New 2-Phenylquinoline Derivatives: Synthesis and Preliminary Evaluation as Antimicrobial Agents

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Abstract: A number of new 2-phenylquinoline derivatives have been synthesized. All the synthesized compounds were evaluated for their antibacterial activity. Most of them showed a good activity against *Escherichia coli* and *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) was determined for tested compounds.

Keywords: 2-Phenylquinoline, antibacterial activity, minimum inhibitory concentration, suzuki coupling.

INTRODUCTION

The dramatically rising prevalence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. In particular, the emergence of multi-drug resistant strains of Gram-positive bacteria pathogens such as methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermis* and vancomycin-resistant *Enterococcus* is a problem of ever-increasing significance [1-5]. One way to counterbalance this challenge is the controlled use of the currently marketed antibiotics; the other is the development of novel antimicrobial agents. Consequently, the search for new antimicrobial agents will always remain an important and challenging task for medicinal chemists.

At present, the role of heterocyclic compounds has become increasingly important in designing new class of structural entities of medicinal importance. Among pharmacologically important heterocyclic compounds, quinoline derivatives are significant compounds in medicinal chemistry.

Quinoline moiety is of great importance to chemists as well as biologists as it is found in a large variety of naturally occurring compounds and also chemically useful molecules having diverse biological activities. A large variety of quinoline derivatives have been used as antimalarial [6], anti-inflammatory [7], anticancer [8], antibiotic [9], antihypertensive [10], tyrokinase PDGF-RTK inhibiting agents [11], and anti HIV [12]. In addition to the medicinal importance, multi-substituted quinolines are valuable synthons used for the preparation of nano- and meso-structures with enhanced electronic and photonic properties [13].

It has been well-established that presence of aryl ring at second position of quinoline moiety gives a very good antibacterial property to the target molecule and plays a significant role in development of new antibacterials [14]. These derivatives were found to be useful biological targets, and at present they attained much attention in the development of new drugs [15]. In addition, they are ideally suited for further modifications to obtain more efficacious antibacterial and antituberculosis agents.

In the course of our ongoing program related to the use of substituted 2-chloro-3-formylquinolines **1**as precursors of different quinoline-containing heterocycles with diverse functionalities [16], we wish to report herein our preliminary results concerning the synthesis of new 2-phenylquinolines and their evaluation as antibacterial agents.

First of all, as an initial attempts, three different series of compounds carrying an amine (series A: **4a-e**), amide (series B: **7a-d**) or ester group (series C: **8a-d**) linked to the 2-phenylquinoline nucleus were synthesized and investigated for their antimicrobial activity. The synthetic pathways adopted for the preparation of the new compounds (series A, B and C) are outlined in schemes **1**–**3**.

The key intermediates 6-methyl-2-phenylquinoline-3-carbaldehyde **2** and 2-chloro-6-methylquinoline-3-carboxylic

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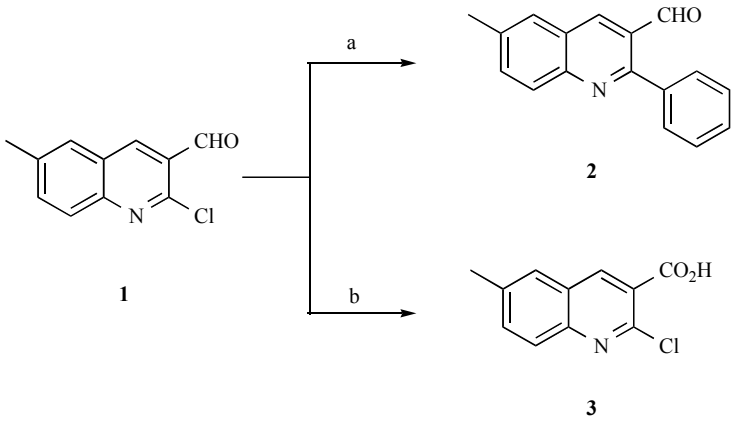
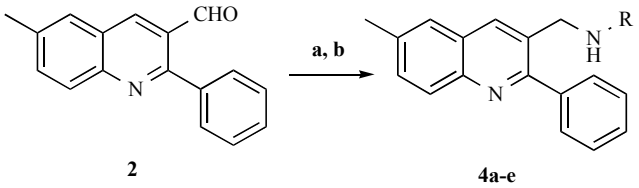


