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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITIES OF HYDRAZONE SCHIFF BASE COMPOUNDS AND ITS DERIVATIVES

(Sintesis, Pencirian dan Aktiviti Antibakteria Sebatian Bes Schiff Hidrazon dan Terbitannya)

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Abstract

Biological activities (e.g. antibacterial) of hydrazone compound have received much attention from the synthetic chemists since last two decades. Herein, we would like to report the synthesis pathways as well as the spectroscopic characterization of three etherified hydrazone Schiff base compound, which were initiated from 2-hydroxyacetophenone. Hydrazone Schiff base compound was obtained through condensation reaction between 2-hydroxyacetophenone with benzhydrazide. Meanwhile the etherified derivatives of hydrazone Schiff base were prepared *via* Williamson ether synthesis under reflux condition. All the synthesized compounds were characterized using Fourier transformation infrared, UV-Vis and ^{1}H nuclear magnetic resonance spectroscopy. In addition, the antibacterial activities of these compounds were also conducted using disc diffusion method against *Bacillus cereus* and *Escherichia coli*. The results are discussed in this present paper.

Keywords: hydrazone Schiff base, etherified derivatives, antibacterial

Abstrak

Aktiviti biologi (contoh: antibakteria) sebatian hidrazon telah menerima banyak perhatian daripada ahli kimia sintetik sejak dua dekad yang lalu. Di sini, kami ingin melaporkan kaedah sintesis serta pencirian spektroskopi tiga hidrazon yang telah di eterifasi, dimulakan dari 2-hidroksiasetofenon. Bes-Schiff hidrazon telah diperolehi melalui tindak balas pemeluwapan antara 2-hidroksiasetofenon dengan benzihidrazida. Sementara itu, terbitan yang telah di eterifasi daripada bes Schiff hidrazon telah disediakan melalui kaedah sintesis eter Williamson di bawah keadaan refluks. Semua sebatian yang disintesis dicirikan menggunakan inframerah transformasi Fourier, spektroskopi UV-Vis dan ^{1}H magnetik nukleus resonan. Di samping itu, aktiviti antibakteria sebatian ini juga dijalankan dengan menggunakan kaedah cakera penyebaran terhadap *Bacillus cereus* dan *Escherichia coli*. Keputusan dibincangkan dalam kajian ini.

Kata kunci: bes Schiff hidrazon, terbitan yang telah dieterifasi, antibakteria

Introduction

Hydrazones generally are prepared by reacting a stoichiometric amount of hydrazide (R-NH-NH_2) and a carbonyl (C=O) compound in suitable solvent under reflux condition [1]. Hydrazone Schiff base compounds and complexes have attracted considerable amount of attention since last two decades due to their pharmaceutical activities such as antiproliferative effect, antimicrobial, antibacterial, antifungal, anti-inflammatory [2], anticonvulsant, antitubercular, antiviral, antioxidative effects and inhibition of tumor growth [3]. These biological activities enable the hydrazone

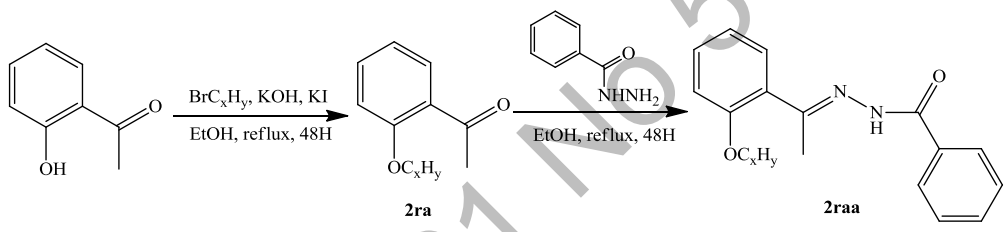
compounds and their complexes are suitable to be used as herbicides, insecticides, nematicides, rodenticides and plant growth regulators [4].

