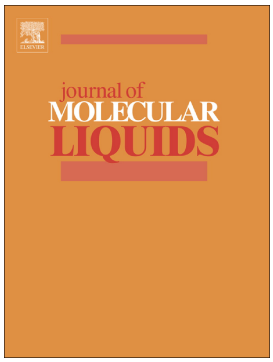

Novel pyridinium based ionic liquids with amide tethers: Microwave assisted synthesis, molecular docking and anticancer studies

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Novel pyridinium based ionic liquids with amide tethers: Microwave assisted synthesis, molecular docking and anticancer studies

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Abstract

The ionic liquids are remarkable molecules with exceptional properties and applications. The microwave assisted green method was used to synthesize (yield, up to 95%) a new series of pyridinium based ionic liquids (ILs); utilizing molecular hybridization of 2- and/or 3-methyl pyridine and several fluorinated phenyls. The characterization of the synthesized ILs was carried out using different spectroscopic techniques (^{1}H, ^{13}C, ^{19}F, ^{31}P, ^{11}B NMR and mass). The DNA binding constants (Kb) of the ionic liquids were in the range of 1.3×10^{3} to 1.52×10^{8} M^{-1}; indicating good binding tendencies of the ionic liquids with DNA. All the ionic liquids intercalated with DNA through minor grooves *via* covalent, intercalative and electrostatic bondings. All the reported ionic liquids showed more than 50% anticancer activities with two lung cancer cell lines *i.e.* A549 and H-1229. Moreover, the maximum proliferation inhibitions were 99.16 to 91.24% for 4a series and 99.23 to 99.69% for 5a series of the ionic liquids. The docking studies indicated quite good binding affinities (-4.6 to -4.9 kcal/mol.) of the reported ionic liquids with DNA. The experimental results of DNA bindings were in good agreement with those of docking ones. The percentage inhibitions were excellent for 4a, 4e and 5n and, hence, these ionic liquids may be the future anti-cancer agents.

Keywords: Pyridinium ionic liquids, Green syntheses, DNA binding, Docking, Anticancer study.

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1. Introduction

The ionic liquids are remarkable molecules having exceptional properties and applications [1]. It is well known that about 50% pharmaceuticals in market are ionic liquids due to the combination of a pharmaceutically active cations and anions making them good and easily soluble drugs in human body. Besides, the negligible vapor pressure, good hydrophobicity, solvent miscibility, polarity, high chemical, thermal and electrochemical stabilities made the ionic liquids as the best pharmaceutical molecules [2]. Therefore, the chemistry of ionic liquids has become a subject of an increasing interest in the organic synthesis [3]. The considerable efforts have been made towards the design and synthesis of these important classes of organic molecules [4-9]. These have been explored extensively for their antibacterial [10], antifungal [11], anti-inflammatory [12] and anticancer [13] properties. The quaternization of sp^2 nitrogen of heterocyclic components is one of the most efficient approaches in the ionic liquids synthesis [3]. Notably, pyridine derivatives are very useful scaffolds gaining wide popularity in the ionic liquids synthesis [14,15].

Cancer is a composite, aggressive, heterogeneous and horrible disease killing millions of people globally [16-22]. As per the GLOBOCAN assessment, 18.1 million new cases and 9.6 million deaths were recorded worldwide in 2018 due to cancer alone [23]. Therefore, there is a great necessity to fight with this lethal disease. Among various modes, chemotherapy is considered as the best approach due to its fast and targeted action [24]. But unfortunately, the chemical compounds show severe toxicities to the normal cells. Besides, the solubility is another problem restricting the use of chemotherapy. Due to these problems, the search of new molecules for the chemotherapy is an area of growing and demanding research. The stratagem of the coherent design of new bioactive molecules has become the goal of many researchers in the

medicinal chemistry. The molecular clubbing of two or more pharmacophore units is the promising approach to develop promising target scaffolds in the drugs design [25,26]. These goals have been molecular hybridization of small and simple cores in one frame work.

The microwave assisted organic synthesis; with green advantages like eco-friendly, good yields and reducing reaction times; is the need of today for organic synthetic chemists [26,27]. The modification of the counter anions to evaluate their synergetic effects in the synthesis of ionic liquids is a subject of great interest [25-36]. The prevailing goals of this study are the development of novel pyridinium ionic liquids as anticancer agents *via* green synthesis, DNA binding, molecular docking and anticancer studies.

2. Experimental section

2.1. Chemicals and reagents

All the chemicals, solvents and reagents used were of analytical grade and used without further purification. These include the following chemicals.

Fine Chemicals: 3-Fluoro-4-methylaniline, 2-fluoro-4-iodoaniline and 2,4,5-trifluoaniline (BDH Chemicals Ltd), 3-methylpyridine, 4-methylpyridine and triethylamine (Sigma Aldrich), chloroacetyl chloride, potassium hexafluorophosphate, sodium tetrafluoroborate and sodium trifluoroacetate (Across).

Solvents: Dichloromethane, ethylacetate, hexane, acetonitrile, chloroform and ethylacetate (Sigma-Aldrich).

2.2 Instruments used

The newly synthesized ILs were fully characterized by ^{1}H, ^{13}C, ^{19}F, ^{31}P, ^{11}B-NMR and MS spectroscopic methods. The melting points of the reported compounds were measured on a melt-temp apparatus (SMP10). TLC was performed on UV fluorescent Silica gel Merck 60 F254

plates and viewed in a UV chamber (254 nm). The eluent used was ethyl acetate-hexane (1:2 v/v). SHIMADZU FT-IR-8400S spectrometer was used for recording IR spectra. The NMR spectra were run with Bruker spectrotometer (400 MHz) with TMS as internal reference. ESI mass spectral data were obtained by a Finnigan LCQ spectrometer. A controllable single-mode microwave reactor (CEM Discovery) was used to carry out the microwave-assisted reactions. The microwave reactor was equipped with a magnetic stirrer as well as pressure, temperature, and power controls and a nitrogen cooling system. The maximum operating pressure of the reactor was 2 x 10^6 Pa with power set as 300 W.

2.3 Synthesis of ionic liquids

The various ionic liquids were synthesized as per the procedure given.

2.3.1 Synthesis of 2-chloro-*N*-(substituted phenyl)acetamides 2a-c

The appropriate fluorinated aniline (3-fluoro-4-methylaniline, 2-fluoro-4-iodoaniline and/or 2,4,5-trifluoaniline) **1a-c** (10 mmol), triethylamine (12 mmol), chloroacetyl chloride (12 mmol) in dichloromethane (40 mL) were allowed to stir at 0 ^{o}C for 2 hr. The reaction was stirred at room temperature for 6 hrs, until the consumption of the starting materials. The resulting solid was filtered off, washed with water and re-crystallized with ethanol to afford the corresponding 2-chloro-*N*-(substituted phenyl)acetamides **2a-c**.

2.3.2 Synthesis of pyridinium ionic liquid halides 4a-f:

To a mixture of 3-methyl and/or 4-methyl pyridine **3a,b** (10 mmol), sodium iodide (10 mmol) and acetonitrile (30 mL) were added with constant stirring and adding with 2-chloro-*N*-(3-fluoro-4-methylphenyl)acetamide, 2-chloro-*N*-(2-fluoro-4-iodophenyl)acetamide and/or 2-chloro-*N*-(2,4,5-trifluorophenyl)acetamide **3a-c** (10 mmol). The mixture was stirred at reflux for 18-20 hrs, until the consumption of the starting material was complete. The solvent was removed

under reduced pressure. The product formed was collected by filtration and/or extracted by chloroform to afford the desired ionic liquids **4a-f**.

2.3.2.1 Microwave procedure

3-Methyl and/or 4-methyl pyridine **3a,b** (1 mmol), sodium iodide (1 mmol), the appropriate amounts of 2-chloro-*N*-(substituted phenyl)acetamides **2a-c** (1 mmol) and acetonitrile (5 mL) were placed in closed borosilicate glass vessel fitted with a silicone cap and exposed to irradiation for 15-20 min using a microwave reactor. The reaction was processed as described in the conventional procedure outlined earlier to give the same pyridinium ionic liquid halides **4a-f**.

2.3.3 Metathesis the synthesis of imidazolium ILs tethering fluorinated counter anion 5a-r:

2.3.3.1 Conventional procedure

A mixture of a suspension of ionic liquids **4a-f** (1 mmol) and the appropriate metal salts potassium hexafluorophosphate, sodium tetrafluoroborate and/or sodium trifluoroacetate (1.1 mmol) in acetonitrile (20 mL) were stirred at reflux for 24 h. After cooling, the reaction mixture was filtered to remove solid metal halide. Acetonitrile was removed under reduced pressure. The product formed was collected by filtration and/or extracted by chloroform to afford the desired ionic liquids **5a-r**.

2.3.3.2 Microwave procedure

Pyridinium halides **4a-f** (1 mmol), the appropriate metal salts (1.1 mmol) and acetonitrile (5 mL) were placed in closed borosilicate glass vessel fitted with a silicone cap and exposed to irradiation for 20 min using a microwave reactor. The reaction was processed as described in the conventional procedure outlined earlier to give the same pyridinium ionic liquids **5a-r**.

2.4 Characterization

The synthesized ionic liquids are characterized by various spectroscopic methods as described below.

