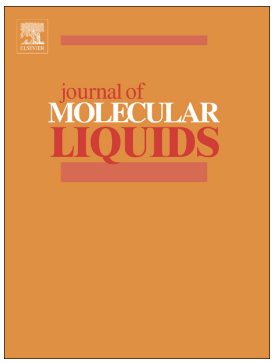

Design, synthesis, in-silico and in-vitro evaluation of di-cationic pyridinium ionic liquids as potential anticancer scaffolds

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Design, Synthesis, *In-silico* and *In-vitro* Evaluation of Di-Cationic Pyridinium Ionic Liquids as Potential Anticancer Scaffolds

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ABSTRACT

An array of dicationic pyridinium ionic liquids (DILs) based hydrazone linkage were designed and synthesized via the quaternization of the appropriate bispyridine hydrazone with different phenacyl halides and led to the formation of halogenated DILs, which undergo metathesis reaction to give the specific task dicationic pyridinium liquids carrying fluorinated counter anions (PF_6^-, BF_4^-, CF_3COO^-). The newly synthesized DILs were well characterized using whole spectroscopic data. The Anticancer evaluation of DILs against breast and colon cancer cell lines revealed that compound **22** appears to be the most active compound in the series with IC_{50} in the two-digit micromolar range. The in-vitro anticancer results were further supported by in-silico molecular docking studies revealing the highest potency of compound **22**. The docking analysis demonstrated good docking score and binding affinities of the synthesized compounds on the target protein PI3Kinase.

Keywords: Ionic liquids synthesis, quaternization, metathesis, dicationic ionic liquids, hydrazones, anticancer activity, molecular docking

1. Introduction

Cancer is one of the most compelling public health concerns worldwide. Based on the most recent statistics released by the World Health Organization (WHO) in 2015, it was estimated that about 8.8 million people passed away from cancer in that year [1]. Chemotherapy remains to be considered as the standard regime for cancer treatment [2, 3]. Despite of the improvements that have been consistently achieved in the field of chemotherapy over the last two decades, the search for new anticancer agents with significantly superior efficacy/safety ratio continues.

Indeed, the major focus of drug discovery has been directed nowadays to develop novel anticancer agents that minimize the toxicity associated with the commonly used chemotherapy.

Yet, one major concern for drug discovery is the ability to turn new chemical moieties into drugs. Many small molecules with very promising features are currently deemed as technically undruggable or at the very least as tremendously difficult to optimize by medicinal chemists [4]. Another infuriating problem is that for medicines which have been successful and have shown significant therapeutic effectiveness, resistance often emerges, as demonstrated lately by crizotinib and vemurafenib [5, 6].

During the last two decades, Ionic liquids (ILs) have emerged as new class of compounds with characteristic properties and the capability to modify their physiochemical properties [7-9]. In fact, ILs become omnipresent in the recent medicinal chemistry [10]. Several studies have reported the exploration of IL as anticancer agents [11-16]. Interestingly, ILs with their adjustable physicochemical properties brought a new prospect of creating new therapeutic agents with tailored anticancer activity and diminished toxicity toward the human normal cells.

An extensive effort has been engaged to produce novel ionic liquids with tuning properties. Recently, a type of ILs namely dicationic ionic liquids (DILs), in which the cation candidates joining together *via* either a rigid or a flexible spacer has been reported [17, 18]. They were often found to have higher melting points than monocationic ILs with the same anion and sometimes are solids at room temperature [17]. The physicochemical properties such as thermal stability, density, viscosity, and solubility behaviours can be tuned to a greater extent in DILs than the monocationic analogues by changing the cation, anion and linker [19]. Therefore, they have high potential to be used in numerous applications SUCH AS DRUG DELIVERY SYSTEMS FOR ADVANCED PHARMACEUTICAL AND TECHNOLOGICAL APPLICATIONS [20-27].

Additionally, hydrazones have attracted increasing interest in organic synthesis [28-30]. Considerable attention have been devoted on the designing and the synthesis of such compounds as inexhaustible resource of new molecules with diverse chemical and biological activities [31-33].

On-to-date demand of pyridine ILs is obvious judging from their endless synthetic value for construction of pharmacological scaffolds. [34-38] Based on these observation and in our endeavour to increase the synergetic effect of some hydrazones based pyridine rings as continuation of our interest in design and synthesis of novel functionalized ionic liquids tethered hydrazone linkage

and their biological potency [39, 40], herein we report an efficient synthetic transformation for designing a new class of dicationic liquids derived from bispyridine hydrazone, which often generated new anticancer scaffold for further optimization.

2. Results and discussions

2.1.Chemistry

The synthetic methods carried out in this work were outlined in scheme 1. The two precursors bispyridine hydrazone **2** and **3** were easily synthesized *via* the thermal condensation of isonicotinohydrazide **1** with nicotinaldehyde and/or isonicotinaldehyde in boiling ethanol in the presence of few drops of hydrochloric acid (Scheme 1) [40].

The strategy adopted for the construction of diionic liquid tethering two pyridinium cations linked *via* a hydrazone spacer involved quaternization and metathesis reaction. Thus, the nucleophilic alkylation of hydrazones **2** and/or **3** with substituted phenacyl bromides in refluxing acetonitrile gave successfully the DILs **4-9** in good yields (88-94 %).

PLEASE INSERT SCHEME 1

The NMR data of the synthesized derivative **7** was taken as model to discuss and confirm the quaternization reaction. Its ^{1}H NMR spectrum showed the two singlets at δ_H 3.91 ppm integrated for six protons of the two methoxy groups (-OCH_3) (Figure 1). The spectrum also showed the presence of the methylene protons (2xNC**H**$_2$) as doublet at δ_H 6.63 ppm. The imine proton (**H**C=N) was observed as two singlets at δ_H 8.81 and 8.86 ppm and integrated as one proton, with ration of 1:3. Same ratio was observed for the N**H** proton splited into two singlets at δ_H 13.11 and 13.28 ppm. Moreover, the pyridinium protons were recorded with the same isomeric ratio to that displayed by N**H** and **H**C=N groups. Such pairing of signals was previously confirmed in our reported work to be due to the *E/cis* and *E/trans* geometrical isomerism about the carbonylamide group (CONH) and the imine bond (**H**C=N) [42, 43]. The extra eight aromatic protons recorded at their usual chemical shifts belonging the two aromatic rings of the phenacyl moiety (See experimental section).

PLEASE INSERT FIGURE 1

The structure of DILs **7** was also supported by its ^{13}C NMR spectrum (Figure 2), which revealed the presence of the methoxy carbons at δ_C 56.34 ppm. The spectrum also showed the appearance of two sets of signals for each peak, supporting the geometrical isomerism.