On the other hand, compounds with long carbon chain i.e. C_8 and above, received much interest from the researchers as they were reported to bear the potential as organogels in nanostructure formation [5], surfactant and in antibacterial [6]. In addition, the existence of the long carbon chain in the structure was reported to support the ability of the molecule to self-assemble into various architectures, enhance lipophilicity and antibacterial properties of the compound [6].

In this project, we report the synthesis of three hydrazone Schiff base compounds bearing carbon chains of C_8H_{17}, $C_{10}H_{21}$ and $C_{12}H_{25}$. The compounds were prepared by reacting etherified ketones with benzhydrazide *via* condensation reaction as shown in scheme 1. Antibacterial study using disc diffusion method was conducted to find the correlation between the presences of long carbon chain with the antibacterial properties of the compound.

Materials and Methods

All ^{1}H Nuclear Magnetic Resonance (NMR) spectra were recorded by 500 MHz of JEOL ECA-500 using $CDCl_3$ and DMSO-d_6 as solvent and TMS as internal standard for ^{1}H NMR. UV-Vis spectra were recorded using Jasco V-630 spectrophotometer. The Fourier Transformation Infrared (FTIR) were recorded using Thermo Scientific Nicolet iS10.



Compound	x	y
2ra8	8	17
2ra10	10	21
2ra12	12	23
2raa8	8	17
2raa10	10	21
2raa12	12	23
2raa	0	1

Scheme 1. Synthesis of hydrazone Schiff base compound and its derivatives

Synthesis of etherified ketone: Synthesis of 2ra8

1-Bromooctane (0.01mol) was added dropwise to the yellow ethanolic solution of 2-hydroxyacetophenone (0.01mol). Pellet of potassium hydroxide (0.01 mol) and powder of potassium iodide (1 g) were added into the mixture prior before mixture was heated under reflux for 48 hours. The resulting solution which appeared in red liquid was cooled, filtered and extracted using water and diethyl ether obtaining a thick yellow liquid. Yield: 60%.

Synthesis of 2ra10

The experimental procedure is similar to that in synthesis of **2ra8** except 1-bromooctane was replaced with 1-bromodecane. Compound **2ra10** was obtained as thick yellow liquid. Yield: 52%.

Synthesis of 2ra12

The experimental procedure is similar to that in synthesis of **2ra8** except 1-bromooctane was replaced with 1-bromododecane. Compound **2ra12** was obtained as thick yellow liquid. Yield: 75%.

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Synthesis of derivatives of hydrazone Schiff base: Synthesis of 2raa8
Benzhydrazide (0.001 mol) was dissolved in 20 ml hot ethanol in a round bottom flask. To the ethanolic solution of benzhydrazide, yellow liquid of **2ra8** (0.001 mol) was added dropwise with constant stirring and heating. The mixture was heated under reflux for 48 hours in ethanol. Resulting yellow liquid was cooled and after 3 days slow evaporation, white solid obtained. Yield: 56%, white solid.

Synthesis of 2raa10
The experimental procedure is similar to that in synthesis of **2raa8** except **2ra8** was replaced with **2ra10** (0.001 mol). Yield: 60%, pale yellow solid.

Synthesis of 2raa12
The experimental procedure is similar to that in synthesis of **2raa8** except **2ra8** was replaced with **2ra12** (0.001 mol). Yield: 45%, yellow solid.

Synthesis of parent compound, 2-hydroxyacetophenonebenzhydrazone, 2raa
The experimental procedure is similar to that in synthesis of **2raa8** except **2ra8** was replaced with 2-hydoxyacetophenone. Yield: 89%, white powder.

