



SYNTHESIS AND CHARACTERISATION OF 4-PROPOXYBENZALDEHYDE SUBSTITUTED HECK-SCHIFF BASE COMPOUND AS SPACER IN ELECTROCHEMICAL DNA SENSOR

(Sintesis dan Pencirian Sebatian 4-Propoksibenzaldehid Gantian Heck-Schiff Bes sebagai
Peruang dalam Sensor DNA Elektrokimia)

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Abstract

Schiff base compound produced from aromatic aldehydes and aromatic amines has been used in a wide range of applications in various fields, including pharmaceutical, life sciences and chemical science (e.g. inorganic and analytical chemistry). It has also been applied in optical and electrochemical sensors to enable the detection of enhanced selectivity and sensitivity of the sensors. These compounds give excellent prospects in sensor study. In this study, Schiff base compound derived from 4-(4-nitrostyryl) aniline and 4-(4-aminostyryl)benzonitrile with 4-propoxybenzaldehyde was synthesised using ethanol as the solvent. All compounds were characterised by using the Fourier transform infrared spectroscopy (FTIR), UV-Vis spectrophotometer and Nuclear Magnetic Resonance (NMR). FTIR showed the formation of C=N (imine) stretching vibrations within the range of 1604.36 – 1606.51 cm⁻¹. In UV-vis, π - π^* electronic transition of the C=N in both compounds was observed within the peak range of 361-385 nm. Meanwhile in the ¹H NMR, the peak of CH=N (Imine) group was found at δ 8.32-8.34 ppm, whereas for ¹³C NMR, the peaks were discovered at δ 158.72, δ 158.80 and 158.72 ppm. The application of the Schiff base as a linker in electrochemical DNA sensor (E-DNA) still has not been studied previously. In this study, two new Schiff base derivatives were synthesised and characterised due to its promising potential in sensors, which prompted its application as a future potential linker in electrochemical DNA sensor.

Keywords: Schiff base, synthesis, spacer, E-DNA sensor

Abstrak

Sebatian Schiff bes yang dihasilkan daripada aromatik aldehid dan aromatik amina telah digunakan dalam pelbagai aplikasi dalam pelbagai bidang termasuk farmaseutikal, sains hayat dan sains kimia termasuk kimia tak organik dan analisis kimia. Ia juga telah digunakan dalam sensor optik dan elektrokimia untuk membolehkan pengesanan meningkatkan pemilihan dan kepekaan sensor. Sebatian ini mungkin memberi prospek yang sangat baik dalam sensor. Sebatian Schiff bes yang diterbitkan daripada 4-(4-nitrostyryl) anilina dan 4-(4-aminostyryl) benzonitril dengan 4-propoksibenzaldehid disintesis menggunakan etanol sebagai pelarut. Kesemua sebatian telah dicirikan oleh spektroskopi inframerah transformasi Fourier (FTIR), UV-Vis spektrofotometer dan Resonans Magnet Nukleus (NMR). FTIR menunjukkan pembentukan C=N (imina) perengangan getaran pada julat 1604.36 – 1606.51 cm⁻¹. Dalam UV-Vis, peralihan elektron π - π^* imina untuk kedua-dua sebatian diperhatikan pada julat puncak 361-385 nm. Sementara itu, puncak ¹H NMR untuk kumpulan CH=N (imina) telah dijumpai pada δ 8.32-8.34 ppm dan untuk ¹³C NMR puncaknya telah dijumpai pada δ 158.80 dan 158.72 ppm. Aplikasi terbitan Schiff bes sebagai penyambung dalam sensor DNA elektrokimia (E-DNA) masih belum dikaji. Dalam kajian ini, dua terbitan Schiff bes yang baru telah disintesis

dan dicirikan kerana ia menjanjikan sensor yang mendorong kita untuk menggunakannya sebagai penyambung potensi masa depan dalam sensor DNA elektrokimia.

Kata kunci: Schiff bes, sintesis, pencirian, peruang, sensor E-DNA

Introduction

A Schiff base is a compound that consists of a carbon-nitrogen double bond, with the nitrogen connected to the aryl or alkyl group [1]. The reaction's name is taken after Hugo Schiff, the inventor [2]. It is produced *via* a condensation reaction between the primary amines and ketones or aldehydes under a specific condition [3]. The general formula for Schiff base is $RHC=N-R_1$ (R, R_1 = alkyl, aryl, cycloalkyl or heterocyclic groups), which is also known as anils, imines or azomethine. Previous research have shown that the presence of a lone pair of electrons in sp^2 hybridised orbital of the nitrogen atom in the Schiff base plays an important role in chemical and biological applications [4].