In addition, the methylene carbons appeared as two signals at δ_C 66.58 and 66.70 ppm, respectively. The presence of *E/cis* and *E/trans* diastereomers of the C=N and C=O groups of the hydrazone linkage were also recorded as two signals at δ_C 160.04 and 164.78 ppm, respectively.

The presence of molecular ion peak at 684.4041 [M^+] in the electron impact mass spectrum of compound **7**, confirmed the quaternization reaction of hydrazone **3** with methoxy phenacyl bromide.

PLEASE INSERT FIGURE 2

The specific task DiILs **10-18** were obtained in satisfactory yields (85-92 %), according to the metathesis protocol. In this step, the bromide anion of the synthesized DILs **4-9** were subjected to anion exchange with an appropriate anions (PF_6^-, BF_4^-, CF_3COO^-), through their thermal treatment with metal salts in acetonitrile resulted in the formation of specific task dicationic pyridinium ionic liquids **10-29**. The introduction of such anion was confirmed by the whole NMR analysis, which revealed that no change in their ^1H and ^{13}C NMR data compared to their corresponding precursors **4-9**. Accordingly, their structures were established based on their ^{31}P, ^{11}B, ^{19}F NMR and mass spectra analyses.

The structure of compound **19** was supported by the ^{31}P and ^{19}F NMR spectra. Its ^{31}P NMR spectrum showed a characteristic multiplet between δ_P -157.39 to -131.04 ppm relative to the phosphorus atom in the PF_6^- anion (Figure 3).

PLEASE INSERT FIGURE 3

On the other hand, the doublet recorded in the ^{19}F NMR spectrum at -69.13 ppm confirmed the bromide (Br^-) anion exchange with hexafluorophosphate anion (PF_6^-) yielding the compound **19** (Figure 4).

PLEASE INSERT FIGURE 4

Moreover, the presence of tetrafluoroborate anion (BF_4^-) as counter anion in the compound **20** was confirmed by the resonance of a multiplet between δ_B (-1.31) to (-1.29) ppm in the ^{11}B spectrum (Figure 5) and two doublets at δ_F -148.18 and -148.12 ppm in its ^{19}F NMR spectrum (Figure 6), respectively.

PLEASE INSERT FIGURE 5

PLEASE INSERT FIGURE 6

PLEASE INSERT FIGURE 7

The singlet observed at δ_F -73.49 ppm in the ^{19}F NMR spectrum being related to the fluorine atom in trifluoroacetate anion form (CF_3COO^-) confirming the formation of DiIL **21** (Figure 7).

2.1. Biological Screening

2.2.1. Anticancer Activity

The *in vitro* anticancer activities of the synthesized compounds were examined against four human cancer cell lines, characterizing tumors of different origins. The human breast adenocarcinoma cell line MCF-7, the human ductal breast epithelial tumor cell line T47D, the human mammary gland/breast tumor cell line MDA231, and the human colorectal adenocarcinoma cell line Caco-2 were incubated in culture media with varying concentrations of the examined compound and the impact of treatment was measured using the MTT assay. The IC_{50} values obtained for the tumor cells under investigation are summarized in Table 1. The results indicate that out of the 26 synthesized derivatives, compounds **8, 22, 23, 24, 25** and **26** exhibited considerable cancer cell growth inhibition against all examined cancer types. Interestingly, compound **22** demonstrates the most potent activity amongst the examined series with IC_{50} value ranging between 59-64 µM on the different cell lines.

PLEASE INSERT TABLE 1

Phosphoinositide 3-kinases (PI3Ks) belongs to the family of lipid kinases and plays a major role in the promotion of cellular growth and survival of various types of cancer [44]. The PI3Ks are the vital regulators of apoptosis in cancer cells. The phosphorylation of phosphatidylinositol is catalyzed by PI3Ks at the 3′-OH position of the inositol ring, producing secondary messenger lipid *i.e* phosphatidylinositol-3,4,5-trisphosphate. The serine/threonine kinase AKT is activated by this trisphosphate, which regulates several signaling pathways in turn, thus, controlling survival of cell, cell proliferation, apoptosis, and motility [45]. PI3Ks are divided into three classes based on their structural features and substrate specificity [46]. The most studied class I PI3Ks are further grouped into 4 isoforms: p110α, p110β, p110δ, and p110γ [48]. PI3KCA gene encoding p110α is the most frequently mutated gene in human tumors and has emerged as an attractive target for tumor therapy [48]. Also, some hydrazone moieties have been reported to exhibit marked PI3kinase p110α inhibition [49].

Thus, in order to explain the anticancer properties of the synthesized ionic liquids on breast cancer (MCF-7, MDA 231 and T47D) and colon cancer (Caco-2) cell lines, *in-silico* molecular docking approach was undertaken to get more insights into the binding mode of the synthesized compounds with PI3K p110α domain (PDB ID: 2RD0). Remarkably, all investigated cell lines have been reported to express PI3Ks [50-53].

2.2.2. In-silico molecular docking studies

Considering the promising *in-vitro* anticancer activities, it was thought worthwhile to predict if the targets have analogous binding mode to the PI3K protein receptor, hence inculcating both *in-silico* and *in-vitro* screening results. In order to determine the best *in-silico* conformation, comparative and automated docking studies of newly synthesized dicationic pyridinium ionic liquids on PI3K as the target protein was performed. To satisfy this purpose, AutoDock 4.0, the Lamarckian genetic algorithm docking program was employed. There are two main programs in AutoDock, one is auto grid which is used to precalculate grid maps of interaction energies for various atom types of ligand with a macromolecule and second one is autodock that performs the docking of the ligand to specified grids. We performed molecular docking on the identified active site residues i.e ILE800, LEU807, LEU814, TYR836, GLY837, CYS838, ILE848 in the p110α PI3K [54]. The above residues are almost 100% identical among the p110 isoforms and are highly conserved in the PI3K family [55]. All the synthesized compounds **2-27** were docked.

Figure. 8 shows the docked images of some selected ligands **13** and **22** including the hydrazones **2** and **3**. Table 2 shows the minimum binding energy and Inhibition constant of all the docked compounds. The docking study revealed that the ligands exhibited bonds with one or more amino acids in the receptor active pocket with good binding energy toward the target protein ranging from -11.88 to -6.37 kJ/mol and calculated inhibition constant ranges from 16.40 nM (nanomolar) to 205.52 uM (micromolar).

PLEASE INSERT FIGURE 8

PLEASE INSERT TABLE 2

3. Experimental

3.1.Chemical characterization and synthesis

Melting points were recorded on a Stuart Scientific SMP1 apparatus (Stuart, Red Hill, UK) and are uncorrected. The NMR spectra were measured with a Bruker spectrometer (600 and 400 Brucker, Fällanden, Switzerland) using Tetramethylsilane (TMS) (0.00 ppm) as an internal standard. High resolution mass spectroscopy (HRMS) was carried out using a LC–MS/MS impact II.