2.4.1 Characterization of 2-chloro-*N*-(substituted phenyl)acetamides 2a-c:

2-Chloro-*N*-(3-fluoro-4-methylphenyl)acetamide (2a)

Brown solid, mp: 155-156 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.17 (s, 2H, NC**H**$_2$), 4.27 (s, 2H, NC**H**$_2$), 7.22 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 7.51-7.54 (m, 1H, Ph-**H**), 10.49 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 14.1 (N**C**H$_2$), 43.9 (N**C**H$_2$), 106.6, 115.3, 119.7, 132.0, 138.3, 159.4, 161.8 (Ph-**C**), 165.2 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = (-115.95 to -115.91) (m, 1F, Ph-**F**). LCMS (M^+) 201.0 found for C_9H_9ClFNO.

2-Chloro-*N*-(2-fluoro-4-iodophenyl)acetamide (2b)

Colorless crystals, mp: 161-162 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 4.45 (s, 2H, NC**H**$_2$), 7.55 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.68-7.75 (m, 2H, Ph-**H**), 10.19 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 43.5 (N**C**H$_2$), 124.8, 125.8, 126.1, 133.9, 152.4, 154.9 (Ph-**C**), 165.7 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -122.17 to -122.12 (m, 1F, Ph-**F**). LCMS (M^+) 312.9 found for $C_8H_6ClFINO$.

2-Chloro-*N*-(2,4,5-trifluorophenyl)acetamide (2c)

Colorless crystals, mp: 118-119 ^{o}C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 4.36 (s, 2H, NC**H**$_2$), 7.69 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.97-8.04 (m, 1H, Ph-**H**), 10.32 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 43.4 (N**C**H$_2$), 106.6, 112.4, 122.9, 144.7, 146.9, 147.2, 150.6 (Ph-**C**), 165.9 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -141.78 to -141.63 (m, 1F, Ph-**F**), -139.14 to -139.03 (m, 1F, Ph-**F**), -125.62 to -125.54 (m, 1F, Ph-**F**). LCMS (M^+) 223.0 found for $C_8H_5ClF_3NO$.

2.4.2 Characterization of pyridinium IL halides 4a-f

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium iodide (4a)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.53 (s, 3H, C**H**$_3$), 5.60 (s, 2H, NC**H**$_2$), 7.20-7.29 (m, 2H, Ph-**H**), 7.51 (d, 1H, *J* = 12.0 Hz, Ph-**H**), 8.15 (dd, 1H, *J* = 8.0 Hz, 16.0 Hz, Ph-**H**), 8.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.89 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.97 (s, 1H, Ph-**H**), 10.88 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 14.1 (**C**H$_3$), 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.5, 115.2, 119.9, 127.3, 132.3, 137.9, 138.5, 144.2, 146.2, 147.1, 159.5, 161.9 (Ph-**C**, **C**=N), 163.7 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = (-115.65 to -115.59) (m, 1F, Ph-**F**). LCMS (M^+)-I^- 259.1 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium iodide (4b)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.52 (s, 3H, C**H**$_3$), 5.70 (s, 2H, NC**H**$_2$), 7.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.71-7.76 (m, 2H, Ph-**H**), 8.15 (dd, 1H, *J* = 4.0, 8.0 Hz, Ph-**H**), 8.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.93 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 9.02 (s, 1H, Ph-**H**), 10.76 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 18.3 (**C**H$_3$), 64.4 (N**C**H$_2$), 89.0, 124.7, 124.9, 125.5, 125.8, 127.3, 133.9, 138.5, 144.2, 144.2, 147.1, 152.2, 154.7 (Ph-**C**, **C**=N), 164.4 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -122.25 to -122.20 (m, 1F, Ph-**F**). LCMS (M^+)-I^- 371.0 found for $C_{14}H_{13}FIN_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium iodide (4c)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.53 (s, 3H, C**H**$_3$), 5.66 (s, 2H, NC**H**$_2$), 7.78 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.98-8.05 (m, 1H, Ph-**H**), 8.15 (dd, 1H, *J* = 4.0, 8.0 Hz, Ph-**H**), 8.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.89 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.97 (s, 1H, Ph-**H**), 10.81 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C= 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.7, 111.7, 112.0, 127.3, 138.6, 144.2, 146.2, 147.2 (Ph-**C**, **C**=N), 164.7 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$):

δ_F = -141.46 to -141.31 (m, 1F, Ph-**F**), -138.92 to -138.81 (m, 1F, Ph-**F**), -126.01 to -125.93 (m, 1F, Ph-**F**). LCMS (M^+)-I^- 281.2 found for $C_{14}H_{12}F_3N_2O^+$.

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-4-methylpyridin-1-ium iodide (4d)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.66 (s, 3H, C**H**$_3$), 5.57 (s, 2H, NC**H**$_2$), 7.20-7.29 (m, 2H, Ph-**H**), 7.51 (dd, 1H, *J* = 4.0, 12.0 Hz, Ph-**H**), 8.06 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 14.1 (**C**H$_3$), 22.0 (**C**H$_3$), 61.7 (N**C**H$_2$), 106.2, 106.5, 115.2, 119.8, 128.2, 132.2, 137.9, 145.8, 159.5, 160.2, 161.9 (Ph-**C**, **C**=N), 164.0 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -115.63 to -115.58 (m, 1F, Ph-**F**). LCMS (M^+)-I^- 259.2 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-4-methylpyridin-1-ium iodide (4e)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.65 (s, 3H, C**H**$_3$), 5.63 (s, 2H, NC**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.71-7.77 (m, 2H, Ph-**H**), 8.06 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 10.76 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C= 22.0 (**C**H$_3$), 61.8 (N**C**H$_2$), 88.6, 124.7, 124.9, 125.5, 125.8, 128.3, 145.8, 152.1, 154.6, 160.3 (Ph-**C**, **C**=N), 164.6 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -122.45 to -122.40 (m, 1F, Ph-**F**). LCMS (M^+)-I^- 371.0 found for $C_{14}H_{13}FIN_2O^+$.

4-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium (4f)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.66 (s, 3H, C**H**$_3$), 5.64 (s, 2H, NC**H**$_2$), 7.78 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.97-8.06 (m, 3H, Ph-**H**), 8.89 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 22.0 (**C**H$_3$), 61.7 (N**C**H$_2$), 106.4, 106.7, 106.9, 111.7, 111.9, 122.7, 128.3, 144.6, 160.3 (Ph-**C**, **C**=N), 165.0 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -141.46 to 141.31 (m, 1F, Ph-**F**), -138.99 to -138.88 (m, 1F, Ph-**F**), -125.99 to -125.91 (m, 1F, Ph-**F**). LCMS (M^+)-I^- 281.2 found for $C_{14}H_{12}F_3N_2O^+$.

2.4.3 Characterization of pyridinium ILs 5a-r

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *tetrafluoroborate* (5a)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.53 (s, 3H, C**H**$_3$), 5.59 (s, 2H, NC**H**$_2$), 7.20-7.29 (m, 2H, Ph-**H**), 7.51 (dd, 1H, *J* = 4.0, 12.0 Hz, Ph-**H**), 8.15 (dd, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 8.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 8.96 (s, 1H, Ph-**H**), 10.83 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 14.1 (**C**H$_3$), 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.2, 106.5, 115.2, 119.9, 127.3, 132.2, 137.8, 138.6, 144.2, 146.2, 147.1, 159.5, 161.9 (Ph-**C**, **C**=N), 163.7 (**C**=O). ^{11}B NMR (128 MHz, DMSO-*d*$_6$): δ_B = -1.32 to -1.31 (m, 1B, **B**F$_4$). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -115.64 to -115.57 (m, 1F, Ph-**F**); -148.23 (d, 4F, B**F**$_4$). LCMS (M^+)-BF_4^- 259.1 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *hexafluoro-phosphate* (5b)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.53 (s, 3H, C**H**$_3$), 5.59 (s, 2H, NC**H**$_2$), 7.20-7.29 (m, 2H, Ph-**H**), 7.51 (dd, 1H, *J* = 4.0, 12.0 Hz, Ph-**H**), 8.15 (dd, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 8.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.96 (s, 1H, Ph-**H**), 10.82 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C= 14.1 (**C**H$_3$), 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.2, 106.5, 115.2, 119.9, 127.3, 132.2, 137.8, 138.6, 144.2, 146.2, 147.1, 159.5, 161.9 (Ph-**C**, **C**=N), 163.7 (**C**=O). ^{31}P NMR (162 MHz, DMSO-*d*$_6$) δ_P = -157.45 to -131.04 (sept, 1P, **P**F$_6$). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -69.18 (d, 6F, P**F$_6$**), -115.62 to -115.57 (m, 1F, Ph-**F**). LCMS (M^+)-PF_6^- 259.1 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *trifluoroacetate* (5c)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.53 (s, 3H, C**H**$_3$), 5.60 (s, 2H, NC**H**$_2$), 7.20-7.29 (m, 2H, Ph-**H**), 7.51 (d, 1H, *J* = 12.0 Hz, Ph-**H**), 8.15 (dd, 1H, *J* = 4.0, 12.0 Hz, Ph-**H**), 8.56 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.89 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.97 (s, 1H, Ph-**H**), 10.87 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 15.3 (**C**H$_3$), 19.5 (**C**H$_3$), 63.5 (N**C**H$_2$), 107.4, 107.7, 116.4, 121.1, 128.5, 133.4, 139.7, 145.4, 147.4, 148.3, 160.7 (Ph-**C**, **C**=N), 164.9 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -72.20 (s, 3F, C**F**$_3$), -114.44 to -114.39 (m, 1F, Ph-**F**). LCMS (M^+)-CH_3COO^- 259.2 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-4-methylpyridin-1-ium *tetrafluoro-borate* (5d)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.18 (s, 3H, C**H**$_3$), 2.66 (s, 3H, C**H**$_3$), 5.57 (s, 2H, NC**H**$_2$), 7.19-7.29 (m, 2H, Ph-**H**), 7.50 (d, 1H, *J* = 12.0 Hz, Ph-**H**), 8.06 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 14.1 (**C**H$_3$), 22.0 (**C**H$_3$), 61.7 (N**C**H$_2$), 106.2, 106.5, 115.2, 119.8, 128.2, 132.2, 137.9, 145.8, 159.5, 160.2, 161.9 (Ph-**C**, **C**=N), 164.0 (**C**=O). ^{11}B NMR (128 MHz, DMSO-*d*$_6$): δ_B = -1.32 to -1.31 (m, 1B, **B**F$_4$). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -115.64 to -115.57 (m, 1F, Ph-**F**); -148.21 (d, 4F, B**F**$_4$). LCMS (M^+)-BF_4^- 259.2 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-4-methylpyridin-1-ium *hexafluoro-phosphate* (5e)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.66 (s, 3H, C**H**$_3$), 5.55 (s, 2H, NC**H**$_2$), 7.19-7.29 (m, 2H, Ph-**H**), 7.50 (dd, 1H, *J* = 4.0, 12.0, Hz, Ph-**H**), 8.06 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.87 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 10.78 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$):

