

Microwave assisted synthesis of *N'*-(Alkanylidene)-2-propylquinoline-4-carbohydrazide derivatives and their antimicrobial potential

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Abstract— The quinoline core is a benzo-fused pyridine which exhibits wide range of therapeutic potentials that have been proven to have remarkable utilities in medicinal chemistry research. Discovery of new antibacterial agents is crucial to counter the challenge of drug-resistant bacterial infections. The aim of this present study is to synthesize *N'*-(alkanylidene)-2-propylquinoline-4-carbohydrazide derivatives **4a-h** in order to evaluate their antimicrobial properties for possible future drug discovery. Pfitzinger synthetic approach was used herein to convert isatin to **1** which upon esterification afforded **2** which was subsequently converted to **3** by hydrazinolysis. Microwave irradiation technique has been used herein as green method to access a series of functionalized *N'*-(alkanylidene)-2-propylquinoline-4-carbohydrazide derivatives **4a-h** in high yields within short time. The proposed structures of synthesized targeted compounds were validated spectroscopically by IR, UV, ¹H-NMR, ¹³C-NMR and DEPT-135 as well as through analytical data. The *in vitro* screening was investigated against six bacterial isolates using agar diffusion method while the minimum inhibitory concentration (MIC) was evaluated using serial dilution method. Out of all the series of **4a-h**, 2-propyl-(*N'*-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)quinoline-4-carbohydrazide **4e** emerged as the most active antimicrobial agent. This targeted quinoline motif **4e** might pave way for new bioactive template from future drug development.

Keywords— Pfitzinger approach, bioactive heterocycle, quinoline, hydrazinolysis, drug design, spectroscopy.

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Introduction

Over the years, the heterocyclic compounds have attracted numerous attentions due to their wide applications in medicinal chemistry research [1]. The heterocyclic template of interest in this present study is quinoline which has the log *P* value of 2.04 and an acidic *pK_b* of 4.85 and a basic *pK_a* of 9.5. Quinoline is a weak tertiary base. It can form salt with acids and displays reactions similar to those of pyridine and benzene. It is nontoxic to humans on oral absorption and inhalation [2]. Several quinoline derivatives isolated from natural resources or prepared synthetically are significant with respect to medicinal chemistry and

biomedical use [3]. Many derivatives of quinoline framework have been prepared using various synthetic approaches such as Skraup [4], Combes [5], Pfitzinger [6], Conrad-Limpach [7], Doebner-Miller [8] and Friedlander [9]. Furthermore, quinoline moieties have been proven to have remarkable utility in medicinal chemistry as one of the most widely used antibiotics and antimalarial in the world. Recently, quinolines and isoquinolines have been reported to possess pharmacological and clinical effects such as antimicrobial [10], antituberculosis [11], anticancer [12], anti-HIV [13], antidiabetic [14], among others. Due to drug resistant prevalence, outbreak of new disease and global health threat, it is conceivable to synthesize a new series of quinoline with hydrazide-hydrazone linker to evaluate their antimicrobial efficacy for possible future drug development.

Materials and Method

General Condition - All chemicals and reagent were obtained from Sigma-Aldrich Chemicals except hydrazine hydrate which was purchased from BDH Chemicals. All the reagents were of analytical grade and were made available by Department of Chemistry, Covenant University. The progress of the reaction was monitored with TLC and the spots visualized using a Varian ProStar 325 UV/vis dual wavelength detector. The ¹H- and ¹³C-NMR spectra were recorded on a NMR Bruker DPX 500 Spectrometer operating at the machine frequencies of 500 MHz and 125 MHz respectively using solvent (CDCl₃ or DMSO-*d*₆) with TMS as internal reference. Melting points were determined with open capillary tube on a Gallenkamp (variable heater) melting point apparatus and were uncorrected. The elemental analysis (C, H, N) of compounds were performed using a Carlo Erba-1108 elemental analyzer. The microwave assisted syntheses were carried out with the aid of CEM Discover Monomode oven operating at frequency of 2450 MHz monitored by a PC computer and temperature control was fixed at 140 °C within the power modulation of 400 W. The melting points were determined using stuart melting point apparatus and were uncorrected.

General procedure for microwave-assisted synthesis of hydrazide-hydrazone (**4a-h**)

2-Propylquinoline-4-carbohydrazide, **3** (3.0 g, 13 mmol) was dissolved in about 10 ml of ethanol in a sealed tube. The resulting mixture was then irradiated at 400 W in microwave oven for a period of 1 to 3 min as the case may be based on the result obtained from the monitored progress of reaction using TLC spotting in dichloromethane (DCM) as eluting solvent. The heated solution was allowed to cool to ambient temperature and filtered to afford the

corresponding hydrazide-hydrazone of quinoline (**4a-h**) in good to excellent yields.

