Synthesis, Characterization, DNA Binding, Anticancer, and Molecular Docking Studies of Novel Imidazolium-Based Ionic Liquids with Fluorinated Phenylacetamide Tethers

Nadjet Rezki, Fawzia Faleh Al-blewi, Salsabeel A. Al-Sodies, Asaad Khalid Alnuzha, Mouslim Messali, Imran Ali, and Mohamed Reda Aouad*

ABSTRACT: Newer imidazolium ionic liquid (IL) halides appending variety of fluorinated phenylacetamide side chains were designed and synthesized through quaternization of 1-methyl and/or 1,2-dimethylimidazole with appropriate 2-chloro-N-(fluorinatedphenyl)acetamides. The resulting ILs were converted to their respective ionic liquid analogues carrying fluorinated counteranions (PF$_6^-$, BF$_4^-$, and/or CF$_3$COO$^-$) 5a–r. All newly synthesized ILs were fully characterized using several spectroscopic experiments such as $^1$H, $^{13}$C, $^{11}$B, $^{19}$F, $^{31}$P NMR, and mass analysis. The synthesized ionic liquids were investigated for their DNA binding and anticancer activities. The obtained DNA binding constants ranged from $1.444 \times 10^5$ to $3.518 \times 10^5$, indicating a reasonably good binding affinity. The percentage of anticancer activities ranged from 48 to 59 with H-1229 cell line, showing quite good anticancer potential. The modeling studies indicated the interactions of the reported molecules with DNA via hydrogen bonds. These were in agreement with those of DNA binding and anticancer results. Briefly, the designed ionic liquids may be used as good anticancer candidates for treating human cancer.

1. INTRODUCTION

The drug discovery is at the cutting edge of the most promising medicinal chemistry. The drug design is at a crossroad, facing growing strategies for the synthesis of new active pharmaceutical ingredients (APIs). These strategies faced several challenges in the development of such scaffolds for effective drug delivery. These challenges are further exacerbated when drug compounds resulted from the combination of simple and active moieties with unique and tunable physicochemical and biological properties. Consequently, the development of potent anticancer agents is a major trend in drug discovery efforts in medicinal chemistry. Ionic liquids (ILs) have been a topic of great interest in organic synthesis owing to their potential pharmaceutical properties and hold an important challenge in medicinal chemistry, especially in the race to synthesize new therapeutic agents or active pharmaceutical ingredients (APIs) tethered such moieties. Generally, ILs are synthesized by combining organic cations such as imidazolium, pyridinium, ammonium, guanidinium, and phosphonium with a wide variety of anions including halides (Cl$^-$, Br$^-$), hexafluoroarsenate (PF$_6^-$), tetrafluoroborate (BF$_4^-$), trifluoroacetate (CF$_3$COO$^-$), bis-(trifluoromethylsulfonyl)amide (NTf$_2$), and dicyanamide (DCA). These classes of ILs are well known as tunable molecules with unique physicochemical properties including low flammability, extremely low vapor pressure at room temperature, high ionic conductivity, and high thermal and chemical stabilities. By modifying the cations and anions with special functional groups, all these properties may be adjustable with fascinating applications such as antiviral, antibacterial, antifungal, anti-inflammatory, and anticancer activities.

In addition, several studies have been devoted to the use of ILs as antitumor agents against several human cancer cells such as breast, brain, colon, lung, liver, osteosarcomas, leukemia, and prostate. In view of encouraging observations and as a continuation of our interest in the development of novel functionalized bioactive ionic liquids, we have anticipated the synthesis of novel imidazolium-ionic liquids carrying fluorinated phenylacetamide. The synthesized ILs were tested with DNA binding and screened for their anticancer activities. Additionally, simulation studies were also investigated to determine the anticancer mechanism.

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2. RESULTS AND DISCUSSION

2.1. Chemistry. A more holistic approach to design the desired imidazolium ionic liquids 4a–f and 5a–r is described in Scheme 1 and comprises quaternization and metathesis reactions.