δ_C = 14.1 (**C**H_3), 22.0 (**C**H_3), 61.7 (N**C**H_2), 106.2, 106.5, 115.2, 119.9, 128.2, 132.3, 137.7, 145.8, 160.2, 161.6 (Ph-**C**, **C**=N), 164.0 (**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6) δ_P = -157.38 to -130.48 (sept, 1P, **P**F_6). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.18 (d, 6F, P**F**$_6$), -115.62 to -115.57 (m, 1F, Ph-**F**). LCMS (M^+)-PF_6^- 259.1 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((3-Fluoro-4-methylphenyl)amino)-2-oxoethyl)-4-methylpyridin-1-ium *trifluoroacetate* (5f)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.19 (s, 3H, C**H**$_3$), 2.66 (s, 3H, C**H**$_3$), 5.56 (s, 2H, NC**H**$_2$), 7.19- 7.29 (m, 2H, Ph-**H**), 7.50 (dd, 1H, *J* = 4.0, 12.0 Hz, Ph-**H**), 8.06 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.87 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C= 14.1 (**C**H_3), 22.0 (**C**H_3), 61.7 (N**C**H_2), 106.2, 106.5, 115.2, 119.8, 128.2, 132.2, 137.9, 145.8, 159.5, 160.2, 161.9 (Ph-**C**, **C**=N), 164.0 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.45 (s, 3F, C**F**$_3$), -115.63 to -115.58 (m, 1F, Ph-**F**). LCMS (M^+)-CH_3COO^- 259.1 found for $C_{15}H_{16}FN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *tetrafluoroborate* (5g)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.52 (s, 3H, C**H**$_3$), 5.64 (s, 2H, C**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.73-7.78 (m, 2H, Ph-**H**), 8.14 (dd, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 8.55 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.96 (s, 1H, Ph-**H**), 10.67 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 18.3 (**C**H_3), 62.4 (N**C**H_2), 88.7, 124.7, 124.9, 125.3, 125.8, 127.3, 134.0, 138.6, 144.2, 146.2, 147.1, 152.1, 154.6 (Ph-**C**, **C**=N), 164.4 (**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = -1.32 to -1.30 (m, 1B, **B**F_4). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -122.62 to -122.57 (m, 1F, Ph-**F**); -148.22 (d, 4F, B**F**$_4$). LCMS (M^+)-BF_4^- 371.0 found for $C_{14}H_{13}FIN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *hexafluoro-phosphate* (5h)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.52 (s, 3H, C**H**$_3$), 5.64 (s, 2H, C**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.73-7.78 (m, 2H, Ph-**H**), 8.14 (dd, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 8.55 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 8.96 (s, 1H, Ph-**H**), 10.67 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 18.3 (**C**H$_3$), 62.4 (N**C**H$_2$), 88.7, 124.7, 124.9, 125.4, 125.8, 127.3, 134.0, 138.6, 144.2, 146.2, 147.1, 154.6 (Ph-**C**, **C**=N), 164.4 (**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6) δ_P = -157.40 to -131.16 (sept, 1P, **P**F$_6$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.17 (d, 6F, P**F$_6$**), -122.60 to -122.55 (m, 1F, Ph-**F**). LCMS (M^+)-PF_6^- 371.0 found for $C_{14}H_{13}FIN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *trifluoroacetate* (5i)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.52 (s, 3H, C**H**$_3$), 5.65 (s, 2H, C**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.73-7.77 (m, 2H, Ph-**H**), 8.14 (dd, 1H, *J* = 4.0, 8.0 Hz, Ph-**H**), 8.55 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.89 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 8.97 (s, 1H, Ph-**H**), 10.67 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 18.3 (**C**H$_3$), 62.4 (N**C**H$_2$), 88.8, 124.7, 124.9, 125.4, 125.8, 127.3, 134.0, 138.6, 144.2, 146.2, 147.1, 154.6 (Ph-**C**, **C**=N), 164.4 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.46 (s, 3F, C**F$_3$**), -122.59 to -122.54 (m, 1F, Ph-**F**). LCMS (M^+)-CF_3COO^- 371.0 found for $C_{14}H_{13}FIN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *tetrafluoroborate* (5j)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.65 (s, 3H, C**H**$_3$), 5.61 (s, 2H, C**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.72-7.77 (m, 2H, Ph-**H**), 8.04 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 8.87 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 10.64 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 22.0 (**C**H$_3$), 61.8

(N**C**H_2), 88.7, 124.9, 125.4, 125.8, 128.3, 134.0, 145.8, 152.1, 154.6, 160.3 (Ph-**C**, **C**=N), 164.6 (**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = -1.32 to -1.30 (m, 1B, **B**F_4). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -122.54 to -122.50 (m, 1F, Ph-**F**); -148.22 (d, 4F, B**F**$_4$). LCMS (M^+)-BF_4^- 371.0 found for $C_{14}H_{13}FIN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *hexafluoro-phosphate* (5k)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.65 (s, 3H, C**H**$_3$), 5.61 (s, 2H, C**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.72-7.77 (m, 2H, Ph-**H**), 8.04 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 8.87 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 10.64 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 22.0 (**C**H_3), 61.8 (N**C**H_2), 88.7, 124.7, 124.9, 125.4, 125.8, 128.3, 134.0, 145.8, 152.1, 154.6, 160.3 (Ph-**C**, **C**=N), 164.6 (**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6) δ_P = -157.38 to -131.10 (sept, 1P, **P**F_6). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.17 (d, 6F, P**F$_6$**), -122.55 to -122.51 (m, 1F, Ph-**F**). LCMS (M^+)-PF_6^- 371.2 found for $C_{14}H_{13}FIN_2O^+$.

1-(2-((2-Fluoro-4-iodophenyl)amino)-2-oxoethyl)-3-methylpyridin-1-ium *trifluoroacetate* (5l)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.65 (s, 3H, C**H**$_3$), 5.62 (s, 2H, C**H**$_2$), 7.57 (d, 1H, *J* = 8.0 Hz, Ph-**H**), 7.72-7.77 (m, 2H, Ph-**H**), 8.06 (d, 2H, *J* = 12.0 Hz, Ph-**H**), 8.87 (d, 2H, *J* = 4.0 Hz, Ph-**H**), 10.64 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 22.0 (**C**H_3), 61.8 (N**C**H_2), 88.8, 124.7, 124.9, 125.4, 125.8, 128.3, 134.0, 145.8, 152.1, 154.6, 160.3 (Ph-**C**, **C**=N), 164.6 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.46 (s, 3F, C**F$_3$**), -122.55 to -122.50 (m, 1F, Ph-**F**). LCMS (M^+)-CF_3COO^- 370.9 found for $C_{14}H_{13}FIN_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium *tetrafluoroborate* (5m)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.53 (s, 3H, C**H**$_3$), 5.66 (s, 2H, C**H**$_2$), 7.77 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.98-8.06 (m, 1H, Ph-**H**), 8.15 (dd, 1H, *J* = 4.0, 8.0 Hz, Ph-**H**), 8.56 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 8.96 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 10.81 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.5, 106.9, 111.8, 112.3, 122.5, 127.3, 138.6, 144.2, 144.6, 146.2, 147.2 (Ph-**C**, **C**=N), 164.7 (**C**=O). ^{11}B NMR (128 MHz, DMSO-*d*$_6$): δ_B = -1.31 to -1.29 (m, 1B, **B**F$_4$). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -126.05 to -125.96 (m, 1F, Ph-**F**), -138.94 to -138.82 (m, 1F, Ph-**F**), -141.48 to -141.32 (m, 1F, Ph-**F**), -148.25 (d, 4F, B**F**$_4$). LCMS (M^+)-BF_4^- 281.1 found for $C_{14}H_{12}F_3N_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium *hexafluoro-phosphate* (5n)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.52 (s, 3H, C**H**$_3$), 5.65 (s, 2H, C**H**$_2$), 7.77 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.97-8.04 (m, 1H, Ph-**H**), 8.14 (dd, 1H, *J* = 4.0, 8.0 Hz, Ph-**H**), 8.55 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 8.95 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 10.81 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.5, 106.9, 111.8, 112.3, 122.5, 127.3, 138.6, 144.2, 144.6, 146.2, 147.2 (Ph-**C**, **C**=N), 164.7 (**C**=O). ^{31}P NMR (162 MHz, DMSO-*d*$_6$) δ_P = -157.39 to -131.06 (sept, 1P, **P**F$_6$). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -69.18 (d, 6F, P**F$_6$**), -126.04 to -125.96 (m, 1F, Ph-**F**), -138.92 to -138.81 (m, 1F, Ph-**F**), -141.44 to -141.36 (m, 1F, Ph-**F**). LCMS (M^+)-PF_6^- 281.2 found for $C_{14}H_{12}F_3N_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium *trifluoroacetate* (5o)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.53 (s, 3H, C**H**$_3$), 5.66 (s, 2H, C**H**$_2$), 7.77 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.97-8.04 (m, 1H, Ph-**H**), 8.15 (dd, 1H, *J* = 4.0, 8.0 Hz, Ph-**H**), 8.56 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 8.88 (d, 1H, *J* = 4.0 Hz, Ph-**H**), 8.96 (s, 1H, Ph-**H**), 10.81 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 18.3 (**C**H$_3$), 62.3 (N**C**H$_2$), 106.5, 106.9, 111.8, 112.3,

