Introduction:

Monomeric pyridinium surfactants are important ingredients of several cosmetic products. They are often utilized as corrosion inhibitor, in emulsion polymerization, flotation of minerals, and in textile processing. Biological applications of these surfaces active agent include their antimicrobial activity, as drug and gene delivery agent.

The overall production of cationic surfactant continues to increase with time and their increasing demand. Major challenge which an industry faces in 21st century is to get the desired molecule not only in cost effective manner but also by environmental friendly means. In coming years the demand of such surfactants are going to increase. Thus, the need of the hour is to synthesize new surfactants from renewable feed stocks via cost effective approach having better surface, and biological properties which can replace the conventional cationic surfactants.

In recent years the over production of glycerol has become a cause of concern. Glycerol is an important by product of biodiesel manufacturing which is produced by transesterfication of vegetable oils. Approximately 10 kg of glycerol is produced for every 100 kg of oil taken for production of biodiesel. With the total production of biodiesel in thousands of tons, huge amount of glycerol is also produced as a byproduct. If glycerol can be utilized for making value added products the cost of B100 type biodiesel can be reduced from US$ 0.63 to US$ 0.35. This enormous amount of glycerol can only be utilized if new value added products are developed which have huge demand in various industries and which can replace existing products. In spite of green origin and overproduction the scientific community had

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failed to explore the potential usefulness of this naturally occurring molecule for making value added products. Thus, utilization of glycerol for the production of surfactants will check loss of energy and resources due to over production of glycerol.
Section 5.1: Synthesis and characterization of hydroxy group containing pyridinium surfactants.

Result and discussion:

Hydroxy group containing pyridinium surfactants have been synthesized starting from α-Olefins using co-bromination protocol. The co-bromination of α-Olefins (1-dodecene (1), 1-tetradecene (2), 1-hexadecene (3), and 1-octadecene (4), with protected glycerol (solketal (6)) at 60 °C, followed by deprotection with 10% HCl, gave chromatographically inseparable isomeric mixtures of β-bromo monoethers of glycerol: 3-(2-bromoalkyloxy)propane-1,2-diol (11a-14a)/3-(1-bromoalkane-2-yloxy)propane-1,2-diol (11b-14b). These β-Bromo glycerol monoethers, on reaction with pyridine (16), gave hydroxyl group containing pyridinium cationic surfactant; 1-(1-(2,3-dihydroxypropoxy)alkane-2-yl)pyridinium bromide (17a-20a)/1-(2-(2,3-dihydroxypropoxy)alkyl)pyridinium bromide (17b-20b).

Initially β-bromo glycerol monoethers were synthesized directly from terminal olefins with green approach utilizing solketal as solvent as well as reactant by co-halogenation reaction. Terminal olefins (1-dodecene (1), 1-tetradecene (2), 1-hexadecene (3) or 1-octadecene (4) on reaction with N-bromosuccinimide (NBS, (5)) and solketal (6) resulted in the formation of chromatographically inseparable positional isomers protected β-bromo monoethers (7a/b-10a/b). The reaction mixture containing positional isomers were directly deprotected with 10% hydrochloric acid into chromatographically inseparable isomeric mixtures of β-bromo monoethers of glycerol: 3-(2-bromoalkyloxy)propane-1,2-diol (11a-14a)/3-(1-bromoalkane-2-yloxy)propane-1,2-diol (11b-14b) and alkyl dibromides as impurity (all designated as (15) in Scheme -5.1). The formation of two isomers indicate that the most probable mechanism involves the formation of a cyclic bromonium ion as intermediate followed by nucleophilic attack of the hydroxyl group from either side as shown in Figure 5.1. β-Bromo glycerol monoethers were quaternized with pyridine to give hydroxyl group containing pyridinium cationic surfactant 1-(1-(2,3-dihydroxypropoxy)alkane-2-yl)pyridinium bromide (17a-20a)/1-(2-(2,3-dihydroxypropoxy)alkyl)pyridinium bromide (17b-20b).
The initial compounds protected β-bromo monoethers (7a/b-10a/b) were purified separately and were characterized by IR and NMR spectroscopy. In the infrared (IR) spectra of (7a/b-10a/b) the stretching for C-Br was observed in the range 667 to 671 cm⁻¹ for the dioxolanes while the C-O stretchings were observed at 1047-1054, 1072-1085, 1112-1115 and 1216-1236 cm⁻¹. The structure revealing ¹³C and ¹H NMR chemical shifts (δ ppm) of the products have been shown in Figure 5.2. The methyl (CH₃) protons attached to C-2 of the 1,3-dioxolane were observed as two singlets at δ 1.36 and δ 1.42 respectively for (7a & 7b). The proton attached to C-5 of the dioxolane ring were observed as a pair of ABX quartets at δ 3.76-3.81 and δ 4.03-4.09 ppm, respectively for both the vicinal protons (Hₐ and Hₙ). The CHBr multiplet at δ 4.04-4.09 was also merged with this signal. Similarly the CH₂-O-CH₂ protons were also observed as a pair of ABX quartets in the range δ 3.52 to 3.58 and δ 3.67-3.74. Clustering of all these signals in a narrow range gave a look of a broad multiplet. In the ¹³C NMR spectrum of (7a/b-10a/b) the C-2 carbon was observed at δ 109.2 to 109.5, while the ring carbon C-5 and C-4 were observed at δ 66.6 and 74.5 respectively. The oxy methyl carbon was observed at δ 71.9 and the C-1 of the alkyl chain was observed at δ 75.8 for isomer suffixed ‘a’ whereas the same carbon was observed at δ 35.12 for isomer suffixed ‘b’. Similarly the C-2 of alkyl chain was observed at δ 53.47 for isomer suffixed ‘a’ and the same was observed at δ 79.6 for isomer suffixed ‘b’. 
Figure 5.2: $^{13}$C and $^1$H Chemical shifts in $\delta$ ppm of (7a) and (7b).
Similarly, the structure of these $\beta$-bromo monoethers (11a/b-14a/b) has also been established by their mass spectra, infrared (IR) spectra, and $^1$H and $^{13}$C nuclear magnetic resonance (NMR) analyses. The IR spectra of the product (11a/b) gave a peak at 3380 cm$^{-1}$, representing the hydroxyl group. The C-O stretchings were observed at 1047, 1072, 1112 and 1216 cm$^{-1}$, along with C-Br stretching at 667.32 cm$^{-1}$. Two chromatographically inseparable positional isomers were formed (as expected) in every instance. The formation of positional isomers have been confirmed by the presence of double signals in both $^1$H NMR and $^{13}$C NMR spectra and was confirmed by $^{13}$C DEPT, 2 D COSY and HETCOR experiments. A multiplet from $\delta$ 3.52 to 4.10 was observed for most of the structure elucidating protons for both types of positional isomers. The $^{13}$C chemical shifts and DEPT experiment were more informative and helpful in establishing the structure of the diols (11a/b-14a/b). The C-1 of alkyl chain appeared at $\delta$ 35.07 to 35.37 for the isomer having the bromide group attached to this carbon as a negative signal, at the same time the C-2 of the alkyl chain was observed at $\delta$ 51.6 to 53.6 as a positive signal indicating the attachment of bromide to this carbon also. CH$_2$-O carbons were observed at 63.73, 63.99, 71.89, 72.17 and 75.54 as the negative signal, whereas, positive signals for carbon attached to the oxygen appeared at $\delta$ 70.55, 70.33 and 79.40. The appearance of double signals for the carbon attached to the bromide and oxygen confirmed formation of positional as well as the structural isomers in all the cases.

