

Synthesis and characterization of alkoxy substituted p-cyano stilbene schiff bases

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Abstract. Schiff bases consist of aromatic have been used in various application including biological, inorganic and analytical chemistry. It also has been applied in optical and electrochemical sensors to enable detection of enhance selectivity and sensitivity of the sensors. These compounds might give excellent prospect in sensor. Schiff bases derived from 4-(4-aminostyryl)benzotrile and three 4-alkoxybenzaldehyde derivatives are synthesis using ethanol as solvent. All compounds were characterized by Fourier transform-infrared spectroscopy (FT-IR), UV-Vis spectrophotometer and Nuclear Magnetic Resonance (NMR). FTIR showed formation of C=N (imine) stretching vibrations at range of 1604 cm⁻¹ to 1609 cm⁻¹. In UV-vis, π - π^* electronic transition of the C=N can be observed at peak range 361 nm. While in the δ_H NMR the peak of CH=N (Imine) group was found at δ 8.32-8.34 ppm and ¹³C NMR for three Schiff bases were discovered at δ 158.72, 158.73 and 158.71 ppm. In previous study there are several types of linker that have been used in E-DNA, especially alkanethiol. In this study, three new Schiff base derivatives have been synthesized and characterized due to it promising in sensor which prompted us to apply its as future potential linker in electrochemical DNA sensor.

Keywords: Synthesis, Characterization, Schiff base, future potential linker in E-DNA

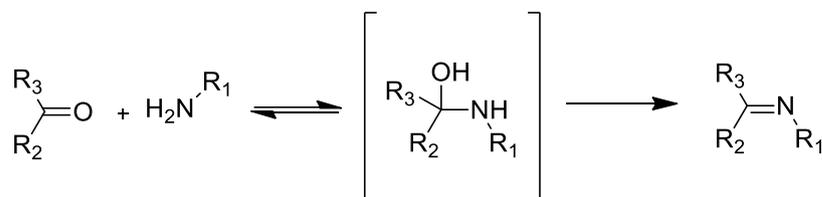
1. Introduction

The chemistry of compound containing Schiff base have been study from ancient years in various field [1]. The compound was discovered by Hugo Schiff, a German Chemist and a Nobel Prize Winner in 1864th [2]. Schiff bases are produced using condensation reaction between ketones or aldehyde with primary amines. The structure of Schiff bases consists of functional group azomethin (-HC=N-) [3]. A Schiff base structurally is a compound that contain nitrogen analogue of an aldehyde or ketone in which an imine or azomethine group replace the carbonyl group (C=O) [4]. Azomethine group is the common structural feature of Schiff base compound with general formula RHC=N-R1, where R and R1 are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. Schiff base compound also known as anils, imines or azomethines. Previous study revealed that the presence



of a lone pair of electron in sp² hybridized orbital of nitrogen atom of the azomethine group is chemically significant and biological importance [5].

During the reaction, the water molecules will be eliminated and can be accelerated using acid as catalyst. Basically, the reaction is run via refluxing the mixture of the carbonyl and an amine using Dean Stark apparatus to get rid of water. The removal of water can avoid the conversion of aminal into imine because it can be reversible. The previous study showed, there are several dehydrating agents to remove water molecules including sodium sulphate and molecular sieves. Besides, there are some in situ method that have been reported involving dehydrating solvents such as tetramethyl orthosilicate or trimethyl orthoformate. In addition, there are various acid catalyst that have been reported including mineral acids (H₂SO₄, HCl), organic acids (p-toluene sulphonic acids, pyridium p-toluene sulphonate), acid resin, montmorillonite or Lewis acid (ZnCl₂, TiCl₄, SnCl₄, BF₃Et₂O, MgSO₄, Mg(ClO₄)₂) [6]. Scheme 1.1 shows the general reaction of Schiff base for imines preparation.



Scheme 1. Schiff base reaction for imine preparation [6].