122.5, 127.3, 138.6, 144.2, 144.6, 146.2, 147.2 (Ph-**C**, **C**=N), 164.7 (**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -75.31 (d, 3F, C**F$_3$**), -126.01 to -125.92 (m, 1F, Ph-**F**), -138.91 to -138.78 (m, 1F, Ph-**F**), -141.46 to -141.31 (m, 1F, Ph-**F**). LCMS (M^+)-CF_3COO^- 281.1 found for $C_{14}H_{12}F_3N_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium *tetrafluoroborate* (5p)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.66 (s, 3H, C**H$_3$**), 5.63 (s, 2H, NC**H$_2$**), 7.77 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.96-8.06 (m, 3H, Ph-**H**), 8.88 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 22.0 (**C**H$_3$), 61.7 (N**C**H$_2$), 106.4, 106.7, 106.9, 111.7, 111.9, 122.7, 128.3, 144.6, 160.3 (Ph-**C**, **C**=N), 165.0 (**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = -1.31 to -1.29 (m, 1B, **B**F$_4$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -125.96 to -125.88 (m, 1F, Ph-**F**), -138.98 to -138.86 (m, 1F, Ph-**F**), -141.46 to -141.33 (m, 1F, Ph-**F**), -148.24 (d, 4F, B**F$_4$**). LCMS (M^+)-BF_4^- 281.0 found for $C_{14}H_{12}F_3N_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium *hexafluoro-phosphate* (5q)

^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 2.66 (s, 3H, C**H$_3$**), 5.63 (s, 2H, NC**H$_2$**), 7.77 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.97-8.06 (m, 3H, Ph-**H**), 8.88 (d, 2H, *J* = 8.0 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 22.0 (**C**H$_3$), 61.7 (N**C**H$_2$), 106.4, 106.7, 106.9, 111.7, 111.9, 122.7, 128.3, 144.6, 160.3 (Ph-**C**, **C**=N), 165.0 (**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6) δ_P = -157.38 to -131.03 (sept, 1P, **P**F$_6$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.17 (d, 6F, P**F$_6$**), -126.03 to -125.94 (m, 1F, Ph-**F**), -139.00 to -138.89 (m, 1F, Ph-**F**), -141.46 to -141.31 (m, 1F, Ph-**F**). LCMS (M^+)-PF_6^- 281.2 found for $C_{14}H_{12}F_3N_2O^+$.

3-Methyl-1-(2-oxo-2-((2,4,5-trifluorophenyl)amino)ethyl)pyridin-1-ium *trifluoroacetate* (5r)

^{1}H NMR (400 MHz, DMSO-*d*$_6$): δ_H = 2.66 (s, 3H, C**H**$_3$), 5.63 (s, 2H, NC**H**$_2$), 7.77 (td, 1H, *J* = 8.0, 12.0 Hz, Ph-**H**), 7.97-8.06 (m, 3H, Ph-**H**), 8.88 (d, 2H, *J* = 6.6 Hz, Ph-**H**), 10.79 (s, 1H, CON**H**). ^{13}C NMR (100 MHz, DMSO-*d*$_6$): δ_C = 22.0 (**C**H$_3$), 61.7 (N**C**H$_2$), 106.4, 106.7, 106.9, 111.7, 111.9, 122.7, 128.3, 144.6, 160.3 (Ph-**C**, **C**=N), 165.0 (**C**=O). ^{19}F NMR (377 MHz, DMSO-*d*$_6$): δ_F = -71.50 (s, 3F, C**F**$_3$), -125.97 to -125.89 (m, 1F, Ph-**F**), -138.96 to -138.85 (m, 1F, Ph-**F**), -141.46 to -141.31 (m, 1F, Ph-**F**). LCMS (M^+)-CF_3COO^- 280.9 found for $C_{14}H_{12}F_3N_2O^+$.

2.5 DNA binding studies

The DNA binding experiments were done in Tris-buffer (10^{-2} M, pH 7.2). Initially, the concentration of DNA was ascertained by recording the absorption spectrum of CT-DNA solution. This solution gave UV absorbance at 230/260 nm (=1.8), indicated free nature of DNA, with ε value equal to 6600 M^{-1} cm^{-1} [37]. The different compounds and DNA solutions were kept at 4 ˚C. The compounds were firstly dissolved in 0.1% DMSO and then diluted with Tris-buffer so that the concentrations become 2.4 × 10^{-4} M. After individual addition of various concentrations (1.2 – 1.5 × 10^{-5} M) of DNA, the absorption spectra were recorded. The binding constants (K_b) were determined with help of Benessi-Hilderbrand equation (1) [38].

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_a - \varepsilon_f) + 1/K(\varepsilon_b - \varepsilon_f) \quad \text{..... (1)}$$

where, absorption coefficients; $\varepsilon_a, \varepsilon_f$, and ε_b are related to A_{obs}/[compound], extinction coefficient for compound in free and extinction coefficient for compound in fully bound form.

The binding constants (K_b) for different compounds were ascertained by slopes and the intercepts of the plots of $[DNA]/(\varepsilon_a - \varepsilon_f)$ $vs [DNA]$.

2.6 Anticancer studies

The anti-proliferative testing of the synthesized compounds was done with two lung cancer cell lines *i.e.* A549 and H-1229 by MTT assay. Briefly, the cells were seeded (density of 8000 cells/well) in a 96 well plate and set to incubate. At about 60-70% confluency, the cells were treated with concentrations (5.0, 10.0, 15 and 20.0 mM) of the reported compounds and allowed to incubate for next 24 hrs. The cells were assayed by adding 15.0 µL (5.0 mg/mL MTT). At 37 ˚C of incubation for 4 hrs, the respective media from each well were aspirated. The cells were re-suspended in 100 µL of DMSO and the plate immediately covered with aluminum foil, followed by gentle shaking on a shaker for about 15 min. Absorbance was recorded at 540 nm [39] and the percent inhibition in proliferation was calculated by the formula in Eq.2

$$\% \text{ Inhibitaion} = [A_{Control} - A_{Sample})/A_{Control}] \times 100 \quad - 2$$

2.7 Docking procedure

The docking studies were performed by Intel dual CPU (1.86 GHz) with Windows XP operating system. The 3D structure of ligand was drawn by using Marwin sketch. The so obtained 3D structure was converted to the pdb file format. Ligand preparation was done by assigning Gastegier charges, merging non-polar hydrogens, and saving it in PDBQT file format using AutoDock Tools (ADT) 4.0 [40]. X-ray crystal structure of DNA (PDB ID: 1bna), was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). Using AutoDock Tools (ADT) 4.0, receptors were saved in PDB file format leaving heteroatoms (water). Gastegier charges were assigned to receptor and saved in PDBQT file format using ADT. Preparation of parameter files for grid and docking was done using ADT. Docking was performed with AutoDock 4.0

(Scripps Research Institute, USA) considering all the rotatable bonds of ligand as rotatable and receptor as rigid [41]. Grid box size of 60 × 80 × 114 Å with 0.375 Å spacing was used that included the whole DNA. Docking to macromolecule was performed using an empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2500000 energy evaluations, a mutation rate of 0.02, and crossover rate of 0.80. Fifty independent docking runs were performed for each ligand and serum-ligand adduct for lowest free energy of binding conformation from the largest cluster and saved in PDBQT format.

3. Results and discussion

3.1 Synthetic of ionic liquids

The selected 2-chloro-*N*-(3-fluoro-4-methylphenyl)acetamide, 2-chloro-*N*-(2-fluoro-4-iodophenyl)acetamide and 2-chloro-*N*-(2,4,5-trifluorophenyl)acetamide **3a-c** used in this study were synthesized *via* the condensation of the appropriate fluorinated aniline **1a-c** with chloroacetyl chloride in the presence of triethylamine as catalyst. The synthetic protocol for all the molecules is shown in scheme 1.

