

The First tetrafluorinated azobenzene-imidazolium ionic conjugates as potential thermotropic liquid crystalline drugs: Self-assembly properties and cytotoxic effects

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ABSTRACT

A new series of tetrafluorinated azobenzene-imidazolium ionic liquid crystals (ILCs) of varying alkyl chain length ($n = 10$ – 18 in even parity) in the imidazolium cationic head group was prepared and characterized using FTIR and NMR spectroscopy, and elemental microanalysis. Their thermal and phase properties were investigated using differential scanning calorimetry (DSC) and polarized optical microscope (POM). Their phase properties and morphology were influenced by fluorination and alkyl chain length. Long alkyl chains were necessary to induce liquid crystalline behaviour in these salts. All homologues were mesogenic, exhibiting stable smectic A phase with broad temperature range. Cytotoxic effects of these ILCs against human cervical cancer (HeLa) cells and normal fibroblasts was evaluated using the MTT assay. Cytotoxicity of these ILCs was dependent on the alkyl chain length in the imidazolium cationic head. All the salts were less toxic towards normal fibroblasts and exhibited 7–27-fold enhancement in cytotoxicity against HeLa cells (24hr IC_{50} ranged between $0.61 \mu M$ – $2.84 \mu M$) as compared to the standard therapeutic drug, Etoposide ($IC_{50} = 25.67 \mu M$). Long homologues ($n = 16, 18$) were selective towards cancer cells with selectivity index ≥ 3 . These thermotropic ILCs could be doped with room-temperature mesomorphic polymers for the formulation of safe and selective anticancer drugs.

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1. Introduction

Liquid crystals (LCs) exist in biological systems, for example glycolipids, nucleic acids and carbohydrates [1]. The biomembrane is a lamellar bilayer that resembles the structure of smectic LCs. The combination of order and mobility in LCs are essential for the survival of living systems which depends on flexibility and structure formation [2]. Hence, LCs have emerged as a scientific discipline in biology and become the basis of many advanced biological and medical applications [3–6]. While many pharmaceutical LCs are lyotropic and they had been extensively investigated in emulsion technology [7], their functions and electronic properties may be greatly influenced and compromised in aqueous media. Hence, it is interesting and appealing to fabricate solvent-free thermotropic LCs. Biomacromolecular thermotropic LCs from DNA and proteins, glycolipids or Ferittin-containing anhydrous thermotropic LCs and

thermotropic LCs drugs such as itraconazole hydrochloride and Cyclosporin were documented [8,9]. These thermotropic LCs drugs could be doped with room-temperature mesomorphic excipient polymers to develop new type of drug delivery systems.

Ionic LCs, on the other hand, are organic salts combining the order and anisotropic properties of LCs with ionic conductivity and fluidity of ionic liquids [10]. Ionic interactions in ILCs induce microspace separation consisting of hydrophilic and hydrophobic domains with enhanced mesophase stability. They are used in a wide variety of applications, such as material science, optoelectric materials, reaction media and catalysis [11–14]. The biological relevance of ILCs, in particular, however, is little known, of which only a few reports were documented [15–17]. Amphiphilic azobenzene incorporating different cationic head groups (guanidinium, ammonium, amidinium and imidazolium) with LC properties were reported [18–19]. Azobenzene is anisotropic and rigid, and often used as mesogenic cores. Azobenzene derivatives have been extensively investigated due to their synthesis versatility and high thermal stability, multi functions and stimuli-responsiveness

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[20,21]. Their biological activities and biomedical applications were also documented. This class of compounds found important applications in drug delivery and cancer therapy [22,23]. On the other hand, imidazole derivatives play a critical role in many biological systems and functions. They constitute a major class of compounds with medicinal and pharmacological properties [24–26]. Imidazolium salts have been vastly investigated as ionic liquids and introduced into ionic liquid crystal systems [27–30]. Due to their amphiphilic character, they showed strong interactions with biological materials. In both azobenzene and imidazolium derivatives, hydrophobicity/lipophilicity was found to increase their biological efficacy [31,32].

Due to the unique chemical and physiological properties of the fluorine atom, fluorination could modulate the chemical and biological properties. The physicochemical properties of liquid crystals could be tuned via fluorination, thus permits the design of mesogens with improved properties for electro-optic applications. Incorporation of fluoro substituents into the mesogenic benzene core or spacer is a feasible route for the synthesis of ionic liquid crystals [33,34]. Fluorination is a well-known strategy for improving drug-like properties and enhance drugs' selectivity, lipophilicity and affinity to target proteins [35–37]. Research and development of fluorine-containing drugs have progressed significantly. Many hitherto antitumor agents used in cancer therapy are fluorinated compounds [11].

While fluorinated imidazolium-ILCs and those with fluorinated anions have been documented [38–41], there is to the best of our knowledge, no reports on fluorinated azobenzene-imidazolium ionic liquid crystals and their cytotoxic effects. In the present study, five new fluorinated azobenzene-imidazolium ionic salts were reported for the first time. The imidazolium cationic head was functionalised with alkyl chain of varying length and linked to the mesogenic azobenzene core via a methylene spacer to induce the formation of mesophases. We investigated the self-assembly properties, cytotoxic effects and selectivity of these salts against human cervical cancer (HeLa) cells.