Schiff base consisting of aryl substituent is more stable and easily synthesised compared to the Schiff base containing alkyl substituent. Furthermore, the Schiff base produced using an aliphatic aldehyde is unstable and readily undergoes polymerisation compared to those produced by aromatics, which are more stable and have an effective conjugation [5]. Schiff bases produced from aromatic aldehydes and aromatic amines have been used in a wide range of applications in various fields, including pharmaceutical, life sciences, and chemical science (e.g. inorganic and analytical chemistry)[2]. Aromatic aldehyde Schiff bases display an outstanding photoluminescence property due to the effect of the conjugation system. It has an excellent possibility in sensor, luminescent devices, and biological devices [6]. Besides, it can improve the selectivity and sensitivity detection of the sensor [7].

The identification and sequencing of DNA molecules have received much attention since Watson and Crick introduced the double helix structure of deoxyribonucleic acid (DNA) in 1953 [8]. The development method of DNA sensor has continued to grow due to possible applications in the field ranging from gene analysis, clinical diagnostics, forensic study and other medical applications [9]. There are many types of DNA biosensor, examples being electrochemical, optical, acoustic, and piezoelectric [10]. Among these, the electrochemical method provides promising benefits due to its simplicity, rapidness, relatively low cost and high sensitivity, which is suitable for the improvement of cheap and portable devices [9].

In electrochemical DNA (E-DNA) sensor, a recognition layer or known as spacer or linker is important to immobilise the DNA on an electrode and it may affect the detection [11]. The previous study has shown that alkanethiol is a common spacer used in the E-DNA sensor, for example, 3-mercaptopropionic acid (MPA), 11-mercaptoundecanoic acid (MUA) and 6-mercapto-1-hexanol (MCH) [12]. The aim of the study is to synthesise two Schiff bases consisting of 4-propoxybenzaldehyde substituted with three carbon length of the alkane chain, followed by chemical characterisation. The synthetic procedures for all synthesised compounds are shown in Scheme 1.

Materials and Methods

Materials and instrumentation

4-vinylaniline, 1-iodo-4-nitrobenzene, 4-iodobenzonitrile, palladium (II) chloride, trimethylamine, 4-hydroxybenzaldehyde, 1-bromopropane, potassium carbonate, potassium iodide, acetone, acetonitrile, hexane, ethyl acetate and ethanol were purchased from Merck, Sigma Aldrich, Acros Organic, Fisher Chemical and HmbG® Chemicals and used without further purification.

Infrared (IR) spectra of all synthesised compounds were recorded using Perkin Elmer 100 Fourier Transform Infrared Spectroscopy using potassium bromide (KBr) pellet within the range of $4000 - 450\text{ cm}^{-1}$. The analysis of UV-Vis spectra was measured by double-beam Spectrophotometer Shimadzu UV-1800 in 1 cm^3 cuvette and acetonitrile as solvent. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were determined by Bruker Avance II 400 spectrometer in deuterated chloroform ($\text{CDCl}_3\text{-d}_6$).

Synthesis of substituted amino stilbene (1): Synthesis of 4-(4-nitrostyryl) aniline (1a)

4-(4-nitrostyryl) aniline (1a) was synthesised via Heck-cross coupling method. 4-Vinyl aniline 90% (2.8 mmol) and 1-iodo-4-nitrobenzene (1.9 mmol) was dissolved in 4 ml of DMF and 3.5 ml trimethylamine, and then refluxed at 70-75 °C for 24 hours in the presence of $\text{PdCl}_2(\text{PPh}_3)_2$ (50 mg). The reaction was monitored by thin layer chromatography (TLC). The product was purified using column chromatography, by utilising hexane: ethyl acetate as an eluent. Red batter powder; Yield 257 mg, 53.3%; FTIR (KBr, ν cm^{-1}): 3463-3390 (N-H stretch), 3027 (aromatic C-H stretch), 1619, 1497 (aromatic C=C stretch), 1585 (alkene C=C stretch), 1340 (N=O nitro), 1181 (C-N stretch); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 3.79 (s, 2H, NH_2), 6.84-7.09 (doublet-doublet, 2H, $\text{HC}=\text{CH}$), 6.11-8.12 (m, 8H, CH, Ar). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 146.34 (Ar-C- NH_2), 145.14 (Ar-C- NO_2), 143.71, 132.46, 127.52, 123.14, 121.45, 114.12 (6 Ar-C), 125.23 (C=C); UV-Vis (Acetonitrile): λ_{max} =251 nm, 410 nm; MS: m/z 240 $[\text{M}^+]$.