Scheme 1. Reagents and conditions: (a) $Pd(PPh_3)_4$, $PhB(OH)_2$, Na_2CO_3 (2M), DME, reflux, 4h; (b) CrO_3 aq., H_2SO_4, acetone, 0°C to rt, 3h.

acid **3**, required for the preparation of the target compounds was obtained from the 2-chloro-3-formylquinoline **1** (scheme 1). The carboxylic acid derivative **3** was prepared by reacting the parent compound **1** with Jones reagent in acetone at 5–10°C. Thus Suzuki coupling of the 2-chloroquinoline-3-carbaldehyde with phenylboronic acid gave the corresponding 2-phenylquinoline derivative **2**.

The 2-phenylquinoline derivatives substituted on their C-3 with an amino group (Series A: **4a-e**; Table **1**) were first prepared by conversion of 6-methyl-2-phenylquinolin-3-carbaldehyde **2** in the presence of appropriate amine in MeOH into corresponding imine, followed by reduction by using $NaBH_4$ in methanol.

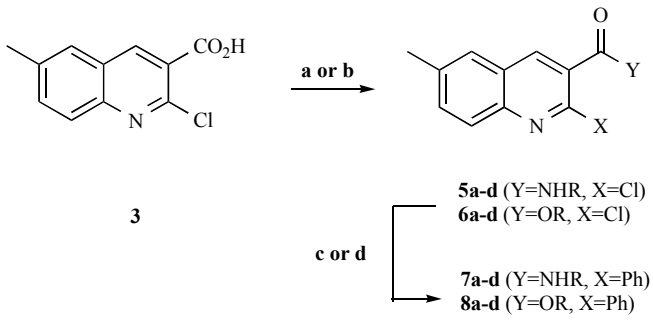


Scheme 2. Reagents and conditions: (**a**) RNH_2, MeOH, 24h; (**b**) $NaBH_4$, MeOH, rt, 3h.

The carboxamide derivatives (Series B: **5a-d**; Table **1**) were prepared by stirring 2-chloroquinolin-3-carboxylic acid **3** with ethyl chloroformate in the presence of triethylamine in chloroform at 5–10°C, followed by addition of the appropriate amine [17]. On the other hand, heating **3** with thionyl chloride gave the 2-chloro-6-methylquinolin-3-carbonyl chloride derivative which was pure enough (TLC) for use in subsequent steps. The acyl chloride was stirred at reflux with alcohol in toluene to give the corresponding 2-chloro-6-methylquinolin-3-carboxylate (Series C: **6a-d**; Table **1**) [18]. The palladium-catalysed cross coupling between phenylboronic acid and halides **5** and **6** gave the corresponding 2-phenylquinoline derivatives **7a–d** and **8a-d** respectively (Scheme **3**).

The X-ray crystallographic analysis of single crystals of **7b** and **8c** confirmed their respective structural assignments (Fig. **1**, **2**).

Numerous studies have demonstrated that the nature of substituents and substitution pattern on the quinoline unit may have a considerable impact on the pharmacological activities [21]. In this context, a new series of compounds were prepared incorporating a nitro group on the quinoline unit. The nitration at C-5 of amides **5a** and **5b**, followed by Suzuki coupling and reduction reaction of the resulting 5-nitroquinoline **10a** and **10b** with PMHS in presence of $Pd(OAc)_2$ gave the corresponding amino derivatives **11a** and **11b** respectively (Scheme **4**).



Scheme 3. Reagents and conditions: (a) for amides (**5**): ethyl chloroformate, Et_3N, RNH_2, $CHCl_3$, 5-10°C; (b) for esters (**6**): (i) $SOCl_2$, reflux, overnight; (ii): ROH, Toluene, 0°C to reflux, 24h; (c) for amides (**7**): $Pd(PPh_3)_4$, $PhB(OH)_2$, Na_2CO_3 (2M), DME, reflux, 4h; (d) for esters (**8**): $Pd(OAc)_2$, $PhB(OH)_2$, K_2CO_3 (2M), PPh_3, DME, reflux, 2h.