2. Materials and methods

Reagents and chemicals used in this study were of analytical grade. They were used as procured. FTIR spectra were recorded on a Perkin Elmer 2000 spectrometer. ^1H - and ^{13}C NMR spectra were recorded using a Bruker ADVANCE 500 MHz spectrometer. Elemental analyses were carried out using a Perkin Elmer 2400 LS Series CHNS/O analyzer. A Stuart Scientific SMP-1 (UK) melting point meter was used to measure the melting points of the synthesized compounds. Phase transition temperatures and enthalpy change values of the compounds were determined using a Perkin Elmer Pyris-1 differential scanning calorimeter at a heating and cooling rate of $5\text{ }^\circ\text{C min}^{-1}$. Liquid crystalline textures of the compounds were studied using a BX53 Olympus microscope equipped with a T95 LinkPad controller and a THMS600 hotstage.

2.1. Cell culture and maintenance

Human cervical adenocarcinoma cancer cell (HeLa) and human normal skin fibroblast cells (Hs27) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). HeLa cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution (Gibco, Grand Island, NY). Hs 27 cells were maintained in Dulbecco's Modified Eagle's Medium High Glucose (DMEM) (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution (Gibco, Grand Island, NY). The cells were grown in a CO_2 incubator at $37\text{ }^\circ\text{C}$.

2.2. Cytotoxicity assay

The *in vitro* cytotoxic activities of the azo-imidazolium salts, **5–9** against HeLa cells and Hs27 cells were determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay [42]. In brief, HeLa (3×10^4 cells/mL) and Hs27 cells (1×10^5 cells/mL) were seeded in a 96-well plate with DMEM supplemented with 10% FBS and 1% penicillin-streptomycin for 24 h. Subsequently, the medium were replaced with DMEM supplemented with 1% FBS and various concentrations of compounds ($0.39\text{--}50\text{ }\mu\text{g/mL}$) for 24 h. Etoposide was used as a positive control while DMSO (0.5%, v/v) was used as a blank control. Upon incubation, $10\text{ }\mu\text{L}$ of MTT solution ($5\text{ }\mu\text{g/mL}$) were added to each well and the cells were further incubated at $37\text{ }^\circ\text{C}$ for 4 h. The medium was then aspirated and $100\text{ }\mu\text{L}$ of DMSO was added to solubilize the water-insoluble purple formazan crystals. The absorbance of each well was recorded by a UV microplate reader at 570 nm and 630 nm (Thermo Scientific Multiskan Spectrum, Thermo Fisher Scientific, Inc., Waltham, MA). The cytotoxicity of the compounds was calculated according to Eq. (1).

$$\text{Cytotoxicity} = \text{Abs}_{\text{compounds}} / \text{Abs}_{\text{blankcontrol}} \times 100\% \quad (1)$$

IC_{50} of the compounds was calculated and expressed as mean $\pm$ standard deviation (SD) while the selectivity index was calculated using Eq. (2). Selectivity index defines the cytotoxic selectivity (i.e. drug safety) for a lead compound against normal cells versus cancer cells [43,44].

$$\text{Selectivityindex(SI)} = \text{IC}_{50}(\mu\text{M})^{\text{normalcell}} / \text{IC}_{50}(\mu\text{M})^{\text{cancercell}} \quad (2)$$