Synthesis of 4-(4-aminostyryl)benzonitrile (1b)

4-(4-aminostyryl)benzonitrile (1b) was synthesised via Heck-cross coupling method. 4-vinyl aniline 90% (2.18 mmol) and 4-aminobenzonitrile (3.27 mmol) was dissolved in 4 ml of DMF and 3.5 ml trimethylamine, and then refluxed at 70-75 °C for 24 hours in the presence of $\text{PdCl}_2(\text{PPh}_3)_2$ (50 mg). The reaction was monitored by TLC. The product was purified using column chromatography, by utilising hexane: ethyl acetate as an eluent. Yellow powder; Yield 257 mg, 58%; FTIR (KBr, ν cm^{-1}): 3447-3369 (N-H stretch), 3033 (aromatic C-H stretch), 1618, 1439 (aromatic C=C stretch), 1594 (alkene C=C stretch), 2224 ($\text{C}\equiv\text{N}$), 1174 (C-N stretch); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 3.76 (2H, NH_2), 6.81- 7.05 (doublet-doublet, 2H, $\text{HC}=\text{CH}$), 6.61-7.52 (8H, CH, Ar). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 146.34 (Ar-C- NH_2), 118.25 ($\text{C}\equiv\text{N}$), 108.60-146.11 (6 Ar-C), 125.23 (C=C); UV-Vis (Acetonitrile): λ_{max} =252 nm, 357 nm; MS: m/z 240 $[\text{M}^+]$.

Synthesis of 4-propoxybenzaldehyde (2)

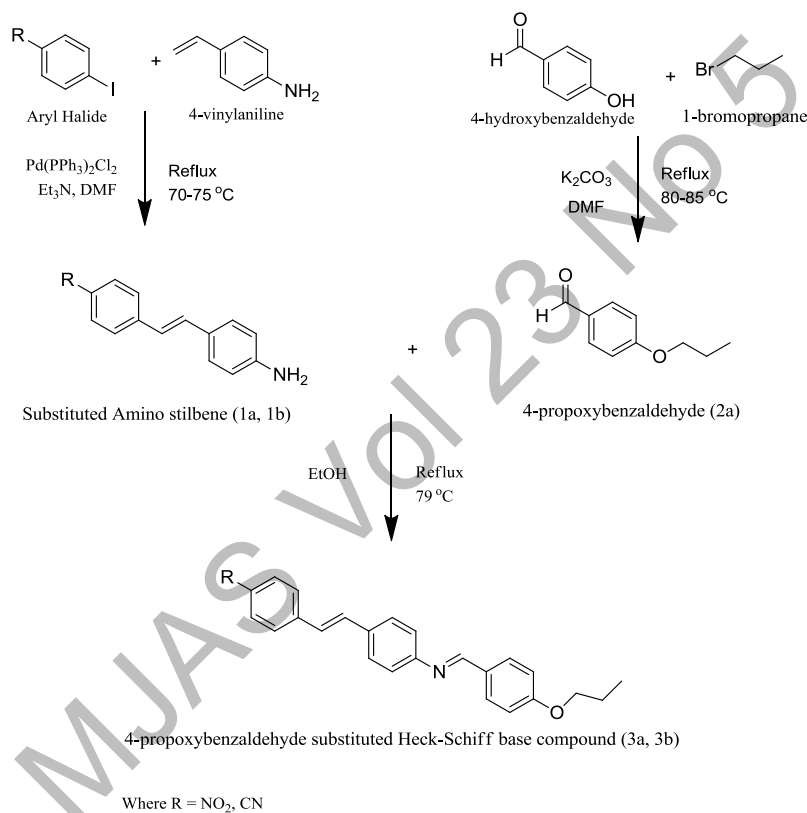
The formation of 4-propoxybenzaldehyde was synthesised via Williamson Ether synthesis. In this reaction, an equimolar mixture of 4-hydroxybenzaldehyde and bromopropane was used. Firstly, 1.5 g of 4-hydroxybenzaldehyde was stirred in 50 ml DMF at the temperature of 65 °C for 30 minutes in the presence of potassium carbonate, K_2CO_3 (1.6 g) and potassium iodide, KI, (1g). After that, bromoalkane was added into the mixture and was stirred continuously at 80-85 °C for 24 hours. The mixture was next extracted using 50 ml of diethyl ether and washed with distilled water repeatedly to remove K_2CO_3 , while the ether layer was washed with 25 ml of 5% NaOH to remove unreacted 4-hydroxybenzaldehyde. Repeated washes were done for the ether layer using distilled water until it became neutral at pH 7. Anhydrous MgSO_4 (1 g) was used to eliminate water contained in the solution. The diethyl ether was removed via rotary evaporator to obtain pure oily transparent pale-yellow liquids of 4-propoxybenzaldehyde. Light Yellow liquid; Yield 55%; FTIR (KBr, ν cm^{-1}): 3075 (aromatic C-H stretch), 2967-2828 (C-H stretch), 1693 (C=O), 1598, 1509 (aromatic C-H stretch), 1474 (CH_2 bend), 1428, 1314 (CH_3 bend), 1254 (C-O stretch); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 0.96 (m, 3H, CH_3), 1.76 (m, 2H, CH_2), 3.91 (m, 2H, CH_2), 6.89-7.74 (m, 4H, CH, Ar), 9.78 (s, 1H, $\text{H}-\text{C}=\text{O}$). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 10.42, 22.41, 69.87 ($\text{CH}_2-\text{CH}_2-\text{CH}_3$), 114.75, 129.76, 131.97, 164.27 (Ar-C), 190.79 (C=O); UV-Vis (Acetonitrile): λ_{max} =218 nm, 274 nm; MS: m/z 164 $[\text{M}^+]$.

