biological activities.[1] These hybridized entities are typically synthetically more accessible than the parent compounds as only the key pharmacologically active groups are necessary. To this end, we envisioned *N*-hydroxyquinolinones, a scarce and a relatively unexplored scaffold in medicinal chemistry as a blend of *N*-hydroxypyridone and quinolone scaffolds and anticipate these to possess the combined biological properties of the parent compounds.

There is a myriad of known *N*-hydroxypyridone-based natural products possessing anti-plasmodial, anti-bacterial, and anti-cancer properties.[2] For example, cordypyridone B (Fig. 1) was reported to display potent anti-plasmodial activity against *Plasmodium falciparum* (K1 strain) with an IC_{50} value of 37 ng/mL[3] as well as display anti-bacterial properties against *Staphylococcus aureus*.[4] Other examples of naturally occurring *N*-hydroxypyridones include akanthomycin, an antibiotic isolated from the fungus *Akanthomycin gracilis*.[4] Moreover, compounds with the *N*-hydroxypyridone motif are widely considered as siderophores and their biological activities have been recognized to originate from their ability to sequester metal ions.[5] For example, pyridoxatin–Fe complex (named terricolin) has been isolated and the absolute structure was elucidated by X-ray crystallographic methods.[6]

On the other hand, the 4-quinolone class of compounds has long been used as broad-spectrum antibiotics since its discovery about 40 years ago.[7] 4-quinolone drugs (such as sitafloxacin) act as anti-bacterials by inhibiting the topoisomerase ligase domain of the bacteria leading to DNA fragmentation and ultimately cell death.[8] Recently, a 4-quinolone compound, ELQ-300 with an IC_{50} value of 14.9 nM against the drug resistant *Plasmodium falciparum* was identified as a lead candidate for the treatment of malaria (Fig. 1).[9] Despite these developments, there is a continual urgent need to develop new and effective anti-malarial and anti-bacterial therapies as the current suite of marketed drugs are becoming ineffective at an alarming rate due to resistance. To date, it is known that the malaria parasite has developed resistance towards the last-line drug artemisinin and threatens malaria control.[10] Similarly, the emergence of bacterial resistance to all the four generations of quinolone antibiotics necessitates the development of new therapies.[8]

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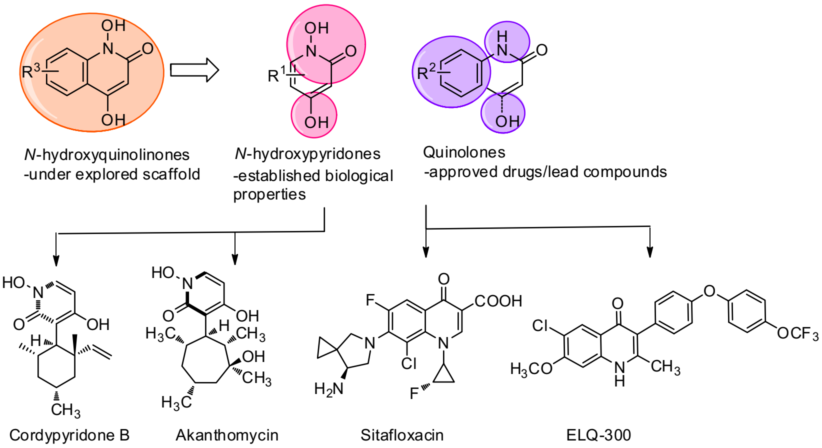
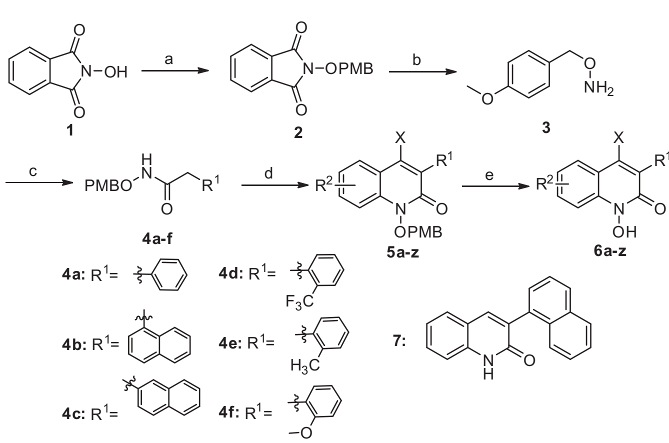


Figure 1. Hybrid approach to *N*-hydroxyquinolinones inspired from *N*-hydroxypyridones and quinolones.