Synthesis of *N'*-(pyridinylmethylene)isonicotino hydrazide derivatives 2 and /or 3. To a mixture of isonicotinic acid hydrazide (**1**) (1 mmol) in ethanol (25 mL) was added 4-pyridinecarboxaldehyde and/or 3-pyridinecarboxaldehyde (1.2 mmol) and few drops of hydrochloric acid, then the mixture was refluxed for 1 h. After cooling, the excess of solvent was removed under reduced pressure. The product formed was recrystallized from ethanol to furnish the desired compounds.

***(E)-N'*-(Pyridin-4-ylmethylene)isonicotinohydrazide (2)**. It was obtained as yellow crystals in 92 % yield; mp: 223–224 °C (Lit. mp: 224–225 ºC) [41]. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 7.57 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 7.71 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 7.82 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 7.90 (dd, 1.75H, *J* = 4 Hz, 8 Hz,, Ar-**H**), 8.14 (s, 0.2H, **H**-C=N), 8.55 (s, 0.8H, **H**-C=N), 8.66 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.74 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.79 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.84 (dd, 1.75H, *J* = 4 Hz, 8 Hz,, Ar-**H**), 12.47 (s, 0.2H, CON**H**), 12.58 (s, 0.8H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 121.32, 121.62, 121.76, 123.28, 140.14, 142.58, 146.21,

149.21, 149.48, 150.38 (Ar-**C**), 162.08, 162.10 (**C**=N, **C**=O). HRMS (ESI) m/z = 226.1414 [M^+].

***(E)-N'*-(Pyridin-3-ylmethylene)isonicotinohydrazide (3)**. It was obtained as pale yellow crystals in 95 % yield; mp: 230–231 ºC (Lit. mp: 232–233 °C).[41] ^{1}H NMR (600 MHz, DMSO-d_6): δ_H = 7.43 (dd, 0.2 H, *J* = 6 Hz, Ar-**H**), 7.52 (dd, 0.8H, *J* = 6 Hz, 12 Hz, Ar-**H**), 7.68 (d, 0.2 H, *J* = 6 Hz, Ar-**H**), 7.84 (dd, 1.8 H, *J* = 6 Hz, 12 Hz, Ar-**H**), 7.89 (d, 0.2H, *J* = 6 Hz, Ar-**H**), 8.15 (s, 0.2 H, **H**-C=N), 8.18 (dd, 0.8 H, *J* = 6 Hz, 12 Hz, Ar-**H**), 8.53 (s, 0.8H, **H**-C=N), 8.57 (d, 0.2H, *J* = 6 Hz, Ar-**H**), 8.65 (dd, 0.8H, *J* = 6 Hz, 12 Hz, Ar-**H**), 8.72 (s, 0.2H, Ar-**H**), 8.75 (d, 0.2H, *J* = 6 Hz, Ar-**H**), 8.81 (dd, 1.8H, *J* = 6 Hz, 12 Hz,Ar-**H**), 8.89 (s, 0.8H, Ar-**H**), 12.20 (s, 0.2H, CON**H**), 12.25 (s, 0.8H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 122.01; 123.58; 124.43; 124.53; 130.29; 130.42; 133.86; 134.10; 140.74; 141.96; 142.43; 146.79; 149.38; 149.40; 150.83; 151.74 (Ar-**C**), 162.23; 168.46 (**C**=N, **C**=O). HRMS (ESI) m/z = 226.1418 [M^+].

General Procedures for the Synthesis of dipyridinium ionic liquids tethered hydrazones 4-9. A mixture of compound **2** and/or **3** (1 mmol) in acetonitrile (30 mL) and the appropriate phenacyl bromide (2.1 mmol) was refluxed for 6-12 h. The solvent was reduced by evaporation under reduce pressure, the precipitate formed was collected by filtration and/or extraction by chloroform to afford the desired dicationic liquids **4-9**.

***1-(2-(4-Methoxyphenyl)-2-oxoethyl)-4-((2-(1-(2-(4-methoxyphenyl-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium bromide* (4)**. It was obtained as syrup in 88 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.92 (s, 6H 2×OC**H**$_3$), 6.58 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.09 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.29 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.40 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.52 (s, 0.25H, **H**-C=N), 8.57 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 8.71 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.78 (s, 0.75H, **H**-C=N), 8.97 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.07 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 9.19 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.28 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 13.37 (s, 0.25H, CON**H**), 13.42 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 56.32 (2×O**C**H$_3$), 66.12; 66.76 (2×N**C**H$_2$), 141.92; 125.13; 126.75; 127.56; 131.25; 131.30; 145.04; 147.23; 147.76; 147.89; 149.83 (Ar-**C**), 160.54; 164.79 (**C**=N, **C**=O), 189.08; 189.46 (2×CH$_2$**C**=O). HRMS (ESI) m/z = 684.3530 [M^+].

***1-(2-(4-Chlorophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazone)methyl)pyridin-1-ium bromide* (5)**. It was obtained as syrup in 90 %

yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.67 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.78 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.13 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.33 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.43 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.54 (s, 0.25H, **H**-C=N), 8.59 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.75 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.83 (s, 0.75H, **H**-C=N), 9.01 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.23 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.31 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 13.39 (s, 0.25H, CON**H**), 13.48 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.41; 67.02 (2×N**C**H$_2$), 124.89; 125.20; 126.85; 127.60; 129.80; 129.84; 130.68; 130.72; 132.72; 132.81; 140.03; 140.11; 145.03; 146.92; 147.22; 147.36; 147.88; 149.91; 149.97 (Ar-**C**), 160.51; 164.79 (**C**=N, **C**=O), 189.08; 189.44 (2×CH$_2$**C**=O). HRMS (ESI) m/z = 693.2168 [M$^+$].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium bromide **(6)**. It was obtained as syrup in 90 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.67 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.33 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.49 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.55 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.58 (s, 0.25H, **H**-C=N), 8.63 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.70 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.73 (s, 0.75H, **H**-C=N), 9.03 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.18 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.25 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 12.90 (s, 0.25H, CON**H**), 12.97 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.60; 67.24 (2×N**C**H$_2$), 122.23; 124.68; 124.98; 126.73; 130.23; 131.20; 138.64; 138.74; 140.02; 143.57; 147.08; 147.82; 150.23; 150.96; 151.10 (Ar-**C**), 159.98; 162.99 (**C**=N, **C**=O), 190.30; 190.67 (2×CH$_2$**C**=O). HRMS (ESI) m/z = 714.3592 [M$^+$].