Thus, the quaternization of sp² nitrogen atom of the substituted imidazoles 1 and/or 2 was carried out through their thermal alkylation by some aromatic acetamide chlorides 3a–c for 2 h to afford the halogenated IL-based imidazolium–amide hybrids 4a–f in 83–90% yields (Table 1).

It should be noted that the fluorinated phenyl acetamide precursors 3a–c have been synthesized via base-assisted nucleophilic acylation of the appropriate fluorinated anilines with chloroacetyl chloride using triethylamine as a basic catalyst and dichloromethane as a solvent.

The structures of the resulted imidazolium iodides 4a–f were elucidated based on their spectroscopic data. Their ¹H NMR spectra showed clearly the appearance of two distinct singlets at 4.64–5.29 and 10.39–10.77 ppm assigned to the NC₃H₂ and NH protons, which confirmed the success of the quaternization reaction. All the remaining protons were recorded in their respective area (see Experimental Section). In addition, the ¹³C NMR spectra were in agreement with the designed structures. They exhibited new signals at 163.75–164.99 and 50.54–51.56 ppm belonging to the acetamide carbonyl (NHCO) and methylene (NCH₂) carbons, respectively.

Table 1. Physical and Analytical Data for the Imidazolium IL Halides 4a–f

| Comp. No | R   | Ar            | mp (°C) | Yield (%) |
|----------|------|---------------|---------|-----------|
| 4a       | H    | –F            | 90-91   | 86        |
|          |      | Colorless crystals |         |           |
| 4b       | CH₃  | –F            | 87-88   | 88        |
|          |      | Colorless crystals |         |           |
| 4c       | H    | –F            | 104-105 | 83        |
|          |      | Colorless crystals |         |           |

| Comp. No | R   | Ar            | mp (°C) | Yield (%) |
|----------|------|---------------|---------|-----------|
| 4d       | CH₃  | –F            | 97-98   | 87        |
|          |      | Colorless needles |       |           |
| 4e       | H    | –F            | 80-81   | 90        |
|          |      | Colorless needles |       |           |
| 4f       | CH₃  | –F            | 74-75   | 85        |
|          |      | Colorless crystals |       |           |

Scheme 1. Synthesis of Ionic Liquids Bearing Imidazole Ring and Fluorinated Phenylacetamide Linkages 4a–f and 5a–r

![Scheme 1](https://dx.doi.org/10.1021/acsomega.9b03468)
The resulting imidazolium iodides 4a−f underwent a metathetical anion exchange via their treatment with appropriate fluorinated metal salts in acetonitrile furnishing on the elaboration of the desired task-specific imidazolium ionic liquids incorporating specific fluorinated anions (BF$_4^-$, PF$_6^-$, and CF$_3$COO$^-$) as counteranions 5a−r (Scheme 1).

The structures of the obtained ILs 5a−r were deduced from their spectroscopic data. It is noticeable that no changes were recorded on the signals that appeared in their $^1$H and $^{13}$C NMR spectra. This confirmed that the exchange occurred only on the counteranion. Thus, the presence of PF$_6^-$ anion was evidenced by the $^{31}$P and $^{19}$F NMR spectra. The presence of a characteristic septet between $-157.43$ and $-131.04$ ppm in $^{31}$P spectra, as well as a diagnostic doublet ranging from $-69.18$ to $-69.16$ ppm in their $^{19}$F NMR spectra, supported the success of the PF$_6^-$ anion exchange (Table 2).

On the other hand, the incorporation of tetrafluoroborate (BF$_4^-$) anions on the specific imidazolium ionic liquids was evidenced based on their $^{31}$B and $^{19}$F NMR spectral data. Their $^{31}$B NMR data showed a diagnostic multiplet near $-1.32$ to $-1.29$ ppm, but their $^{19}$F NMR spectra exhibited two characteristic doublets around $-148.23$ to $-148.14$ ppm. However, the structures of the imidazolium ionic liquids, bearing the trifluoroacetate (CF$_3$COO$^-$) as a counter anion, were deduced based on their $^{19}$F NMR spectra and showed a distinct singlet around $-73.67$ to $-73.52$ ppm attributed to such an anion. Additionally, all the spectra showed variable multiplets ranging from $-141.60$ to $-118.31$ ppm belonging to the aromatic fluorine atoms of the substituted phenyl rings (see Experimental Section).