Synthesis of Alkoxy substituted amino stilbene-Schiff base compound (3): 4-(4-((4-propoxybenzylidene)amino)styryl)benzonitrile anilines (3a)

Firstly, 157 mg of precursor 4-(4-nitrostyryl) aniline was fully dissolved in the ethanol and mixed with 0.1 ml 4-propoxybenzaldehyde. The mixture was refluxed at 79-80 °C for 2 hours and the solvent was reduced to half using a dean stalk. The reaction was monitored by thin layer chromatography (TLC). A precipitate was formed and recrystallised using acetonitrile. Yellow solid; Yield 51%; FTIR (KBr, ν cm^{-1}): 2969-2877 (C-H stretch), 1604 ($\text{C}\equiv\text{N}$), 1584 (C=C alkene), 1340 (N=O nitro), 1259 (C-N), 1164 (C-O stretch); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 0.99 (m, 3H, CH_3), 1.77 (m, 2H, CH_2), 3.92 (m, 2H, CH_2), 6.91, 7.19, 7.51, 7.56, 7.77, 8.15 (d, 12H, Ar), 7.05, 7.16 (doublet-doublet, 2H, $\text{HC}=\text{CH}$), 8.34 (s, 1H, $\text{HC}=\text{N}$). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 9.47, 21.49, 68.69 ($2\text{CH}_2-\text{CH}_3$), 113.73, 120.54, 123.15, 126.94, 127.84, 129.63, 131.86, 132.56, 143.00, 145.59, 151.76, 161.08 (Ar-C), 124.44, 125.70 (C=C), 158.80 (C=N); UV-Vis (Acetonitrile): λ_{max} =240 nm, 290 nm and 385 nm; MS: m/z 386 $[\text{M}^+]$.