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-3-((2-(1-(2-(4-methoxyphenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium bromide **(7)**. It was obtained as yellow crystals (EtOH) in 89 % yield; mp: 86-88 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.91 (s, 6H 2×OC**H**$_3$), 6.63 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.09 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.32 (dd, 0.25H, *J* = 8 Hz, Ar-**H**), 8.41 (dd, 0.75H, *J* = 8 Hz, Ar-**H**), 8.57 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.74 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 8.81 (s, 0.2H, **H**-C=N), 8.86 (s, 0.8H, **H**-C=N), 9.07 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.12 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.20 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.31 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.32 (s, 0.25H, Ar-**H**), 9.56 (s, 0.75H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.28 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 56.34 (2×O**C**H3), 66.58; 66.70 (2×N**C**H$_2$), 114.94; 126.68; 126.71; 126.78; 127.56; 128.46; 131.30; 134.18; 144.02; 144.54;

145.33; 147.24; 147.83; 147.85 (Ar-**C**), 160.04, 164.78 (**C**=N, **C**=O), 189.10, 189.33 (2×CH_2**C**=O). HRMS (ESI) m/z = 684.4014 [M^+].

1-(2-(4-Chlorophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium bromide **(8)**. It was obtained as colorless crystals (EtOH) in 92 % yield; mp: 94-95 ºC. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.66 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.13 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.33 (dd, 0.25H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.41 (dd, 0.75H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.58 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.75 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 8.81 (s, 0.2H, **H**-C=N), 8.85 (s, 0.8H, **H**-C=N), 9.06 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.11 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.20 (d, 0.25H, *J* = 8 Hz, Ar-**H**), 9.30 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.32 (s, 0.2H, Ar-**H**), 9.56 (s, 0.8H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.27 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 66.89; 66.98 (2×N**C**H_2), 126.76; 127.65; 128.32; 128.54; 129.81; 129.83; 130.71; 132.73; 132.80; 134.23; 140.03; 140.11; 144.35; 144.58; 145.22; 147.19; 147.31; 147.83; 148.01 (Ar-**C**), 160.04; 164.78 (**C**=N, **C**=O), 190.08; 190.29 (2×CH_2**C**=O). HRMS (ESI) m/z = 693.2262 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium bromide **(9)**. It was obtained as colorless crystals in 94 % yield; mp: 98-99 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.67 (d, 4H, *J* = 4 Hz, 2×NC**H**$_2$), 8.34 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.42 (dd, 0.75H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.51 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.59 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.67 (s, 0.2H, **H**-C=N), 8.76 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 8.85 (s, 0.8H, **H**-C=N), 9.03 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.08 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.18 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.27 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.29 (s, 0.2H, Ar-**H**), 9.54 (s, 0.8H, Ar-**H**), 13.14 (s, 0.25H, CON**H**), 13.38 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 67.12; 67.25 (2×N**C**H_2), 124.70; 126.67; 126.82; 128.50; 128.58; 13.24; 130.27; 134.29; 138.56; 138.74; 144.69; 145.11; 147.15; 147.85; 148.16; 151.15 (Ar-**C**), 160.05; 160.09 (**C**=N, **C**=O), 190.28; 190.49 (2×CH_2**C**=O). HRMS (ESI) m/z = 714.4627 [M^+].

General procedure for the synthesis of task specific dipyridinium ILs 10-27. A mixture of ionic liquid **4-9** (1 mmol) and metal salts: potassium hexafluorophosphate, sodium tetrafluoroborate, and/or sodium trifluoroacetate (2.5 mmol) in acetonitrile (20 ml) was refluxed for 16 h. After cooling, the product was isolated by extraction or filtration to give the dicationic liquids **10-27**.

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-4-((2-(1-(2-(4-methoxyphenyl-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium hexafluorophosphate **(10)**. It was obtained as pale yellow crystals (EtOH) in 87 % yield; mp: 100-102 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.91 (s, 6H 2×OC**H₃**), 6.57 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.07 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.29 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.40 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.52 (s, 0.25H, **H**-C=N), 8.57 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 8.71 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.78 (s, 0.75H, **H**-C=N), 8.97 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.07 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 9.19 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.26 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 12.95 (s, 0.25H, CON**H**), 12.98 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 56.31 (2×O**C**H$_3$), 66.11; 66.56 (2×N**C**H$_2$), 141.91; 125.11; 126.74; 127.56; 131.24; 131.30; 145.08; 147.23; 147.76; 147.90; 149.83 (Ar-**C**), 160.54; 164.84 (**C**=N, **C**=O), 189.08; 189.45 (2×CH$_2$**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6): δ_P = (-157.39) to (-131.04) (m, 2P, 2×**P**F$_6$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.13 (d, 12F, 2×P**F₆**). HRMS (ESI) m/z = 814.5801 [M$^+$].

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-4-((2-(1-(2-(4-methoxyphenyl-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium tetrafluoroborate **(11)**. It was obtained as white needless (EtOH) in 86 % yield; mp: 92-93 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.92 (s, 6H 2×OC**H₃**), 6.54 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.09 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.28 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.38 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.52 (s, 0.25H, **H**-C=N), 8.58 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.69 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.73 (s, 0.75H, **H**-C=N), 8.94 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.05 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 9.17 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.25 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 13.37 (s, 0.25H, CON**H**), 13.42 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 56.31 (2×O**C**H$_3$), 66.12; 66.76 (2×N**C**H$_2$), 141.92; 125.13; 126.75; 127.56; 131.25; 131.30; 145.04; 147.23; 147.76; 147.89; 149.83 (Ar-**C**), 160.54; 164.79 (**C**=N, **C**=O), 189.08; 189.46 (2×CH$_2$**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = (-1.31) to (-1.29) (m, 2B, 2×**B**F$_4$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -148.20, -148.15 (2d, 8F, 2×B**F**$_4$). HRMS (ESI) m/z = 698.1808 [M$^+$].

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-4-((2-(1-(2-(4-methoxyphenyl-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium trifluoroacetate **(12)**. It was obtained as syrup in 90 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.92 (s, 6H 2×OC**H₃**), 6.58 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.09 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.30 (d, 0.5H, *J* = 8 Hz, Ar-**H**), 8.40 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.53 (s, 0.25H, **H**-C=N), 8.58 (d, 1.5H, *J* = 4 Hz, Ar-

H), 8.72 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 8.79 (s, 0.75H, **H**-C=N), 8.98 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.07 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.20 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.28 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 13.37 (s, 0.25H, CON**H**), 13.43 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 56.32 (2×O**C**H$_3$), 66.11; 66.75 (2×N**C**H$_2$), 141.91; 125.13; 126.75; 127.56; 131.25; 131.30; 145.03; 147.23; 147.75; 147.89; 149.83 (Ar-**C**), 160.54; 164.85 (**C**=N, **C**=O), 189.09; 189.45 (2×CH$_2$**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.78 (s, 6F, 2×C**F$_3$**). HRMS (ESI) m/z = 750.5615 [M^+].

