Microwave-assisted Döbner Synthesis of 2-Phenylquinoline-4-carboxylic Acids and their Antiparasitic Activities.

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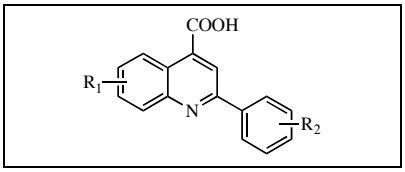

A series of twelve substituted 2-phenylquinoline-4-carboxylic acids analogous to antimalarial and antileishmanial natural products was developed *via* the Döbner reaction employing microwave irradiation (MW). The products were obtained in moderate yields in 0.5-3 minutes and nine of them were evaluated *in vitro* against the parasites responsible for malaria, leishmaniasis and trypanosomiasis diseases (WHO, Switzerland). Four compounds exhibited activity against *Trypanosoma cruzi* and another two resulted active against *Plasmodium falciparum* and *Leishmania infantum*, respectively.

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INTRODUCTION

Quinoline derivatives represent a major class of heterocycles, and a number of preparations have been known since the late 1800s. The quinoline ring system occurs in various natural products, especially in alkaloids. In 1820, quinine was isolated as the active ingredient from the bark of Cinchona trees and replaced the crude bark for the treatment of malaria and its synthetic derivative chloroquine (CQ) was the prime therapy for nearly half a century [1]. *Plasmodium falciparum*, the cause of most deadly variety of malaria, is now CQ-resistant in nearly all regions of the globe. However, in the search of new candidates, this drug remains the one to emulate [2].

Chimanine alkaloids, simple 2-substituted quinolines isolated from the bark of *Galipea longiflora* trees of the Rutaceae family, are effective against the parasites *Leishmania* sp., which are the agents of leishmaniasis, a protozoan disease of the tropical areas in South America. Two of these compounds had the same efficacy as the reference antimonial drug currently administered by the parenteral route. On the basis of the results obtained in studies with 2-substituted quinolines, the World Health Organization (WHO) has established as a priority their ability to provide oral treatment for cutaneous leishmaniasis [3,4].

On the other hand, despite the progress made in *Trypanosoma cruzi* biochemistry and physiology, the chemotherapy of Chagas´ disease (trypanosomiasis) is deficient and no medication is adequate in the chronic phase [5].

2-Phenylquinoline-4-carboxylic acid also known as cincophen acid (or atophan) and its derivatives have shown a variety of biological effects [6]. In 1946, the synthesis of a large family of substituted 2-phenyl-4-quinolinemethanols from cincophens as possible antimalarials, was reported. Seven of the appropriate cincophens were made through the corresponding isatins by the Pfitzinger method and the other twelve were made by the Döbner-Miller synthesis. Of the two methods, the Pfitzinger gave by far the best yields but it was preferred when the materials were readily available [7].

Ten years ago, a series of eight 2-arylquinoline-4-carboxamides was achieved in good yields employing solid phase synthesis as well as a multi-component condensation approach based on Döbner reaction [6]. More recently, the preparation of quinaldines and lepidines by Döbner-Miller reaction under thermal and microwave irradiation (MW) conditions using phospho-tungstic acid was reported [8].

The microwave-assisted organic synthesis (MAOS) without the use of catalyst or solvent is particularly eco-friendly and has the advantages of short reaction time and high yields [9]. Moreover, the utility of multi-component reactions (MCRs) in preparing libraries to screen is well appreciated [10].

RESULTS AND DISCUSSION

Herein, the synthesis of twelve 2-arylquinoline-4-carboxylic acids (**4a-l**) prepared *via* Döbner reaction [11] under MW is described (Scheme 1). The one-pot reactions of pyruvic acid (**1**) and substituted anilines (**2**) and benzaldehydes (**3**) were carried out in a MW domestic oven adapted for the use of a reflux condenser, at constant power (400 W). All the reactions proceed to completion between 0.5 and 3 min (Table 1), instead of the 2 h with conventional heating and a longer time did not increase the yields.