Finally, the structure of hydroxyl group containing pyridinium surfactants was characterized by elemental analysis, IR, NMR, Mass spectroscopy. Initially, the CHNO elemental analysis of the new cationic surfactants revealed almost accurate composition of carbon, hydrogen, nitrogen and oxygen present in the salt (17a/b-20a/b). The C% for (17a/b to 20a/b) was found to be 57.62, 59.27, 60.83 and 62.23 respectively which lies extremely close to those calculated values. Similarly N% was found to be 3.41, 3.20, 2.98 and 2.79 for (17a/b to 20a/b). The percent of oxygen and hydrogen also lie very close to those calculated values.

The characterization of synthesized surfactants were further done by Mass spectroscopy TOF MS ES+. The parent ion peak of (17a/b) observed at 338 (100% intensity), 339.2 (26%), 340.2 (2%) for calculated 339.2 molecular weight having molecular formula C$_{20}$H$_{36}$NO$_3$+. Similarly the parent ion peak having 100% intensity for (18a/b), (19a/b) and (20a/b) were observed at 366.3, 394.4 and 422.4 respectively.
which matched the calculated value. The IR spectra of the products gave a broad peak at 3388-3427 cm\(^{-1}\) for –OH group. The C-O stretching were observed at 1047-1048 and 1110-1112 cm\(^{-1}\).

Two multiplets were observed at a chemical shift of \(\delta\) 4.74-4.79 and 5.14-5.18 for -O-CH\(_2\)-CH-N\(^+\)C\(_5\)H\(_5\) and C\(_3\)H\(_3\)N\(^+\)CH\(_2\) respectively in the \(^1\)H NMR spectra of (17a/b-20a/b), suggests the presence of two positional isomers. Figure 5.3 shows \(^1\)H NMR spectra of gemini pyridinium surfactant (19a/b). The integration ratio of the two signals which denotes two protons each is helpful in establishing the amount of each isomer present. The ring protons of pyridine were observed at \(\delta\) 8.13-8.19, 8.58 and 9.33ppm.

![Figure 5.3: \(^1\)H NMR spectra of pyridinium surfactant (19a/b).](image)

The \(^{13}\)C NMR spectroscopy further helped to establish the structure of these compounds. The sp\(^3\) carbons for terminal methyl were observed at \(\delta\) 14.05-14.11 ppm while the sp\(^3\) carbons for CH\(_2\) were observed in the range of \(\delta\) 22.60 - 31.91 ppm.
Figure 5.4: $^{13}$C NMR spectra of pyridinium surfactant (19a/b).

Figure 5.5: $^{13}$C DEPT NMR spectra of pyridinium surfactant (19a/b).
However the sp\(^3\) carbons directly linked to a heteroatom, oxygen and nitrogen was observed at δ 63.97-64.01 ppm. **Figure 5.4** and **5.5** shows \(^{13}\)C NMR spectra and DEPT NMR spectra of pyridinium surfactant (19a/b), respectively. The CH-O carbon was observed at δ 78.21-78.33. The CH-N carbon was observed at δ 78.55-78.64. The assignment of chemical shifts to various protons and carbons has been done on the basis of DEPT (Distortionless enhanced polarization transfer), 2D HETCOR (Hetronuclear chemical shift correlation).

**Figure 5.6:** \(^1\)H-\(^{13}\)C 2D HETCOR NMR spectra of pyridinium surfactant (19a/b).

**Experimental:**

**Materials and Methods:** 1-Dodecene, 1-tetradecene, 1-hexadecane, 1-octadecene and ethidium bromide were purchased from Sigma Aldrich. N-bromosuccinimide (NBS) was purchased from Central Drug House, New Delhi, India. (2,2-dimethyl-1,3-
dioxolan-4-yl)methanol (solketal) was prepared by a previously reported method by refluxing glycerol in 1:1 acetone and petroleum ether in the presence of catalytic amount of p-toluenesulfonic acid monohydrate. N-bromosuccinimide (NBS), p-toluenesulfonic acid monohydrate, chloroform, hexane, ethyl acetate, diethyl ether and acetone were purchased from Central Drug House, New Delhi, India. Pyridine was purchased from Qualigens fine chemicals, Mumbai, India. Agarose.

FAB mass spectra of initials were recorded on a JEOL (Japan), model SX-102/DA-6000 mass spectrometer/data system using argon/xenon (6kV, 10 mA) as the FAB gas. Mass spectra of final surfactants were recorded on Waters Q-Tof Micromass. IR spectrum was recorded as a thin neat film on a Shimadzu model FT-IR 8400s (Kyoto, Japan) instrument. $^1$H and $^{13}$C NMR were recorded on a JEOL (Japan), model FT-NMR 300 MHz system (AL-300) and BRUKER AVANCE II 400 MHz system at the Punjab University, Chandigarh, India as a solution in CDCl$_3$, using tetramethylsilane (TMS) as an internal standard. Elemental analyses were recorded on a Thermo Electron (U.K.) made Flash EA 1112 Series CHNSO analyzer.