1-(2-(4-Chlorophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-chloro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium hexafluorophosphate) **(13)**. It was obtained as pale yellow crystals (EtOH) in 91 % yield; mp: 78-80 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.62 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.78 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.11 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.31 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.41 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.53 (s, 0.25H, **H**-C=N), 8.59 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.73 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.85 (s, 0.75H, **H**-C=N), 9.01 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.23 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.27 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 13.39 (s, 0.25H, CON**H**), 13.48 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.41; 67.02 (2×N**C**H$_2$), 124.89; 125.20; 126.85; 127.60; 129.80; 129.84; 130.68; 130.72; 132.72; 132.81; 140.03; 140.11; 145.03; 146.92; 147.22; 147.36; 147.88; 149.91; 149.97 (Ar-**C**), 160.51; 164.79 (**C**=N, **C**=O), 189.08; 189.44 (2×CH$_2$**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6): δ_P = (-157.39) to (-131.04) (m, 2P, 2×**P**F$_6$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.15 (d, 12F, 2×P**F$_6$**). HRMS (ESI) m/z = 823.3839 [M^+].

1-(2-(4-Chlorophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium tetrafluoroborate **(14)**. It was obtained as syrup in 90 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.63 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.78 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.12 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.31 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.41 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.54 (s, 0.25H, **H**-C=N), 8.59 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.73 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.79 (s, 0.75H, **H**-C=N), 8.98 (d, 0.5H, *J* = 8 Hz, Ar-**H**), 9.07 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.20 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.29 (d, 1.5H, *J* = 8 Hz, Ar-**H**), 13.38 (s, 0.25H, CON**H**), 13.44 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.39, 67.01 (2×N**C**H$_2$), 124.87; 125.20; 126.82; 127.60; 129.80; 130.67; 130.71; 132.71; 132.80; 140.03; 140.12; 145.04; 147.21; 147.34; 147.89; 149.93; 149.98 (Ar-**C**), 160.53; 164.79 (**C**=N, **C**=O), 190.06; 190.42 (2×CH$_2$**C**=O). ^{11}B

NMR (128 MHz, DMSO-d_6): δ_B = (-1.30) to (-1.28) (m, 2B, 2×**B**F_4). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -148.21 and -148.16 (2d, 8F, 2×B**F**$_4$). HRMS (ESI) m/z = 707.0516 [M^+].

1-(2-(4-Chlorophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium trifluoroacetate **(15)**. It was obtained as yellow crystals (EtOH) in 89 % yield; mp: 78-79 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.67 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.78 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.13 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.33 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.43 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.54 (s, 0.25H, **H**-C=N), 8.59 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.75 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.84 (s, 0.75H, **H**-C=N), 9.01 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.23 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.31 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 13.39 (s, 0.25H, CON**H**), 13.49 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.41, 67.02 (2×N**C**H$_2$), 124.89; 125.19; 126.84; 127.60; 129.80; 129.84; 130.69; 130.72; 132.72; 132.81; 140.03; 140.11; 145.03; 146.91; 147.22; 147.35; 147.88; 149.91; 149.97 (Ar-**C**), 160.51; 164.79 (**C**=N, **C**=O), 190.08; 190.44 (2×CH$_2$**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -74.05 (s, 6F, 2×C**F$_3$**). HRMS (ESI) m/z = 759.4248 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium hexafluorophosphate **(16)**. It was obtained as syrup in 92 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.67 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.33 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.49 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.55 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.58 (s, 0.25H, **H**-C=N), 8.63 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.70 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.73 (s, 0.75H, **H**-C=N), 9.03 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.18 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.25 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 12.90 (s, 0.25H, CON**H**), 12.97 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.59; 67.23 (2×N**C**H$_2$), 122.21; 124.69; 124.73; 124.98; 126.72; 130.22; 130.27; 138.64; 138.74; 147.08; 147.82; 150.66; 151.00; 151.11 (Ar-**C**), 159.96; 162.99 (**C**=N, **C**=O), 190.30; 190.67 (2×CH$_2$**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6): δ_P = (-148.61) to (-139.82) (m, 2P, 2×**P**F_6). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.15 (d, 12F, 2×P**F$_6$**). HRMS (ESI) m/z = 844.4078 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium tetrafluoroborate **(17)**. It was obtained as syrup in 86 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.68 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.80 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.33 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.49 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.55 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.60 (s, 0.25H, **H**-C=N), 8.63 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.70 (d, 1.5H, *J* = 4 Hz, Ar-

H), 8.74 (s, 0.75H, **H**-C=N), 9.03 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.20 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.27 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 12.91 (s, 0.25H, CON**H**), 12.98 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.60, 67.23 (2×N**C**H_2), 122.23; 124.67; 124.98; 126.72; 127.47; 130.23; 130.28; 138.64; 138.74; 140.00; 143.58; 147.07; 147.81; 150.39; 150.96; 151.09 (Ar-**C**), 159.97; 163.00 (**C**=N, **C**=O), 190.30; 190.66 (2×CH_2**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = (-1.31) to (-1.29) (m, 2B, 2×**B**F_4). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -148.21 and -148.16 (2d, 8F, 2×B**F**$_4$). HRMS (ESI) m/z = 728.1631 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-4-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium trifluoroacetate **(18)**. It was obtained as yellow crystals (ETOH) in 85 % yield; mp: 91-93 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.64 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 4 Hz, Ar-**H**), 8.32 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.49 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.56 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 8.58 (s, 0.25H, **H**-C=N), 8.63 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.69 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 8.86 (s, 0.75H, **H**-C=N), 9.01 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 9.17 (d, 0.5H, *J* = 4 Hz, Ar-**H**), 9.24 (d, 1.5H, *J* = 4 Hz, Ar-**H**), 12.92 (s, 0.25H, CON**H**), 12.95 (s, 0.75H, CON**H**). ^{13}C NMR (150 MHz, DMSO-d_6): δ_C = 66.60, 67.24 (2×N**C**H_2), 122.23; 124.68; 124.98; 126.73; 130.23; 131.20; 138.64; 138.74; 140.02; 143.57; 147.08; 147.82; 150.23; 150.96; 151.10 (Ar-**C**), 159.98; 162.99 (**C**=N, **C**=O), 190.30; 190.67 (2×CH_2**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.61 (s, 6F, 2×C**F**$_3$). HRMS (ESI) m/z = 780.5815 [M^+].