Schiff base consist of aryl substituents are more stable and readily synthesized compared to those with alkyl substituent which is relatively unstable. Moreover, Schiff base from aliphatic aldehydes are unstable and readily polymerizable compared to those with aromatic aldehyde that have effective conjugation which make it more stable [7]. Nowadays, several of Schiff base compound have been synthesized and their complexation characteristics have been studied due to its great flexibility and diverse structural aspects [8]. Schiff bases comprise of aromatic have been used in many applications in field of biological, inorganic and analytical chemistry. The presence of organic reagents is required in application of many new analytical devices as essential compounds of the measuring system. It is applied in optical and electrochemical sensors, as well in chromatographic method. The advantages of Schiff base in sensor are improved the selectivity and sensitivity of the detection [9]. In addition, aromatic aldehyde Schiff bases display great photoluminescence properties. It might be good to use in sensor, luminescence, electroluminescent devices and biological [10].

Many advance technologies have been developed which provide us tools for cultivate new technique for monitor biorecognition and interaction between solid and in solution over past 15 years [11]. Biosensor is known as an analytical device which covert biological response into the signal [12]. It consists of two main parts which is a bioreceptor and a transducer. Bioreceptor act as a biological compound that will identify the target analyte. Transducer part which also known as physicochemical detector component that will transform the recognition event into signal that can be measured. There are many types of biosensors including; optical biosensor, resonance biosensor, thermal biosensors, electrochemical biosensor, bioluminescence sensors, nuclei acid-based biosensors and nanobiosensors [13]. Electrochemical DNA sensor gained popularity due to their great advantages that offer simplicity, rapidness, low cost and high sensitivity compared to others method for DNA detection [14]. It has become one of the trusted tools for variety applications including diagnosis, reliable forensic analysis,

environmental monitoring, and biological research due to its benefits [15]. Nowadays, there are many kinds of working electrode that have been applied in electrochemical DNA sensor including mercury electrode, carbon electrode, gold electrode, indium tin oxide electrode and chemically modified electrode. The properties of electrode can strongly affect the efficiency of DNA hybridization on the surface of electrode, especially in term of material types, surface modification and surface area [16]. Previous study shows the common linkers are used in E-DNA is alkanethiol due to its simplicity and ease of preparation [17]. Most of researcher have modified their electrode using 6-mercapto-1-hexanol (MCH) [18], 3-mercaptopionic acid (MPA) [19] and 11-mercaptoundecanoic acid (MUA) [19, 20].

The aim of the study is to synthesize three Schiff bases consist of alkoxy substituted with different length of alkane chain, followed by characterization. The synthetic procedures for all synthesized compounds were showed in scheme 2. In the future work, all three Schiff base derivatives will be applied as potential linker in E-DNA sensor due to its excellent prospect in sensor. These compounds might be excellent linker as it consists of cyano functional group and alkyl chain to chemically binding with gold electrode and phosphate group in DNA. The study about the attachment is still in progress.

2. Experiment

2.1. Materials and Instrumentation

All chemical and solvents were all purchased from Merck, Sigma Aldrich, Acros Organic, Fisher Chemical and HmbG® Chemicals and were used without further purification.

Infrared (IR) spectra of all synthesized compound were recorded on Perkin Elmer 100 Fourier Transform Infrared Spectroscopy using potassium bromide (KBr) pellet in the range 4000 – 450 cm^{-1} . The analysis of UV spectra were measured by double beam Spectrophotometer Shimadzu UV-1800 in 1 cm^3 cuvette and acetonitrile as solvent. δ_{H} and ^{13}C nuclear magnetic resonance (NMR) spectra were determined by Bruker Avance II 400 spectrometer in deuterated chloroform ($\text{CDCl}_3\text{-d}_1$).