***N'*-(Butan-2-ylidene)-2-propylquinoline-4-carbohydrazide (4a).** Microwave-assisted reaction of **3** (3.0 g, 13 mmol) with butan-2-one (1.16 ml, 13 mol) for 2 min afforded *N'*-(butan-2-ylidene)-2-propylquinoline-4-carbohydrazide, **4a**. Yield 3.25 g (82 %). UV-Vis.: λ_{\max} (nm)/ log ϵ_{\max} (mol⁻¹ cm⁻¹): 203 (4.04), 225 (4.08), 251 (4.10), 254 (4.38), 305 (4.11). IR (KBr, cm⁻¹) $\bar{\nu}$: 3415 (N-H of hydrazide), 2981 (CH aliphatic), 1633 (C=C), 1140 (C-N hydrazide), 982 (=C-H bending), 612 (Ar-H). ¹H-NMR (500 MHz, DMSO-d₆) δ_{H} : 7.75-7.73 (d, J = 10.0 Hz, 2H, Ar-H), 7.52 (s, 1H, Ar-H), 7.29-7.26 (dd, J₁ = 10.0 Hz, J₂ = 12.50 Hz, 2H, Ar-H), 5.80 (s, 1H, NH), 3.73-3.72 (m, 2H, CH₂), 3.31-3.25 (q, J = 8.0 Hz, 2H, CH₂CH₃), 3.17-3.11 (q, J = 8.2 Hz, 2H, CH₂CH₃), 2.40 (s, 3H, CH₃-C=N), 1.12-1.08 (t, J = 8.0 Hz, 3H, CH₃CH₂), 1.02-0.99 (t, J = 8.0 Hz, 3H, CH₃CH₂). ¹³C-NMR (125 MHz, DMSO-d₆) δ_{C} : 173.2 (C=O), 158.4, 151.2, 141.9, 135.0, 132.3, 127.4, 120.8, 117.0, 116.8, 110.0, 41.3, 29.3, 25.3, 20.2, 15.1, 10.1 (CH₃) ppm. DEPT 135 (125 MHz, DMSO-d₆) δ_{C} : Positive signals are 141.9 (CH), 135.0 (CH), 132.3 (CH), 117.0 (CH), 116.8(CH), 20.2 (CH₃), 15.1 (CH₃), 10.1 (CH₃). Negative signals are 41.3, 29.3, 25.3 (CH₂) ppm.

***N'*-(Pentan-2-ylidene)-2-propylquinoline-4-carbohydrazide (4b).** Microwave-assisted reaction of **3** (3.0 g, 13 mmol) with pentan-2-one (1.38 ml, 13 mmol) for 2 min afforded *N'*-(pentan-2-ylidene)-2-propylquinoline-4-carbohydrazide, **4b**. Yield 3.16 g (76%). UV-Vis.: λ_{\max} (nm)/ log ϵ_{\max} (mol⁻¹ cm⁻¹): 225 (4.10), 250 (4.31), 265 (4.45), 275 (4.75), 317 (4.61). IR (KBr, cm⁻¹) $\bar{\nu}$: 3358 (N-H of hydrazide), 3151 (C-H aromatic), 2925 (C-H aliphatic), 2805 (C-H aliphatic), 1683 (C=O of hydrazide), 1603 (C=C aromatic), 1589 (C=N of hydrazone), 1467 (CH₃ deformation), 1248 (C-N), 982 (=C-H bending), 749 (Ar-H). ¹H-NMR (500 MHz, DMSO-d₆) δ_{H} : 7.77-7.75 (d, J = 10.0 Hz, 2H, Ar-H), 7.75 (s, 1H, Ar-H), 7.28-7.26 (dd, J₁ = 10.0 Hz, J₂ = 12.50 Hz, 2H, Ar-H), 4.76 (s, 1H, NH), 3.53-3.48 (q, J = 8.5 Hz, 2H, CH₂CH₃), 3.34-3.29 (q, J = 8.9 Hz, 2H, CH₂CH₃), 2.40 (s, 3H, CH₃-C=N), 2.08 (m, 2H, CH₂), 1.86 (m, 2H, CH₂), 1.27-1.24 (t, J = 8.9 Hz, 3H, CH₃CH₂), 1.10-1.06 (t, J = 8.5 Hz, 3H, CH₃CH₂). ¹³C-NMR (125 MHz, DMSO-d₆) δ_{C} : 173.1 (C=O), 158.8, 151.3, 141.9, 135.0, 132.3, 127.4, 120.8, 117.0, 116.8, 110.0, 41.3, 33.3, 29.3, 25.3, 20.2, 15.1, 10.1 (CH₃) ppm. DEPT 135 (125 MHz, DMSO-d₆) δ_{C} : Positive signals are 141.9 (CH), 135.0 (CH), 132.3 (CH), 117.0 (CH), 116.8(CH), 20.2 (CH₃), 15.1 (CH₃), 10.1 (CH₃). Negative signals are 41.3, 33.3, 29.3, 25.3 (CH₂) ppm.