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-3-((2-(1-(2-(4-methoxyphenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono)methyl)pyridin-1-ium hexafluorophosphate **(19)**. It was obtained as yellow crystals (EtOH) in 90 % yield; mp: 102-104 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.92 (s, 6H 2×OC**H**$_3$), 6.67 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.09 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.30 (dd, 0.25H, *J* = 8 Hz, Ar-**H**), 8.39 (dd, 0.75H, *J* = 4 Hz, Ar-**H**), 8.52 (s, 0.2H, **H**-C=N), 8.57 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.70 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.79 (s, 0.8H, **H**-C=N), 9.05 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.06 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.16 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.27 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.32 (s, 0.2H, Ar-**H**), 9.53 (s, 0.8H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.23 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 56.33 (2×O**C**H3) 66.68, 66.71 (2×N**C**H_2), 114.92; 126.66; 126.68; 126.76; 128.46; 131.25; 131.28; 134.20; 144.16; 144.59; 145.24; 147.23; 147.27; 147.85; 147.93 (Ar-**C**), 160.08; 164.79 (**C**=N, **C**=O), 189.10; 189.32 (2×CH_2**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6): δ_P = (-157.39) to (-

131.04) (m, 2P, 2×**P**F_6). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.13 (d, 12F, 2×P**F$_6$**). HRMS (ESI) m/z = 814.5142 [M^+].

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-3-((2-(1-(2-(4-methoxyphenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono)methyl)pyridin-1-ium tetrafluoroborate **(20)**. It was obtained as syrup in 90 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.91 (s, 6H 2×OC**H$_3$**), 6.61 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.08 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.31 (dd, 0.25H, *J* = 8 Hz, Ar-**H**), 8.40 (dd, 0.75H, *J* = 8 Hz, Ar-**H**), 8.57 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.72 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.79 (s, 0.2H, **H**-C=N), 8.83 (s, 0.8H, **H**-C=N), 9.07 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.10 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.18 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.29 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.30 (s, 0.2H, Ar-**H**), 9.55 (s, 0.8H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.26 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 56.33 (2×O**C**H3), 66.58; 66.70 (2×N**C**H$_2$), 114.92; 126.67; 126.77; 127.57; 128.46; 131.26; 134.17; 144.05; 144.55; 145.31; 147.23; 147.28; 147.83 (Ar-**C**), 160.04; 164.78 (**C**=N, **C**=O), 189.10; 189.33 (2×CH$_2$**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = (-1.31) to (-1.29) (m,2B, 2×**B**F_4). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -148.17, -148.12 (2d, 8F, 2×B**F**$_4$). HRMS (ESI) m/z = 898.1998 [M^+].

1-(2-(4-Methoxyphenyl)-2-oxoethyl)-3-((2-(1-(2-(4-methoxyphenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium trifluoroacetate **(21)**. It was obtained as colorless needless (EtOH) in 88 % yield; mp: 82-84 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 3.92 (s, 6H 2×OC**H$_3$**), 6.55 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.22 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.08 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.30 (dd, 0.25H, *J* = 8 Hz, Ar-**H**), 8.39 (dd, 0.75H, *J* = 8 Hz, Ar-**H**), 8.51 (s, 0.2H, **H**-C=N), 8.56 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.69 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.77 (s, 0.8H, **H**-C=N), 9.07 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.12 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.15 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.25 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 9.32 (s, 0.2H, Ar-**H**), 9.52 (s, 0.8H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.28 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 56.34 (2×O**C**H3), 66.58; 66.70 (2×N**C**H$_2$), 114.94; 126.68; 126.71; 126.78; 127.56; 128.46; 131.30; 134.18; 144.02; 144.54; 145.33; 147.24; 147.83; 147.85 (Ar-**C**), 160.04; 164.78 (**C**=N, **C**=O), 189.10; 189.33 (2×CH$_2$**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.49 (s, 6F, 2×C**F$_3$**). HRMS (ESI) m/z = 750.5984 [M^+].

1-(2-(4-Chlorophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium hexafluorophosphate **(22)**. It was obtained as syrup in 89 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.57 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d,

4H, *J* = 8 Hz, Ar-**H**), 8.12 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.30 (dd, 0.25H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.40 (dd, 0.75H, *J* = 8 Hz, Ar-**H**), 8.57 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.69 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 8.72 (s, 0.8H, **H**-C=N), 8.79 (s, 0.2H, **H**-C=N), 9.03 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.07 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.13 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.23 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.32 (s, 0.2H, Ar-**H**), 9.51 (s, 0.8H, Ar-**H**), 13.10 (s, 0.25H, CON**H**), 13.15 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 66.96; 66.99 (2×N**C**H$_2$), 126.73; 127.73; 128.32; 128.51; 129.82; 129.85; 130.71; 132.70; 132.78; 134.26; 140.08; 140.15; 144.35; 144.65; 145.08; 147.15; 147.31; 147.87; 148.15 (Ar-**C**), 160.11; 164.78 (**C**=N, **C**=O), 190.05; 190.27 (2×CH$_2$**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6): δ_P = (-157.39) to (-131.04) (m, 2P, 2×**P**F$_6$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.13 (d, 12F, 2×P**F$_6$**). HRMS (ESI) m/z = 823.5163 [M$^+$].

***1-(2-(4-Chlorophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-chloro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium tetrafluoroborate* (23)**. It was obtained as white crystals (EtOH) in 87 % yield; mp: 78-80 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.64 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.12 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.33 (dd, 0.25H, *J* = 8 Hz, Ar-**H**), 8.41 (dd, 0.75H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.58 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.74 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 8.81 (s, 0.2H, **H**-C=N), 8.83 (s, 0.8H, **H**-C=N), 9.06 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.08 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.18 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.28 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.31 (s, 0.2H, Ar-**H**), 9.55 (s, 0.8H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.25 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 66.87; 66.98 (2×N**C**H$_2$), 126.76; 127.66; 128.54; 129.82; 129.84; 130.68; 130.70; 132.72; 132.79; 134.22; 140.04; 140.11; 144.39; 144.59; 145.20; 147.19; 147.29; 147.84; 148.02 (Ar-**C**), 160.06; 164.09 (**C**=N, **C**=O), 190.08; 190.30 (2×CH$_2$**C**=O). ^{11}B NMR (128 MHz, DMSO-d_6): δ_B = (-1.30) - (-1.28) (m, 2B, 2×**B**F$_4$). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -148.17 and -148.12 (2d, 8F, 2×B**F$_4$**). HRMS (ESI) m/z = 707.1026 [M$^+$].