Although the *N*-hydroxypyridone-based natural products are promising anti-plasmodial candidates, their abundance in nature is low and their molecular complexity and presence of multiple stereo-centers make them less attractive to be considered as drug candidates. There are few reports on the total syntheses of these natural products but the crucial *N*-hydroxylation step relies on the use of Vedejs reagent[11] (containing highly carcinogenic hexa-methylphosphoramide coordinated to molybdenum) and the tedious purification procedures associated with the removal of residual molybdenum contamination makes those routes undesirable particularly on a production scale. Hence, we sought to develop a new route to *N*-hydroxyquinolinones, which could avoid the use of Vedejs reagent and at the same time be amenable for library synthesis. Herein, we report a one-pot palladium catalyzed cascade of amidation/cyclization sequence to construct a 26-member library of *N*-hydroxyquinolinone derivatives and report their in vitro anti-plasmodial, anti-bacterial and iron-chelation properties.

The detailed synthetic route to *N*-hydroxyquinolinones is depicted in Scheme 1. Our strategy commenced with the synthesis of protected *N*-hydroxyphthalimide **2** by treating *p*-methoxybenzyl chloride with *N*-hydroxyphthalimide (**1**).[12] Treatment of **2** with hydrazine monohydrate furnished 87% of *O*-(4-methoxybenzyl) hydroxylamine[13] (**3**), which was subsequently treated with 6 different commercially available α-arylacetyl chlorides to afford 6 different *N*-((4-methoxybenzyl)oxy)-2-arylacetamides (**4a**–**f**) in 68–88% yield. Gratifyingly, the coupling of the protected *N*-hydroxyamides **4** with the benzaldehydes or benzoates under palladium catalyzed Buchwald-type C–N bond formation followed by a tandem cyclodehydration furnished the quinolinones (**5a**–**z**) in good yields. Various substituents on **4** including 2-methyl phenyl, 2-trifluromethyl phenyl, 2-methoxy phenyl, 1-naphthyl, 2-naphthyl were tolerated under the reaction conditions above. In these studies, $Pd_2(dba)_3$ (0.25% equiv) was used as a palladium source in the presence of Cs_2CO_3 as a base and Xantphos (5% equiv) as a ligand.



Scheme 1. Reagents and conditions: (a) PMBCl, DMF, Et_3N, 90 °C; (b) hydrazine monohydrate, DMF, MeOH, 60 °C; (c) aryl acetyl chloride, DCM, Et_3N, rt; (d) substituted 2-bromobenzaldehyde or methyl 2-bromobenzoate, Cs_2CO_3, $Pd_2(dba)_3$, Xantphos, toluene, 110 °C; (e) TFA, anisole, DCM, rt.

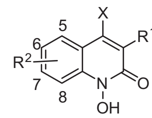
Although this kind of coupling–cyclization sequence with amides has been documented,[14] to the best of our knowledge this is the first method wherein hydroxylamine-derived amides were shown to be amenable for the C–N bond formation reaction, thus opening up an easy and direct access to *N*-hydroxyquinolinone scaffolds. Following the above procedure compounds **5a**–**z** were synthesized. Subsequently, the final compounds **6a**–**z** were obtained by treating compounds **5a**–**z** with trifluoroacetic acid in dichloromethane.[15] Additionally, the quinolinone **7** was synthesized by adopting the reported protocol[14] and used as a reference to understand the importance of the –*N*-OH functionality during biological evaluation. All of these compounds were evaluated for their biological activities.

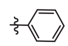
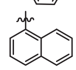
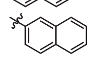
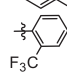
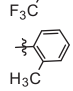
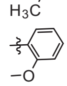
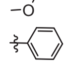
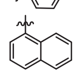
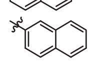
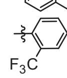
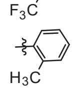
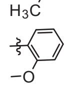
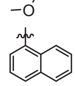
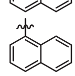
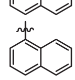
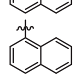
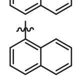
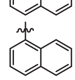
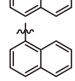
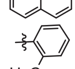
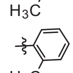
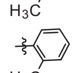
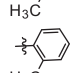
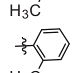
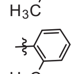
The 27 synthesized compounds were screened for their in vitro anti-plasmodial activities. Their MBC (minimum bactericidal concentration) values were also determined. The results are summarized in Table 1.

The anti-plasmodial screening was conducted with chloroquine sensitive 3D7 strain using a well established in vitro maturation assay.[16] Most of these compounds displayed moderate anti-plasmodial activities. It is noteworthy that compounds **6n**, **6q**, **6r**, and **6u** show potent anti-plasmodial activity (1.1–1.4 μM) as compared to that of the natural product cordypyridone B (0.8 μM). The quinolinone **7** is not active within the range tested suggesting the importance of the –*N*-OH functionality for the biological activity, as compared to **6h**. Compounds with a hydroxyl group at the 4-position of the heterocyclic ring were less active as compared to the ones without a –OH group (compounds **6a**–**f** vs compounds **6g**–**l**) suggesting that the –OH group is not necessary for the activity. The presence of the methoxy group at the 7-position of the quinolinone ring significantly decreased the anti-plasmodial activities, as shown by compounds **6m**, **6n**, **6o** and **6t**. Electron-withdrawing or electron-donating groups have subtle effects on the activities when present on C-5 or C-6 of the fused aryl ring as shown by compounds **6n** and **6p**, **6q** and **6r**.