4-(4-((4-propoxybenzylidene)amino)styryl)benzonitrile anilines (3b)

139 mg of precursor 4-(4-aminostyryl)benzonitrile was fully dissolved in the ethanol and mixed with 0.1 ml 4-propoxybenzaldehyde. The mixture was refluxed for at 79-80 °C for 2 hours and the solvent was reduced to half using a dean stalk. The reaction was monitored by TLC and the precipitate formed was recrystallised using acetonitrile. Yellow solid; Yield 46 %; FTIR (KBr, ν cm^{-1}): 3025 $\nu(\text{C-H})$ aromatic, 2967-2879 $\nu(\text{C-H})$, 2224 $\nu(\text{C}\equiv\text{N})$, 1604 $\nu(\text{C}=\text{N})$, 1584 $\nu(\text{C}=\text{C})$ alkene, 1249 $\nu(\text{C-N})$, 1164 $\nu(\text{C-O})$, 843 (C-H out of plane bend); ^1H NMR (400 MHz, CDCl_3 , ppm) δ : 0.98 (3H, CH_3), 1.76, 3.91 (6H, CH_2), 6.90, 7.15, 7.47, 7.50, 7.54, 7.76 (12H, Ar), 6.98, 7.12 (doublet-doublet, 2H, $\text{HC}=\text{CH}$), 8.32 (1H, $\text{HC}=\text{N}$). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 9.47, 21.48, 68.67 ($2\text{CH}_2\text{-CH}_3$), 109.32, 113.72, 120.49, 126.81, 127.85, 129.61, 130.93, 131.46, 132.68, 140.95, 151.56, 161.04 (Ar-C), 124.44, 125.70 (C=C), 158.72 (C=N), 118.07 (C $\equiv$ N); UV-Vis (Acetonitrile): λ_{max} =238 nm, 279 nm and 361 nm; MS: m/z 366 [M^+].



Scheme 1: Synthesis procedures for 4-propoxybenzaldehyde substituted Heck-Schiff base compound

Results and Discussion

Fourier transform infrared spectroscopic analysis

The FTIR spectra of compounds 3a and 3b are presented in Figure 1. The spectra showed similar vibration types except for the main functional group of every compound. Based on the spectra, C-H stretching for aromatic for all compounds was observed around 3024.27 - 3025.13 cm^{-1} [13]. Meanwhile, C-H stretching for aliphatic appeared in the range of 2877.11 - 2969.09 cm^{-1} . For the imine group band, C=N, it was clearly observed at 1604.36 - 1606.51 cm^{-1} confirming the formation of Schiff base [14]. In addition, the absorption band of aliphatic alkene occurred within the range of 1582.82 - 1585.38 cm^{-1} . The FTIR spectrum of 3a shows the stretching of N=O (NO_2) asymmetric at the region ranging from 1509.23 - 1519.05 cm^{-1} . Furthermore, N=O (NO_2) symmetric was confirmed by the peak at the range between 1335.12 cm^{-1} - 1340.88 cm^{-1} [13]. Meanwhile, for spectrum 3b, the presence of the

cyano group in the compound was confirmed by the strong absorption peak at 2224.90 cm^{-1} that was assigned as $\text{C}\equiv\text{N}$ stretching [15]. The C-O stretching for compounds 3a and 3b both was discovered at 1259.76 cm^{-1} and 1250.70 cm^{-1} , respectively [16].