***1-(2-(4-Chlorophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-chlorophenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl) hydrazono) methyl)pyridin-1-ium trifluoroacetate* (24)**. It was obtained as white crystals (EtOH) in 90 % yield; mp: 93-94 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.64 (d, 4H, *J* = 8 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.12 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.33 (dd, 0.25H, *J* = 8 Hz, Ar-**H**), 8.41 (dd, 0.75H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.58 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.74 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 8.81 (s, 0.2H, **H**-C=N), 8.83 (s, 0.8H, **H**-C=N), 9.06 (d, 1H, *J* = 4 Hz,

Ar-**H**), 9.08 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.18 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.28 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.31 (s, 0.2H, Ar-**H**), 9.55 (s, 0.8H, Ar-**H**), 13.11 (s, 0.25H, CON**H**), 13.25 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 66.87; 66.98 (2×N**C**H_2), 126.76; 127.66; 128.54; 129.82; 129.84; 130.68; 130.70; 132.72; 132.79; 134.22; 140.04; 140.11; 144.39; 144.59; 145.20; 147.19; 147.29; 147.84; 148.02 (Ar-**C**), 160.06; 164.09 (**C**=N, **C**=O), 190.08; 190.30 (2×CH_2**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.86 (s, 6F, 2×C**F$_3$**). HRMS (ESI) m/z = 759.4167 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium hexafluorophosphate **(25)**. It was obtained as syrup in 90 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.65 (d, 4H, *J* = 4 Hz, 2×NC**H**$_2$), 8.34 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.42 (dd, 0.75H, *J* = 8 Hz, Ar-**H**), 8.51 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.66 (s, 0.2H, **H**-C=N), 8.71 (d, 0.25H, *J* = 8 Hz, Ar-**H**), 8.76 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.84 (s, 0.8H, **H**-C=N), 9.06 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.07 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.16 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.26 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.29 (s, 0.2H, Ar-**H**), 9.54 (s, 0.8H, Ar-**H**), 13.14 (s, 0.25H, CON**H**), 13.38 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 67.24; 67.28 (2×N**C**H_2), 124.71; 126.67; 126.83; 128.57; 128.58, 13.26; 130.27; 134.29; 138.63; 138.73; 144.74; 145.08; 147.85; 148.15; 148.16, 151.15 (Ar-**C**), 160.04; 160.09 (**C**=N, **C**=O), 190.28; 190.50 (2×CH_2**C**=O). ^{31}P NMR (162 MHz, DMSO-d_6): δ_P = (-157.39) to (-131.04) (m, 2P, 2×**P**F_6). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -69.14 (d, 12F, 2×P**F$_6$**). HRMS (ESI) m/z = 844.4387 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium tetrafluoroborate **(26)**. It was obtained as pale yellow crystals (EtOH) in 88 % yield; mp: 99-100 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.58 (d, 4H, *J* = 4 Hz, 2×NC**H**$_2$), 7.79 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.12 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.30 (dd, 0.25H, *J* = 4 Hz, 8 Hz, Ar-**H**), 8.40 (dd, 0.75H, *J* = 8 Hz, Ar-**H**), 8.57 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.69 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.73 (s, 0.8H, **H**-C=N), 8.77 (s, 0.2H, **H**-C=N), 9.05 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.07 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.14 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.24 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.26 (s, 0.2H, Ar-**H**), 9.52 (s, 0.8H, Ar-**H**), 13.09 (s, 0.25H, CON**H**), 13.15 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 67.24; 67.28 (2×N**C**H_2), 124.71; 126.67; 126.83; 128.57; 128.58; 13.26; 130.27; 134.29; 138.63; 138.73; 144.74; 145.08; 147.85; 148.15; 148.16; 151.15 (Ar-**C**), 160.04; 160.09 (**C**=N, **C**=O), 190.28; 190.50 (2×CH_2**C**=O). ^{11}B NMR (128 MHz,

DMSO-d_6): δ_B = (-1.31) to (-1.29) (m,2B, 2×**B**F_4). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -148.17, -148.12 (2d, 8F, 2×B**F**$_4$). HRMS (ESI) m/z = 728.1147 [M^+].

1-(2-(4-Nitrophenyl)-2-oxoethyl)-3-((2-(1-(2-(4-nitro phenyl)-2-oxoethyl)pyridin-1-ium-4-carbonyl)hydrazono) methyl)pyridin-1-ium trifluoroacetate **(27)**. It was obtained as syrup in 88 % yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H = 6.65 (d, 4H, *J* = 4 Hz, 2×NC**H**$_2$), 8.34 (d, 4H, *J* = 4 Hz, *J* = 8 Hz, Ar-**H**), 8.43 (dd, 0.75H, *J* = 4 Hz, *J* = 8 Hz, Ar-**H**), 8.51 (d, 4H, *J* = 8 Hz, Ar-**H**), 8.61 (s, 0.2H, **H**-C=N), 8.69 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 8.73 (d, 1.75H, *J* = 4 Hz, Ar-**H**), 8.79 (s, 0.8H, **H**-C=N), 9.07 (d, 1H, *J* = 4 Hz, Ar-**H**), 9.09 (d, 1H, *J* = 8 Hz, Ar-**H**), 9.16 (d, 0.25H, *J* = 4 Hz, Ar-**H**), 9.26 (d, 1.75H, *J* = 8 Hz, Ar-**H**), 9.27 (s, 0.2H, Ar-**H**), 9.54 (s, 0.8H, Ar-**H**), 13.12 (s, 0.25H, CON**H**), 13.24 (s, 0.75H, CON**H**). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C = 67.24; 67.28 (2×N**C**H$_2$), 124.71; 126.67; 126.83; 128.57; 128.58; 130.26; 130.27; 134.29; 138.63; 138.73; 144.74; 145.08; 147.85; 148.15; 148.16; 151.15 (Ar-**C**), 160.04; 160.09 (**C**=N, **C**=O), 190.28; 190.50 (2×CH$_2$**C**=O). ^{19}F NMR (377 MHz, DMSO-d_6): δ_F = -73.87 (s, 6F, 2×C**F**$_3$). HRMS (ESI) m/z 780.5824 [M^+].

3.2. Biological screening

Cell Lines and Culture Conditions. The human breast adenocarcinoma cell line MCF-7, the human ductal breast epithelial tumor cell line T47D, the human mammary gland/breast tumor cell line MDA231, the human Colorectal Adenocarcinoma cell line Caco-2, were purchased from ATCC. Cell lines were cultured in high glucose Dulbecco's modified eagle medium (DMEM) (Invitrogen, USA) containing 10 % heat inactivated fetal bovine serum (HI-FBS) (Invitrogen), 2 mmol L^{-1} of L-glutamine, 50 U mL^{-1} of penicillin and 50 μg mL^{-1} of streptomycin. Cell lines were maintained at 37 °C in a 5 % CO_2 atmosphere of 95% humidity.