### Table 2. Physical and Analytical Data of the Imidazolium ILs Carrying Fluorinated Counteranions 5a−r

| Comp. No | R   | Ar         | Y        | mp (°C) | Yield (%) |
|----------|-----|------------|----------|---------|-----------|
| 5a       | H   | F          | PF$_6^-$ | 75-76   | 87        |
| 5b       | H   | F          | BF$_4^-$ | Syrup   | 89        |
| 5c       | H   | F          | CF$_3$COO | 66-67   | 90        |
| 5d       | CH$_3$ | F         | PF$_6^-$ | 69-70   | 91        |
| 5e       | CH$_3$ | F         | BF$_4^-$ | Syrup   | 92        |
| 5f       | CH$_3$ | F         | CF$_3$COO | Syrup   | 90        |
| 5g       | H   | F          | PF$_6^-$ | 95-96   | 84        |
| 5h       | H   | F          | BF$_4^-$ | Syrup   | 87        |
| 5i       | H   | F          | CF$_3$COO | 87-88   | 85        |
| 5j       | CH$_3$ | F         | PF$_6^-$ | 86-87   | 88        |
| 5k       | CH$_3$ | F         | BF$_4^-$ | Syrup   | 85        |
| 5l       | CH$_3$ | F         | CF$_3$COO | Syrup   | 83        |
| 5m       | H   | F          | PF$_6^-$ | Syrup   | 89        |
| 5n       | H   | F          | BF$_4^-$ | Syrup   | 91        |
| 5o       | H   | F          | CF$_3$COO | Syrup   | 87        |
| 5p       | CH$_3$ | F         | PF$_6^-$ | Syrup   | 90        |
| 5q       | CH$_3$ | F         | BF$_4^-$ | Syrup   | 92        |
| 5r       | CH$_3$ | F         | CF$_3$COO | Syrup   | 87        |

2.2. DNA Binding Study. DNA is among the most significant pharmaceutical targets of anticancer medications.28−30 Thus, studying the interactions of target compounds with DNA is important to get an indication about their anticancer impacts and imaginable mechanisms of action. In general, a compound and DNA formed covalent and noncovalent bonds. Through covalent bonding, a labile compound is switched by a nitrogen atom of the DNA base,
such as \( N^7 \) of guanine, in which the interactions like electrostatic, intercalation, and groove binding are conceivable in non-covalent binding.\(^{31}\) Meanwhile, The change in the wavelength or absorption or both is characteristic of the interactions and intercalative modes including strong stacking interactions between DNA base pairs and aromatic chromophores.\(^{32}\) It is supposed that bathochromism means breakage of the secondary structure of DNA and hypochromism involves covalent binding. Moreover, considerable red shifts revealed that the compounds were coordinated to DNA via \( N^7 \) position of guanine.\(^{33}\) On the other hand, no or slight shifts in UV spectra are indicative of an outside groove binding, generally with minor hyperchromicity.

In this study, the absorption bands of the tested ionic liquids 4a–f and 5a–r are recorded in the absence and presence of DNA. The spectra for 4a are shown in Figure 1 as the representative one. The UV–vis spectral data for ILs 4a–f and 5a–r are summarized in Table 3. The DNA spectra for the remaining compounds are provided in the Supporting Information. Obviously, the absorption spectra of the compounds revealed the presence of absorption bands around 200 nm. The trivial shifts of the bands were assigned to 240–260 nm by adding DNA owing to the intraligand \( \pi \rightarrow \pi^* \) transitions.\(^{34,35}\) These minor shifts of the bands pointed to bathochromic shifts of all the compounds, which confirmed their interactions with DNA. Additionally, hypochromaticities were recorded for all the compounds with the addition of DNA in different concentrations (1.45 \( \times \) 10\(^{-4} \) to 1.0 \( \times \) 10\(^{-7} \) M). Such changes are obvious evidence of the formation of DNA adducts.\(^{36}\) It is believed that these hypochromic shifts are probably due to the covalent and noncovalent bonds, which were observed in all target compounds.\(^{37}\)