In our investigations, the designing of halogenated pyridinium ionic liquids was studied and found to be easily obtained *via* the alkylation of 3-methyl and/or 4-methylpyridine with the appropriate fluorinated phenylacetamide chlorides **2a-c,** in refluxing acetonitrile in the presence of sodium iodide, under both conventional and microwave conditions.

The structural elucidation of the new pyridinium iodides **4a-f** was carried out on the basis of their ^{1}H, ^{13}C, ^{19}F NMR and mass spectrometric data. The ^{1}H, ^{13}C NMR spectra of all the synthesized ILs **4a-f** agreed with their assigned structures. Thus, their ^{1}H NMR spectra revealed the presence of two diagnostic singlets between 5.57-5.70 ppm and 10.76-10.88 ppm attributed

to the NC**H**$_2$ and CON**H** protons of the acetamide linkage, respectively. The spectra also exhibited extra aromatic protons in their appropriate chemical shifts (see experimental section). In their ^{13}C-NMR spectra, the appearance of new signal in the aliphatic region at 61.7-64.4 ppm assigned to the -NCH_2 carbon and a diagnostic amide carbonyl carbon at 163.7-165.0 ppm provided additional evidences for the success of the quaternization reaction. The ^{19}F NMR results were also in the agreement with the proposed structures. Thus, the ^{19}F NMR spectra of ILs **4a**, **4b**, **4d** and **4e** showed a multiplet between -122.45 to -115.58 ppm belonging to one aromatic fluorine atom. In contrast, three multiplets were recorded in the ^{19}F NMR spectra of ILs **4c** and **4f** from -141.46 to -125.91 ppm attributed to three aromatic fluorine atoms; thus, confirming the presence of trifluorophenyl group. The structures, yields and reaction times in conventional and microwave assisted syntheses are given in Table 1.

Under the optimized conventional versus metathesis conditions, the reaction of the pyridinium IL halides **4a-f** with three fluorinated metal salts (including $NaBF_4$, KPF_6 and CF_3COONa) led to the formation of task specific ionic liquids tethering fluorinated counter anions **5a-r** (Table 2). The composition and structure of the designed ILs **5a-r** were confirmed by usual spectroscopic experiments such as ^{1}H, ^{13}C, ^{19}F, ^{31}P, ^{11}B NMR and mass analysis. No changes were recorded on the ^{1}H and ^{13}C NMR spectra of **5a-r** as compared to their corresponding halogenated ionic liquids **4a-f**. However, their structures were efficiently established from their ^{19}F, ^{31}P, ^{11}B NMR and Mass analysis. Thus, the significant doublets (at -148.25 to -148.21 ppm) and multiplet (at -1.32 to -1.29 ppm) were recorded on the ^{19}F and ^{11}B NMR spectra, respectively; assigning to the tetrahydroborate anion (BF_4^-). These results confirmed the success of the metathethical reaction. On the other hand, the ^{31}P and ^{19}F NMR spectra exhibited characteristic septets and doublets near (-157.45 to -131.03 ppm) and (-69.18 to

-69.17 ppm), respectively, belonging to the PF_6^- anion as counter anion. In addition, the fluorine atoms of CF_3COO^- anion were observed as a distinct singlet ranging between -75.31 to -71.50 ppm in their ^{19}F NMR analysis. These observations supported the halide anion exchange with trifluoroacetate anion (CF_3COO^-). The remaining multiplets signals recorded in all ^{19}F NMR spectra were between -141.48 and -114.39 ppm; attributing to the aromatic fluorine atoms. Further structural elucidation was confirmed by their mass spectral analyses, which was in accordance with the proposed structures.

3.2 DNA binding studies of ionic liquids

All the absorption experiments involving interactions of the reported compounds with CT-DNA were executed in tris-buffer having physiological pH. The UV spectra of CT-DNA in buffer showed two absorbance peaks at 260 and 280 nm in the ratio of 1.9:1 that indicated protein free nature of DNA [42]. Using molar absorption coefficient ε_{260} = 6600 L mol^{-1} cm^{-1}, the concentration of DNA was determined by UV absorption [43]. For elimination of DNA absorbance an equal amount of DNA was added to both the reference and the other solutions. The absorption titration experiments were conducted by varying the concentration of DNA with the fixed concentrations of the compounds.

DNA is one of the important pharmacological targets of drugs in various diseases particularly cancer. Although, there are several techniques used to study the drugs and DNA interactions but, absorption spectroscopy is simple and powerful tool. The absorption spectra of the most active ionic liquids **4a, 4e** and **5n** are given in **Figure 1** while the others are reported in the supplementary information. In the absence of CT-DNA, the compounds exhibited absorbance in the range of 260-300 nm in tris-buffer at physiological pH. With the addition of the different concentrations ($1.2 – 1.5 \times 10^{-5}$ M) of CT-DNA to the fixed concentration of compounds (2.4 ×

10^{-5} M), the absorption intensity of the compounds showed hypochromism with slight and moderate changes in the shape and position of absorption bands. Generally, hyperchromism and hypochromism are the spectral features observed during the spectrophotometric titration of small molecules with CT-DNA [44-47]. Hypochromism is usually characterized by non-covalently intercalative bindings of the compounds with DNA [48], whereas hypochromism associated with non-intercalative binding modes; most probably electrostatic or hydrogen bondings [49]. Furthermore, the hyperchromism of the compounds indicated bindings to DNA it its grooves. The binding constants of the reported compounds were calculated by using modified Benesi–Hildebrand equation [50].

In the present study, almost all the compounds showed small red shifts. The absorption spectra of compound **4a** in both the absence [2.4×10^{-4} M] and the presence [$1.8\text{-}3.8 \times 10^{-4}$ M] of CT-DNA are shown in **Figure 1**. It is clear from the absorption spectra that moderate bathochromism and hypochromism at 270 nm were observed when **4a** was titrated with various concentrations of CT-DNA. These spectral changes indicated that **4a** had been interacted with CT-DNA *via* non-covalently intercalations, which might be due to the stacking interactions between the two chromophores. Similarly, for compound **4d**, the absorption peak at 269 nm was observed, but with addition of various DNA concentrations, the absorption peak underwent strong hypochromism and slight bathochromism. In case of other compounds (**4b, 4c, 4e, 4f** and **5a-r**) the similar changes (hypochromic and bathochromic shifts) were observed that showed the strong interactions of the compounds with CT-DNA. The intrinsic DNA binding constants were calculated for all the compounds **4a-f** and **5a-r** and ranged from 1.3×10^{3} to 1.52×10^{8} M^{-1} (**Table 3**). In addition to the hypochromism and bathochromism changes, the isosbestic points were also appeared in DNA binding spectra for some compounds (**4e, 4f, 5b** and **5h**). These were

also the indications of the moderate bindings of the compounds with DNA. Finally, from the above observed spectral changes in all the compounds and high values of K_b suggested that the compounds have strong affinity with CT-DNA. These can be considered as non-covalent binders [51-53].

3.3 Anticancer activities of ionic liquids

In vitro percentage inhibitions of two lung cancerous cells *i.e.* A549 and H-1229 were studied at 5.0, 10.0, 15.0 and 20.0 mM concentrations of the reported compounds. The results are given in Figures 2 and 3, respectively. Originally, the molecules were dissolved in 0.1 % DMSO and natural cells with DMSO were utilized as vehicle control. Then accumulation of the molecules was waited till the control cells reached at inactive phase. The cells were counted after 24 hrs. For all the compounds, the proliferation inhibitions for both the cells were augmented with increasing amount of the compounds. The results indicated the significant inhibition in cancer cell proliferations. The two sets of the compounds i.e. 4a-f and 5a-r were screened. The maximum percentage inhibitions of 4a-f series with A549 and H-1229 cell lines were 99.16% (4a) and 91.24%, (4e), respectively. The order of the anticancer activities with these cell lines was 4a > 4e > 4b > 4d > 4c and 4e > 4c > 4a > 4f > 4b > 4d. The maximum percentage inhibitions of 5a-r series with A549 and H-1229 cell lines were 99.23% (5n) and 99.69% (5n), respectively. The order of the anticancer activities with these cell lines was 5n > 5f > 5h > 5i > 5g > 5a > 5k > 5m > 5e > 5d > 5c > 5p > 5b > 5i > 5g > 5j > 5o > 5r and 5n > 5p > 5q > 5h > 5c > 5g > 5f > 5r > 5m > 5k > 5a > 5o > 5i > 5j > 5i > 5d > 5b > 5e. All the compounds showed more than 50% inhibition of the lung cancer cell lines. Moreover, the maximum proliferation inhibitions were 99.16 to 91.24% for 4a series and 99.23 to 99.69% for 5a series of the ionic

liquids. The percentage inhibition was found to be excellent (4a, 4e and 5n) and, hence, these ionic liquids may be the future molecules for treating cancer.