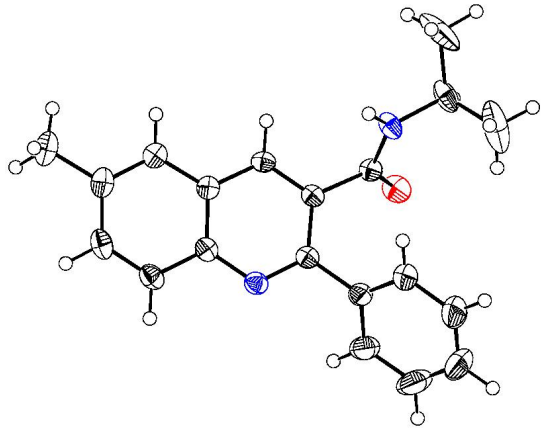


Fig. (1). ORTEP plot of the X-ray crystal structure of **7b.** Displacement ellipsoids are drawn at the 50% probability level [19].

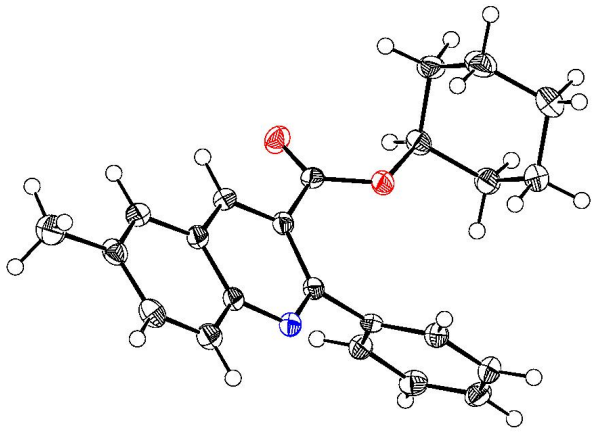


Fig. (2). ORTEP plot of the X-ray crystal structure of **8c.** Displacement ellipsoids are drawn at the 50% probability level [20].

Compounds of series (**4**, **7**, **8**, **10**, **11**) has been tested for their antimicrobial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Klebsiella pneumoniae* (ATCC-700603) and *Salmonella thipymurium* (ATCC-07095) using the disk-diffusion method [22].

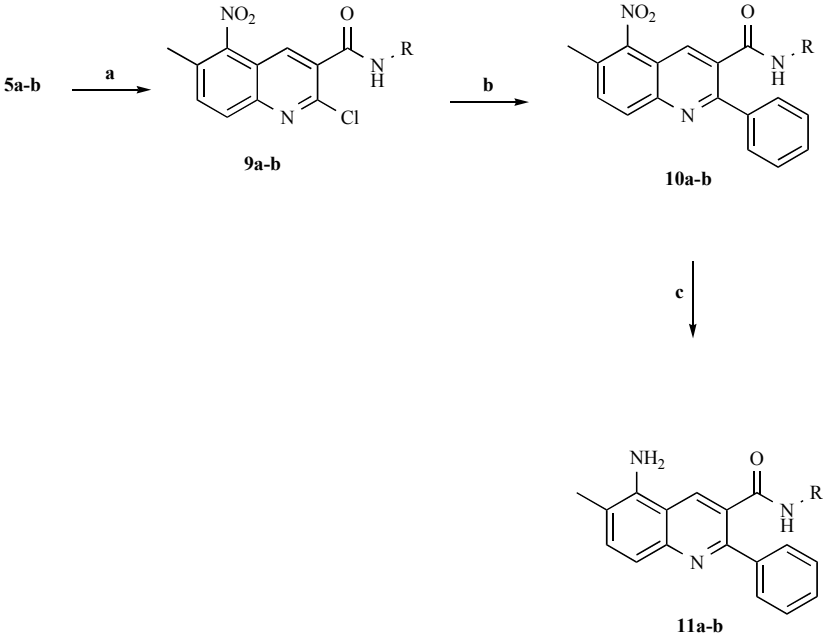
The disk-diffusion method was performed as follows: the bacterial suspension was spread on the surface of a Mueller-Hinton agar. Paper disks (6mm) were charged with 50µg of the tested compounds (**4a-11b**) and were placed on the agar surface. Two standard drugs were used for comparison (Gentamicin and Chloramphenicol). After overnight incubation at 37°C, inhibition zones were measured with a ruler and the zones were recorded in millimeter. The results of the antibacterial screening of the tested compounds are summarized in Table **1**.