**Synthesis:** 1-dodecene (1; 3.36 g, 20 mmol), 1-tetradecene (2; 3.92 g, 20 mmol), 1-hexadecene (3; 4.48 g, 20 mmol), or 1-octadecene (4; 5.04 g, 20 mmol) was added to stirred solution of $N$-bromosuccinimide (5; 3.56 g, 20 mmol) and solketal (6; 7.92 g, 60 mmol) at 60°C. The reaction was stirred for 6 hours at 60°C. The progress of the reaction was monitored by thin layer chromatography and the equilibrium stage was obtained in 6 Hours. The crude reaction mixture was cooled to 25°C. Hexane (50 ml) was added to the reaction mixture and it was stirred for 5 minutes and filtered to remove the precipitated succinimide. The filtrate was collected in a separating funnel and washed 4 times with 50 ml of water followed by removal of hexane by rotary flash evaporator. The reaction mixture was treated with 20 ml of 10% HCl and vigorously stirred for 3 hours. The content was transferred to a separating funnel. 100 ml chloroform and water was added. The organic layer was washed till the pH of the water becomes 7. The organic layer was then dried over anhydrous sodium sulphate. The filtration followed by evaporation and subsequent fractionation on a silica gel (60-120 mesh) column chromatography using hexane, hexane: ethyl acetate mixture (at ratios of 100:00 to 70:30; the stepwise increasing-polarity elution method) yielded, first, unreacted olefins then dibromides (5-10%), the traces of the dioxolanes and then

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the respective glycerol monoethers (11a/b-14a/b) in 70 to 75% isolated yield as a pure fraction. This yield was calculated on the basis of the amount of α-olefins that actually reacted. The conversion per batch was 75-80%. The intermediate compounds 1,3- dioxolanes (7a/b-10a/b) were isolated separately through column chromatography after the removal of the succinimide and were characterised through spectroscopic techniques. Each individual glycerol monoethers (11a/b-14a/b) was taken in a round bottom flask and warmed to 50°C. Pyridine (16; 1.89g, 24 mmol) was then added and the mixture was stirred at 100°C for 10 hours. The reaction mixture was allowed to cool to 25°C. The crude reaction mixture (solid) was suspended in 40 ml of diethyl ether and stirred for 15 minutes at 0 to -5 °C. The suspended material was filtered to remove excess of pyridine and unreacted reactant. The step was repeated with diethyl ether and acetone at 0 to -5 °C to get white to off white product. The product was vacuum dried at 35°C in a rotary flash evaporator to get hydroxyl group containing pyridinium surfactants (17a/b-20a/b).
Synthesis and Properties of Hydroxy Group Containing Pyridinium Surfactants

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\begin{align*}
\text{RCH=CH}_2 \quad &+ \quad \text{[5]} \quad + \quad \text{[6]} \\
& \xrightarrow{60^\circ C} \\
\text{Br} \quad \text{R-CH-CH}_2 \quad \text{and} \quad \text{Br} \quad \text{R-CH-CH}_2 \\
\text{O} \quad \text{O} \quad \text{[7a-10a]} \quad \text{[7b-10b]} \\
& \xrightarrow{10\% \text{ aq HCl}} \quad \text{Room Temperature} \quad 3 \text{ hours} \\
\text{Br} \quad \text{R-CH-CH}_2 \quad \text{and} \quad \text{Br} \quad \text{R-CH-CH}_2 \\
\text{O} \quad \text{O} \quad \text{OH} \quad \text{OH} \quad \text{[15]} \\
\text{[11a-14a]} \quad \text{[11b-14b]} \\
\text{py}[16] \quad &\xrightarrow{100^\circ C} \quad 10 \text{ hours} \\
\text{R-CH-CH}_2 \quad \text{and} \quad \text{R-CH-CH}_2 \\
\text{O} \quad \text{O} \quad \text{OH} \quad \text{OH} \quad \text{[17a-20a]} \quad \text{[17b-20b]} \\
\begin{align*}
7, 11 & \& 17 \quad \text{R} = -(\text{CH}_2)_3-\text{CH}_3 \\
8, 12 & \& 18 \quad \text{R} = -(\text{CH}_2)_{11}-\text{CH}_3 \\
9, 13 & \& 19 \quad \text{R} = -(\text{CH}_2)_{13}-\text{CH}_3 \\
10, 14 & \& 20 \quad \text{R} = -(\text{CH}_2)_{15}-\text{CH}_3
\end{align*}
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Scheme 5.1
4-((2-bromododecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (7a)/ 4-((1-bromododecan-2-yl oxy)methyl)-2,2-dimethyl-1,3-dioxolane (7b): Slightly yellow liquid. 300 MHz $^1$H NMR (CDCl$_3$, TMS) $\delta$ ppm: 0.85 (dt, 6H, 2 × terminal–CH$_3$), 1.2-1.3 (br. s, 32 H chain CH$_2$), 1.36 (s, 6H, 2 × –CH$_3$ attached to C-2 of dioxolane ring), 1.42 (s, 6H, 2 × –CH$_3$ attached to C-2 of dioxolane ring), 1.58 (m, 4H, 2 × CH$_2$ (C-3 of aliphatic chain)), 3.40-3.42 (m, 2H, CH$_2$-Br), 3.50 (m, 1H, BrCH$_2$-CHO-), 3.52-3.58 (m, 4H, 2 × -OC$_H$$_a$H$_b$-), 3.67-3.74 (m, 2H, -OC$_H$$_a$H$_b$-CHBr), 3.76-3.81 (m, 2H, 2 × -OCH$_a$H$_b$(proton attached to C-5 of the ring)), 4.05-4.10 (m, 3H, CH$_3$-Br, 2 × -OC$_H$$_a$H$_b$-(proton attached to C-5 of the ring)), 4.26-4.29 (m, 2H, 2 × –C$_H$-(methine proton of C-4 of the ring)). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) $\delta$ ppm: 14.05 (+ve, terminal 2 × CH$_3$), 22.64 - 34.88 (–ve, chain CH$_2$), 26.34 & 26.68, (+ve, 2 × CH$_3$), 35.14 (–ve, CH$_2$Br), 35.54 (+ve, CHBr), 66.64, 66.91, (–ve, C-5 of the ring), 70.72, 71.00 (–ve, 2 × -OCH$_2$-CHBr), 74.56 (+ve, 2 × C-4 of the ring), 75.87 (–ve, -OCH$_2$-CHBr), 79.70 (–ve, BrCH$_2$-CHO-), 109.29, 109.41 (2 × C(CH$_3$)$_2$ observed only in $^{13}$C). IR (CHCl$_3$) cm$^{-1}$: 667.32, 1054, 1085, 1155, 1217. ESI-MS positive ions m/z: 401.2 and 403.2 (M$^+$+Na and M$^+$+2+Na) parent ion.