3.4 Docking studies of ionic liquids

The attempts were made to determine the interactions and mechanism of action of the reported molecules with DNA. The different binding affinities obtained are given in Table 4. Basically, the binding study of only organic molecules with DNA is carried out. The present article describes twenty-four compounds but the basic organic structures are of six different types **4a-f**. Therefore, the binding study was carried out with six structures only. The binding affinities of the ionic liquids with DNA ranged from -4.6 to -4.9 kcal/mol because of the different structures of the organic moieties. Besides, the hydrogen bond formation with DNA was also based on the different structures of the molecules. The hydrogen bonds formed with DNA were one (**4a)**, two (**4b,c)** and three (**4d-f)**. After hydrogen bond formation study, the hydrophobic interactions were seen using ligplot software. The common residues of DNA involved with ionic liquids were dc9, dc13, dc14, dc15, dg10, dg14, dg16, c1, c2, c3, c4, c5, c6, c7, c8, c7, c9, c10, c11, c13, c14 and c15. The interactions models of the most active compound (4a) is given in Figure 4, while the rest in supplementary information. It is clear from the docked models that all the compounds preferred DNA minor grooves. During the process of DNA interaction, it was also observed that all the compounds oriented themselves in such a fashion that their active sites were inside the minor grooves. Overall, the experimental results of DNA binding were in good agreement with those of docking studies. It is very important to mention here that all twenty-four ionic liquids have only six different organic moieties while these differ with respect to the anionic parts. In spite of only six organic moieties, all the twenty-four compounds showed the different anticancer activities. It is due to the fact that the different

ionization of ionic liquids provided different concentrations of six organic moieties. It is similar to the different interactions of *cis*- and *trans*-platin isomers [54].

4. Significance of microwave assisted synthesis of ionic liquids

It was noticeable that the excellent yields obtained were 92-95 % within short reaction times (15-20 min), when the quaternization of the same picoline **3a** and/or **3b** was carried out under MWI (Table 1). Microwave irradiated (MWI) versus thermal metathetical anion exchange was also occurred smoothly; when the designed pyridinium IL halides **4a-f** were treated with the appropriate fluorinated metal salts ($NaBF_4$, KPF_6 and CF_3COONa). This led to the formation of task specific ionic liquids tethering fluorinated counter anions **5a-r**. Under the thermal conditions, 24 hrs were required to complete the anion exchange furnishing the formation of **5a-r** in 80-86 % yields. The general metathesis reaction worked well in very short time (20 min) for the anion exchange using MWI and yielded the target task ionic liquids **5a-r** in excellent yields (up to 97 %; Table 2).

5. Conclusion

Really, ionic liquids are remarkable molecules with excellent properties and applications. The reported ionic liquids were obtained in good yields (up to 95%) with microwave assisted green approach syntheses. The DNA binding constants (Kb) were in the range of 1.3×10^3 to 1.52×10^8 M^{-1}. All the ionic liquids intercalated with DNA through the minor grooves via covalent, intercalative and electrostatic bindings. All the reported ionic liquids showed more than 50% anticancer activities with two lung cancer cell lines *i.e.* A549 and H-1229. Moreover, the maximum proliferation inhibitions were 99.16 to 91.24% for 4a series and 99.23 to 99.69% for 5a series of the ionic liquids. The docking studies indicated quite good binding affinities (-4.6 to -4.9 kcal/mol.) of the reported ionic liquids with DNA. The experimental results of DNA

bindings were in good agreement with those of docking studies. The percentage inhibition was found to be excellent (4a, 4e and 5n) and, hence, these ionic liquids may be the future molecules for treating cancer.

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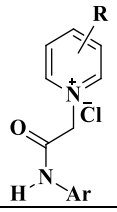
Table 1: Microwave versus conventional syntheses of halogenated pyridinium ionic liquids tethering aromatic acetamide linkage (**4a-f)**



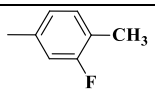
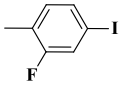
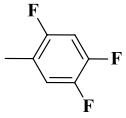
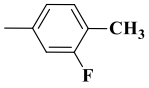
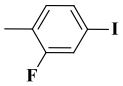
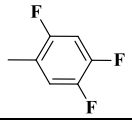
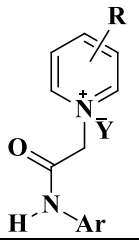
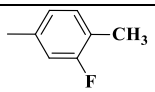
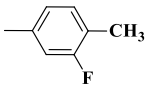
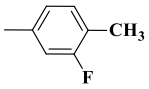
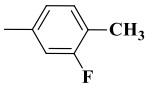
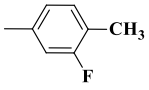
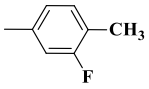
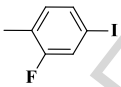
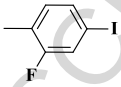
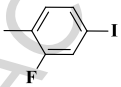
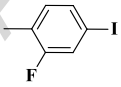
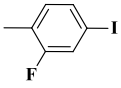
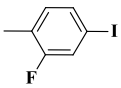
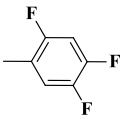
Comp. No	R	Ar	Mp (ºC)	CP		MWI	
				Time (h)	Yield (%)	Time (min)	Yield (%)
4a	**3-CH$_3$**		87-88 Colorless crystals	20	80	20	92
4b	**3-CH$_3$**		75-76 Colorless crystals	18	81	15	92
4c	**3-CH$_3$**		Syrup	18	83	15	94
4d	**4-CH$_3$**		82-81 Colorless crystals	20	82	20	93
4e	**4-CH$_3$**		71-72 Colorless crystals	18	84	15	94
4f	**4-CH$_3$**		Syrup	18	84	15	95

Table 2: Microwave versus conventional syntheses of task specific ionic liquids tethering fluorinated counter anions **5a-r**.



Comp. No	R	Ar	Y	Mp (ºC)	CM		MWI	
					Time (h)	Yield (%)	Time (min)	Yield (%)
5a	**3-CH$_3$**		BF_4	Syrup	24	84	20	95
5b	**3-CH$_3$**		PF_6	70-71 Colorless crystals	24	81	20	93
5c	**3-CH$_3$**		CF_3COO	63-64 Colorless crystals	24	80	20	91
5d	**4-CH$_3$**		BF_4	Syrup	24	85	20	95
5e	**4-CH$_3$**		PF_6	66-67 Colorless crystals	24	81	20	93
5f	**4-CH$_3$**		CF_3COO	58-59 Colorless crystals	24	81	20	92
5g	**3-CH$_3$**		BF_4	Syrup	24	85	20	95
5h	**3-CH$_3$**		PF_6	55-56 Colorless crystals	24	82	20	92
5i	**3-CH$_3$**		CF_3COO	Syrup	24	83	20	93
5j	**4-CH$_3$**		BF_4	Syrup	24	86	20	96
5k	**4-CH$_3$**		PF_6	53-54 Colorless crystals	24	82	20	92
5l	**4-CH$_3$**		CF_3COO	Syrup	24	83	20	94
5m	**3-CH$_3$**		BF_4	Syrup	24	86	20	96

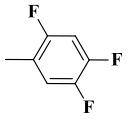
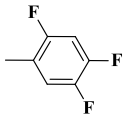
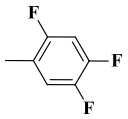
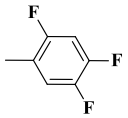
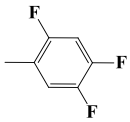
5n	**3-CH$_3$**		PF_6	Syrup	24	84	20	94
5o	**3-CH$_3$**		CF_3COO	Syrup	24	85	20	95
5p	**4-CH$_3$**		BF_4	Syrup	24	86	20	97
5q	**4-CH$_3$**		PF_6	Syrup	24	85	20	95
5r	**4-CH$_3$**		CF_3COO	Syrup	24	85	20	95

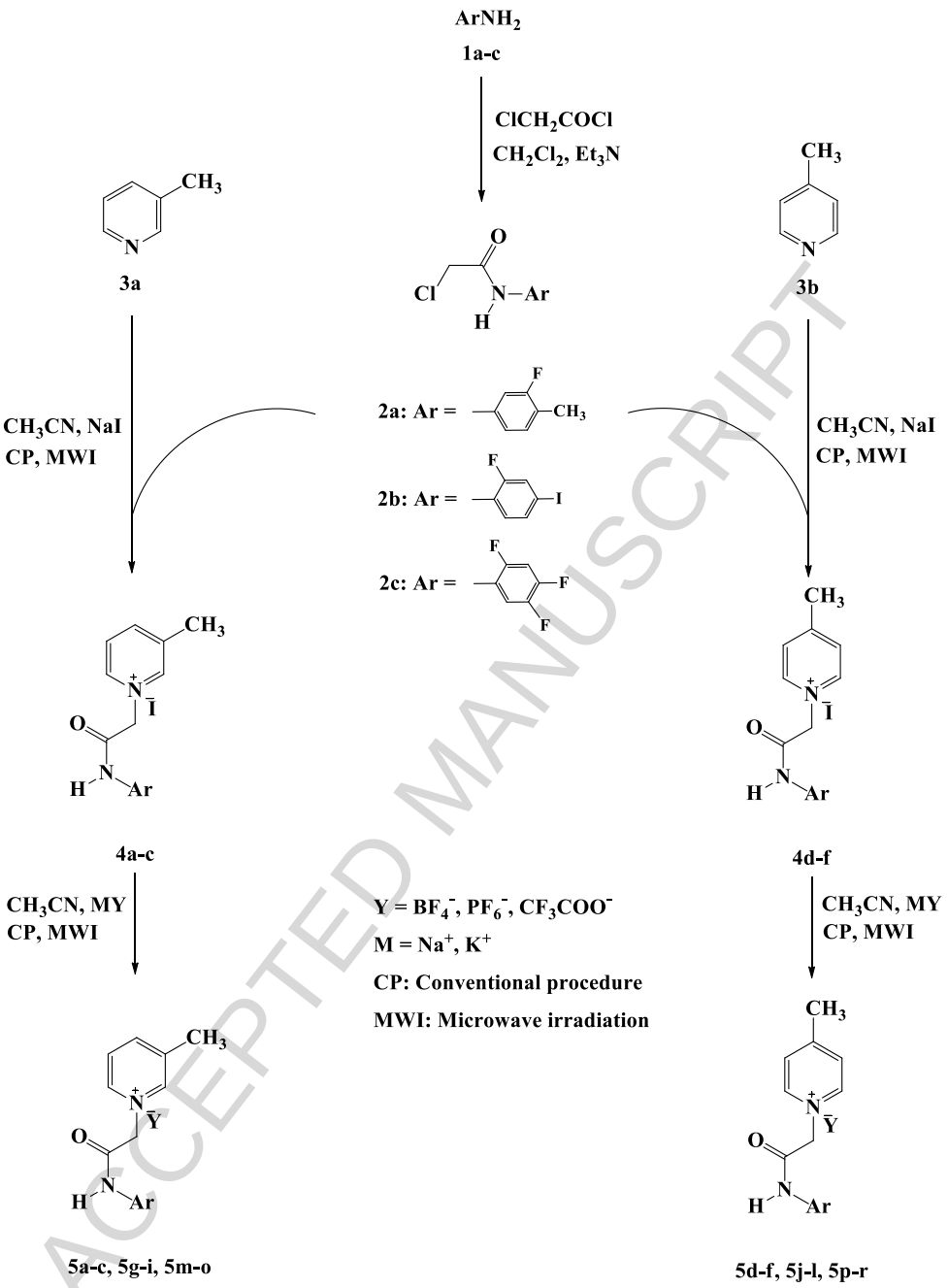
Table 3. The UV absorption properties of **4a-f, 5a-r** compounds.