Most of the compounds tested were found to have good antibacterial activities against *Staphylococcus aureus* and *Escherichia coli*, however, in most case, no or moderate activities were observed against *Pseudomonas aeruginosa*. The compounds of the library C (**8a-d**) don't show any inhibitory activity against *Pseudomonas aeruginosa* and *Escherichia coli* but have significant inhibition effect on the growth of bacteria like *Staphylococcus aureus*, *Salmonella typhimurium* and *Klebsiella pneumoniae*. The best results were observed with amidoquinolines (series B) which were showed the broadest antibacterial activity.

The data (Table **1**) indicate that the introduction of the nitro (compounds **10a-b**) or amine group (compounds **11a-b**) on the quinoline ring affect the antimicrobial activity. Comparison of biological activities of **10** and **11** with the corresponding no substituted ones **7a-b** show that these substitutions reduce the antibacterial activity.

To confirm the antibacterial activities of the synthesized compounds, the MICs tests were carried out. Bacterial inoculums were prepared by dilution of an overnight broth culture to give the equivalent of 10^6 cell/mL approximately. The MICs values (µg/mL) of each compound after 1 day of exposure are shown in Table **1**.

In conclusion, new series of 2-phenylquinoline with diverse functionalities were synthesized using an appropriate synthetic routes. All the target compounds were evaluated for their *in vitro* antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhimurium*. These prepared quinoline derivatives have shown moderate to significant antibacterial activities. The minimum inhibitory concentration (MIC) was determined for the tested compounds.



Scheme 4. Reagents and conditions: (**a**) H_2SO_4, HNO_3, 5-10°C to rt 1h; (**b**) $Pd(PPh_3)_4$, $PhB(OH)_2$, Na_2CO_3 (2M), DME, reflux, 4h; (**c**) $Pd(OAC)_2$, PMHS, KF, H_2O, THF, rt, 3h.

Table 1. ***In Vitro*** **Antibacterial Activity of Compounds 4, 7, 8, 10, 11**

Compounds	R	Yield[a] (%)	MIC(μg/ml) (zones of inhibition in mm)				
			Escherichia coli	***Staphylococcus aureus***	***Pseudomonas Aeruginosa***	***Klebsiella pneumoniae***	***Salmonella Typhimurium***
4a	Isopropyl	79	200 (8)	50 (32)	>200 (10)	>200 (8)	No inhibition
4b	Pentyl	84	>200 (8)	>200 (17)	No inhibition	>200 (08)	No inhibition
4c	Cyclohexyl	73	100 (13)	50 (16)	200 (08)	200 (13)	>200 (10)
4d	Benzyl	61	No inhibition	>200 (12)	No inhibition	No inhibition	No inhibition
4e	Tolyl	70	100 (16)	100 (15)	>200 (10)	>200 (10)	>200 (12)
7a	Butyl	83	>200 (12)	100 (22)	No inhibition	No inhibition	No inhibition
7b	Isopropyl	84	100 (10)	200 (18)	No inhibition	>200 (08)	No inhibition
7c	Benzyl	75	100 (14)	50 (18)	>200 (12)	No inhibition	100 (18)
7d	Pentyl	79	200 (08)	200 (14)	200 (08)	200 (08)	200 (No inhibition)
8a	Ethyl	78	No inhibition	200 (13)	No inhibition	>200 (12)	>200 (15)
8b	Hexyl	76	No inhibition	100 (10)	No inhibition	>200 (08)	200 (No inhibition)
8c	Cyclohexyl	86	No inhibition	200 (16)	No inhibition	>200 (08)	>200 (10)
8d	Cyclopentyl	68	No inhibition	100 (13)	No inhibition	100 (12)	>200 (08)
10a	Butyl	75	200 (08)	200 (16)	No inhibition	No inhibition	No inhibition
10b	Isopropyl	84	200 (08)	200 (16)	No inhibition	No inhibition	No inhibition
11a	Butyl	80	200 (10)	100 (14)	No inhibition	No inhibition	No inhibition
11b	Isopropyl	85	>200 (10)	100 (13)	>200 (08)	>200 (10)	No inhibition
Gentamicin			< 4 (25)	< 4 (21)	< 4 (25)	< 4 (15)	< 4 (20)
Chloramphenicol			< 8 (30)	< 8 (18)	ND	< 8 (20)	< 8 (25)