2.2. Synthesis of 4-(4-aminostyryl)benzonitrile (1)

4-(4-aminostyryl)benzonitrile (**1**) was synthesized by using 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. The solution was refluxed at 70-75 $^{\circ}\text{C}$ for 24 h in the presence of $\text{PdCl}_2(\text{PPh}_3)_2$ (0.043 mmol) and was monitored by TLC. The product was purified using column chromatography using hexane: ethyl acetate as an eluent. Yellow powder; Yield 257 mg, 58%; IR (KBr, ν cm^{-1}): 3447-3369 $\nu(\text{N-H})$, 3033 $\nu(\text{C-H})$ aromatic, 1618, 1439 $\nu(\text{C=C})$ aromatic, 1594 $\nu(\text{C=C})$ alkene, 2224 $\nu(\text{C}\equiv\text{N})$, 1174 $\nu(\text{C-N})$; δ_{H} 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 (C \equiv N), 108.60-146.11 (6 Ar-C), 125.23 (C=C); UV-Vis (Acetonitrile): λ_{max} =252 nm, 360 nm; MS: m/z 240 [M+].

2.3. Synthesis of 4-alkoxybenzaldehyde (2a, 2b, 2c)

The formation of 4-alkoxybenzaldehyde was synthesized using general method of Williamson Ether synthesis. This synthesis involved reaction between equimolar mixture of 4-hydroxybenzaldehyde and bromoalkane in the presence of potassium carbonate, K_2CO_3 and potassium iodide, KI. 4-hydroxybenzaldehyde (1.5g) and 50 ml of DMF was stirred at temperature 65 $^{\circ}\text{C}$ for 30 min in the presence of K_2CO_3 and potassium iodide, KI. After that, bromoalkane was added into the mixture and was kept stirred under 80-85 $^{\circ}\text{C}$ for 24 h. The reaction was monitored by TLC. The mixture was transferred into a 500 ml of separatory funnel and 50 ml of diethyl ether was added. The reaction mixture was washed with distilled water repeatedly to remove the K_2CO_3 . 25 ml of 5% NaOH was used to removed unreacted 4-hydroxybenzaldehyde by washing the ether layer. Then repeatedly washed the ether layer with distilled water until it became neutral to pH paper and used anhydrous MgSO_4 to eliminate water. The diethyl ether was evaporated via rotary evaporator to give transparent pale-yellow liquids [20].

2.3.1 4-propoxybenzaldehyde (2a). Light Yellow liquid; Yield 55%; IR (KBr, ν cm^{-1}): 3075 $\nu(\text{C-H})$ aromatic, 2967-2828 $\nu(\text{C-H})$, 1693 $\nu(\text{C=O})$, 1598, 1509 $\nu(\text{C=C})$ aromatic, 1474 (CH_2 bend), 1428, 1314 (CH_3 bend), 1254 $\nu(\text{C-O})$; δ_{H} NMR (400 MHz, CDCl_3 , ppm) δ : 0.96 (3H, CH_3), 1.76 (2H, CH_2), 3.91 (2H, CH_2), 6.89-7.74 (4H, CH, Ar), 9.78 (1H, H-C=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): $\lambda_{\text{max}}=218$ nm, 274 nm; MS: m/z 164[M+].

2.3.2 4-(hexyloxy)benzaldehyde (2b). Light Yellow liquid; Yield 60%; IR (KBr, ν cm^{-1}): 3074 $\nu(\text{C-H})$ aromatic, 2932-2734 $\nu(\text{C-H})$, 1691 $\nu(\text{C=O})$, 1602, 1509 $\nu(\text{C=C})$ aromatic, 1464 (CH_2 bend), 1427, 1393 (CH_3 bend), 1255 $\nu(\text{C-O})$; δ_{H} NMR (400 MHz, CDCl_3 , ppm) δ : 0.91 (3H, CH_3), 1.34 (4H, $\text{CH}_2\text{-CH}_2$), 1.45 (2H, CH_2), 1.81 (2H, CH_2), 4.02 (2H, CH_2), 6.97-7.82 (4H, CH, Ar), 9.86 (1H, H-C=O). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 12.97, 21.54, 24.61, 28.00, 30.49, 67.42 ($\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_2\text{-CH}_3$), 113.74, 1128.76, 1130.94, 163.27 (Ar-C), 189.73 (C=O); UV-Vis (Acetonitrile): $\lambda_{\text{max}}=219$ nm, 275 nm; MS: m/z 206[M+].