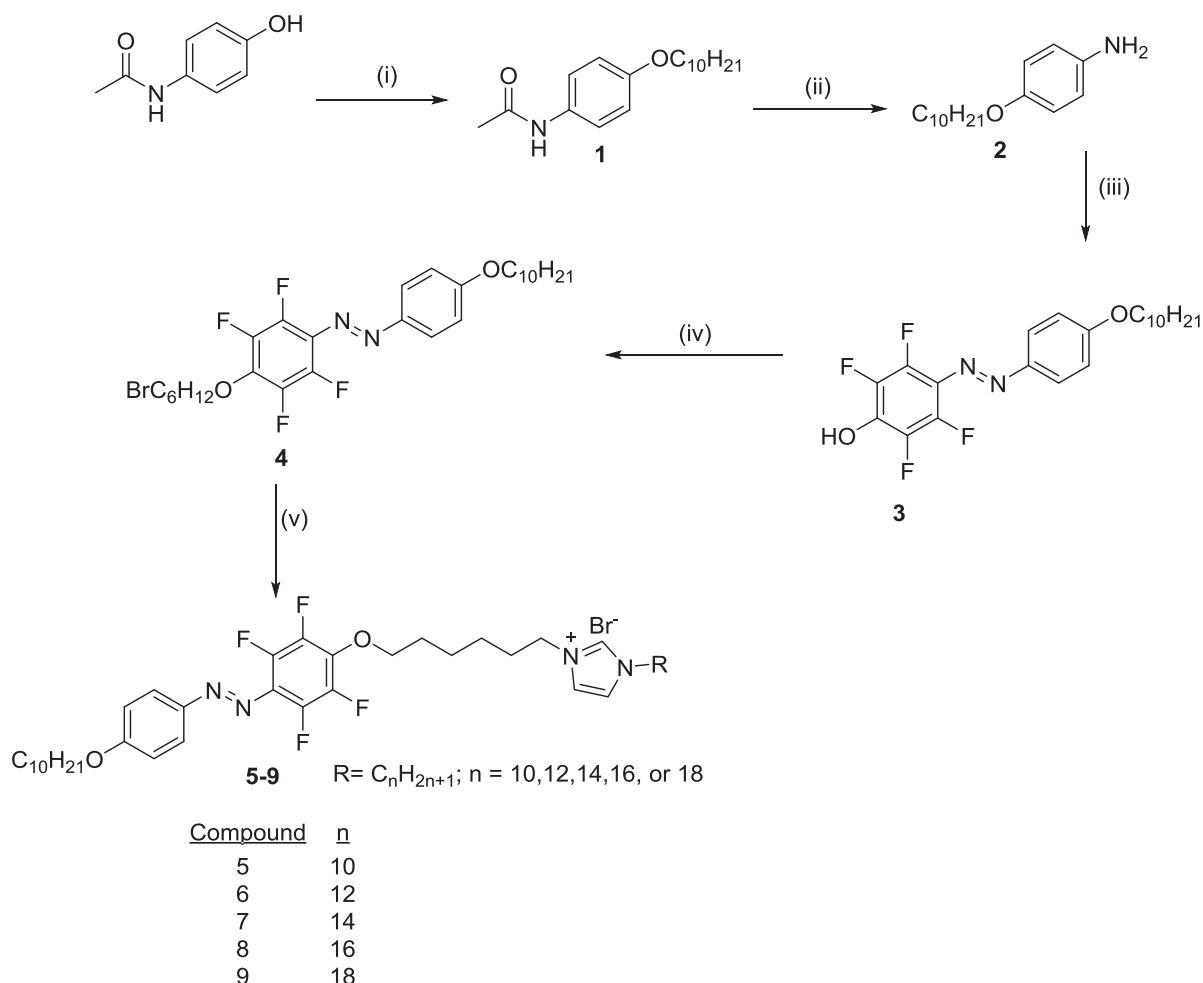
2.3. Synthesis of compounds 3–9

The new azobenzene derivatives, **3–9** discussed herein were prepared following the procedure as outlined in Scheme 1. 4-Acetamidophenol was O-alkylated with 1-bromodecane to produce N-[4-(decyloxy)phenyl]acetamide, **1**, which was further refluxed with alcoholic sodium hydroxide to give 4-(decyloxy)aniline, **2**. Compound **2** was diazotized with sodium nitrite and concentrated HCl at $0\text{ }^\circ\text{C}$, followed by treatment with 2,3,5,6-tetrafluorophenol in the presence of sodium carbonate to obtain the monoalkylated azobenzene intermediate, **3**. Compound **3** was reacted with 1,6-dibromohexane, to yield the dialkylated azobenzene intermediate, **4**. The azobenzene-imidazolium salts, **5–9** were obtained upon reaction of compound **4** with N-alkylated imidazoles. The crude products were recrystallized from diethyl ether and characterized using FTIR, ^1H -NMR and ^{13}C -NMR spectroscopy and elemental analysis.

3. Results and discussion

3.1. FTIR spectroscopy

The FTIR spectra of compounds **3–9** showed characteristic bond stretching within the expected regions: the *para*-substituted phenyl rings, ν (C=C) around 845 cm^{-1} , ν (C-N) within $1155\text{--}1162\text{ cm}^{-1}$, ν (C-O) ether within $1252\text{--}1254\text{ cm}^{-1}$, ν (N=N) within $1499\text{--}1509\text{ cm}^{-1}$, ν (C=C) aromatic within $1603\text{--}1642\text{ cm}^{-1}$, and ν ($\text{C}_{\text{sp}^3}\text{-H}$) at 2853 and 2920 cm^{-1} which conformed to the reported values [45]. In the monoalkylated azobenzene intermediate, **3**, the O-H stretching was observed at 3383 cm^{-1} and bands at 1509 cm^{-1} and 1379 cm^{-1} could be assigned to the N=N and C-F stretching, respectively (Fig. 1(a)). Upon alkylation of **3** to form the dialkylated azobenzene intermediate, **4**, the band ascribable to the O-H stretching disappeared and that of the C-Br was observed at 565 cm^{-1} (Fig. 1(b)). The successful incorporation of the imidazolium moiety into azobenzene core to yield the salts, **5–9**



Scheme 1. Synthesis of azobenzene intermediates, **3-4**, and tetrafluorinated azobenzene-imidazolium salts, **5-9**. Reagents and conditions: (i) K₂CO₃/acetone, 1-bromodecane, reflux 24 h; (ii) NaOH/ethanol, reflux 24 h; (iii) cooled HCl conc.(aq); cooled NaNO₂(aq), stirred for 1 h; cooled 2,3,5,6-tetrafluorophenol and Na₂CO₃(aq), stirred 2 h at 0–5 °C; (iv) K₂CO₃/acetone, 1,6-dibromohexane, reflux 6 h; (v) N-alkyl imidazole/acetone, reflux for 48 h.

was confirmed by the observation of the Csp²-H band at between 3052–3071 cm⁻¹ [46] and disappearance of the C-Br stretching band at 565 cm⁻¹ (Fig. 1(c)).

3.2. NMR Spectroscopy

The molecular structures of all synthesised compounds were further elucidated using the ¹H- and ¹³C NMR spectroscopy. The following spectral discussion is based on **3**. As shown in Fig. 2, signals attributable to the aromatic protons were observed as two doublets, at 7.03 ppm (*J* = 9.1 Hz) and 7.77 ppm (*J* = 9 Hz), each integrated to two protons. The oxymethylene protons are more deshielded and their signals were shifted downfield as compared to that of other methylene protons in the terminal chains. This was observed as a triplet at 4.07 ppm (*J* = 8 Hz), whilst that of other methylene protons were observed as three multiplets within 1.27 ppm–1.82 ppm. Signal corresponding to the methyl protons were observed as a triplet at 0.88 ppm (*J* = 7.0 Hz). In the ¹H NMR spectrum of the dialkylated intermediate, **4** (Fig. 3), characteristic peaks as those discussed for **3** were observed within the expected regions. In addition, two additional triplets at 4.28 ppm (*J* = 6.4 Hz) and 3.41 ppm (*J* = 6.8 Hz) attributable to the oxymethylene protons of the fluorinated benzene ring, and methylene protons attached to the brominated carbon (-BrCH₂), respec-