a: Yield of isolated product.
ND: Not determined.

CONFLICT OF INTEREST

Declared none.

ACKOWLEDGEMENTS

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REFERENCES

[1] Dalhoff, A. Quinolone resistance in *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Development during therapy and clinical significance. *Infection*, **1994**, *22*(9), S111-S121.

[2] Lee, V.; Hecker, S. Antibiotic resistance versus small molecules, the chemical evolution. *J. Med. Res. Rev.,* **1999**, *19*(6), 521-542.

[3] Livermore, D. Antibiotic resistance in staphylococci. *Int. J. Antimicrob. Agents*, **2000**, *16*, S3-10.

[4] Poole, K. Multidrug resistance in Gram-negative bacteria. *Curr. Opin. Microbiol.,* **2001**, *4*(5), 500-508.

[5] Abbanat, D.; Macielag, M.; Bush, K. Novel antibacterial agents for the treatment of serious Gram-positive infections. *Expert Opin. Investig. Drug,* **2003**, *12*(3), 379-399.

[6] Nasveld, P.; Kitchener, S. Treatment of acute vivax malaria with tafenoquine. *Trans. R.Soc. Trop. Med. Hyg.*, **2005**, *99*(1), 2-5.

[7] Leatham, P.A.; Bird, H. A.; Wright, V.; Seymour, D.; Gordon, A. A double blind study of antrafenine, naproxen and placebo in osteoarthrosis. *Eur. J. Rhumatol. Inflamm.,* **1983**, *6*(2), 209-211.

[8] Denny, W.A.; Wilson, W.R.; Ware, D.C.; Atwell, G.J.; Milbank, J.B.; Stevenson, R.J. Anti-cancer 2,3-dihydro-1H-pyrrolo[3,2-f]quinoline complexes of cobalt and chromium. *U. S. Patent*. **2006**, 7064117.

[9] Mahamoud, A.; Chevalier, J.; Davin-Regli, A.; Barbe, J.; Pages, J. M. Quinoline derivatives as promising inhibitors of antibiotic efflux pump in multidrug resistant enterobacter aerogenes isolates. *Curr. Drug Targ.,* **2006**, *7*(7), 843-847.

[10] Muruganantham, N.; Sivakumar, R.; Anbalagan, N.; Gunasekaran, V.; Leonard, J.T. Synthesis, anticonvulsant and antihypertensive activities of 8-substituted quinoline derivatives. *Biol. Pharm. Bull.,* **2004**, *27*(10), 1683-1687.

[11] Maguire, M.P.; Sheets, K.R.; McVety, K.; Spada, A.P.; Zilberstein, A. A new series of PDGF receptor tyrosine kinase inhibitors: 3-substituted quinoline derivatives. *J. Med. Chem*., **1994**, *37*(14), 2129-2137.

[12] (a) Wilson, W.D.; Zhao, M.; Patterson, S.E.; Wydra, R.L.; Janda, L.; Strekowski, L.; Schinazi, R.F. Design of RNA interactive anti-HIV-1 agents: unfused aromatic intercalators. *Med. Chem. Res.,* **1992**, *2*, 102-110. (b) Strekowski, L.; Mokrosz, J.L.; Honkan, V.A.; Czarny, A.; Cegla, M.T.; Patterson, S.E.; Wydra, R.L.; Schinazi, R.F. Synthesis and quantitative structure-activity relationship analysis of 2-(aryl or heteroaryl)quinolin-4-amines, a new class of anti-HIV-1 agents. *J. Med. Chem.,* **1991**, *34*(5), 1739-1746.