Antimicrobial activity assay. The general sensitivity testing of the titled compounds **4a-h** against six organisms was monitored in comparison with that of gentamicin clinical standard using agar diffusion method as described by a standard method [15]. The six organisms comprised of three gram positive (*Staphylococcus aureus*, *Bacillus licheniformis* *Micrococcus varians*) and three gram negative (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) organisms.

Result and Discussion

Quinoline is a heterocyclic scaffold of paramount importance to the human race. In view of our current trust in the microwave-assisted organic synthesis [16] and various findings mentioned earlier, there is merit in developing a facile route for the formation of quinoline incorporated with hydrazide-hydrazone templates via microwave synthetic approach to investigate the antimicrobial properties of such targeted library. The Pfitzinger reaction of pentan-2-one with isatin afforded **1** which was esterified to furnish **2** which was subsequently treated with hydrazine hydrate to give carbohydrazide **3** as shown in Scheme 1a. The head group modification of the free NH₂ of carbohydrazide **3** was achieved by simple condensation of the reactive intermediate **3** with aliphatic and alicyclic ketones to afford **4a-h** in good to excellent yields (Scheme 1b). Although, the precursor and reactive intermediates were synthesized via conventional heating method under reflux, the final targeted products **4a-h** were synthesized using microwave assisted methods (MAM). From Table 1, it was observed that preparation of the quinoline products **4a-h** was highly favoured under microwave method in term of higher product yields (70-94%) and shorter reaction times (1-3 min). Thermodynamic justification for faster reaction rate under microwave could be explained by the works of Gude *et al.*, who established by Arrhenius equation, that the reaction rate under microwave was 1000 fold higher than that from conventional heating approach [17]. The melting point of the compounds ranged from 255 °C for **4h** to > 300 °C for **4e**. The colour of the titled products was identified to be white for **4e**, black for **4h**, red for **4f** while the rest of the compounds were brown in colour. The result of the elemental analysis for C, H, N showed consistence and agreement between % calculated and % observed with not more than ±0.20 difference.

<<<<<Scheme 1>>>>>

<<<<<Table 1>>>>>

The characterization was carried out using FT-IR, UV, ¹H- and ¹³C-NMR analyses. Infrared spectra of the compounds (**4a-h**) showed absorption bands due to the stretching vibrational bands at 3415-3244 cm⁻¹, 3151-3010 cm⁻¹, 2981-2805 cm⁻¹, 1715-1668 cm⁻¹, 1638-1603 cm⁻¹ and 1589-1575 cm⁻¹ which depicted the presence of N-H, C-H of aromatic, C-H of aliphatic, C=O, C=C and C=N functional entities respectively while the electronic transition in UV-vis spectra of the same titled products gave rise to wavelength (λ_{\max}) ranging from 203 nm for **4a** to 320 nm for **4d**. The result of ¹H-NMR spectral of quinoline-based hydrazide-hydrazone **4a-h** showed the presence of aromatic protons downfield at δ 7.75-7.19 ppm while the shielded protons appeared upfield at δ 3.73 to 0.89 ppm, but the N-H protons of hydrazide was observed at δ 6.01-4.76 ppm. The antibacterial screening of the titled hydrazide-hydrazones **4a-h** alongside that if clinical standard gentamicin was investigated using agar diffusion method [15] and the zones

of inhibition was recorded in millimeter (Table 2). The most active antibacterial agent was 2-propyl-(*N*-(1,7,7-trimethyl bicyclo[2.2.1]heptan-2-ylidene)quinoline-4-carbohydrazide, **4e** with broad zones of inhibition ranging from 30-40 mm. Overall, we have been able to show that head group modification of alkanylidene is a viable route for the synthesis of quinoline antibacterial analogues bearing hydrazide-hydrazone linker. Further modifications of hydrazone side chain to include diversified alkyl chain lengths are currently under investigation as well as linkage of tail regions with increased activity profiles. Disclosure of the full tail region library and minimum inhibitory concentration (MIC) testing will be presented in due course.