tively, were observed for **4**. Incorporation of the imidazolium moiety in **5-9** gave rise to additional peaks in their ¹H-NMR spectra. Spectral characteristics similar to those as discussed for its precursor, **4** were observed in **5**. Proton peaks in the imidazolium moiety was observed as: A singlet at 10.81 ppm, and two triplets at 7.24 ppm and 7.20 ppm which corresponded to the acidic proton attached to C2 and N-CH, respectively; as well as two triplets at 4.42 (*J* = 7.4 Hz), and 4.34 ppm (*J* = 7.5 Hz) ascribable to the methylene protons. Formation of the salts, **5-9** was substantiated by ¹³C NMR spectroscopy. According to Scheme 1, compound **5** contained 43 carbons but due to overlap of peaks, only 32 signals were observed in its ¹³C NMR spectrum. Signals of methyl carbons were observed at 14.12 ppm, while those of methylene carbons in the alkyl chains and spacer were observed within 31.90 ppm–22.68 ppm. Peaks at 50.05 ppm and 50.28 ppm could be assigned to the two nitrogenated carbons, while those at 68.53 ppm and 75.09 ppm could be attributed to the oxymethylene carbons. Signals of the alkenyl carbons were seen at 121.30 ppm and 121.37 ppm while that of the acidic carbon, C2 of the imidazolium ring was seen at 138.09 ppm. Aromatic carbons of the azobenzene moiety were observed within 162.98 ppm–114.83 ppm, which was in good agreement with that reported in literature [47,48]. The C-F signals were observed as two multiplets at 140 and 142 ppm (Fig. 4).