Antibacterial evaluation of the compounds
The assay was carried out on the parent compound (**2raa**) and the derivatives (**2raa8 – 2raa10**) using disc diffusion method [7]. One colony of *Bacillus cereus* ATCC 33019 from a streak plate was inoculated in 20 ml of Luria-Bertani broth. After 16 hours of incubation, the optical density of the inoculums was measured and further diluted to achieve McFarland standard of 0.5. Using sterile cotton swab, agar plate was uniformly swabbed with diluted inoculums of the bacteria before sterile filter paper (6 mm) which were impregnated with different concentrations of **2raa** and the derivatives **2raa8-2raa10** (0.16%-0.2%) using DMSO as solvent were placed on the agar. The measurements of the inhibitory zones (mm) were taken after 24 hours of incubation. The procedures were conducted in another set for *Escherichia coli* ATCC 35150 and were performed in triplicate.

Results and Discussion

FTIR spectroscopy
The parent compound namely, 2-hydroxyacetophenonebenzhydrazone (**2raa**) was prepared by reacting 2-hydroxyacetophenone with benzhydrazide under reflux using ethanol as solvent, whereas for the preparation of derivatives of hydrazone Schiff base compound, the starting material, 2-hydroxyacetophenone, was reacted with bromoalkane of C8, C10 and C12 carbons chain in the presence KOH and KI to obtain etherified ketones **2ra8 – 2ra12** (Scheme 1). The presence of long carbon chain in the compounds were indicated by the presence of C-H alkane stretching band in range of 2925.26 and 2852.54 cm^{-1} in IR spectrum [8]. The OH stretch that presence in the starting material ketone is no longer detected in the etherified ketone, **2ra8 – 2ra12** (Figure 1).

The etherified ketones, **2ra8 – 2ra12** were then reacted with benzhydrazide under reflux using ethanol as the solvent to obtain the derivatives of hydrazone Schiff base compounds, **2raa8 – 2raa12**. The formation of the hydrazone Schiff base compounds were supported by the presence of stretching band at 3204cm^{-1}attributed to the presence of N-H moiety in the hydrazone Schiff base [3]. On top of N-H band, the appearance of C=N stretching at 1600 cm^{-1} also supported the formation of hydrazone Schiff base [3]. The major difference between parent ligand and the derivatives is the presence of alkane stretch at 2922.23 to 2825.42 cm^{-1}[8].

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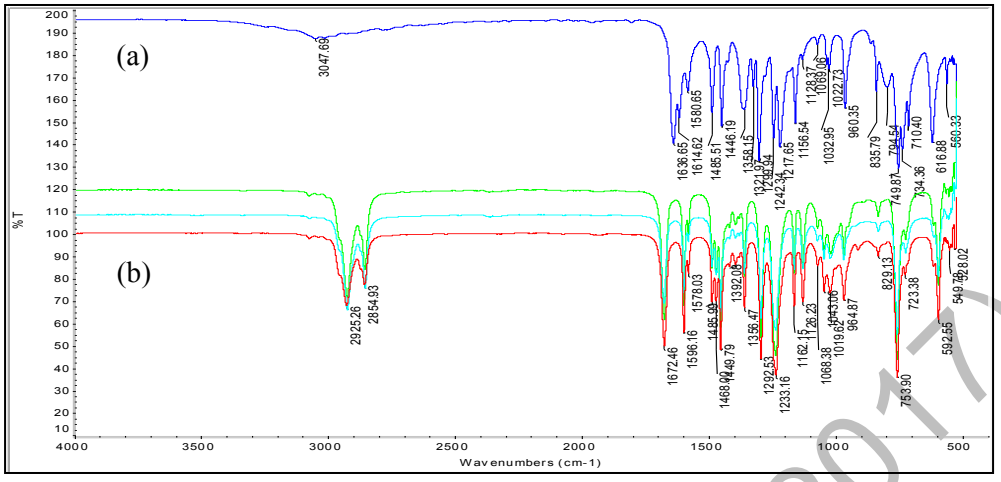

Figure 1. Infrared spectra of (a)starting material, 2-hydroxyacetophenone, (b)etherified 2-hydroxyacetophenone, **2ra8 – 2ra12**