<<<<<Table 2>>>>>

Conclusion

The present study has demonstrated that the implemented synthetic methodology under microwave condition was a highly efficient approach for accessing a variety of substituted quinoline hydrazide-hydrazones in good to excellent yields. The broad spectrum of biological activities noticed in the *in vitro* screening of the titled compounds **4a-h** showed that they might pave way towards harnessing diversified moieties of this class for future drug discovery.

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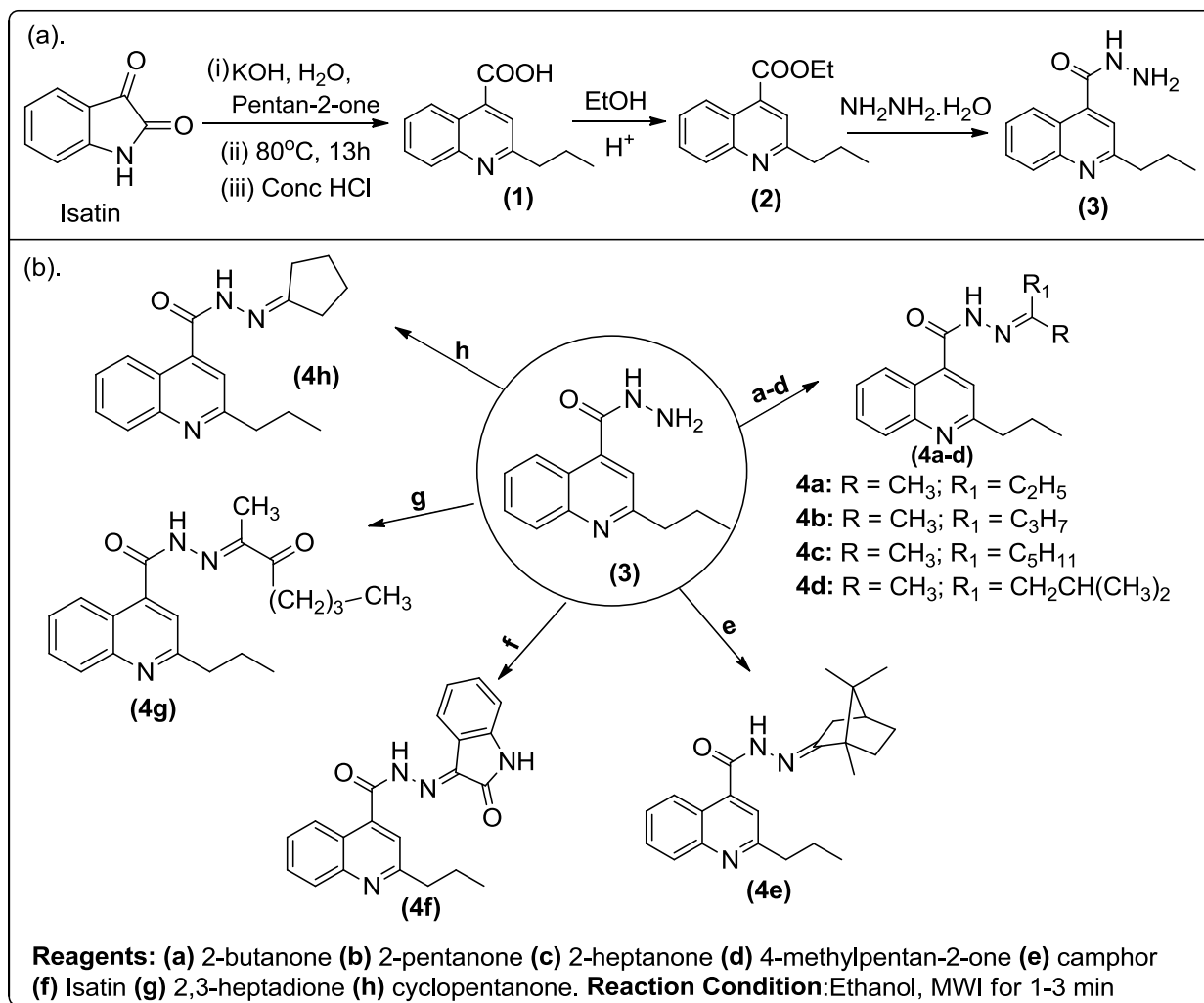
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Scheme 1: (a) Synthesis of carbohydrazone precursor, 3. (b) Synthesis of hydrazone-hydrazones 4a-h