Furthermore, the values of DNA binding constants for all ILs range from 4.543 \( \times \) 10\(^{4} \) to 3.518 \( \times \) 10\(^{5} \), signifying a good interaction with DNA. The regression analysis was investigated by origin software for DNA binding readings. The resulted correlation coefficient \( (R^2) \) for each compound is also given in the respective figure and showed that almost all compounds interpolated through the minor groove with Ct-DNA. These results are bolstered by the accessible literature.\(^{38}\) The literature referred that the compounds forming adducts with DNA through minor grooves and hydrophobic interactions as well as hydrogen bonds make major contribution adducts.\(^{39,40}\) All these facts are well supported by the simulation studies.

### Table 3. UV–Vis Data for Compounds 4a–f and 5a–r

| comp. no. | \( A_f \) | \( A_b \) | \( \Delta \lambda_{max} \) (nm) | % Hypochromism | \( K_b \) (M\(^{-1} \)) |
|----------|---------|---------|-----------------|----------------|-----------------|
| 4a       | 1.311   | 1.123   | 0.188           | 14             | 1.825 \( \times \) 10\(^3 \) |
| 4b       | 2.608   | 2.154   | 0.086           | 4              | 2.124 \( \times \) 10\(^3 \) |
| 4c       | 1.594   | 1.422   | 0.172           | 10.7           | 2.547 \( \times \) 10\(^3 \) |
| 4d       | 1.384   | 1.204   | 0.18            | 13             | 2.568 \( \times \) 10\(^3 \) |
| 4e       | 0.553   | 0.503   | 0.05            | 9              | 1.524 \( \times \) 10\(^3 \) |
| 4f       | 0.029   | 0.025   | 0.004           | 13.7           | 2.356 \( \times \) 10\(^3 \) |
| 5a       | 1.359   | 1.145   | 0.214           | 15.7           | 2.739 \( \times \) 10\(^3 \) |
| 5b       | 0.13    | 0.113   | 0.017           | 13             | 1.832 \( \times \) 10\(^3 \) |
| 5c       | 0.120   | 0.132   | 0.012           | 1              | 1.506 \( \times \) 10\(^3 \) |
| 5d       | 0.218   | 0.226   | 0.008           | 3.6            | 1.444 \( \times \) 10\(^3 \) |
| 5e       | 0.028   | 0.029   | 0.001           | 3.5            | 5.566 \( \times \) 10\(^4 \) |
| 5f       | 2.350   | 2.261   | 0.089           | 3.7            | 1.693 \( \times \) 10\(^3 \) |
| 5g       | 1.520   | 1.424   | 0.096           | 6              | 2.5 \( \times \) 10\(^3 \) |
| 5h       | 1.745   | 1.549   | 0.196           | 11             | 1.928 \( \times \) 10\(^3 \) |
| 5i       | 0.987   | 0.882   | 0.105           | 10             | 2.716 \( \times \) 10\(^3 \) |
| 5j       | 0.023   | 0.026   | 0.003           | 13             | 2.40 \( \times \) 10\(^3 \) |
| 5k       | 0.997   | 0.945   | 0.052           | 5              | 1.829 \( \times \) 10\(^3 \) |
| 5l       | 1.265   | 1.153   | 0.112           | 8.8            | 1.935 \( \times \) 10\(^3 \) |
| 5m       | 0.449   | 0.469   | 0.02            | 4              | 1.802 \( \times \) 10\(^3 \) |
| 5n       | 0.591   | 0.523   | 0.068           | 11.5           | 4.543 \( \times \) 10\(^4 \) |
| 5o       | 1.126   | 1.074   | 0.052           | 4.6            | 3.518 \( \times \) 10\(^3 \) |
| 5p       | 0.145   | 0.150   | 0.005           | 3              | 2.303 \( \times \) 10\(^3 \) |
| 5q       | 0.141   | 0.151   | 0.01            | 7              | 3.109 \( \times \) 10\(^3 \) |
| 5r       | 1.549   | 1.305   | 0.244           | 15.7           | 3.032 \( \times \) 10\(^3 \) |