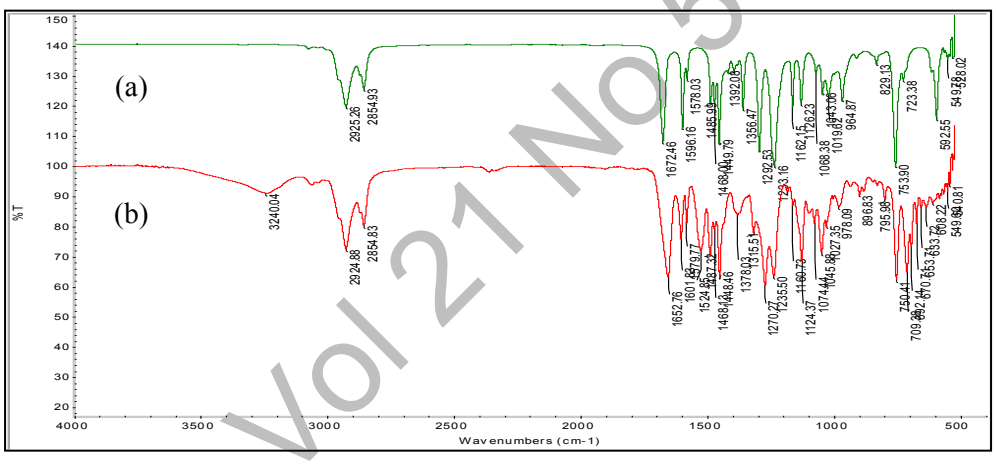

Figure 2. Infrared spectra of (a) etherified ketone, **2ra8** and (b) derivatives of Schiff base, **2raa8**

Table1. Summary of IR spectra stretches of the compounds

	ν(NH) cm^{-1}	*ν*(alkane) cm^{-1}	*ν*(C=O) cm^{-1}	*ν*(C=N) cm^{-1}	*ν*(N-N) cm^{-1}
2-octyloxyacetophenone (**2ra8**)	-	2925.26, 2854.93	1672.46	-	-
2-decyloxyacetophenone (**2ra10**)	-	2923.25, 2853.57	1673.00	-	-
2-dodecyloxyacetophenone (**2ra12**)	-	2922.31, 2852.54	1673.50	-	-
2-octyloxyacetophenone benzhydrazone (**2raa8**)	3240.04	2925.88, 2854.83	1652.76	1601.80	896.00
2-decyloxyacetophenone benzhydrazone (**2raa10**)	3239.09	2923.60, 2853.69	1654.94	1602.20	897.00
2-dodecyloxyacetophenone benzhydrazone (**2raa12**)	3248.21	2922.23, 2825.42	1655.74	1600.40	881.00
2-hydroxyacetophenone benzhydrazone (**2raa**)	3205.95	-	1647.71	1604.13	894.00

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UV-Vis

The UV-Visible electronic spectra of parent compound, **2raa** and derivatives **2raa8 – 2raa12** were measured at room temperature in ethanol solution within range 200 to 800 nm. The data is summarized in Table 2. UV-Vis spectrum for **2raa** showed 2 peaks (Figure 3) at 283 and 328 nm which attributed toπ to π* and n to π* transitions indicating the presence of benzene ring and amino C=N respectively [7].