4-((2-bromotetradecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (8a)/ 4-((1-bromotetradecan-2-yl oxy)methyl)-2,2-dimethyl-1,3-dioxolane (8b): Slightly yellow viscous liquid. 300 MHz $^1$H NMR (CDCl$_3$, TMS) $\delta$ ppm: 0.88 (dt, 6H, 2 × terminal–CH$_3$), 1.2-1.3 (br. s, 40 H chain CH$_2$), 1.36 (s, 6H, 2 × –CH$_3$ attached to C-2 of dioxolane ring), 1.42 (s, 6H, 2 × –CH$_3$ attached to C-2 of dioxolane ring), 1.57 (m, 4H, 2 × CH$_2$ (C-3 of aliphatic chain)), 3.39-3.42 (m, 2H, CH$_2$-Br), 3.49 (m, 1H, BrCH$_2$-CHO-), 3.52-3.58 (m, 4H, 2 × -OCH$_2$H$_b$-), 3.67-3.74 (m, 2H, -OCH$_2$H$_b$-CHBr), 3.76-3.82 (m, 2H, 2 × -OCH$_a$H$_b$(proton attached to C-5 of the ring)), 4.03-4.09 (m, 3H, CH$_2$Br, 2 × -OCH$_a$H$_b$(proton attached to C-5 of the ring)), 4.25-4.27 (m, 2H, 2 × –CH-(methine proton of C-4 of the ring)). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) $\delta$ ppm: 14.19 (+ve, terminal 2 × CH$_3$), 22.69 - 34.78 (–ve, chain CH$_2$), 25.40, 26.79 (+ve, 2 × CH$_3$), 35.22 (–ve, CH$_2$Br), 53.65 (+ve, CHBr), 66.71, 66.98, (–ve, C-5 of the ring), 70.87, 71.15 (–ve, -OCH$_2$-CHBr), 74.64 (+ve, 2 × C-4 of the ring), 75.87 (–ve, -OCH$_2$-CHBr), 79.79 (+ve, BrCH$_2$-CHO-), 109.42, 109.53 (2 × C(CH$_3$)$_2$ observed only in $^{13}$C). IR (CHCl$_3$) cm$^{-1}$: 669.25, 1028, 1053, 1115, 1234. ESI-MS positive ions m/z: 429.2 and 431.2 (M$^+$+Na and M$^+$+2+Na) parent ion.
4-((2-bromohexadecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (9a)/ 4-((1-bromohexadecan-2-oyoxy)methyl)-2,2-dimethyl-1,3-dioxolane (9b): Slightly yellow viscous liquid. 300 MHz $^1$H NMR (CDCl$_3$, TMS) $\delta$ ppm: $\delta$ 0.85 (dt, 2 x terminal–CH$_3$), 1.2-1.3 (br. s, 48 H chain CH$_2$), 1.36 (s, 6H, 2 x –CH$_3$ attached to C-2 of dioxolane ring), 1.42 (s, 6H, 2 x –CH$_3$ attached to C-2 of dioxolane ring), 1.58 (m, 4H, 2 x CH$_2$ (C-3 of aliphatic chain)), 3.39-3.42 ( m, 2H, CH$_2$-Br), 3.50 (m, 1H, BrCH$_2$-CHO-), 3.52-3.58 ( m, 4H, 2 x -OCH$_2$H$_5$), 3.67-3.74 ( m, 2H, -CHBr), 3.76-3.82 (m, 2H, -OCH$_2$H$_5$-(proton attached to C-5 of the ring)), 4.04-4.09 (m, 3H, CHBr, 2 x -OCH$_2$H$_5$-(proton attached to C-5 of the ring)), 4.25-4.27 ( m, 2H, 2 x –CBr-(methine proton of C-4 of the ring)). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) $\delta$ ppm: 14.03 (+ve, terminal 2 x CH$_3$), 20.16 - 34.12 ( –ve, chain CH$_2$), 25.18 (+ve, 4 x CH$_3$ ), 35.12 ( –ve, CH$_2$Br), 51.63 (+ve, -CHBr), 66.62, 66.89, ( –ve, C-5 of the ring), 70.72, 71.00 ( –ve, -OCH$_2$-), 74.56 (+ve, 2 x C-4 of the ring), 76.58 ( –ve, -OCH$_2$CHBr) 79.76 (+ve, BrCH$_2$-CHO-), 109.27, 109.28 (2 x C(CH$_3$)$_2$ observed only in $^{13}$C). IR (CHCl$_3$) cm$^{-1}$: 668.46, 1047, 1072, 1112, 1216. ESI-MS positive ions m/z: 457.2 and 459.2 (M$^+$+Na and M$^+$+2+Na) parent ion.