\(^a\) % Hypochromism (H%) = \([\text{change in } \lambda_{max}/A_f] \times 100\), where \( A_f \) and \( A_b \) are the absorbances of free and bound compounds, respectively, \( K_b \) = binding constants, \( \lambda_{max} \) = \( \lambda_{max} \) (free), \( \lambda_{f} \) = \( \lambda_{max} \) (bound to DNA).

### 2.3. Anticancer Study

In vitro percentage inhibitions of lung cancer cell line H-1229 were used to assess the anticancer profiles of the tested ILs at 5.0, 10.0, 15.0, and 20.0 mM concentrations. The results are represented in Figure 2.

Initially, the tested molecules 4a–f, 5a–5f, 5g–5l, and/or 5m–5r were dissolved in DMSO (0.1%), and natural cell by DMSO was used as vehicle control. The resulting mixtures were accumulated until the control cells touched an inactive phase. After 24 h, the cells were counted. The proliferation inhibitions were clearly enhanced with the increasing amount of compounds. The results exhibited a noteworthy inhibition in cancer cell proliferation. The maximum percentage of inhibitions were in the range of 48–59%. The inhibition ranges

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**Figure 1.** DNA binding study of compound 4a.
of 4a–f, 5a–5l, and 5m–5r compounds were 50–59, 48–56, 50–55, and 52–56%, respectively. The percentage inhibitions were almost the same in all four series. The variations in the percentage inhibition were observed with the dissociation tendencies of the reported compounds. It was observed that highly dissociated molecules exhibited higher anticancer activities. This is probably because highly dissociated molecules provided a high concentration of positive ionic

Table 4. Simulation Studies Results of the Reported Compounds with DNA

| comp.no. | no. of H bonds | bond length (Å) | affinity in (kcal/mol) | hydrophobic interaction |
|----------|----------------|----------------|------------------------|------------------------|
| 4a       | 1              | .263/A/DG10/O6 & ‘H’ of −CONH: gp. (2.2) | −4.4 | C1::dc15&dc9, C2::dc9, C3::dt8&dc9, C4::dc9, C5::O6, C8::dc15&dg14, C10::dg14&dc15, C12::O, N1::O6 |
| 4b       | 2              | .263/A/DG10/O6 & ‘H’ of −CONH: gp. (2.4) | −4.3 | C2::dg14&O6, C4::dg14, C5::O6, C7::dc15, C8::dc15, C9::dc15, C10::dg14&dc15, C13::O6, N2::dc15, O::dc9 |
| 4c       | 3              | .263/A/DG10/O6 & ‘H’ of −CONH: gp. (2.3) | −4.4 | C2::dt8&dc9, C3::dc9, C5::O6, C6::dg14, C7::dg14, C8::dc15, C10::dc15, C12::dc15&O6, N2::dc15 |
| 4d       | 1              | .263/A/DG10/O6 & ‘H’ of −CONH: gp. (2.5) | −3.9 | C3::dc9, C4::dt8&dc9, C6::O6, C7::dg14, C8::dg14, C11::dc9&O6, C13::O6, N3::dg10&O6 |
| 4e       | 1              | .263/A/DG10/O6 & ‘H’ of −CONH: gp. (2.1) | −4.4 | C1::dc9&dc15, C3::O6, C6::dg14&dc15, C8::dg14&dc15, C9::dc9&dt8, C10::dt8, C12::O6 |
| 4f       | 2              | .263/A/DG10/O6 & ‘H’ of −CONH: gp. (2.6) | −4.3 | C1::dg14&O6, C4::O6, C5::dc15, C6::dc15, C7::dc15, C8::dc15&dg14, C10::dg14, C13::dc9&O6, N2::dc15 |
liquid parts to the DNA, leading to the higher binding with DNA and hence more activity. In the same manner, it was observed that bulky molecules showed low anticancer activities due to the steric effect. Thus, large-size molecules might have restricted their interactions with DNA. Overall, the total percentage inhibitions of 48–59% are quite good, and the reported molecules may be considered as potential anticancer agents for lung cancer.