2.3.3 4-(nonyloxy)benzaldehyde (2c). Light Yellow liquid; Yield 64%; IR (KBr, ν cm^{-1}): 2927-2733 $\nu(\text{C-H})$, 1692 $\nu(\text{C=O})$, 1598, 1509 $\nu(\text{C=C})$ aromatic, 1468 (CH_2 bend), 1428, 1393 (CH_3 bend), 1251 $\nu(\text{C-O})$; δ_{H} NMR (400 MHz, CDCl_3 , ppm) δ : 0.80 (3H, CH_3), 1.23 (10H, 5 CH_2), 1.38 (2H, CH_2), 1.72 (2H, CH_2), 3.95 (2H, CH_2), 6.89-7.74 (4H, CH, Ar), 9.79 (1H, H-C=O). ^{13}C -NMR (400 MHz, CDCl_3 , ppm) δ : 13.07, 21.64, 24.94, 28.05, 28.22, 28.32, 28.48, 30.84, 67.42 (8 $\text{CH}_2\text{-CH}_3$), 113.74, 128.77, 130.94, 163.27 (Ar-C), 189.72 (C=O); UV-Vis (Acetonitrile): $\lambda_{\text{max}}=219$ nm, 275 nm; MS: m/z 248[M+].

2.4 Synthesis of 4-(4-((4-xbenzylidene)amino)styryl)benzonitrile (3a, 3b, 3c)

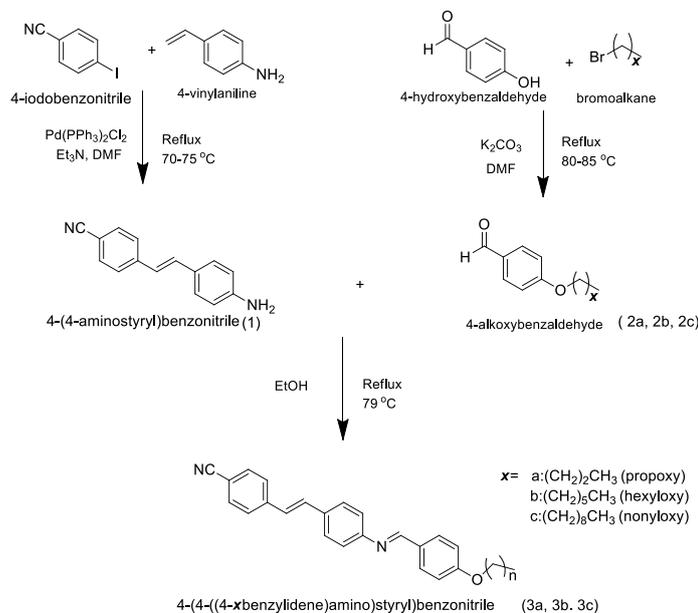
4-(4-aminostyryl)benzonitrile product was dissolved in the ethanol and it will be treated with 4-alkoxybenzaldehyde. Then, the mixture was stirred under reflux for 2 hours and the solvent was reduced to half by using dean stalk. The reaction was monitored by thin layer chromatography (TLC). Precipitate was formed and recrystallized using acetonitrile.

2.4.1 4-(4-((4-propoxybenzylidene)amino)styryl)benzonitrile anilines, (3a). Yellow solid; Yield 46 %; IR (KBr, ν cm^{-1}): 3025 $\nu(\text{C-H})$ aromatic, 2967-2879 $\nu(\text{C-H})$, 2224 $\nu(\text{C}\equiv\text{N})$, 1604 $\nu(\text{C=N})$, 1584 $\nu(\text{C=C})$ alkene, 1249 $\nu(\text{C-N})$, 1164 $\nu(\text{C-O})$, 843 (C-H out of plane bend); δ_{H} 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, HC=CH), 8.32 (1H, HC=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): $\lambda_{\text{max}}=288$ nm, 385 nm; MS: m/z 386 [M+].