[13] Aggarwal, A.K.; Jenekhe, S.A. New conjugated polyanthrazolines containing thiophene moieties in the main chain. *Macromolecules,* **1991**, *24*(25), 6806-6807. (b) Zhang, X.; Shetty, A.S.; Jenekhe, S.A. Electroluminescence and photophysical properties of polyquinolines. *Macromolecules,* **1999**, *32*(22), 7422-7429. (c) Jenekhe, S.A.; Lu, L.; Alam, M.M. New conjugated polymers with donor-acceptor architectures: Synthesis and photophysics of carbazole–quinoline and phenothiazine-quinoline copolymers and

oligomers exhibiting large intramolecular charge transfer. *Macromolecules,* **2001,** *34*(21), 7315-7324.

[14] Nilsson, J.; Nielsen, E.; Liljefors, T.; Nielsen, M.; Sterner, O. Azaflavones compared to flavones as ligands to the benzodiazepine binding site of brain $GABA_A$ receptors. *Bioorg. Med. Chem. Lett.,* **2008**, *18*(21), 5713-5716. (b) Jason, M.E.; Robert, W.C.; Mark, C.; Gary, G.C.; Peter, H.H.; Brian, A.J.; MacLeod, A.; Rose, M.; Georgina, M.L.; Elena, M.; Fraser, M.; Michael, R.; Inmaculada, R.; Michael, G.N.R.; Bindi, S.; Kwei, L.T.; Brian, W. *N*′,2-Diphenylquinoline-4-carbohydrazide based NK_3 receptor antagonists. *Bioorg. Med. Chem. Lett.,* **2006**, *16*(22), 5748-5751.

[15] Simeon, M.; John, N.; Georgia, L.; Eleni, Vasiliki, K.G.; Dimitrios T.; Pavlos, N. Nikolaos, C.; Georgios, T. Penetration of linezolid into sternal bone of patients undergoing cardiopulmonary bypass surgery. *Int. J. Antimicrob. Agents*, **2007**, *29*(6), 742-744. (b) Kaila, N.; Janz, K.; DeBernardo, S.; Bedard, P.W.; Camphausen, R.T.; Tam, S.; Tsao, D.H.H.; Keith J.C.; Nutter, C.N.; Shilling, A.; Sciame, R.Y.; Wang, Q. Synthesis and biological evaluation of quinoline salicylic acids as P-selectin antagonists. *J. Med. Chem.,* **2007**, *50*(1), 21-39. (c) Andersen, E.K.; Lundt, B.F.; Jorgensen, A.S.; Braestrup, C. Oxadiazoles as bioisosteric transformations of carboxylic functionalities II. *Eur. J. Med. Chem.,* **1996**, *31*(5), 417-425.

[16] Benzerka, S.; Bouraiou, A.; Bouacida, S.; Roisnel, T.; Belfaitah, A. 2-(2-Chloro-6,7-dimethylquinolin-3-yl)-2,3-dihydroquinolin-4(1*H*)-one. *Acta Cryst.* **2011**, E*67*, o2084-o2085. (b) Hayour, H.; Bouraiou, A.; Bouacida, S.; Berrée, F.; Carboni, B.; Roisnel, T.; Belfaitah, A. Synthesis and X-ray structures of new cycloalka[*e*]pyrano[2,3-*b*]pyridine derivatives: novel tacrine analogues. *Tetrahedron Lett*., **2011**, *52*, 4868-4871. (c) Bouraiou, A.; Berrée, F.; Bouacida, S.; Carboni, B.; Debache, A.; Roisnel,T.; Belfaitah, A. Efficient syntheses of new chromone- and chromanequinoline hybrids and their aza-analogs. *Lett. Org. Chem*., **2011**, *8*, 474-477. (d) Bouraiou, A.; Debache, A.; Rhouati, S.; Belfaitah, A.; Benali-Cherif, N.; Carboni, B. Synthesis of some new 3-pyrrolidinylquinoline derivatives *via* 1,3-dipolar cycloaddition of stabilized azomethineylide to quinolinyl α, β-unsaturated ketones. *Open Org. Chem. J.,* **2010**, *4*, 1-7.