Compound codes	λ_{max} free (nm)	λ_{max} bound (nm)	$\Delta\lambda_{max}$ (nm)	% Hypochromism[a]	K_b[b] (M^{-1})
4a	270	284	14	35	4.90×10^5
4b	267	268	1	53	7.70×10^5
4c	270	273	3	50	7.40×10^5
4d	269	269	-	35	6.30×10^5
4e	261	265	4	10	4.60×10^4
4f	285	290	5	31	1.52×10^5
5a	257	259	2	50	7.10×10^7
5b	263	270	7	2.2	1.30×10^3
5c	269	271	2	34	4.60×10^5
5d	267	268	1	2.4	2.90×10^4
5e	267	268	1	53	1.50×10^5
5f	259	261	2	22	5.40×10^4
5g	266	267	1	55	1.52×10^8
5h	266	266	-	23	7.90×10^4
5i	268	272	4	49	7.60×10^6
5j	271	273	2	45	4.10×10^5
5k	265	265	-	14	6.30×10^5
5l	266	267	1	55	8.40×10^7
5m	269	273	4	39	1.52×10^5
5n	269	271	2	34	5.60×10^5
5o	268	273	5	54	8.0×10^7
5p	265	266	1	43	2.10×10^5
5q	265	266	1	8.9	6.30×10^4
5r	265	267	2	36	4.60×10^6

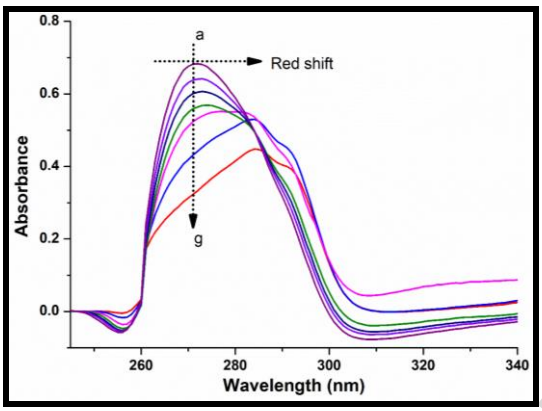
where, [a] % Hypochromism = $[A_f - A_b)/A_f] \times 100$, [b] Binding constants.

Table 4. The docking studies of the ionic liquids with DNA.

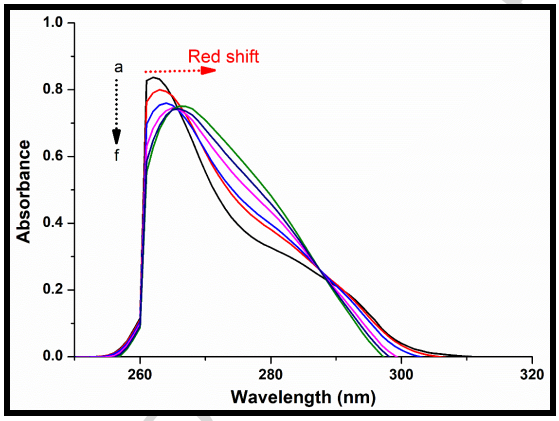
S.No.	Compounds Name	No. of H-Bond	Bond length (A°)	Hydrophobic interaction	Affinity in (kcal/mol)
1	**4a**	1	H of -CONH- group &.263/A/DG`10/O6 (2.0 A°)	dc15::C7&C9 dg14::C7 dg10::O6 dc9::C13&C9 dt8::C8	-4.6
2	**4b**	2	H of -CONH- group &.263/A/DG`10/O6 (2.4 A°) O of -CONH- group &.218/B/DG`14/N7 (3.1 A°)	dg14::C4&C10 dc15::C1,C2,C9&C14 dc9::C3&C5	-4.7
3	**4c**	2	H of -CONH- group &.624/B/DG`14/OP2 (2.8 A°) O of -CONH- group &.624/B/DG`14/OP2 (3.2 A°)	dt8::C1 dc15::C5,C8,C3,C2&C7 dg14::O,C5,C9&C14 dg16::C1 dc9::C3&C1 dc13::C10	-4.7
4	**4d**	3	H of -CONH- group &.263/A/DG`10/O6 (2.4 A°) O of -CONH- group &.218/B/DG`14/N7 (3.4 A°) O of -CONH- group &.263/A/DG`10/O6 (3.2 A°)	dc9::C13,C9&C10 dc14::C12 dc15::C15,C11,C4,C6&C7	-4.9
5	**4e**	3	H of -CONH- group &.263/A/DG`10/O6 (2.3 A°) O of -CONH- group &.263/A/DG`10/O6 (3.2 A°) O of -CONH- group &.218/B/DG`14/N7 (3.5 A°)	dc9::C1&C5 dc15::C10,C3,C4&C6 dg14::C9&C2	-4.9
6	**4f**	3	H of -CONH- group &.263/A/DG`10/O6 (2.4 A°) O of -CONH- group &.218/B/DG`14/N7 (3.3 A°) O of -CONH- group &.263/A/DG`10/O6 (3.5 A°)	dg14::C2&C8 dc15::C3,C1,C7&C14 dc9::C5&C11	-4.9



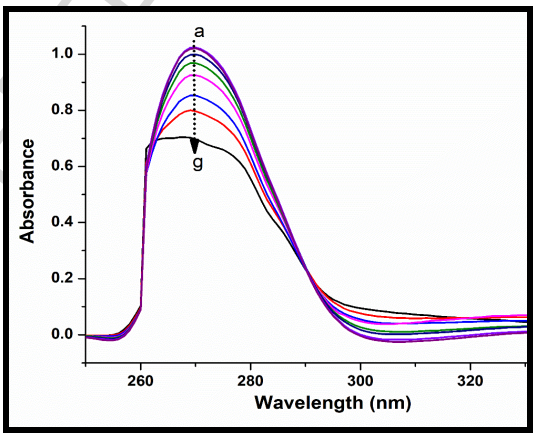
Scheme 1: Microwave irradiated (MWI) versus conventional syntheses of pyridinium ionic liquids carrying fluorinated phenylacetamide linkage for 4a-f and 5a-r.



(I)



(II)



(III)

Figure: 1. DNA binding spectra of **4a (I), 4e (II) and 5n (III)** in the absence and presence of different DNA concentrations. Arrow shows decrease in absorbance [Compound] = 2.4×10^{-5} M, [DNA] = 1.2 - 3.8×10^{-5} M.