2.4.2 4-(4-((4-hexyloxybenzylidene)amino)styryl)benzonitrile, (3b). Yellow solid; Yield 48.3%; IR (KBr, ν cm^{-1}): 3025 $\nu(\text{C-H})$ aromatic, 2935-2871 $\nu(\text{C-H})$, 2224 $\nu(\text{C}\equiv\text{N})$, 1605 $\nu(\text{C=N})$, 1583 $\nu(\text{C=C})$ alkene, 1257 $\nu(\text{C-N})$, 1164 $\nu(\text{C-O})$, 843 (C-H out of plane bend); δ_{H} NMR (400 MHz, CDCl_3 , ppm) δ : 0.84 (3H, CH_3), 1.28, 1.41, 1.73, 3.95 (10H, CH_2), 6.90, 7.17, 7.48, 7.52, 7.56, 7.77 (12H, Ar), 6.99, 7.13 (doublet-doublet, 2H, HC=CH), 8.34 (1H, HC=N). ^{13}C NMR (400 MHz, CDCl_3 , ppm) δ : 13.01, 21.57, 24.66, 28.11, 30.54, 67.22 (5 $\text{CH}_2\text{-CH}_3$), 109.33, 113.73, 120.49, 126.81, 127.84, 129.61, 130.93, 131.46, 132.68, 140.96, 151.57, 161.06 (Ar-C), 124.89, 125.73 (C=C), 158.73 (C=N), 118.07 (C \equiv N); UV-Vis (Acetonitrile): $\lambda_{\text{max}}=286$ nm, 387 nm; MS: m/z 428 [M+].

2.4.3 4-(4-((4-nonyloxybenzylidene)amino)styryl)benzonitrile (3c). Yellow solid; Yield 66 %; IR (KBr, ν cm^{-1}): 3040 $\nu(\text{C-H})$ aromatic, 2923-2853 $\nu(\text{C-H})$, 2222 $\nu(\text{C}\equiv\text{N})$, 1609 $\nu(\text{C=N})$, 1585 $\nu(\text{C=C})$ alkene, 1258 $\nu(\text{C-N})$, 1162 $\nu(\text{C-O})$, 846 (C-H out of plane bend); δ_{H} NMR (400 MHz, CDCl_3 , ppm) δ :

0.81 (3H, CH₃), 1.21, 1.39, 1.73, 3.94 (16H, 8CH₂), 6.90, 7.15, 7.47, 7.50, 7.55, 7.76 (12H, Ar), 6.98, 7.12 (doublet-doublet, 2H, HC=CH), 8.32 (1H, HC=N). ¹³C NMR (400 MHz, CDCl₃, ppm) δ; 13.09, 21.65, 24.99, 28.15, 28.23, 28.36, 28.50, 30.85, 67.23 (8CH₂-CH₃), 109.34, 113.73, 120.49, 126.81, 127.85, 129.61, 130.94, 131.46, 132.68, 140.97, 151.58, 161.07 (Ar-C), 124.89, 125.73 (C=C), 158.71 (C=N), 118.07 (C≡N); UV-Vis (Acetonitrile): λ_{max}=289 nm, 388 nm; MS: m/z 470 [M+].