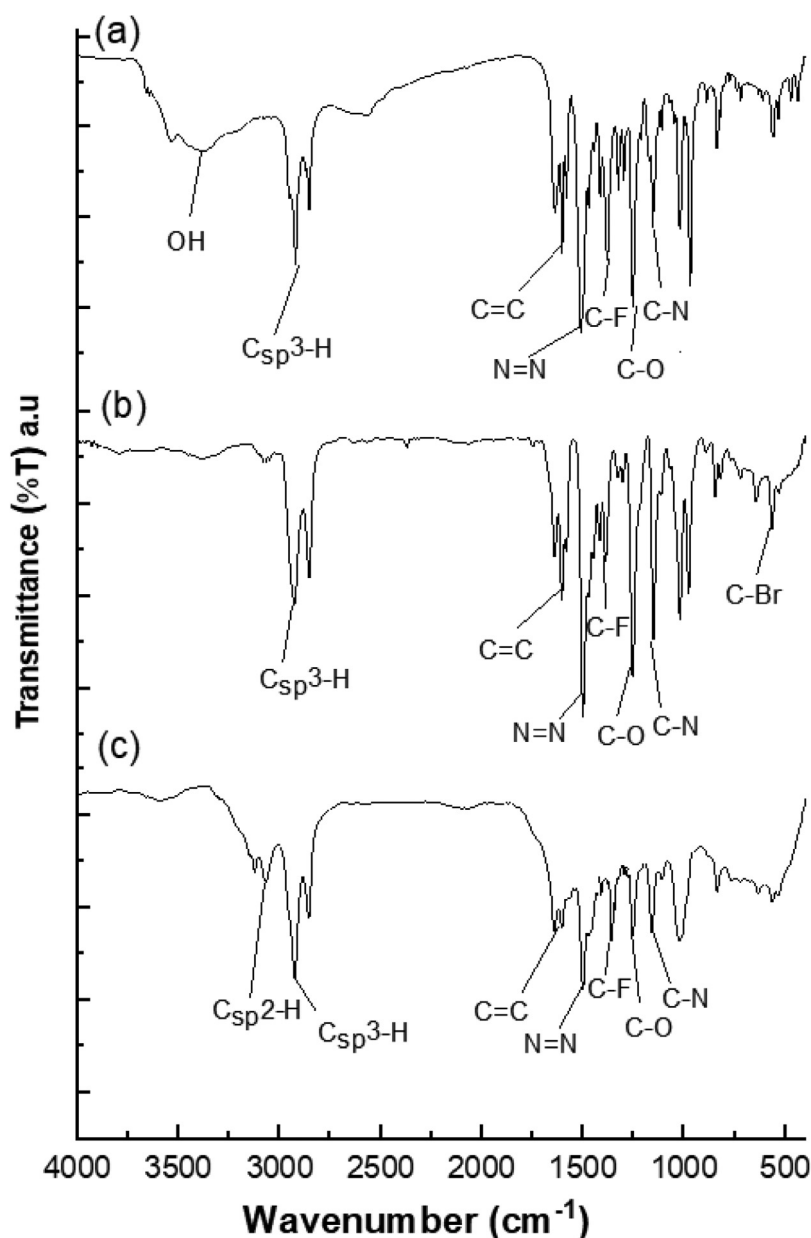


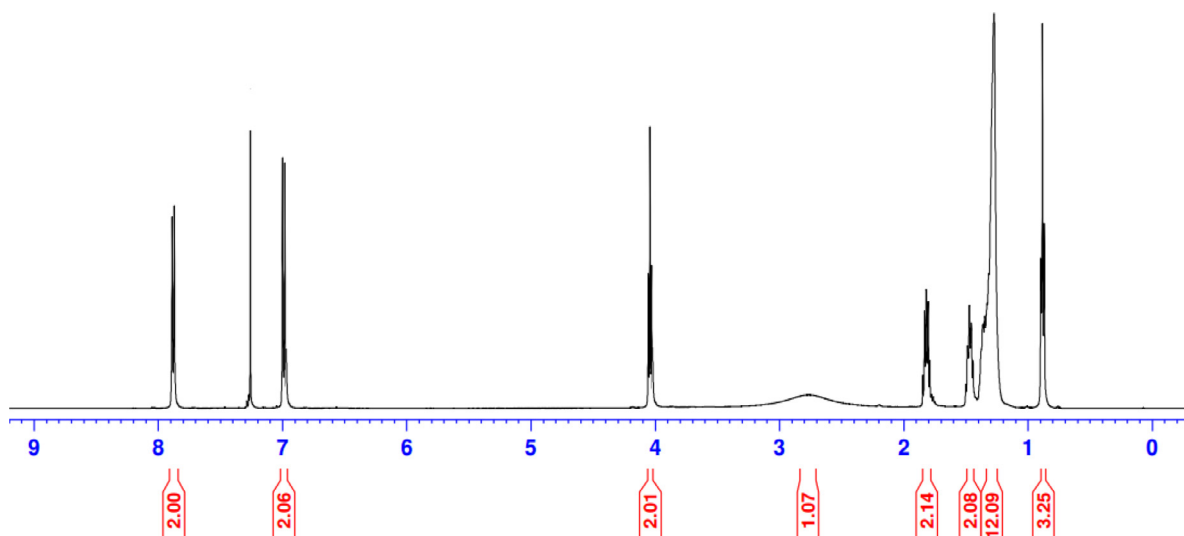
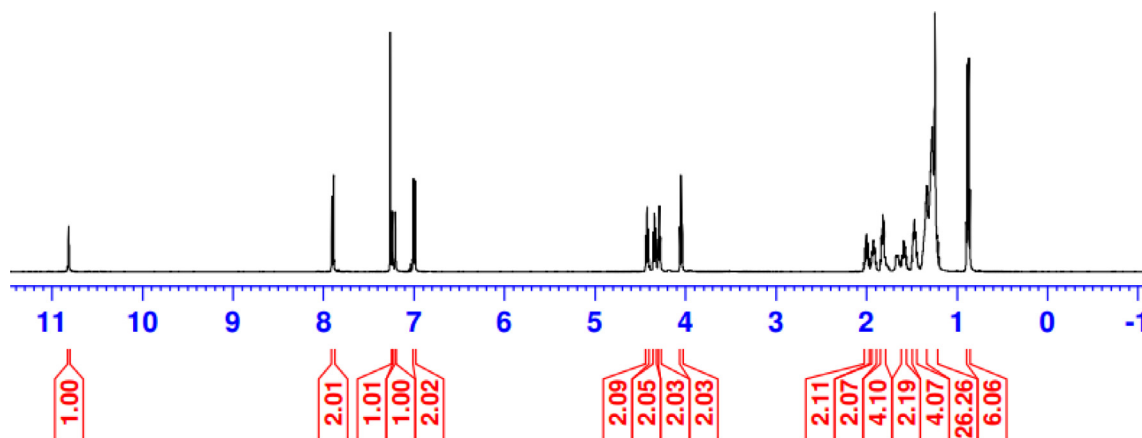
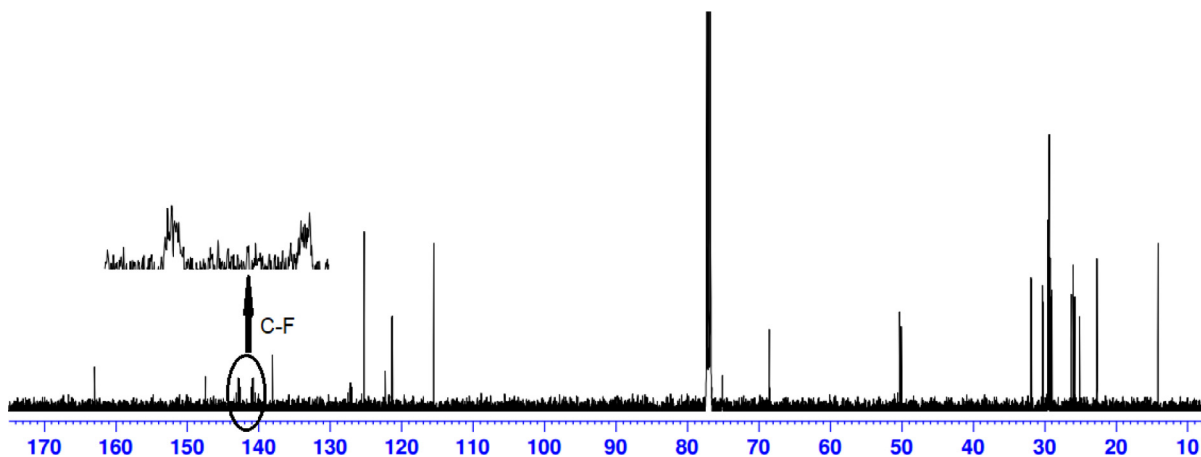
Fig. 1. FTIR spectra of compounds (a) **3**, (b) **4** and (c) **5**.