Scheme 1

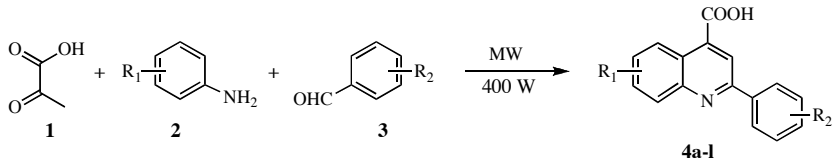
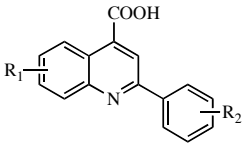


Table 1



Entry	R_1	R_2	Product	Time (s)	Yield (%)	mp (Lit.) (ºC)
1	H	H	**4a**	240	12	208-210 (209-210)[12a]
2	H	4-OCH_3	**4b**	60	55	195-196
3	H	4-OH	**4c**	30	11	191-192
4	H	4-Cl	**4d**	120	24	142-144
5	H	2-OH	**4e**	30	10	37-40
6	H	2-NO_2	**4f**	30	10	176-178 (253-255)[12b]
7	8-Cl	H	**4g**	30	18	85-87
8	7-Cl	H	**4h**	30	63	191-192 (244-246)[12c]
9	6-Cl	H	**4i**	60	37	147-149 (243)[12d]
10	6-F	H	**4j**	18	23	134-136
11	7-CF_3	H	**4k**	60	13	127-129
12	7-CH_3	H	**4l**	90	12	217-218

Compounds **4a**, **4f**, **4h** and **4i** have been known for many years and the reaction times for their preparation were long and the yields were usually low [12]. However, they were made without application to the malaria problem.

In a previous paper [13], we have reported the synthesis and antiparasitic activity of a closely related quinoline series. Four of these compounds exhibited some activities: two of them against leishmaniasis, one against malaria and another one against both malaria and Chagas´disease. These results prompted us to synthesize this new quinoline series, possessing also a chloro atom and a phenyl ring as substituents in the quinoline skeleton. The replacement of chloro for fluor, methyl or trifluoromethyl moieties could improve the activity, taking in account recognized antimalarial agents, such as mefloquine and amodiaquine. The substitution in position 2 could resemble Chimanine alkaloids.

Nine of the synthesized products were selected for a primary *in vitro* screening at Tropical Disease Research (TDR) Program, World Health Organization (WHO, Switzerland). Compounds **4e**, **4f** (with a nitro group) and **4i** were refused. Compound **4a** resulted moderately active against *T. cruzi* meanwhile **4b** exhibited moderate activity against *P. falciparum* (Table 2) [14]. The remaining compounds were tested at University of Antwerp (Tables 3 and 4). Compounds **4c**, **4d**, **4g**, **4h**, **4j** and **4l** lacked of antimalarial and antileishmanial activities but **4k** resulted moderately active against *L. infantum* (Table 3). In addition, compounds **4d**, **4h** and **4l** were evaluated against *T. cruzi* and they exhibited some activity (Table 4) [15]. However, all their IC_{50} values are quite far from the reference drugs and the named compounds were not selected for further screening.

As an approach for establishing structure-activity relationships, electron-donating groups at position 4 of the 2-phenyl moiety improved antimalarial activity and the OCH_3 was the best. The activity against leishmaniasis was better when a CF_3 group was attached at position 7 of the quinoline ring, meanwhile for Chagas´ disease the same position could be substituted either with electron-attracting or donating groups. These results suggest that compound **4k** could also be active against *T. cruzi*.

In conclusion, microwave-assisted solvent-free reactions were employed to develop a series of 2-phenyl-quinoline-4-carboxylic acids. Although the yields were not high, the starting materials are available and inexpensive and the method offers quite short reaction times. The promising biological evaluation encouraged us to enlarge this series in order to modulate the antichagasic activity and diminish their toxicity. Furthermore, the synthesis of new derivatives of compound **4k** could be interesting against leishmaniasis.

Table 2

Antiprotozoal activity of compounds **4a** and **4b** (IC_{50} values are given in μg/mL)[a]

Compound	*P. falciparum* chloroquine 0.04	*L. donovani* miltefosine 0.2	*T. cruzi* benznidazole 0.25	*T. b. rhodesiense* melarsoprol 0.005	cytotoxicity L6 cell line podophyllotoxin 0.007
4a	> 5	> 30	13.8	> 90	35.6
4b	3.1	22.4	> 30	35.25	75.6

[a] IC_{50} = 50 % growth inhibition.