Scheme 2. Synthetic procedures for the 4-(4-((4-xbenzylidene)amino)styryl)benzonitrile

3. Results and Discussion

3.1. Infrared (IR) Spectroscopic Analysis

The FTIR spectra of synthesized compounds 3a, 3b and 3c are shown in Figure 1. The structure of all compound was similar and only differ in length chain of alkoxy. The spectra showed that the band of CH stretch for aromatic in range 3025-3040 cm⁻¹. The peak observed at 2853 – 2967 cm⁻¹ show the presence of C-H stretching of alkyl chain in the compound. Absorption band of cyano group (C≡N) was discovered at peak range of 2222-2224 cm⁻¹. The characteristics absorption bands of carbon-nitrogen double band of the imine group (-HC=N-) of compound 3a, 3b and 3c were presence in range 1604, 1605 and 1609 cm⁻¹ respectively [21, 22]. Another band around 1583-1585 cm⁻¹ assigned to olefinic double bonds (-CH=CH-) [21]. Appearance of band at 1249-1258 cm⁻¹ indicate the existence of C-O stretching [20].

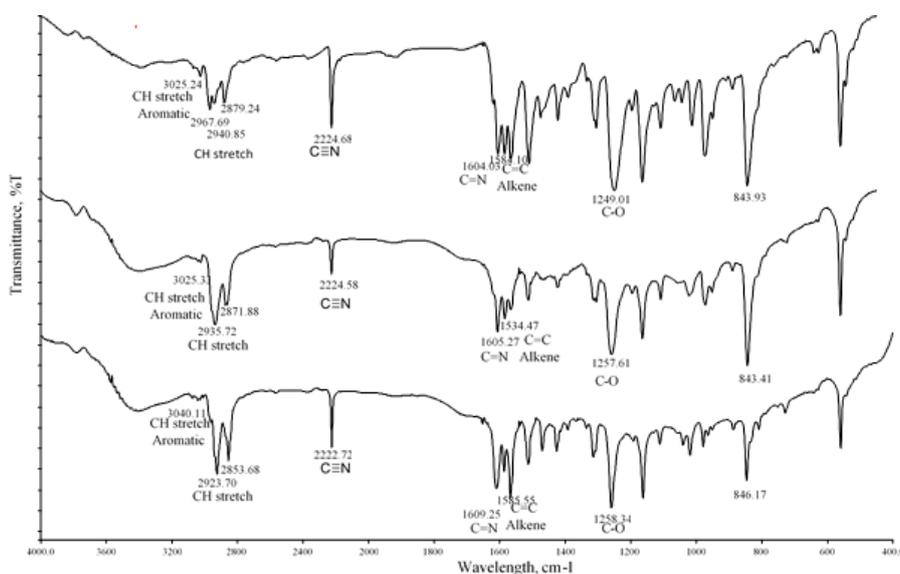


Figure 1. FTIR spectra of compound 3a-3b

3.2. Ultraviolet-visible (UV-vis) spectroscopy

The Ultraviolet-visible (UV-vis) spectroscopy analysis of compound 3a-3b was done by using acetonitrile (1×10^{-5} M) as a solvent with cut-off point at 190.0 nm (Figure 2). The ultraviolet-visible electronic spectra of three compound showed the presence π - π^* electronic transition of C=C (aromatic) at the wavelength 227 nm to 238 nm. While, band at wavelength 361 nm indicate to the π - π^* electronic transition of the C=N group [23].

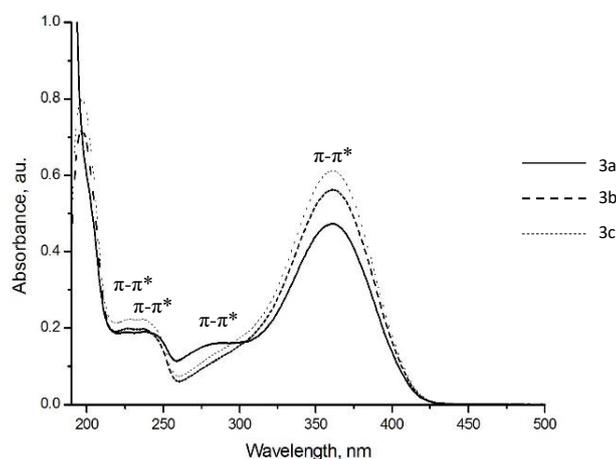


Figure 2. UV-vis spectrum for 3a-3b

3.3. δ_H and ^{13}C Nuclear Magnetic Resonance (NMR)