3.3. Thermal and Phase Transitional Properties

The phase transition temperatures and associated enthalpies of compounds **3–9** determined by DSC are collated in [Tables 1](#). All transitional data reported for compounds were obtained from the second heating cycle. Both the melting and clearing temperatures of the salts were chain length dependent which indicated the dominant effect of the hydrophobic interactions during phase transitions. [Fig. 5](#) shows the representative DSC thermogram of compound **7**. It could be inferred from the relatively large enthalpy change that, a considerable structural change occurred on melting due to the breakup of a 3D, ordered crystal lattice. However, a small enthalpy change upon clearing implied the overcome of van der Waals forces between alkyl chains in the imidazolium cationic head. The SmA phase was stabilized with elongation of the alkyl chain. Increased mesophase range from 20 °C (**5**, $n = 10$) to 40 °C (**9**, $n = 18$) was observed. Upon increasing the chain length, the viscous liquids of the azobenzene-imidazolium salts also exhib-

ited a great tendency to supercool before crystallizing. This difference in phase transition temperatures between heating and cooling could be due to slow phase transition kinetics and insufficient crystal nucleation that suppressed the crystallization of viscous liquids [49].

Nature of mesophase was characterized using POM. The influence of alkyl chain in the imidazolium moiety and fluorine atoms on the liquid crystalline behaviour of the azobenzene derivatives was evident. It could be inferred that two long alkyl chains are necessary to induce and sustain a mesophase. The mono-alkylated azobenzene intermediate, **3** was non-mesogenic whilst its dialkylated analogue, **4** was a low-melting nematogen. The formation of the phase was evident based the schlieren texture observed under crossed polarizers ([Fig. 6\(a\)](#)). The low phase stability and transition temperatures could be due to larger intermolecular distance caused by the polar and steric effects of the lateral fluoro substituents that increased molecular breadth and repulsive forces that reduced longitudinal polarisability and efficiency of

Fig. 2. ^1H NMR spectrum of compound **3** (in CDCl_3).Fig. 3. ^1H NMR spectrum of compound **5** (in CDCl_3).Fig. 4. ^{13}C NMR spectrum of compound **5** (in CDCl_3).

molecular packing. Incorporation of long alkyl chains into the imidazolium cationic head induced amphiphilic properties and altered the mesomorphic behaviour of the azobenzene-imidazolium salts, **5–9**. The mesomorphic range increased with increasing alkyl chain length. The combination of outboard lateral dipoles from the ether oxygens and lateral fluoro substituents as well as aggregation of compatible parts, micro segregation of incompatible parts

and minimization of volume in the bulk of these ionic mesogens had led to the formation of a lamellar SmA phase [40,50]. The non-covalent attraction between the polar, cationic imidazolium head and glass surface had resulted a homeotropic alignment of the mesogens with the hydrophobic tails arranged perpendicular to and away from the glass surface, and hence, dark domains were seen [38]. The co-existence of the dark domains and fan texture

Table 1

Phase transition temperatures ($^{\circ}\text{C}$) and enthalpy change values (kJ mol^{-1}) of the intermediates, **3–4** and salts **5–9** determined by DSC at a heating and cooling rate of $5^{\circ}\text{C min}^{-1}$.

Compound	Transition temperatures, $^{\circ}\text{C}$ (ΔH , kJ mol^{-1})	
	Heating	Cooling
3	Cr 51.06 (38.59) Iso	No crystalline phase observed upon cooling
4	Cr 61.31(47.70) Iso	Iso 56.48 (-1.07) N 47.44 (-39.50) Cr
5 (n = 10)	Cr 65.46 (26.60) SmA 85.53 (4.66) Iso	Iso 74.17 (-8.44) SmA 64.04 (-23.65) Cr
6 (n = 12)	Cr 69.14 (19.24) SmA 92.09 (12.15) Iso	Iso 80.00 (-11.91) SmA 64.98 (21.73) Cr
7 (n = 14)	Cr 82.44 (33.59) SmA 110.87 (0.79) Iso	Iso 109.20 (-0.64) SmA 76.97 (-37.50) Cr
8 (n = 16)	Cr 69.69 (0.77) Cr ₁ 88.93 (36.25) SmA 124.37 (1.12) Iso	Iso 123.01 (-1.14) SmA 73.23 (-36.91) Cr ₁ 57.64 (-0.82) Cr
9 (n = 18)	Cr 92.97 (45.73) SmA 132.81 (1.87) Iso	Iso 131.01 (-1.14) SmA 72.98 (-47.37) Cr

Note: Cr = crystal; Cr₁ = sub-crystal; SmA = smectic A; Iso = isotropic.