Table 3

Antiprotozoal activity of compounds **4c, 4g, 4j and 4k** (IC_{50} values are given in μg/mL)[a]

Compound	*P. falciparum* chloroquine 0.026	*L. infantum* pentostam 2.4	*T. cruzi* Benznidazole	*T. b. rhodesiense* suramin 0.13	cytotoxicity L6 cell line tamoxifen 4.9
4c	> 17	> 17	nt	> 17	> 17
4g	> 18.1	> 18.1	nt	> 18.1	> 18.2
4j	> 18.1	> 18.1	nt	> 18.1	> 18.8
4k	> 20.3	8.6	nt	2.2	> 20.3

[a] IC_{50} = 50 % growth inhibition; nt: not tested.

Table 4

Antiprotozoal activity of compounds **4d, 4h** and **4l** (IC_{50} values are given in μg/mL)[a]

Compound	*P. falciparum* artemether 0.038	*L. infantum* pentostam 2.4	*T. cruzi* nifurtimox 0.57	*T. b. rhodesiense* suramin 0.061	cytotoxicity MRC-5 cell line niclosamide 0.87
4d	> 18.16	>18.2	> 18.2	13.3	> 18.2
4h	> 19.95	> 19.9	> 19.9	> 19.9	> 19.9
4l	> 16.85	> 16.9	> 16.9	> 16.9	> 16.9

[a] IC_{50} = 50 % growth inhibition; nt: not tested.

EXPERIMENTAL

Melting points were determined in a capillary Electrothermal 9100 SERIES-Digital apparatus and are given uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at r.t. using a Bruker 200 MHz spectrometer with TMS as the internal standard. The chemical shifts (δ) are given in ppm. Infrared spectra were recorded on a FT Perkin Elmer Spectrum One from KBr discs. Analytical TLC were performed on DC-Alufolien Kiesegel 60 F_{254} Merck. Microwave-assisted reactions were carried out in a household MW oven BGH-QUICK Chef 15240. This apparatus was modified for laboratory applications adapting an external Liebig condenser.

General procedure for compounds 4a-4l. A mixture of aniline (4.1 mmoles), benzaldehyde (3.9 mmoles) and pyruvic acid (4.3 mmoles) placed in a 50 mL round-bottomed flask was subjected to MW irradiation (400 W). After completion of the reaction (TLC), the reaction mixture was allowed to cool and the residual semisolid crystallized. When this did not occur, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with water, 5 % HCl (10 mL) and brine (10 mL). This was then dried (Na_2SO_4) and concentrated *in vacuo* to give a solid product which was triturated with EtOH.

2-Phenylquinoline-4-carboxylic acid (4a). This compound was obtained as white needles, mp 208-210 ºC; IR: 3311 (OH), 1678 (CO), 746, 694 (CH arom.) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.07-8.15 (m, 10 H, arom.) ppm. ^{13}C NMR (DMSO-d_6): δ 109.74, 116.63, 120.18, 121.43, 124.36, 126.70, 127.66, 128.61, 128.67, 128.92, 131.72, 137.09, 137.91, 141.94, 166.46 ppm. *Anal.* calcd. for $C_{16}H_{11}NO_2$: C, 77.10; H, 4.45; N, 5.62. Found: C, 76.90; H, 4.56; N, 5.50.

2-(4-Methoxyphenyl)-quinoline-4-carboxylic acid (4b). This compound was obtained as white powder, mp 195-196 ºC; IR: 3313 (OH), 3061 (CH), 1678 (CO), 1649 (CN), 1034, 1251 (COC), 778, 748 (CH arom.) cm^{-1}; ^{1}H NMR ($DCCl_3$): δ 3.75 (s, 3 H, OCH_3), 5.64 (d, 1 H, arom.), 6.07 (d, 1 H, arom.), 6.68-7.54 (m, 7 H, arom.) ppm. *Anal.* calcd. for $C_{17}H_{13}NO_3$: C, 73.11; H, 4.69; N, 5.02. Found: C, 73.28; H, 4.38; N, 5.40.