4-((2-bromooctadecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane (10a)/ 4-((1-bromooctadecan-2-oyoxy)methyl)-2,2-dimethyl-1,3-dioxolane (10b): White waxy liquid. 300 MHz $^1$H NMR (CDCl$_3$, TMS) $\delta$ ppm: 0.85 (dt, 2 x terminal–CH$_3$), 1.2-1.3 (br. s, 56 H chain CH$_2$), 1.36 (s, 6H, 2 x –CH$_3$ attached to C-2 of dioxolane ring), 1.42 (s, 6H, 2 x –CH$_3$ attached to C-2 of dioxolane ring), 1.58 (m, 4H, 2 x CH$_2$ (C-3 of aliphatic chain)), 3.39-3.42 ( m, 2H, CH$_2$-Br), 3.49 (m, 1H, BrCH$_2$-CHO-), 3.52-3.58 ( m, 4H, 2 x -OCH$_2$H$_5$), 3.67-3.74 ( m, 2H, -OCH$_2$H$_5$-CHBr), 3.76-3.82 (m, 2H, 2 x -OCH$_2$H$_5$-(proton attached to C-5 of the ring)), 4.05-4.09 ( m, 3H, CHBr, 2 x -OCH$_2$H$_5$-(proton attached to C-5 of the ring)), 4.28-4.31 (m, 2H, 2 x –CBr-(methine proton of C-4 of the ring)). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) $\delta$ ppm: 14.14 (+ve, terminal 2 x CH$_3$), 22.63 - 34.85 ( –ve, chain CH$_2$), 25.18 (+ve, 4 x CH$_3$ ), 35.12 ( –ve, CH$_2$Br), 51.63 (+ve, -CHBr), 66.62, 66.89, ( –ve, C-5 of the ring), 70.72, 71.00 ( –ve, -OCH$_2$-), 74.56 (+ve, 2 x C-4 of the ring), 76.58 ( –ve, -OCH$_2$CHBr) 79.76 (+ve, BrCH$_2$-CHO-), 109.27, 109.28 (2 x C(CH$_3$)$_2$ observed only in $^{13}$C). IR (CHCl$_3$) cm$^{-1}$: 667.32, 1054, 1081, 1155, 1236, 3380. ESI-MS positive ions m/z: 485.2 and 487.2 (M$^+$+Na and M$^+$+2+Na) parent ion.
3-(2-bromododecyloxy)propane-1,2-diol (11a)/ 3-(1-bromododecan-2-yloxy)propane-1,2-diol (11b): Yellowish white translucent viscous liquid, Yield 74.17%. 300 MHz $^1$H NMR (CDCl$_3$, TMS) δ ppm: 0.85 (dt, 2 × –CH$_3$), 1.2-1.3 (br. s, 32 H chain CH$_2$), 1.50-1.60 (m, 2H, -CH$_2$-CH$_2$-CHBr), 1.82 (m, 2H, -CHO-CH$_2$-), 2.35 (bs, 2H, 2 × OH-1), 2.75 (bs, 2H, 2 × OH-2), 3.38-3.52 (m, 3H, -CH$_2$Br, BrCH$_2$-CHO-), 3.54-3.76 (m, 10H, 2 × –OC$_2$H$_4$H$_6$OH, -OCH$_2$-CHBr), 3.87 (m, 2H, 2 × -CHBr). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) δ ppm: 14.03 (+ve, terminal CH$_3$), 22.56-32.94 (-ve, chain CH$_2$), 35.07 (-ve, C$_2$H$_2$Br), 53.80 (+ve, CHBr), 63.75, 63.83 (-ve, C1 CH$_2$-O), 70.57, 70.76 (+ve, C2 CH-O), 71.08, 72.41 (-ve, C3 CH$_2$-O), 75.54 (-ve, -OCH$_2$-CHBr), 79.40 (+ve, BrCH$_2$-CH-O). IR (CHCl$_3$) cm$^{-1}$: 667.32, 1054, 1085, 1114, 1220, 3380. ESI-MS positive ions m/z: 361.2 and 363.2 (M$^+$+Na and M$^+$+2+Na) parent ion.

3-(2-bromotetradecyloxy)propane-1,2-diol (12a)/ 3-(1-bromotetradecan-2-yloxy)propane-1,2-diol (12b): Yellowish white translucent viscous liquid, Yield 70.30%. 300 MHz $^1$H NMR (CDCl$_3$, TMS) δ ppm: δ 0.88 (t, 2 × –CH$_3$), 1.2-1.3 (br. s, 40 H chain CH$_2$), 1.50-1.60 (m, 2H, -CH$_2$-CH$_2$-CHBr), 1.81 (m, 2H, -CHO-CH$_2$-), 2.86 (bs, 2H, 2 × OH-1), 2.86 (bs, 2H, 2 × OH-2), 3.31-3.51 (m, 3H, -CH$_2$Br, BrCH$_2$-CHO-), 3.55-3.76 (m, 10H, 2 × –OC$_2$H$_4$H$_6$OH, -OCH$_2$-CHBr), 3.87 (m, 2H, 2 × -CHBr). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) δ ppm: 14.10 (+ve, terminal CH$_3$), 22.56-32.94 (-ve, chain CH$_2$), 35.07 (-ve, CH$_2$Br), 53.80 (+ve, CHBr), 63.75, 63.83 (-ve, C1 CH$_2$-O), 70.57, 70.76 (+ve, C2 CH-O), 71.08, 72.41 (-ve, C3 CH$_2$-O), 75.54 (-ve, -OCH$_2$-CHBr), 79.40 (+ve, BrCH$_2$-CH-O). IR (CHCl$_3$) cm$^{-1}$: 667.32, 1054, 1085, 1114, 1220, 3380. ESI-MS positive ions m/z: 389.2 and 391.2 (M$^+$+Na and M$^+$+2+Na) parent ion.