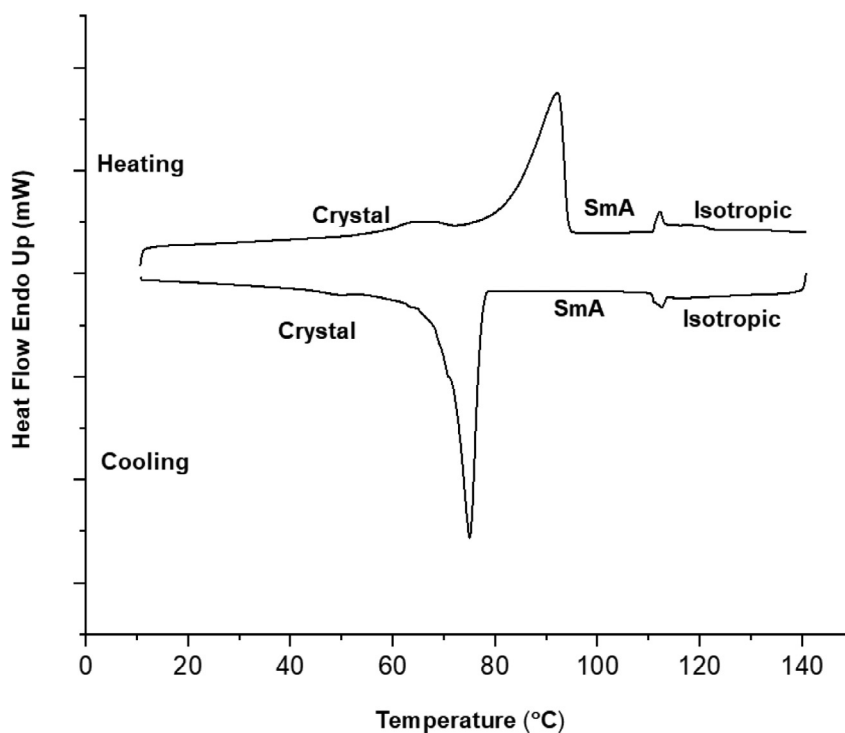


Fig. 5. A DSC thermogram of compound **7**.

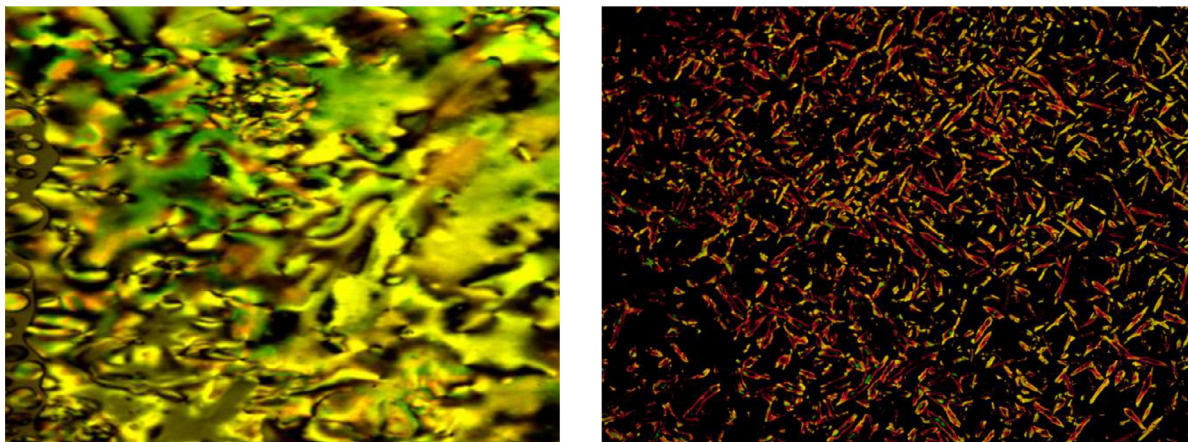


Fig. 6. Photomicrographs showing (a) schlieren texture of N phase (compound **4**) and (b) co-existence of dark domains and fan texture of SmA phase (compound **6**) during cooling.

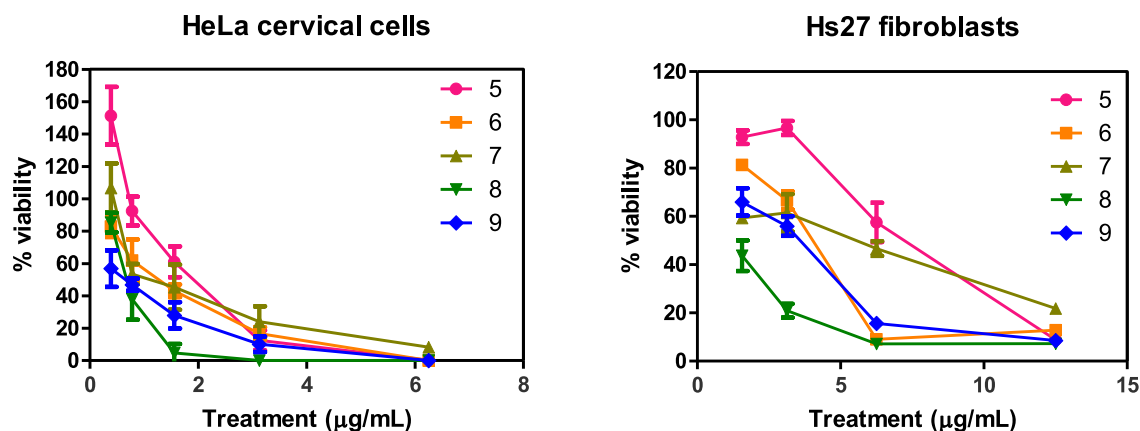


Fig. 7. The cytotoxicity of compounds 5–9 against HeLa cervical cells and Hs27 fibroblasts. The compounds showed dose-dependent manner in reduction of cell viability.

Table 2

The half maximal inhibitory concentration (IC_{50}) and selectivity index of the tetrafluorinated azo-imidazolium salts, 5–9 and Etoposide on Hela cells and Hs27 fibroblasts at 24 h as determined using the MTT assay.

Compound	$IC_{50} \pm SD$ (μ g/mL)HeLa cells	IC_{50} (μ M)HeLa cells	$IC_{50} \pm SD$ (μ g/mL)Hs27 cells	IC_{50} (μ M)Hs27 cells	Selectivity index (SI)
5 (n = 10)	2.26 ± 0.08	2.84	6.74 ± 0.46	8.45	2.98
6 (n = 12)	1.17 ± 0.22	1.42	3.30 ± 0.05	4.00	2.82
7 (n = 14)	1.51 ± 0.32	1.77	3.18 ± 0.44	3.72	2.11
8 (n = 16)	0.64 ± 0.18	0.72	1.92 ± 0.08	2.18	3.02
9 (n = 18)	0.56 ± 0.12	0.61	2.15 ± 0.11	2.37	3.89
Etoposide	15.11 ± 1.23	25.67	> 50	> 85	> 3.31

from cooling the isotropic melt confirmed the presence of this smectic phase (Fig. 6(b)).