Table 2. Electronic absorption spectra data of the hydrazone Schiff base 2raa and derivatives

Compound	λ_m(nm)
2raa	283, 328
2raa8	273, 375
2raa10	274, 375
2raa12	275, 375

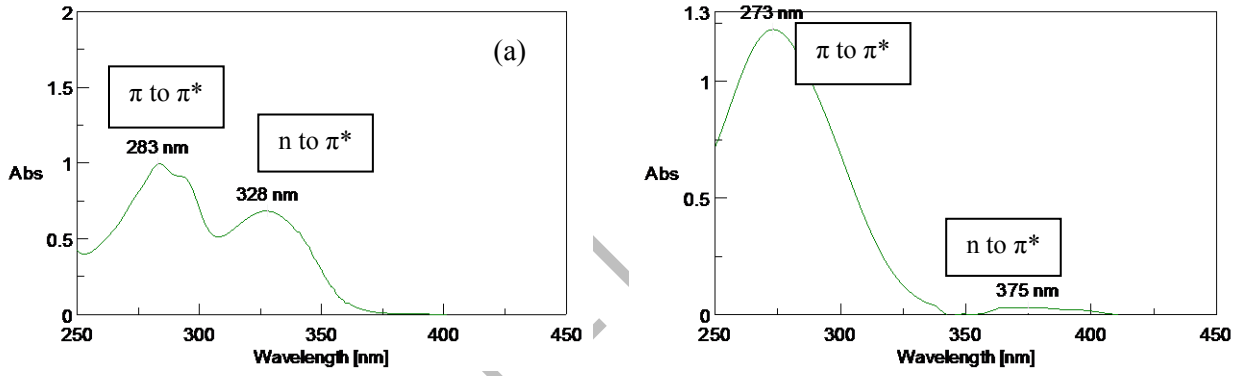

Figure 3. Electronic spectra of (a) parent compound (**2raa**), (b) derivative of Schiff base compound (**2raa8**)

Comparing the spectra of parent ligand and the derivatives, the peak of the derivatives shifted to a higher wavelength which is from 283 to 375 nm indicating the presence of long carbon chain in the moiety. It is known that the presence of electron donating group as substituent in aromatic molecule has significant effect on the absorption band in electronic spectra [8]. Long carbon chain is a donating group in which it decreased the energy gap between LUMO and HOMO in molecular orbital of azomethine C=N hence resulting in lower energy, increasing the wavelength as shown in the electronic spectra of the derivatives.

Nuclear magnetic resonance spectroscopy

Comparing the NMR spectra of starting material, 2-hydroxyacetophenone and etherified ketone, **2ra8 – 2ra12**, the presence long carbon chains in the **2ra8 – 2ra12** moiety were indicated by the presence of signals in range of 4.04 – 0.82 ppm [6] and disappearance of OH signal of hydroxyl group at meta position of starting material. Etherified ketones, **2ra8 – 2ra12** were reacted with benzhydrazide to form the derivatives of hydrazone Schiff base, **2raa8 – 2raa12** which were indicated by the presence of aromatic proton in the range of 7.56 – 6.97 ppm with the total number of nine protons [6] representing two benzene rings in the structure of the derivatives and also the presence of NH signal at 8.90 ppm [9]. The summary was tabulated in Table 3.

Table 3. Summary of significant ^{1}H NMR spectral data for compounds

Compounds	Chemical Shifts, δ(ppm)				
	OH	NH	CH aromatic	CH_3	Alkane
2ra8	-	-	7.71 – 6.91 (d, t)	2.61 (s)	4.01 (t) 1.82 – 0.85 (d, t)
2ra10	-	-	7.73 – 6.93 (d, t)	2.61 (s)	4.02 (t),1.83 – 0.87 (d, t)
2ra12	-	-	7.22 – 6.88 (d, t)	2.60 (s)	4.0 (t), 1.84 – 0.84 (d, t)
2raa8	-	8.9 (s)	7.56 – 7.01 (d, t)	2.39 (s)	3.99 (t), 1.72 – 0.84 (d, t)
2raa10	-	8.93 (s)	7.56 – 6.97 (d, t)	2.39 (s)	3.98 (t), 1.71 – 0.74 (d, t)
2raa12	-	8.93 (s)	7.56 – 7.01 (d, t)	2.38 (s)	3.97 (t), 1.78 – 0.84 (d, t)
2raa	13.37 (s)	11.34 (s)	7.96 – 6.91 (d, t)	3.35 (s)	13.37 (s)