3-(2-bromohexadecyloxy)propane-1,2-diol (13a)/ 3-(1-bromohexadecan-2-yloxy)propane-1,2-diol (13b): White viscous liquid, Yield 70.37%. 300 MHz $^1$H NMR (CDCl$_3$, TMS) δ ppm: 0.85 (dt, 6H, 2 × –CH$_3$), 1.2-1.3 (br. s, 48 H chain CH$_2$), 1.50-1.60 (m, 2H, -CH$_2$-CH$_2$-CHBr), 1.82 (m, 2H, -CHO-CH$_2$-), 2.21 (bs, 2H, 2 × OH-1), 2.79 (bs, 2H, 2 × -CHOH), 3.37-3.53 (m, 3H, -CH$_2$Br, BrCH$_2$-CHO-), 3.55-3.76 (m, 10H, 2 × –OC$_2$H$_4$H$_6$OH, -OCH$_2$-CHBr), 3.87 (m, 2H, 2 × -CHBr), 4.10 (m, 1H, -CHBr). 75 MHz $^{13}$C/DEPT NMR (CDCl$_3$) δ ppm: 14.17 (+ve, terminal CH$_3$), 22.66-35.06 (-ve, chain CH$_2$), 35.33 (-ve, CH$_2$Br), 53.74 (+ve, CHBr), 63.87 (-ve, C1 CH$_2$-O), 70.59 (+ve, C2 CH-O), 71.01, 72.42 (-ve, C3 CH$_2$-O), 75.54 (-ve,
3-(2-bromooctadecyloxy)propane-1,2-diol (14a) / 3-(1-bromohexadecan-2-yloxy)propane-1,2-diol (14b): White waxy solid, Yield 73.02%. 300 MHz 1H NMR (CDCl₃, TMS) δ ppm: 0.85 (t, 6H, 2 × –C₃H₃), 1.2-1.3 (br. s, 56 H chain C₂H₂), 1.50-1.60 (m, 2H, -CH₂-CH₂-CHBr), 1.79 (m, 2H, -CHO-CH₂-), 2.23 (bs, 2H, 2 × OH-1), 2.63 (bs, 2H, 2 × OH-2), 3.33-3.48 (m, 3H, -C₂H₂Br,BrCH₂-C₂H₂), 3.81 (m, 2H, 2 × -C₂H₂OH), 4.04 (m, 1H, -C₅HBr). 75 MHz 13C/DEPT NMR (CDCl₃) δ ppm: 14.12 (+ve, terminal C₃H₃), 22.61-31.88 (ve, chain C₂H₂), 63.30, 63.67 (C₃ (ve, -C₂H₂OH)), 64.01 (–ve, 2 × C, -O-CH₂-CH-N⁺C₃H₅, C₃H₅N⁺CH₂), 70.72, 71.61 (+ve, C₂ -CHOH), 71.09, 72.12 (–ve, C₁ -CH₂O), 78.26, 78.60 (+ve, C₃H₅N⁺CH₂-CHO-, C₃H₅N⁺CH₂-), 128.02 (+ve, 2 × C-3,5 of N⁺C₃H₅), 145.63 (+ve, 2 × C-4 of N⁺C₃H₅), 145.96 (+ve, 2 × C-2,6 of N⁺C₃H₅). IR (CHCl₃) cm⁻¹: 669, 1047, 1074, 1108, 1215, 3390. ESI-MS positive ions m/z: 445.2 and 447.2 (M⁺+Na and M⁺+2Na) parent ion.

1-(1-(2,3-dihydroxypropoxy)dodecan-2-yl)pyridinium bromide (17a) / 1-(2-(2,3-dihydroxypropoxy)dodecyl)pyridinium bromide (17b): Off white solid, Yield 62.72%. 300 MHz 1H NMR (CDCl₃, TMS) δ ppm: 0.87 (dt, 6H, 2 × C₃H₃), 1.25 (br s, 30H, C₂H₂ chain), 1.39 (br s, 4H, C₅H₅N⁺CH₂-CH₂), 1.55 (br s, 2H, β to C₅H₅N⁺CH₂-CH₂), 2.31 (br s, 3H, 3 × -OH), 3.42-3.67 (m, 8H, 2 × -O-CH₂H₅OH, 2 × CH-CH₂H₅-O-), 3.73 (m, 2H, 2 × -CHOH), 3.91 (br s, 3H, -OH, C₅H₅N⁺CH₂-CH₂), 4.69-4.75 (q, 2H, -O-C₂H₂-CH-N⁺C₅H₅), 5.12-5.16 (m, 2H, C₅H₅N⁺CH₂), 8.12-8.20 (merged q, 4H, 2 × py-H-3,5), 8.57 (t, 2H, J = 7.8 Hz, 2 × pyH-4), 9.27 (merged t, 4H, 2 × pyH-2,6). 75 MHz 13C/DEPT NMR (CDCl₃) δ ppm: 14.09 (+ve, terminal CH₃), 22.64-31.88 (–ve, chain CH₂), 63.30, 63.67 (C₃ (–ve, -CH₂-OH)), 64.01 (–ve, 2 × C, -O-CH₂-CH-N⁺C₃H₅, C₃H₅N⁺CH₂), 70.72, 71.61 (+ve, C₂-CHOH), 71.09, 72.12 (–ve, C₁ -CH₂O), 78.26, 78.60 (+ve, C₃H₅N⁺CH₂-CHO-, C₃H₅N⁺CH₂-), 128.02 (+ve, 2 × C-3,5 of N⁺C₃H₅), 145.63 (+ve, 2 × C-4 of N⁺C₃H₅), 145.96 (+ve, 2 × C-2,6 of N⁺C₃H₅). IR (CHCl₃) cm⁻¹: 3427, 3388, 2923, 2854, 1636, 1488, 1108, 1048. ESI-MS positive ions m/z: 338.2, 339.2, 340.2. Elemental analysis % calculated (found): C, 57.41; H, 8.67; N, 3.35; O, 11.47 (C, 57.62; H, 8.61; N, 3.41; O, 11.56).
1-(1-(2,3-dihydroxypropoxy)tетradecan-2yl)pyridinium bromide (18a) / 1-(2-(2,3-
dihydroxypropoxy)tetradecyl)pyridinium bromide (18b): White solid, Yield 60.87%. 300 MHz $^1$H NMR (CDCl$_3$, TMS) $\delta$ ppm: 0.87 (t, 6H, 2 × CH$_3$), 1.25 (br s, 3H, CH$_2$ chain), 1.39 (4H, C$_3$H$_7$N$^+$CH-CH$_2$, -CHO-CH$_2$-), 1.56 (br s, 2H, $\beta$ to C$_3$H$_7$N$^+$CH-), 3.39-3.67 (m, 8H, 2 × -CH$_3$H$_3$OH, 2 × CH-CH$_3$H$_3$O-), 3.72 (m, 2H, 2 × -CHOH), 3.90 (br s, 2H, C$_3$H$_7$N$^+$CH$_2$-CHO-, C$_3$H$_7$N$^+$CH-), 4.17 (br s, 2H, 2 × -OH), 4.25 (br s, 2H, 2 × -OH), 4.71-4.75 (q, 2H, -O-C$_5$H$_9$), 4.74-4.79 (m, 2H, 2 × -O-C$_5$H$_9$), 4.25 (br s, 2H, 2 × -OH), 4.71-4.75 (q, 2H, -O-C$_5$H$_9$), 4.74-4.79 (m, 2H, 2 × -O-C$_5$H$_9$). IR (CHCl$_3$) cm$^{-1}$: 3415, 3350, 2923, 2852, 1635, 1488, 1112, 1049. ESI-MS positive ions m/z: 366.3, 367.3, 368.3. Elemental analysis % calculated (found): C, 59.18; H, 9.03; N, 3.14; O, 10.75 (C, 59.27; H, 9.99; N, 3.20; O, 10.82).