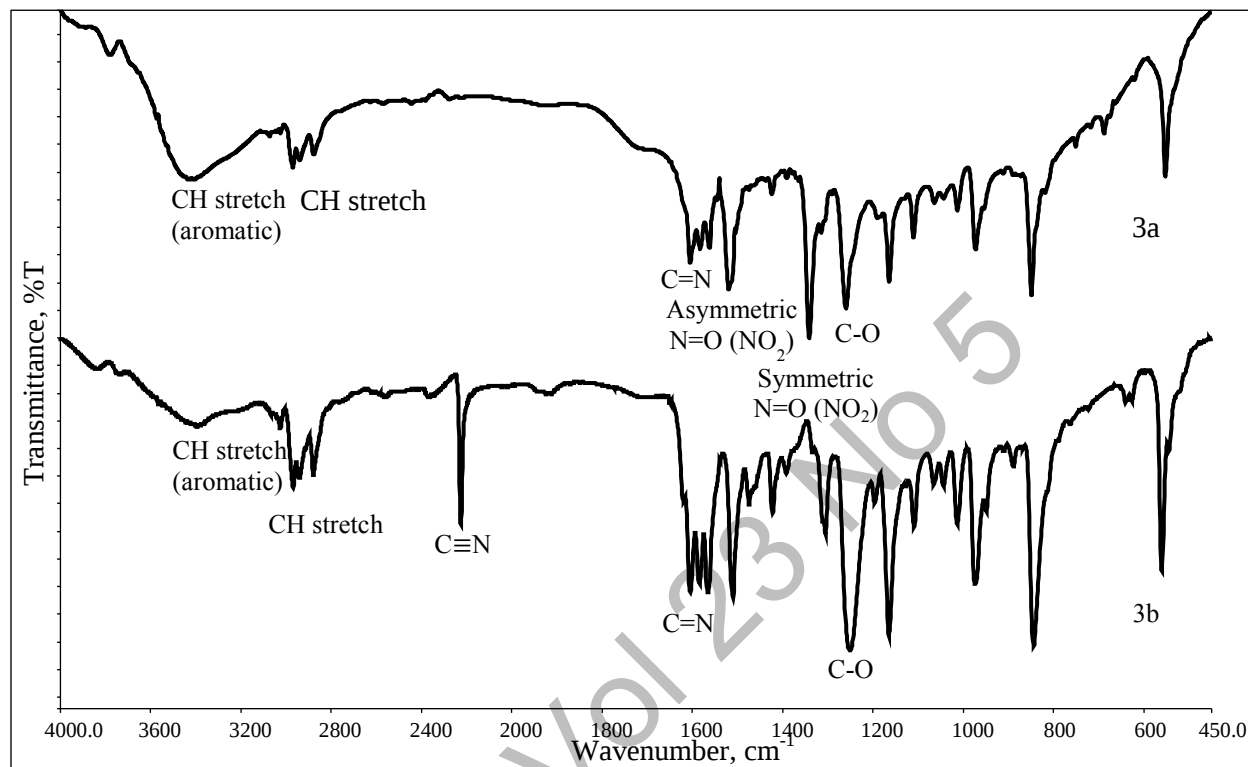


Figure 1. FTIR spectra of compounds 3a and 3b

Ultraviolet-visible (UV-vis) spectroscopy

The UV-Vis spectra of compounds 3a and 3b both are presented in Figure 2. The UV-vis spectroscopy analysis of compounds 3a-3b was done by using acetonitrile ($1 \times 10^{-5}\text{ M}$) as a solvent with a cut-off point at 190.0 nm . The electronic absorption band spectra of compound 3a and 3b showed three bands. The first two bands were indicated to transition $\pi\text{-}\pi^*$ of aromatic rings band at λ_{max} 240 nm and λ_{max} 290 nm [17]. The other peaks appeared at transition $\pi\text{-}\pi^*$ of azomethine band at λ_{max} 385 nm [18]. Meanwhile, the electronic band spectra for compound 3b showed three bands. The spectra showed the presence of $\pi\text{-}\pi^*$ electronic transition of $\text{C}=\text{C}$ (aromatic) at the wavelength λ_{max} 238 nm and λ_{max} 279 nm . Additionally, the absorption appeared at 361 nm indicated to the transition of the azomethine band.