Table 1. Physico-chemical properties of the synthesized compounds (4a-h)

Compd No	Molecular formula	Mol. Wt.	Yield (%)	Melting pt (°C)	Colour	Elemental analysis (%)		
						Calcd.	(Found)	
						C	H	N
4a	C ₁₇ H ₂₁ N ₃ O	283.87	82	258 (s)	Brown	72.11(72.06)	7.52(7.47)	14.92(14.83)
4b	C ₁₈ H ₂₃ N ₃ O	297.39	76	261-264	Brown	72.65(72.70)	7.74(7.80)	14.09(14.13)
4c	C ₂₀ H ₂₇ N ₃ O	325.46	85	263-265	Brown	73.71(73.81)	8.27(8.36)	13.04(12.91)
4d	C ₁₉ H ₂₅ N ₃ O	311.42	70	265-268	Brown	73.12(73.28)	8.15(8.09)	13.58(13.49)
4e	C ₂₃ H ₂₉ N ₃ O	363.50	77	>300	White	75.88(76.00)	8.16(8.04)	11.66(11.56)
4f	C ₂₁ H ₁₈ N ₄ O	358.39	94	>300	Red	70.38(70.21)	5.06(4.94)	15.63(15.48)
4g	C ₂₀ H ₂₅ N ₃ O ₃	339.43	81	290-291	Brown	70.77(71.01)	7.42(7.59)	12.38(12.55)
4h	C ₁₈ H ₂₁ N ₃ O	295.38	89	255 (s)	Black	73.19(72.99)	7.17(6.97)	14.23(14.39)

Comp No = Compound No; Mol. Form. = Molecular Formular; Mol. Wt. = Molecular Weight; (s) = Sharp.

Table 2. The result of sensitivity testing of **4a-h** with zones of inhibition in mm

Comp	Organisms used and Z.O.I. (mm)					
	<i>S. aureus</i>	<i>B. licheniformis</i>	<i>M. varians</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>P. aeruginosa</i>
4a	39.00 ± 0.10	38.00 ± 0.09	33.00 ± 0.10	30.00 ± 0.09	R	29.00 ± 0.09
4b	28.00 ± 0.09	23.00 ± 0.09	23.00 ± 0.09	15.00 ± 0.09	13.00 ± 0.08	24.00 ± 0.09
4c	18.00 ± 0.08	31.00 ± 0.09	24.00 ± 0.09	25.00 ± 0.09	18.00 ± 0.08	26.00 ± 0.09
4d	38.00 ± 0.11	13.00 ± 0.08	14.00 ± 0.08	11.00 ± 0.09	13.00 ± 0.08	20.00 ± 0.09
4e	40.00 ± 0.10	33.00 ± 0.10	33.00 ± 0.09	30.00 ± 0.09	31.00 ± 0.10	38.00 ± 0.09
4f	25.00 ± 0.08	16.00 ± 0.09	11.00 ± 0.08	15.00 ± 0.09	14.00 ± 0.08	14.00 ± 0.08
4g	19.00 ± 0.08	8.00 ± 0.08	8.00 ± 0.08	3.00 ± 0.09	32.00 ± 0.10	4.00 ± 0.08
4h	42.00 ± 0.11	11.00 ± 0.09	14.00 ± 0.08	4.00 ± 0.09	17.00 ± 0.09	8.00 ± 0.08
GTM	23.00 ± 0.08	15.00 ± 0.09	18.00 ± 0.09	25.00 ± 0.09	25.00 ± 0.09	R

P. aeruginosa = *Pseudomonas aeruginosa*, *S. aureus* = *Staphylococcus aureus*, *E. coli* = *Escherichia coli*, *B. licheniformis* = *Bacillus licheniformis*, *P. vulgaris* = *Proteus vulgaris*, *M. varian* = *Micrococcus varian*. GTM. = Gentamicin. R = Resistance. Mean ± Standard deviation of triplicate measurements.

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	Dr. Olayinka Oyewale AJANI has established himself both as an academic & a researcher in the areas of Organic Synthesis and Medicinal Chemistry. Dr. Ajani specializes in organic synthesis and medicinal chemistry research. He is a recipient of many prestigious awards some of which are German DAAD Short Research Visit Fellowship, TWAS Research Grant for individual Scientist & 2009 CAS-TWAS Fellowship Award.
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