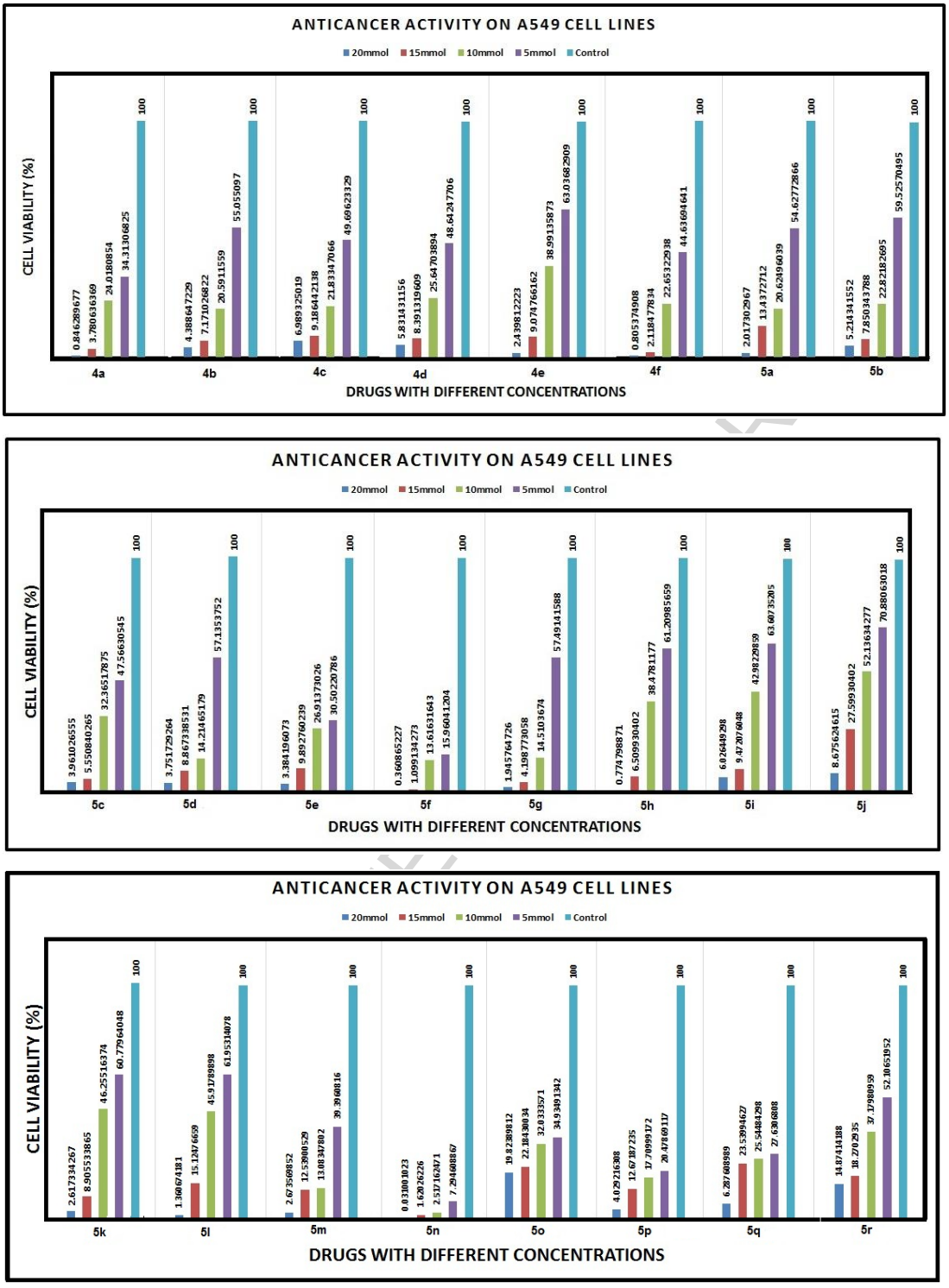


Figure 2: Anticancer activities of 4a-f and 5a-r series of ionic liquids with A-549 cancer lines.

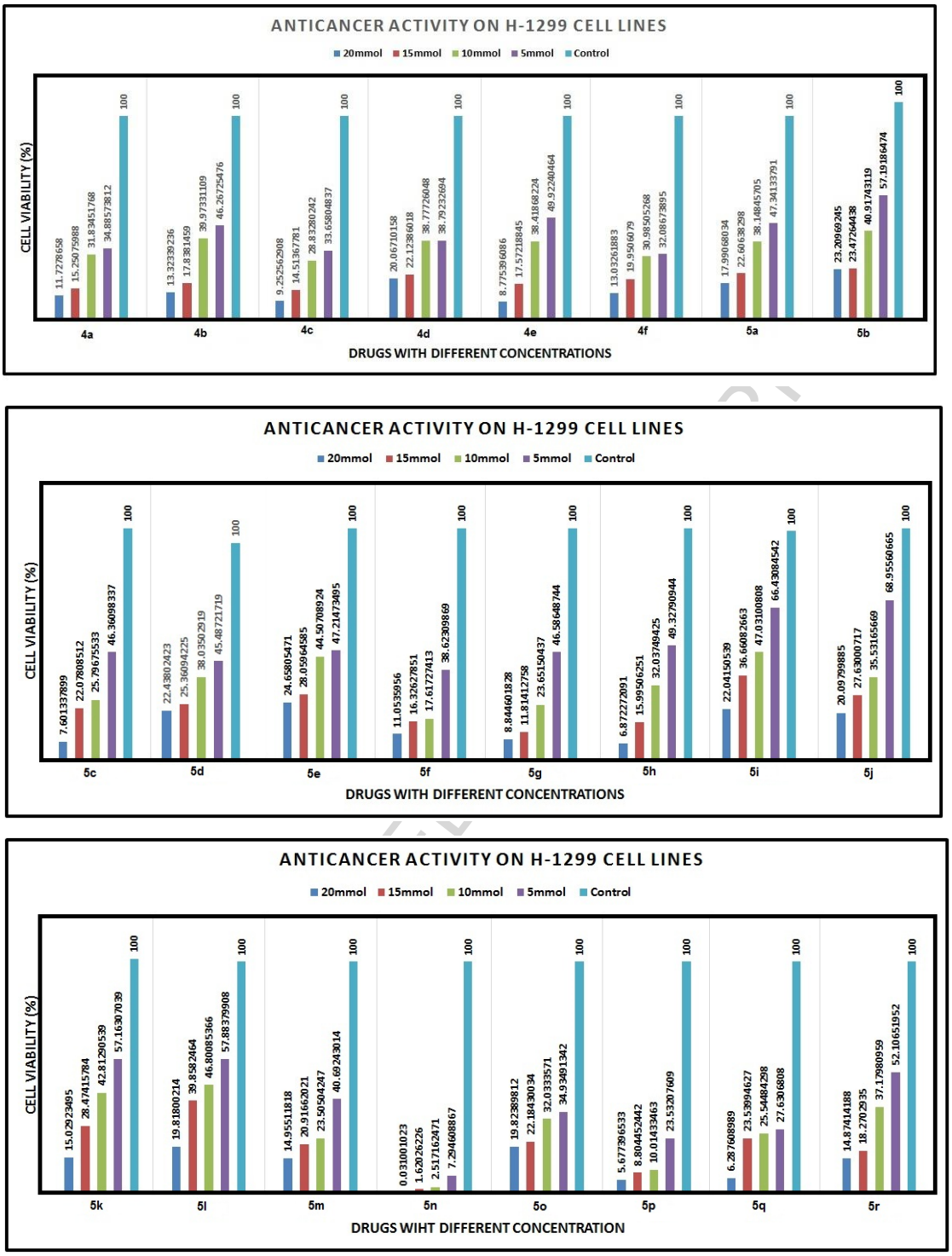


Figure 3: Anticancer activities of 4a-f and 5a-r series of ionic liquids with H-1299 cancer lines.

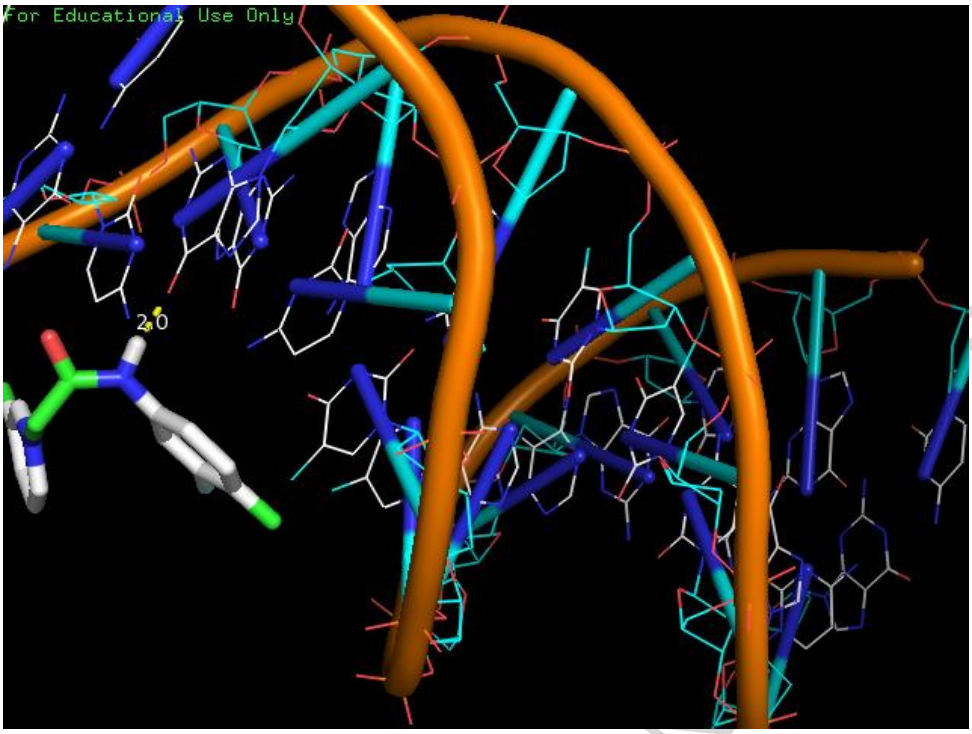


Figure 4: Docking model of 4a compound showing various interactions between atoms of 4a and residues of DNA.

Highlights

- Green microwave assisted synthesis of ionic liquids (86%).

- Characterization of ionic liquids by spectroscopic methods.

- DNA binding and docking studies of ionic liquids.

- Anticancer activities of ionic liquids.

- Novel pyridinium based ionic liquids may be used as anticancer agents.