The MBC values of these compounds were determined against *Escherichia coli* and *Staphylococcus aureus*. Compound **6b** is not active towards *E. coli* but displayed mild activity towards *S. aureus*,

Table 1
In vitro anti-plasmodial and anti-bacterial activities of targeted compounds



Compounds	X	R^1	R^2	MBC (mM)		Anti-plasmodial IC_{50} (μM)
				E. coli	*S. aureus*	
6a	OH		H	4.0	NA	NA
6b	OH		H	NA	1.7	NA
6c	OH		H	NA	NA	NA
6d	OH		H	NA	NA	NA
6e	OH		H	NA	NA	NA
6f	OH		H	NA	NA	NA
6g	H		H	NA	NA	14
6h	H		H	NA	NA	2.6
6i	H		H	NA	3.6	2.9
6j	H		H	NA	3.4	5.9
6k	H		H	NA	4.1	3.0
6l	H		H	1.9	3.8	7.6
6m	H		6,7-OCH_2O–	NA	NA	4.2
6n	H		6-OCH_3	NA	NA	1.3
6o	H		6-OCH_3 7-OCH_3	NA	NA	5.0
6p	H		5,6-OCH_2O–	NA	3.1	1.9
6q	H		6-F	NA	NA	1.1
6r	H		5-F	NA	NA	1.4
6s	H		6-OH	3.4	NA	2.5
6t	H		6,7-OCH_2O–	NA	NA	4.6
6u	H		6-OCH_3	NA	3.6	1.3
6v	H		6-OCH_3 7-OCH_3	NA	NA	5.5
6w	H		5,6-OCH_2O–	NA	NA	3.6
6x	H		6-F	NA	NA	4.8
6y	H		5-F	NA	NA	5.0

(*continued on next page*)

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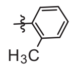
Table 1 (*continued*)

Compounds	X	R^1	R^2	MBC (mM)		Anti-plasmodial IC_{50} (μM)
				E. coli	*S. aureus*	
6z	H		6-OH	1.9	3.8	3.5
7				NA	NA	NA
Cordypyridone B				NT	NT	0.8

NA: not active up to the maximum concentration tested (for MBC: up to 1024 μg/ml, anti-plasmodial against 3D7 strain: up to 5092 ng/ml); NT: not tested.

Table 2
Chelation ability of selected *N*-hydroxyquinolinones

Compounds	50% CA (mM)	Compounds	50% CA (mM)
6b	0.28	**6u**	0.28
6n	0.25	**6x**	0.29
6q	0.24	**6y**	0.28
6r	0.24	**7**	NA
EDTA	0.13		

CA: chelation ability; NA: no chelation observed up to the maximum concentration tested.

indicating that gram positive bacterial may be susceptible to compound **6b**. Four compounds (**6i**, **6j**, **6k**, **6l**) indicate effective killing against *Staphylococcus aureus*, whereas for compound **6z**, no difference in the activities of these compounds towards gram negative or gram positive bacteria was observed. Although these compounds are not potent anti-bacterials, the results are encouraging and provide further clues towards further discovery of new antibiotics.

Compounds containing *N*-hydroxypyridone motif are well recognized for their metal ion chelating properties[17,18] and hence one would also expect *N*-hydroxyquinolinones to chelate metal irons such as iron(II) ion. This has implications on the manifestation of their biological properties, including anti-oxidant properties. To this end, 9 compounds were selected to test their iron(II) chelation abilities following the assay reported by Selvakumar et al.[19] The results are shown in Table 2. Eight *N*-hydroxyquinolinones show similar iron chelation abilities, while the quinolinone **7** was not able to chelate iron within the range tested. The *N*-hydroxyquinolinones have a narrow range of chelation abilities as reported by the 50% CA values, that is, a range from 0.24 mM to 0.29 μM. EDTA was used as control and its 50% chelation ability was determined to be 0.13 mM. Thus the iron chelation assay shows that *N*-hydroxyquinolinones are promising iron chelating agents, though this ability is relatively insensitive to the nature of the substituents studied.

In summary, we have implemented a new route for the facile synthesis of a library of *N*-hydroxyquinolinones based on a one-pot palladium catalyzed Buchwald-type C–N amidation/dehydrocyclization sequence. The design of these compounds was inspired from the naturally occurring cordypyridones and therapeutically valuable quinolones. These new set of compounds exhibited promising anti-plasmodial activities, some of which are as potent as cordypyridone B. These compounds have the advantages that they are simple to synthesize and scale-up. Their anti-bacterial as well as the iron(II)-chelating abilities were evaluated and discussed.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.12.014.

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