s: singlet, d: duplet, t: triplet

Antibacterial activities

Disc diffusion method was used to evaluate the antibacterial properties of the hydrazone Schiff base compound, **2raa** and its long carbon chain derivatives, **2raa8 – 2raa12**. Gram positive bacteria, *Bacillus cereus* ATCC 33019 and gram-negative bacteria, *Escherichia coli* ATCC 35150 were used in this study. Compounds **2raa** and **2raa8 – 2raa12** were dissolved into the concentration of 0.16 to 0.020% using DMSO and the diameters of inhibition of the compounds were measured in mm unit. Inhibitions less than 10 mm is considered weak and insignificant, whereas more than 10mm is considered moderate and more than 16 mm is considered significant [10]. For *Bacillus cereus*, the derivatives of Schiff base, **2raa8 – 2raa12** showed better antibacterial properties than the parent compound (**2raa**) at all concentrations (Table 4).

Table 4. Results of antibacterial properties of compounds against *Bacillus cereus* ATCC 33019

Compound	Diameter of Inhibition Zone (mm)				
	Concentrations of Compounds in DMSO				
	DMSO only	0.16%	0.08%	0.04%	0.02%
2raa	-	10	-	-	-
2raa8		15	12	11	10
2raa10		15	10	10	10
2raa12		11	-	-	-

The azomethine group of Schiff base compound is known to possess antibacterial properties as the group have the ability to form H bond with the intracellular components of the bacteria and interfere with the normal cell activity thus lead to the cell death [11]. The presence of long chain in moiety of the compound has increased the lipophilicity of the compound thus assist in the penetration through the peptidoglycan of the bacteria [6]. Synergistically, the effect of these two factors has enhanced the antibacterial properties of the compound. At the concentration of 0.16%, **2raa8** and **2raa10** showed better inhibition than **2raa12**. The antibacterial activity was found to increase with longer chain carbon until certain length where it reached cutoff effect where long chain no longer serves it purpose (as antibacterial agent) [6]. Hence, we can see that at 12 carbon compound namely **2raa12** has weaker inhibition compared to **2raa8** and **2raa10** at 0.16%. **2raa12** also showed insignificant inhibition at 0.02 – 0.08% concentration of compound. Meanwhile antibacterial screening against *Escherichia coli* showed that at all concentrations the incorporation of long chains into the compound (Table 5) has increased the antibacterial activities compared to the parent compound (**2raa**).

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Table 5. Results of antibacterial properties of compounds against *Escherichia coli* ATCC 35150

Compound	Diameter of Inhibition Zone (mm)				
	Concentrations of Compounds in DMSO				
	DMSO only	0.16%	0.08%	0.04%	0.02%
2raa	-	-	-	-	-
2raa8		11	10	10	-
2raa10		16	-	-	-
2raa12		16	14	12	11

Conclusion

Hydrazone Schiff base compound and its long derivatives were successfully synthesized and characterized using ^{1}H NMR, UV-Vis and FTIR. Antibacterial activities of the compounds were evaluated against *Bacillus cereus* and *Escherichia coli* using disc diffusion method where long carbon chain derivatives of hydrazone Schiff base (**2raa8 – 2raa12**) showed considerable antibacterial properties compared to the parent compound (**2raa**). The cooperative effect in between the ability of azomethine group of Schiff base compound to disrupt the normal processes of the bacterial cell by forming H bond with the intracellular components of the bacterial cell and the enhancement in the lipophilicity of the compound by the incorporation of long carbon chain into the moiety of the Schiff base compound that allow easy penetration through the peptidoglycan of the bacterial cell has increased the antibacterial properties of derivatives of the hydrazone Schiff base compound. This can be observed through the antibacterial activities result where the zone inhibition of Schiff base compound was increase upon incorporation of long carbon chain into the moiety of the compound.

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