2.4. Docking Study. It is important to mention here that out of 24 compounds, the basic organic moieties are 6 in 4a–f. It is very clear that DNA docking study is carried out with organic moieties. Therefore, the docking study was carried out with six moieties of 4a–f molecules. The obtained binding energies of the reported imidazolium ILs with DNA are summarized in Table 4 and were in the range from −3.9 to −4.4 kcal/mol. It is noticeable that the binding energies were variable due to the structure variation of the tested imidazolium compounds. Also, these structural differences also caused variation in hydrogen bond formation with DNA. Hydrogen bondings were found responsible for six moieties with DNA binding, one hydrogen bond was formed between DNA and compounds 4a, 4d, and 4e, while two hydrogen bonds were formed between DNA and compound 4b. However, three hydrogen bonds were formed between DNA and compound 4c. The hydrophobic interactions were also responsible for DNA interaction with the reported compounds. All the hydrophobic interactions with DNA are shown in Table 4. It was found that the DNA fragments involved with imidazolium were dc9, dc13, dc15, dg10, dg14, dc15, and dt8. The representative model of DNA binding with compound 4a is shown in Figure 3, while the rest are given in the Supporting Information.

Figure 3. Docking model of compound 4a with DNA.

4. EXPERIMENTAL SECTION

4.1. General Methods. Melting points were measured with a Stuart Scientific SMP1 apparatus and were uncorrected. All synthesized compounds were fully characterized by 1H, 13C, 19F, 31P, and 11B NMR spectroscopy and HRMS. TLC was performed on aluminum plates silica gel (Kieselgel, 0.25 mm, 60 F254, Merck, Germany) and spots were visualized by ultraviolet (UV) light absorption using ethyl acetate/hexane as a developing solvent system. The NMR spectra were recorded with a 400 MHz Bruker NMR spectrometer. Chemical shifts (δ) were expressed in ppm using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectroscopy (HRMS) was carried out using an LC-MS/MS impact II.

4.2. Synthesis and Characterization of 2-Chloro-N-(substituted phenyl)acetamides 3a–c. Chloroacetyl chloride (12 mmol) was added dropwise under stirring to a solution of appropriate fluorinated aniline 2a–c (10 mmol) and triethylamine (12 mmol) in dichloromethane (40 mL) for 2 h at 0 °C. Then, the stirring was continued at room temperature for 6 h. The obtained precipitate was filtered, washed with water, and recrystallized from ethanol to afford the desired phenylacetamides 3a–c.

4.2.1. 2-Chloro-N(4-fluorophenyl)acetamide (3a). Colorless crystals, mp: 128–129 ºC (lit. mp: 130 ºC).12 1H NMR (400 MHz, DMSO-d6): δH = 4.34 (s, 2H, NCH2), 7.55–7.60 (m, 2H, Ar-H), 7.79 (d, J = 8.0 Hz, 2H, Ar-H), 10.38 (s, 1H, CONH). 13C NMR (100 MHz, DMSO-d6): δC = 43.82 (NCH2), 116.25, 121.97, 124.51, 135.68, 147.26, 158.70 (Ar-C), 164.87 (C=O). 19F NMR (377 MHz, DMSO-d6): δF = −118.57 to −118.59 (m, 1F, Ar-F).

4.2.2. 2-Chloro-N(2-fluorophenyl)acetamide (3b). Colorless crystals, mp: 119–121 ºC (lit. mp: 120–122 ºC).13 1H NMR (400 MHz, DMSO-d6): δH = 4.41 (s, 2H, NCH2), 7.21–7.30 (m, 3H, Ar-H), 7.82–7.90 (m, 1H, Ar-H), 10.24 (s, 1H, CONH). 13C NMR (100 MHz, DMSO-d6): δC = 43.5 (NCH2), 116.36, 123.24, 125.23, 135.67, 146.32, 155.35 (Ar-C), 165.16 (C=O). 19F NMR (377 MHz, DMSO-d6): δF = −124.74 to −124.67 (m, 1F, Ar-F).