Table 3.1. represent the δ_H spectra of compound 3a-3b. The spectra display triplet signal moieties of CH_3 of alkyl chain at δ 0.98, 0.84 and 0.81 ppm for compound 3a, 3b and 3c, while the moieties of CH_2 of alkyl chain were observed at δ 1.21 to 3.94 ppm. Aromatic protons of the benzene ring resonated at δ 6.90–7.76 ppm. Doublet-doublet signal of C=C of alkene was discovered at δ 6.98-6.99 ppm and δ

7.12-7.13 ppm for all compound. Finally, the signals at δ 8.34- 8.32 ppm were attributed to the imine proton which show the evidence of the Schiff base.

Table 1 δ_H NMR spectral data of compound **3a-3c**

Compound	CH ₃	C-H (Alkyl chain)	C-H (Aromatic)	C=C (Alkene)	CH=N
3a	0.98	1.76, 3.91	6.90, 7.15, 7.47, 7.50, 7.54, 7.76	6.98, 7.12	8.32
3b	0.84	1.28, 1.41, 1.73, 3.95	6.90, 7.17, 7.48, 7.52, 7.56, 7.77	6.99, 7.13	8.34
3c	0.81	1.21, 1.39, 1.73, 3.94	6.90, 7.15, 7.47, 7.50, 7.55, 7.76	6.98, 7.12	8.32

The ^{13}C NMR of compound **3a**, **3b** and **3c** in CDCl_3 are used as complementary technique for approving the structure of the prepared Schiff bases as shown in Table 3.2. C-C of alkyl chain resonance clearly identified at δ 9.47- 68.67 ppm. The C=C (alkene) were appeared at δ 124.28- 124.29 ppm and δ 125.73 ppm. The resonance of aromatic ring was recorded in the range δ 109.32 - 161.07 ppm. Resonance at δ 118.06-118.07 assign for $\text{C}\equiv\text{N}$ in the compound. The resonance of C=N (imine) can be seen at δ 158.72, 158.73 and 158.71 ppm prove that compound **3a-3c** were successfully synthesized.

Table 2 ^{13}C NMR spectral data of compound **3a-3c**

Compound	C-C (Alkyl chain)	C-C (Aromatic)	C=C (Alkene)	C=N (Imine)	$\text{C}\equiv\text{N}$
3a	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
3b	13.01, 21.57, 24.66, 28.11, 30.54, 67.22	109.33, 113.73, 120.49, 126.81, 127.84, 129.61, 130.93, 131.46, 132.68, 140.96, 151.57, 161.06	124.89, 125.73	158.73	118.07
3c	13.09, 21.65, 24.99, 28.15, 28.23, 28.36, 28.50, 30.85, 67.23	109.34, 113.73, 120.49, 126.81, 127.85, 129.61, 130.94, 131.46, 132.68, 140.97, 151.58, 161.07	124.89, 125.73	158.71	118.06

4. Conclusion

In conclusion, three Schiff's bases: 3a, 3b and 3c were successfully synthesized by condensation reaction between primary amine and aldehyde and characterized by UV, FTIR, δ_{H} -NMR, ^{13}C -NMR spectroscopies and Mass spec. All three Schiff base compounds consist of aromatic substituent which make the compounds more stable. These compounds were believed to give great prospect in sensor which it can enhance selectivity and sensitivity in sensor detection. In future work, all three compounds will be applied as potential linker in E-DNA sensor for cancer detection to improve the sensitivity of the sensor. Besides, we hope the compound can be excellent linker as it consists of cyano functional group and alkyl chain to chemically binding with gold electrode and phosphate group in DNA.

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Acknowledgments

We would like to thank Universiti Malaysia Terengganu and Ministry of Higher Education (MOHE) for research grant FRGS vot number 59383 for giving us financial support and opportunity to be involved in this research