1-(1-(2,3-dihydroxypropoxy)hexadecan-2yl)pyridinium bromide (19a) / 1-(2-(2,3-
dihydroxypropoxy)hexadecyl)pyridinium bromide (19b): White solid, Yield 68.92%. 400 MHz $^1$H NMR (CDCl$_3$, TMS) $\delta$ ppm: 0.87 (t, 6H, 2 × CH$_3$), 1.29 (br s, 46H, CH$_2$ chain), 1.39 (br.s, 7H, 3 × -OH, C$_3$H$_7$N$^+$CH-CH$_2$, -CHO-CH$_2$-), 1.58 (m, 2H, $\beta$ to C$_3$H$_7$N$^+$CH-), 3.38-3.66 (m, 8H, 2 × -CH$_3$H$_3$OH, 2 × CH-CH$_3$H$_3$O-), 3.73 (m, 2H, 2 × -CHOH), 3.88 (br s, 3H, -OH, C$_3$H$_7$N$^+$CH$_2$-CHO-, C$_3$H$_7$N$^+$CH-), 4.74-4.79 (q, 2H, -O-C$_5$H$_9$-CHN$^+$C$_3$H$_3$), 5.14-5.18 (m, 2H, C$_3$H$_7$N$^+$CH$_2$), 8.13-8.19 (merged q, 4H, 2 × H-3,5), 8.58 (t, 2H, J = 7.9 Hz, 2 × H-4), 9.33 (merged t, 4H, 2 × H-4), 9.33 (merged q, 4H, 2 × H-3,5), 8.58 (t, 2H, J = 7.9 Hz, 2 × H-4), 9.33 (merged t, 4H, 2 × H-4). 100 MHz $^{13}$C/DEPT NMR (CDCl$_3$) $\delta$ ppm: 14.11 (+ve, terminal CH$_3$), 22.67-31.91 (−ve, chain CH$_2$), 63.33 & 63.69 (−ve, C3 (-CH$_2$-OH), 63.99 (−ve, 2 × C, -O-CH$_2$-CH-N$^+$C$_3$H$_3$, C$_3$H$_7$N$^+$CH$_2$), 70.80 & 71.63 (+ve, C2 -CHO), 71.11 & 72.12 (−ve, C1 -CH$_2$O), 78.33 & 78.64 (+ve, C$_3$H$_7$N$^+$CH$_2$-CHO-, C$_3$H$_7$N$^+$CH-), 128.04 (+ve, 2 × C-3,5 of N$^+$C$_3$H$_3$), 145.64 (+ve, 2 × C-4 of N$^+$C$_3$H$_3$), 146.03 (+ve, 2 × C-2,6 of N$^+$C$_3$H$_3$). IR (CHCl$_3$) cm$^{-1}$: 3413, 3390, 2923, 2852, 1636, 1467, 1110, 1047. ESI-MS positive ions m/z: 394.4, 395.4, 396.4. Elemental analysis % calculated (found): C, 60.75; H, 9.35; N, 2.95; O, 10.12 (C, 60.83; H, 9.41; N, 2.98; O, 10.07).
Synthesis and Properties of Hydroxy Group Containing Pyridinium Surfactants

1-(1-(2,3-dihydroxypropoxy)octadecan-2yl)pyridinium bromide (20a) / 1-(2-(2,3-dihydroxypropoxy)octadecyl)pyridinium bromide (20b): Yield 66.2%, white solid.

300 MHz $^1H$ NMR (CDCl$_3$, TMS) δ ppm: 0.87 (dt, 6H, 2 × CH$_3$), 1.25 (br s, 54H, CH$_2$ chain), 1.39 (br s, 4H, C$_3$H$_5$N$^+$CH-CH$_2$), 1.66 (br s, 6H, 4 × –OH, β to C$_3$H$_5$N$^+$CH-), 3.40-3.70 (m, 8H, 2 × -C$_2$H$_4$OH, 2 × CH–C$_2$H$_4$-O-), 3.76 (m, 2H, 2 × -CHOH), 3.93 (br s, 2H, C$_3$H$_5$N$^+$CH$_2$-CHO-, C$_3$H$_5$N$^+$CHI-), 4.73 (m, 2H, -O-CH$_2$-CH-N$^+$C$_3$H$_5$), 5.18-5.20 (m, 2H, C$_3$H$_5$N$^+$CH$_2$), 8.08-8.15 (merged q, 4H, 2 × H-3,5), 8.51 (t, J = 7.5 Hz, 2 × H-4), 9.32 (merged d, 4H, 2 × H-2,6).

$^{75}$ MHz $^{13}$C/DEPT NMR (CDCl$_3$) δ ppm: 14.05 (+ve, terminal CH$_3$), 22.60-31.84 (−ve, chain CH$_2$), 63.25 & 63.61(−ve, C3 (-CH$_2$-OH), 63.97 (−ve, 2 × C, -O-CH$_2$-CH-N$^+$C$_3$H$_5$, C$_3$H$_5$N$^+$CH$_2$), 70.67 & 71.56 (+ve, C2 -CHOH), 71.03 & 72.06 (−ve, C1 -CH$_2$O), 78.21 & 78.55 (+ve, C$_3$H$_5$N$^+$CH$_2$-CHO-, C$_3$H$_5$N$^+$CH-), 127.91 (+ve, 2 × C-3,5 of N$^+$C$_3$H$_5$), 145.57 (+ve, 2 × C-4 of N$^+$C$_3$H$_5$), 145.89(+ve, 2 × C-2,6 of N$^+$C$_3$H$_5$). IR (CHCl$_3$) cm$^{-1}$: 3407, 3388, 2920, 2850, 1635, 1465, 1110, 1048. ESI-MS positive ions m/z: 422.4, 423.4, 424.4. Elemental analysis % calculated (found): C, 62.14; H, 9.63; N, 2.79; O, 9.55 (C, 62.23; H, 9.59; N, 2.85; O, 9.61).
Section 5.2: Evaluation of surface and biological properties of hydroxy group containing pyridinium surfactants.