4.2.3. 2-Chloro-N(2,4,5-trifluorophenyl)acetamide (3c). Colorless crystals, mp: 116–117 ºC (lit. mp: 118–119 ºC).14 1H NMR (400 MHz, DMSO-d6): δH = 4.34 (s, 2H, NCH2), 7.65–7.78 (m, 1H, Ar-H), 8.21–9.11 (m, 1H, Ar-H), 10.30 (s, 1H, CONH). 13C NMR (100 MHz, DMSO-d6): δC = 43.05 (NCH2), 106.86, 123.32, 135.76, 146.21, 156.69 (Ar-C), 165.84 (C=O). 19F NMR (377 MHz, DMSO-d6): δF = −164.77 to −164.62 (m, 1F, Ar-F), −139.91 to −139.74 (m, 1F, Ar-F), −125.60 to −125.51 (m, 1F, Ar-F).
4.3. General Quaternization Procedure for the Synthesis of Imidazolium IL Halides 4a–f. 2-Chloro-N-(4-fluorophenyl)acetamide, 2-chloro-N-(4-fluorophenyl)acetamide, and/or 2-chloro-N-(2,4,5-trifluorophenyl)acetamide 3a–c (10 mmol) were added with stirring to a solution of 1-methyl and/or 1,2-dimethyl imidazole 1a,b (10 mmol) in acetonitrile (30 mL). Then, the mixture was refluxed for 2 h, until the completion of the reaction, as indicated by TLC (silicagel, hexane-ethyl acetate). The excess of the solvent was reduced under reduced pressure. The obtained product was filtered and/or extracted by chloroform to yield the desired ionic liquids 4a–f.

NB: Sodium iodide (10 mmol) was added during the synthesis of compounds 4a, 4b, 4e, and 4f.

The characterization of the imidazolium ILs 4a–f is reported in the Supporting Information.

4.4. General Metathesis Procedure for the Synthesis of Imidazolium ILs Carrying Fluorinated Counteranions 5a–r. To a mixture of imidazolium IL halides 4a–f (1 mmol) in acetonitrile (20 mL) was added suitable metal salts: potassium hexafluorophosphate, sodium tetrafluoroborate, and/or sodium trifluoroacetate (1.1 mmol) at room temperature under stirring. The reaction mixture was heated under reflux for 24 h. Then, the reaction mixture was cooled and filtered to eliminate solid metal halide. The acetonitrile was evaporated to afford quantitatively the desired imidazolium ionic liquids 5a–r.

The characterization of the imidazolium ILs 5a–r is reported in the Supporting Information.

4.5. DNA Binding Studies. The DNA binding experiments were carried out in Tris buffer (10^{-2} M, pH 7.2). Initially, the concentration of DNA was adjusted by recording the absorption spectrum of the CT-DNA solution. This solution showed UV absorbance at 230/260 nm (=1.8), indicating the free nature of DNA, with \( \varepsilon \) value equal to 6600 M^{-1} cm^{-1}. The different solutions of the tested compounds and DNA were prepared and kept at 4 °C. First, the compounds were dissolved in DMSO (0.1%) and then diluted with Tris buffer till their concentrations became 2.4 \times 10^{-4} M. Then, DNA was added in various concentrations (1.2–1.5 \times 10^{-5} M) to record the respective absorption. Eventually, the binding constants (\( K_b \)) were calculated according to the Benesi–Hilderbrand eq 1.55

\[
\frac{[DNA]}{(\varepsilon_a - \varepsilon_t)} \, \text{(1)}
\]

where absorption coefficients \( \varepsilon_{a} \), \( \varepsilon_{t} \), and \( \varepsilon_{b} \) are related to \( A_{abs} / [\text{compound}] \), extinction coefficient for the compounds in free as well in fully bound form. The binding constants (\( K_b \)) for different compounds were determined from slopes and the intercepts of the plots of

\[
\frac{[DNA]}{(\varepsilon_a - \varepsilon_t)} \, \text{vs} \, [DNA] \}
\]