3.4. Cytotoxicity evaluation

The potential toxicity and selectivity of the amphiphilic azobenzene-imidazolium salts on human cervical cancer (HeLa) cells and human skin fibroblasts (Hs27) were evaluated using the MTT assay. The cytotoxic effect of the salts at various concentrations was expressed as the half maximal inhibitory of concentration (IC_{50}), of which the lower the IC_{50} value, the stronger the cytotoxic effect, were calculated from the dose–response curve (Fig. 7).

The results of the cytotoxicity evaluation against HeLa cells and normal fibroblasts (Hs27) are shown in Table 2. The salts exhibited excellent inhibition potentials against HeLa cells and decreased their viability in a dose-dependent manner. IC_{50} values of the salts after 24 h incubation ranged between 0.6 μ M for 9 (n = 18) to 2.8 μ M for 5 (n = 10), which are several order of magnitude lower than that of Etoposide (IC_{50} = 25.7 μ M), a standard therapeutic drug used as the positive control in this experiment. It is noteworthy to mention that the azobenzene-imidazolium ILCs, 5–9 were generally less toxic towards normal fibroblasts (indicated by larger IC_{50} values). Long homologues (n = 16 & 18) had a broader safety window with selectivity index ≥ 3 , thus indicating these compounds did not affect the viability of normal fibroblasts and were highly selective towards HeLa carcinomas [51,52]. The selectivity towards HeLa cells could be arranged in a descending order: 9 > 8 > 7 > 6 > 5 > Etoposide. This trend clearly indicated the influence of alkyl chain length on cytotoxicity.

Generally, cytotoxicity effect of organic compounds could be directly correlated with their lipophilic properties [53,54]. In the present study, we found a positive correlation between the alkyl chain length and cytotoxicity wherein the ILCs with longer alkyl chains had higher cytotoxicity against the HeLa cells and normal fibroblasts, which was consistent with the results in previous reports [55–57]. It was reported that the affinity of imidazolium ILs

towards phospholipid bilayers increases as a function of the hydrocarbon chain length of the cations. The polar and hydrophilic imidazolium head group can act on bio membranes via electrostatic interaction and increases cellular membrane permeability as they are structurally similar to cationic surfactants [58–60]. It has been documented that it was easier for ILs with long alkylated cations to disrupt phospholipid bilayers. In addition, it was evident that long alkyl chain attached to N1 in the imidazolium moiety enhanced surface activity of biological membranes and mesogenic characteristics of 1-alkylimidazolium cations in aqueous solution [61]. The introduction of fluorine also generally confers increased lipophilicity. Therefore, we surmise that the in vitro cytotoxicity of the azo-imidazolium ILCs appears to be governed mainly by the side chain in the imidazolium cationic head. The long-chain ILCs (higher lipophilicity), 7–9 (n = 14–18) were expected to have higher effectiveness of interaction with the apolar tails of cell phospholipids as compared to their shorter homologues, 5 & 6 (n = 10 & 12, respectively). The former could penetrate into the cell membrane more easily and induced a more pronounced perturbation and weakening of the cell membrane [56,62] that eventually led to cell death as evidenced by their low IC_{50} values.

4. Conclusion

A new series of tetrafluorinated azobenzene-imidazolium ionic liquid crystals were synthesized and characterized. Phase transitional properties of the synthesized compounds were investigated using POM and DSC. Growth inhibition potential of the ILCs against human cervical cancer (HeLa) cells and human skin fibroblasts (Hs27) was evaluated using the MTT assay. The chain length in the cationic head group and fluorination were found to influence the mesomorphic behaviour and phase morphology of the homologues. All homologues were mesogenic, exhibiting stable smectic A phase with broad temperature range which increased with alkyl chain length. All the salts were qualified as ionic liquids which melted below 100 °C. The cytotoxicity of the ILCs was gov-

erned mainly by the side chain in the imidazolium cationic head. All the ILCs exhibited excellent inhibition potentials against HeLa and Hs27 cells in a dose-dependent manner. Long-chain homologues had enhanced lipophilic capacity, improved membrane permeability, and hence resulted in higher cytotoxicity. These ILCs were generally less toxic towards normal fibroblasts, and hence selective towards cancer cells with long homologues having selectivity index ≥ 3 . IC₅₀ ranged between 0.6 μ M for the longest homologue, **9** ($n = 18$) and 2.8 μ M for its shortest counterpart, **5** ($n = 10$). Their cytotoxic effect was 7 to 27 times stronger than that of the anticancer agent, Etoposide (IC₅₀ = 25.7 μ M). Our results suggested that long-chain tetrafluorinated azobenzene-imidazolium ILCs may represent new alternatives to fabricate safe and selective thermotropic liquid crystalline anticancer drugs when doped with room-temperature mesomorphous polymers in thermotropic LCs driven drug delivery systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Halimah Funmilayo Babamale: Investigation, Data curation, Formal analysis. **Nelson Jeng-Yeou Chear:** Methodology, Validation, Visualization, Writing – review & editing. **Rosenani A. Haque:** Visualization, Writing – review & editing. **WanSinn Yam:** Supervision, Conceptualization, Methodology, Funding acquisition, Validation, Writing – original draft.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.molstruc.2022.132470](https://doi.org/10.1016/j.molstruc.2022.132470).

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