2-(4-Hydroxyphenyl)-quinoline-4-carboxylic acid (4c). This compound was obtained as dark yellow powder, mp 191-192 ºC; IR: 3435 (OH), 2916 (CH), 1603 (CO), 1286 (CO), 841 (CH arom.) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.46-8.61 (m, 9 H, arom.), 9.77 (s, 1 H, OH) ppm. *Anal.* calcd. for $C_{16}H_{11}NO_3$: C, 72.45; H, 4.18; N, 5.28. Found: C, 72.59; H, 3.90; N, 5.10.

2-(4-Chlorophenyl)-quinoline-4-carboxylic acid (4d). This compound was obtained as pale yellow powder, mp 142-144 ºC; IR: 3346 (OH), 3060 (CH), 1742 (CO), 1656 (CN), 1121 (CO), 754, 696 (CH arom.) cm^{-1}; ^{1}H NMR ($DCCl_3$): δ 6.00 (d, 2 H, Ph), 6.05 (d, 2 H, Ph), 6.99-7.51 (m, 5 H, arom.) ppm. *Anal.* calcd. for $C_{16}H_{10}ClNO_2$: C, 67.74; H, 3.55; N, 4.94. Found: C, 68.03; H, 3.37; N, 5.00.

2-(2-Hydroxyphenyl)-quinoline-4-carboxylic acid (4e). This compound was obtained as orange powder, mp 37-40 ºC; IR: 3336 (OH), 2911 (CH), 1616 (CO), 1273 (CO), 753 (CH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.95-7.67 (m, 9 H, arom.), 8.96 (s, 1 H, OH), 13.10 (s, 1 H, COOH) ppm. *Anal.* calcd. for $C_{16}H_{11}NO_3$: C, 72.45; H, 4.18; N, 5.28. Found: C, 72.15; H, 3.92; N, 4.93.

2-(2-Nitrophenyl)-quinoline-4-carboxylic acid (4f). This compound was obtained as white powder, mp 176-178 ºC; IR: 3314 (OH), 3056 (CH), 1692 (CO), 1656 (CN), 1523, 1346 (NO_2), 746 (CH) cm^{-1}; ^1H NMR ($DCCl_3$): δ 6.30-8.03 (m, 9 H, arom.) ppm. *Anal.* calcd. for $C_{16}H_{10}N_2O_4$: C, 65.31; H, 3.43; N, 9.52. Found: C, 65.48; H, 3.51; N, 9.88.

8-Chloro-2-phenylquinoline-4-carboxylic acid (4g). This compound was obtained as yellow needles, mp 85-87 ºC; IR: 3467 (OH), 3067 (CH), 1705 (CO), 1269 (CO), 760, 698 (CH) cm^{-1}; ^1H NMR ($DCCl_3$): δ 7.52-7.60 (m, 4 H), 7.92-7.94 (m, 1 H), 8.33-8.36 (m, 2 H), 8.64 (s, 1 H), 8.78-8.82 (m, 1 H) ppm. Anal. calcd. for $C_{16}H_{10}ClNO_2$: C, 67.74; H, 3.55; N, 4.94. Found: C, 67.44; H, 3.26; N, 4.67.

7-Chloro-2-phenylquinoline-4-carboxylic acid (4h). This compound was obtained as pale yellow powder, mp 191-192 ºC; IR: 3319 (OH), 3025 (CH), 1678 (CO), 1582 (CN), 1264 (CO), 780, 679 (CH) cm^{-1}; ^1H NMR ($DCCl_3$): δ 5.66 (d, 1 H), 6.11 (d, 1 H), 6.69-7.39 (m, 6 H), 7.70 (t, 1 H) ppm. *Anal.* calcd. for $C_{16}H_{10}ClNO_2$: C, 67.74; H, 3.55; N, 4.94. Found: C, 67.61; H, 3.39; N, 5.12.

6-Chloro-2-phenylquinoline-4-carboxylic acid (4i). This compound was obtained as pale yellow powder, mp 147-149 ºC; IR: 3328 (OH), 3066 (CH), 1760 (CO), 1670 (CN), 822, 696 (CH) cm^{-1}; ^1H NMR ($DCCl_3$): δ 5.65 (d, 1 H), 6.05 (d, 1 H), 6.38 (d, 1 H), 7.17-7.49 (m, 6 H) ppm. *Anal.* calcd. for $C_{16}H_{10}ClNO_2$: C, 67.74; H, 3.55; N, 4.94. Found: C, 68.03; H, 3.75; N, 4.65.