4.6. Anticancer Studies. The antiproliferative activity of the target imidazolium ILs was performed against lung cancer cell line H-1229 using the MTT assay. Thus, the cells were seeded (density of 8000 cells/well) in a 96-well plate and incubated. Around 60–70% confluency, the treatment of the cells with different concentrations of the reported compounds (5.0, 10.0, 15, and 20.0 mM) was carried out, followed by incubation for 24 h. The cells were assayed by adding 15.0 µL of 5.0 mg/mL MTT. Then, the respective media from each well were aspirated at 37 °C of incubation for 4 h. In 100 µL of DMSO, the cells were resuspended and the plate was instantly covered with aluminum foil and shook gently for 15 min. Absorbance was measured at 540 nm and the percent inhibition of cellular proliferation was calculated using eq 2.

\[
\% \text{ inhibition} = \left( \frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad (2)
\]

4.7. Docking Study. The docking readings were conducted using Windows XP operating system on Intel dual CPU (1.86 GHz). Marvin sketch was used in the drawing of the ligand 3D structure, which was transformed to the PDB file format. Ligand preparation was achieved by using AutoDock Tool (ADT) 4.0 version to allocate Gastegier charges, nonpolar hydrogens were merged, and then saved in the PDBQT file format. X-ray DNA crystal structure (PDB ID: 1bna) was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb). The receptors were saved in the PDB file format using AutoDock Tools (ADT) 4.0, leaving heteroatoms (water). Besides, Gastegier charges were allocated to the receptor and then saved employing previous tools in the PDBQT file format. Preparation of parameter files for grid and docking was performed by means of ADT. Docking was carried out with AutoDock 4.0 (Scripps Research Institute) considering conformationally all the rotatable bonds of the ligand as rotatable and receptor as rigid.53 Grid box size of 60 × 80 × 114 Å³ with 0.375 Å spacing was used that encompassed the whole DNA. Macromolecule docking was done using an empirical-free energy function and Lamarckian genetic algorithm (LGA), with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy assessments, a crossover rate of 0.80, and a mutation rate of 0.02. For each ligand and receptor–ligand adduct, at least 50 independent docking runs were performed for the lowest free energy of binding confirmation from the largest cluster and then saved in the PDBQT format.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03468.

Characterization of all newly synthesized compounds as well their respected NMR and mass spectra; DNA binding results and docking model of all newly synthesized compounds (PDF)

AUTHOR INFORMATION

Corresponding Authors

Nadjet Rezki — Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia; Department of Chemistry, Faculty of Sciences, University of Sciences and Technology Mohamed Boudiaf, Laboratoire de Chimie and Electrochimie des Complexes Metalliques (LCECM) USTO-MB, Oran 31000, Algeria; Phone: +966537268682; Email: nadjetrezki@yahoo.fr

Mohamed Reda Aouad — Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia; Department of Chemistry, Faculty of Sciences, University of Sciences and Technology Mohamed Boudiaf, Laboratoire de Chimie and Electrochimie des Complexes Metalliques (LCECM) USTO-MB, Oran 31000, Algeria; orcid.org/0000-0002-6876-4096; Phone: +966540953537; Email: mr_aouad@yahoo.fr
Authors
Fawzia Falah Al-Blewii – Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia
Salsabeel A. Al-Sodies – Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia
Asaad Khalid Alnuzha – Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia
Mouslim Messali – Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia
Imran Ali – Department of Chemistry, Faculty of Science, Taibah University, Al-Madinah Al-Munawarah 30002, Saudi Arabia; Department of Chemistry, Jamia Millia Islamia (A Central University), New Delhi 110025, India

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03468

Author Contributions
All authors discussed the results and commented on the manuscript.

Notes
The authors declare no competing financial interest.

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