Result and discussion:

a) Evaluation of surface properties: The critical micelle concentration (cmc) and affinity to reduce surface tension at cmc of these hydroxyl group containing pyridinium surfactants (17a/b-20a/b) have been determined by surface tension measurements (Table – 5.1). The presence of two hydroxyl groups impart polarity and there is possibility of significant hydrogen bonding in the aqueous system, such type of bonding favors aggregation of surfactant monomer to form micelle. A general trend was observed as the cmc decreased with the increase in chain length. The affinity to reduce surface tension by these surfactants decreased with increase in hydrophobic alkyl chain length. Figure 5.7 shows plot of decrease in surface tension with increase in surfactant concentration for pyridinium surfactants (17a/b-20a/b) and TTAB.

![Surface Tension versus concentration plot of pyridinium surfactants 17a/b-20a/b and TTAB.](image)

Figure 5.7: Surface Tension versus concentration plot of pyridinium surfactants 17a/b-20a/b and TTAB.

Table 5.1: Surface tension, haemolytic activity and IC<sub>50</sub> values of pyridinium surfactants 17a/b-20a/b

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<sup>a</sup> Ratio of isomers determined by integration value of chemical shift at δ 4.74-4.79 and 5.14-5.18 by NMR.  
<sup>b</sup> cmc obtained by Du Nouy ring tensiometer.

The cmc of these new hydroxy groups containing pyridinium surfactants have been found to be lower compared to conventional cationic surfactants tetradecyltrimethyl ammonium bromide (TTAB) and hexadecylpyridinium bromide (HDPB).

**b) Evaluation of DNA binding properties:** DNA binding capability of these new hydroxy groups containing pyridinium surfactants was evaluated through simple agarose gel electrophoresis. This binding capability of the new surfactants to DNA increased with increase in hydrophobic alkyl chain length and has been found to be better compared to tetradecyltrimethyl ammonium bromide (TTAB) and hexadecylpyridinium bromide (HDPB). This important property can be exploited for DNA extraction methods as well as for gene delivery. It was found that all surfactants were able to bind plasmid DNA at 10mM concentration. However it was observed that binding capability increased with increase in alkyl chain length. Surfactant (17a/b) and (18a/b) were unable to bind plasmid DNA at 100µM and 1mM concentrations while surfactant (19a/b) was able to bind at 1mM concentration and no binding was observed at 100µM. Surfactant (20a/b) was able to bind at 100 µM as well as 1mM concentration (**Figure – 5.8**). It was also observed that presence of glycerol moiety in the surfactant molecule increased its DNA binding capability as all the molecule synthesized were able to bind DNA at concentration level of 10mM but none of the two reference molecules was able to bind DNA at the same concentration although slight retardation was observed in case of hexadecylpyridinium bromide (HDPB).
c) Evaluation of cytotoxicity and hemolytic activity: Low toxicity of a molecule is a prerequisite for its biomedical applications such as gene and drug delivery and also environmental aspect such as biodegradability. The cytotoxicity of these cationic surfactants was assessed on C6 glioma cells. Interestingly the results of these new molecules explicate direct relation between the alkyl chain length of the compounds and their cytotoxicity. Pyridinium surfactant (17a/b) was found to be the least toxic having IC$_{50}$ value of 35.99μM whereas (20a/b) was found to be the most toxic among the series of pyridinium cationics synthesized and reported in the present study having IC$_{50}$ value of 8.43 μM. Further the evaluation of haemolytic activity of these surfactants correlate the trend of increase in the toxicity with increase in alkyl chain length as evident with MTT assay results (Table-5.1). Again the most toxic of all the surfactants synthesized was (20a/b) causing rupture of the red blood cells at 60 μM concentration of the surfactant. The presence of two hydroxyl groups imparts polarity and is responsible for increase in hydrophilic character of the molecule, which correspondingly reduces its toxicity. Keeping the hydrophilic part constant and subsequently increasing the alkyl chain length caused increase in toxicity. All these new surfactants synthesized were less toxic than commercially available cationic surfactants tetradecyltrimethyl ammonium bromide (TTAB) and hexadecylpyridinium bromide (HDPB).
Experimental:

Materials and Methods: Tris buffer and glycerol were purchased from Sisco research laboratory Pvt. Ltd, Mumbai, India. Ethidium Bromide, TTAB and HDPB were purchased from Sigma Aldrich, USA. Plasmid DNA pUC 18 was purchased from Bangalore GeNei, Bangalore, India. Triply distilled autoclaved water was used in all experiments.

Surface tension measurements: Critical micelle concentration (cmc) and Surface tension attained at cmc was determined using CSC (Central scientific Co., Inc, USA) Du Nouy interfacial tensiometer using platinum-iridium ring (circumference – 5.992 cm) at 25 ± 0.1 °C. The tensiometer was calibrated using triple distilled water. The surfactant solution was aged for 12 hours prior to determination of surface activity.

Agarose Gel Electrophoresis: pDNA and surfactants were loaded with 5µL glycerol into 1% Agarose gel containing 2µL of ethidium bromide (0.5 mg/mL) at different concentration. Electrophoresis was carried out at 100 V in Tris buffer for 30 min. The DNA band was visualized under UV transillumination Alpha Imager HP (Alpha Innotech Corporation, USA) Photographs were taken using Alpha Imager.

Cytotoxicity and Hemolytic activity: The MTT based cytotoxicity test was used to evaluate all the four glycerol based cationic surfactants and the test was carried out on C6 glioma (cancerous brain cell line). Cells were seeded in 96 -well flat bottom microplates at the density 2.5-3.0 X 10^4 per ml, 100 µl per well and were allowed to grow for 24 hours. The compounds dissolved in double distilled water were sterilized using 0.22µ Millipore filter and were added to culture media over a concentration range of 0.1 µM to 100 µM. The cytotoxicity of the compounds was assessed after 24 hours of exposure. Assessment was made using Muliskan PLUS plate reader (Labsystem). The statistical analysis was performed using Sigma stat 3.5.1 and Sigma plot 11.0

Haemolytic activity of the compounds was assessed on Human red blood cells (2% in PBS). Concentration range of the compounds was 1µM to 1mM. Human red blood cells were kept in incubation for 2 hours and the amount of haemoglobin released was determined using Muliskan PLUS plate reader (Labsystem) at 540 nm. The cytotoxicity and haemolysis experiments were done twice in triplicates.