6-Fluoro-2-phenylquinoline-4-carboxylic acid (4j). This compound was obtained as pale yellow powder, mp 134-136 ºC; IR: 3348 (OH), 3067 (CH), 1760 (CO), 1658 (CN), 1218 (CO), 782, 697 (CH) cm^{-1}; ^1H NMR ($DCCl_3$): δ 6.02 (d, 1 H), 6.32 (d, 1 H), 7.02-7.40 (m, 7 H) ppm. *Anal.* calcd. for $C_{16}H_{10}FNO_2$: C, 71.91; H, 3.77; N, 5.24. Found: C, 72.18; H, 3.49; N, 5.06.

2-phenyl-7-trifluoromethylquinoline-4-carboxylic acid (4k). This compound was obtained as white needles, mp 127-129 ºC; IR: 3330 (OH), 3086 (CH), 1680 (CO), 1655 (CN), 1329, 1164, 1133 (CF_3), 794, 691 (CH) cm^{-1}; ^1H NMR ($DCCl_3$): δ 5.73 (d, 1 H), 6.15 (d, 1 H), 6.82 (s, 1 H), 7.20-7.43 (m, 4 H), 7.71 (d, 1 H), 7.92 (s, 1 H) ppm. *Anal.* calcd. for $C_{17}H_{10}F_3NO_2$: C, 64.36; H, 3.18; N, 4.41. Found: C, 64.52; H, 3.40; N, 4.38.

7-Methyl-2-phenylquinoline-4-carboxylic acid (4l). This compound was obtained as white powder, mp 217-218 ºC; IR: 3346 (OH), 3030 (CH), 2915, 2851 (CH_3), 1706 (CO), 1599 (CN), 1224 (CO), 749, 702 (CH) cm^{-1}; ^1H NMR (DMSO): δ 2.56 (s, 3 H, CH_3), 7.53-7.60 (m, 4 H), 7.96 (s, 1 H), 8.26-8.29 (m, 2 H), 8.39 (s, 1 H), 8.56 (d, 1 H) ppm. *Anal.* calcd. for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.27; H, 5.00; N, 4.99.

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[14] The *in vitro* protocols and activity criteria can be found at WHO website (www.who.int/tdr/grants/workplans/pdf). For antimalarial activity (K1 strain is used), if the IC_{50} is >5 μg/mL, the compound is classified as inactive. If the IC_{50} is 0.5–5 μg/mL, the compound is classified as moderately active. If the IC_{50} is <0.5 μg/mL, the compound is classified as active and is further evaluated using two strains, K1 and NF54. For Chagas disease, if the IC_{50} is >30 μg/mL, the compound is classified as inactive. If the IC_{50} is between 2 and 30 μg/mL, the compound is classified as moderately active. If the IC_{50} is <2 μg/mL, the compound is classified as active and is further evaluated in an *in vivo* screen. Regarding cytotoxicity, if the IC_{50} is > 90 μg/mL, the compound is classified as not cytotoxic. If the IC_{50} is between 2 and 89 μg/mL, the compound is classified as moderately cytotoxic and if the IC_{50} is < 2 μg/mL, the compound is classified as cytotoxic.

[15] Antwerp University, LMPH, standard procedures used for the TDR *in vitro* screening. For antimalarial activity GHA strain is used and for antileishmanial activity MHOM/MA(BE)/67 strain is used. The compound is classified as inactive when the IC_{50} is higher than 16 μg/mL. When IC_{50} lies between 16 and 1 μg/mL, the compound is regarded as being moderately active. When the IC_{50} is lower than 1 μg/mL, the compound is classified as active and is evaluated in a secondary screening. For Chagas disease, Trypanosoma cruzi β galactosidase strain is used. The compound is classified as inactive when the IC_{50} is higher than 16 μg/mL. When IC_{50} lies between 16 and 1 μg/mL, the compound is regarded as being moderate active. When the IC_{50} is lower than 1 μg/mL, the compound is classified as highly active and is further evaluated in a secondary screening. For toxicity, the compound is classified as non-toxic when the IC_{50} is higher than 32 μg/mL or μM. Between 16 and 4 μg/mL, the compound is regarded as moderately toxic. When the IC_{50} is lower than 4 μg/mL, the compound is classified as highly toxic.