[17] General experimental procedure: To a stirred solution of 2-chloroquinoline-3-carboxylic acid **3** (1 mmol) and triethylamine (2mmol) in chloroform (2mL) was added dropwise at 5-10°C, ethyl chloroformate (1mmol). The mixture was stirred at rt for 30 min before the addition of the appropriate amine (1mmol). After further stirring at rt for 4h, the mixture was extracted with dichloromethane**.** The organic layer was washed, dried over anhydrous Na_2SO_4, filtered and the solvent evaporated. The resultant solid was purified by crystallization using CH_2Cl_2/di-isopropylether to give the pure corresponding amide (Serie B). Selected data for *N-isopropyl-6-methyl-2-phenylquinolin-3-carboxamide* **7b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.98 (d, *J*=7.7 Hz, 1H), 7.66-7.39 (m, 7H), 5.12 (br, 1H), 4.00 (dq, *J* =7.7, 6.5 Hz, 1H), 2.48 (s, 3H), 1.18 (d, *J*=6.5 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$): δ 167.3, 155.2, 146.7, 139.8, 137.2, 137.1, 133.4, 129.7, 2x129.1, 2x129.0, 128.7, 126.8, 126.4, 41.9, 2x22.1, 21.7. Anal. Calcd. for $C_{20}H_{20}N_2O$: C, 78.92; H, 6.62; N, 9.20; found: C, 78.89; H, 6.63; N, 9.24.

[18] General experimental procedure: To 2-chloro-6-methylquinoline-3-carboxylic acid (1mmol) was added, at rt under nitrogen, $SOCl_2$ (2 mL) and the mixture was stirred at 80 °C for 24 h. After removal of the solvent and the excess of $SOCl_2$ under vacuum, the acyl chloride was recovered immediately and after cooling to 0°C an appropriate alcohol (2mmol) dissolved in toluene (5mL), was added over a period of 30 min. The mixture was further stirred at 110 °C for 24 h. After removal of the solvent under vacuum, the remaining acid was extracted with a solution of $NaHCO_3$. The resultant residue was purified by crystallization using CH_2Cl_2/di-isopropylether to give the pure product. Selected data for *cyclohexyl 6-methyl-2-phenylquinolin-3-carboxylate* **8c**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.00 (d, *J*=7.7 Hz, 1H), 7.60-7.39 (m, 7H), 4.80-4.76 (m, 1H), 2.48 (s, 3H), 1.76-1.02 (m, 10H).^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7, 157.3, 146.9, 141.0, 138.2, 137.2, 133.8, 129.1, 2x128.6, 2x128.3, 128.2, 126.9, 126.0, 125.9, 74.1, 2x31.1, 25.2, 2x23.5, 21.6. Anal. Calcd. for $C_{23}H_{23}NO_2$: C, 79.97; H, 6.71; N, 4.05; found: C, 79.95; H, 6.69; N, 4.17.

[19] Benzerka, S.; Bouraiou, A.; Bouacida, S.; Roisnel, T.; Belfaitah, A. N-Isopropyl-6-methyl-2-phenylquinoline-3-carboxamide. *Acta Cryst*., **2010**, E*66*, o2304-o2305.

[20] Crystallographic data (excluding structure factors) for compounds **2a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC832565. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

[21] Metwally, K. A.; Abdel-Aziz, L. M.; Lashine, E. M.; Husseiny M. I.; Badawya, R. H. Hydrazones of 2-aryl-quinoline-4-carboxylic acid hydrazides: Synthesis and preliminary evaluation as antimicrobial agents. *Bioorg. Med. Chem*., **2006**, *14*(24), 8675.

[22] Couvalin, P.; Leclerc, R.; Bingen, E.”*Antibiogramme*”, 2nd Ed.