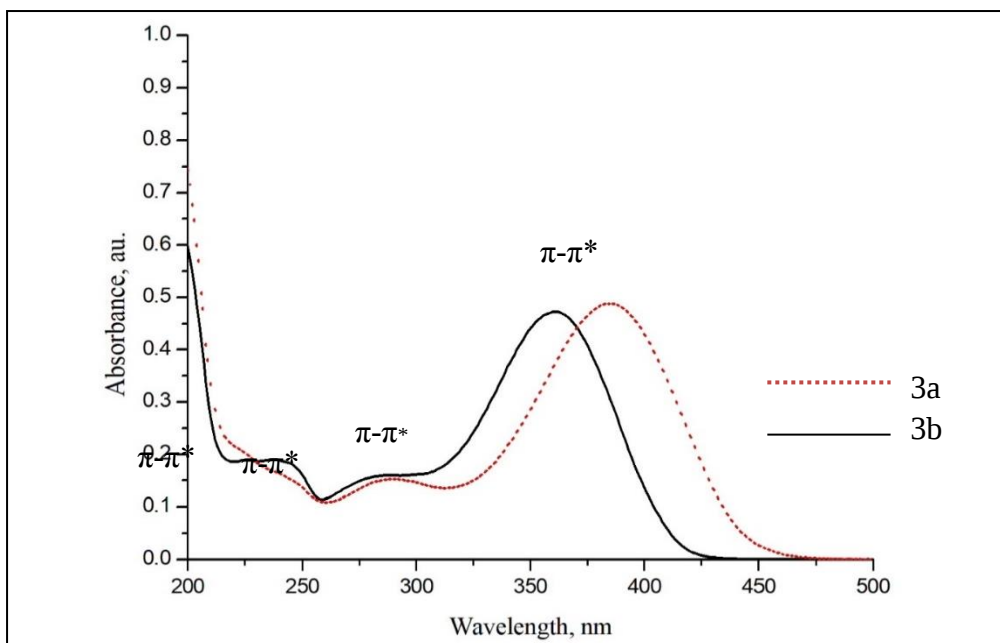


Figure 2: UV-vis spectra of compound 3a and 3b

¹H and ¹³C nuclear magnetic resonance

¹H and ¹³C nuclear magnetic resonance (NMR) analysis confirmed the identity of all synthesised Schiff bases in order to identify their structure. The azomethine group (CH=N) of synthesised compounds 3a and 3b both was discovered in the far downfield at δ 8.34 and 8.32 [18]. Furthermore, the ¹H NMR spectra data showed the presence of alkyl chain proton (C-H) observed at the upfield region within the range δ 1.76 to 3.92 ppm. Meanwhile, the signal moieties for the CH₃ of alkyl chain for compounds 3a and 3b were observed at 0.99 and 0.98, respectively. The aromatic protons of the benzene ring for all compounds resonated at region δ 6.90-8.15 ppm. In addition, the proton signal for the doublet-doublet of the vinyl bond was discovered around δ 6.98-7.16 ppm for all compounds [20].

Table 1. ¹H NMR spectral data of compounds 3a and 3b

Compound	CH ₃	C-H (Alkyl chain)	C-H (Aromatic)	C=C (Alkene)	CH=N
3a	0.99	1.76, 3.92	6.91, 7.19, 7.50, 7.56, 7.77, 8.15	7.05, 7.16	8.34
3b	0.98	1.76, 3.91	6.90, 7.15, 7.47, 7.50, 7.54, 7.76	6.98, 7.12	8.32

Table 3.2 shows the ¹³C NMR of compounds 3a and 3b both in CDCl₃ used as a complementary technique for approving the structure of the prepared Schiff bases. The resonance of the C-C alkyl chain was clearly observed at δ 9.46-68.67 ppm. For the aromatic ring, the resonance carbon was found at the range between δ 109.32-161.08 ppm. Furthermore, the carbon resonance for the (C=C) alkene of all compounds obviously appeared in the region δ 124.28-124.44 ppm and δ 125.70-125.73 ppm. The carbon NMR showed the chemical shift for the azomethine group (C=N) at δ 158.80 ppm and δ 158.72 ppm. In addition, for the 3b compound, the carbon resonance of C $\equiv$ N was identified at δ 118.07 ppm.

Table 2. ^{13}C NMR spectral data of compounds 3a and 3b

Compound	C-C (Alkyl Chain)	C-C (Aromatic)	C=C (Alkene)	C=N (Imine)	C≡N
3a	9.46, 21.49, 68.68	113.73, 120.54, 123.15, 126.94, 127.84, 129.63, 131.86, 132.56, 143.00, 145.59, 151.76, 161.08	124.44, 125.70	158.80	-
3b	9.47, 21.48, 68.67	109.32, 113.72, 120.49, 126.81, 127.85, 129.61, 130.93, 131.46, 132.68, 140.95, 151.56, 161.04	124.28, 125.73	158.72	118.07

Conclusion

Two 4-propoxybenzaldehyde substituted Heck-Schiff bases: 3a and 3b were successfully synthesised by a condensation reaction between 4-(4-nitrostyryl) aniline and 4-(4-aminostyryl)benzonitrile with 4-propoxybenzaldehyde. All synthesised compounds were characterised by UV, FTIR, ^1H NMR, ^{13}C -NMR spectroscopies and mass spectrometry. The 4-propoxybenzaldehyde substituted the Heck-Schiff base compounds consisting of the aromatic substituent, which made the compounds more stable. These compounds are believed to yield a great prospect in sensors, as they could enhance the selectivity and sensitivity in sensor detection. In future work, the compounds should be applied as a potential linker in the E-DNA sensor for cancer detection to improve the sensor sensitivity. Besides, the compound is hoped to be an excellent linker as it consists of nitro and cyano functional groups and alkyl chain to chemically bind with a gold electrode and phosphate group in the DNA.

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