Cell proliferation by MTT assay. Viable cell number was verified using the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. Purple formazan product is yielded from the metabolically active cells via reduction of the yellow tetrazolium dye [MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] and released into the intracellular compartment. Afterward, the quantity of the formed formazan salt was determined by measuring it absorbance at wavelength 490. Cells were seeded at density of $8x10^3$ to ensure exponential growth throughout the experimental period and a linear relationship between absorbance and cell number.

Anti-proliferative assay. The examined compounds were first dissolved in a volume of DMSO to provide a final concentration of 1 M stock solution. This stock solution was further diluted in culture media to yield the various concentrations used in this study. Final concentration of DMSO was maintained constant in wells and never exceeded 0.1%. Cells were treated with the various concentrations, in three triplicates for each concentration, and incubated at 37 °C in a 5 % CO_2 incubator, for 48 h. At the end of the treatment time, MTT assay was carried out as previously described [39].

***In-silico* molecular docking studies.** In order to find out the more effective inhibitors out of the synthesized compounds by the structure based drug designing for PI3K, *in-silico* molecular docking approach was employed. The 3D atomic coordinates of the ligands were created using ACD/Labs-Chemsketch 12.0 software. Their geometries were cleaned and generated as the corresponding pdb ligand files. 2RD0 (PDB ID) was analyzed as a most suitable pure three-dimensional crystal structure of p110α subunit of PI3K retrieved from the protein data bank (PDB) (Source: www.rcsb.org/pdb/). The Graphical User Interface program namely ‘‘Auto-Dock Tools (ADT, 1.5.6)’’ was used in preparing, running and analyzing the docking simulations. Energy of the molecules was minimized using Dundee PRODRG2 server [56]. The docking analyses of the energy minimized compounds were carried out by means of the Autodock v4. 0 program [57]. The ligand and the receptor protein were treated using the united-atom approximation, Kollman united-atom charges were assigned and only polar hydrogens were added to the receptor protein. Gasteiger charge was assigned to the ligands, non-polar hydrogens were merged and the internal degrees of freedom and torsions were set. We have made the grids and adjusted the number of points in X, Y, Z-axis in order to cover the entire active site of protein. Electrostatic and affinity maps for all the atom types present were computed with a grid spacing of 0.375 Å. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers in populations of 150 individuals with 0.02 mutation rate in 10 docking trials with cross-over rate of 0.8. Other docking parameters were set to the software’s default values. The results were evaluated by sorting the different compounds with respect to the binding energy predicted. A cluster analysis which is based on root mean square deviation values, that takes the starting geometry as the reference, was performed and the conformation of the more populated cluster possessing the lowest energy was considered to be the most trustable solution. The lowest binding energy and the inhibitory constant (ki) values of the analyzed ligands were extracted

from this statistical mechanical analysis. The ligand protein interaction was visualized using UCSF Chimera 1.11.2.

4. Conclusion

This study explores the designing and the synthesis of dicationic pyridinium (DILs) incorporating the hydrazone linkage starting from bis-pyridine hydrazones through their quaternization reaction with several phenacyl bromide. The resulted ionic liquids undergo metathesis process *via* the introduction of some fluorinated anions yielding the task-specific dicationic pyridinium ionic liquids. The structures of newly synthesized DILs were evidenced by conventional spectroscopic experiments. The obtained compounds were screened for their *in-vitro* anticancer properties. Compound 22 is the most active against all tested cell lines. These results were further supported by *in-silico* studies revealing good docking scores and lesser binding energies towards PI3Kinase as target protein. The targeted ligands exhibited bonds with one or more amino acids in the active pocket site of the receptor with good binding energies toward the target protein. Compound 22 manifested highest Minimum binding energy score i.e -11.88 kJ/mol and lowest concentration of 16.40 nM as calculated inhibition constant. Therefore, the *in-silico* analysis propounds the importance of evaluating the prediction accuracy of scoring functions employed in AutoDock and unveiled excellent correlation between binding free energy, calculated inhibition constant (Ki) and IC_{50} values. Hence, this investigation validated the importance of drug design studies and viability of computational tools in correlating experimental values with the computational ones.

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Scheme 1. Synthetic strategies for the synthesis of di-cationic pyridinium hydrazone ionic liquids **4-27**.

Figure 1. ^{1}H NMR spectrum of compound **7**.

Figure 2. ^{13}C NMR spectrum of compound **7**.

Figure 3. ^{31}P NMR spectrum of compound **19**.

Figure 4. ^{19}F NMR spectrum of compound **19**.

Figure 5. ^{11}B NMR spectrum of compound **20**.

Figure 6. ^{19}F NMR spectrum of compound **20.**

Figure 7. ^{19}F NMR spectrum of compound **21.**

Figure 8. Docking results of some selected compounds 2, 3, 13 and 22 with hydrogen bonding with one or another amino acid present in the active site of PI3K (PBD:2RD0).

Table 1. Anticancer activities against four examined cell lines. Data are expressed as IC_{50} (μM) ±SD.*

Compound No	IC_{50} T47D	IC_{50} MCF7	IC_{50} MDA231	IC_{50} Caco-2
2	192±17	211±19	188±13	196±19
3	165±13	189±16	175±15	171±11
4	>300	>300	>300	>300
5	>300	>300	>300	>300
6	>300	>300	>300	>300
7	156±16	154±15	149±9	161±14
8	83±9	88±7	82±5	89±8
9	105±8	99±7	97±6	103±14
10	>300	>300	>300	>300
11	>300	>300	>300	>300
12	>300	>300	>300	>300
13	>300	>300	>300	>300
14	>300	>300	>300	>300
15	>300	>300	>300	>300
16	>300	>300	>300	>300
17	>300	>300	>300	>300
18	>300	>300	>300	>300
19	163±9	169±17	158±8	156±11
20	112±13	109±11	113±12	110±9
21	161±16	173±12	169±10	165±17
22	62±6	59±2	59±4	64±3
23	71±4	77±5	68±5	72±5
24	86±9	89±8	91±5	84±3
25	96±4	95±6	96±5	98±7
26	94±6	93±4	99±5	101±8
27	102±11	110±12	108±9	105±10

***** Each experiment was repeated 3 times. IC_{50} concentrations were obtained from the dose–response curves using Graph Pad Prism Software 5 (San Diego California USA, www.graphpad.com)

Table 2. Binding Energy and Inhibition Constant of the docked compounds 2-27.

Compound No	Minimum binding energy (kJ mol^{-1})	Inhibition Constant (ki)
2	-6.37	19.55 uM
3	-6.80	10.35 uM
4	-10.68	32.45 uM
5	-10.56	94.32 uM
6	-9.78	48.10 uM
7	-10.00	15.07 uM
8	-11.25	48.10 nM
9	-9.53	175.23 nM
10	-9.55	165.51 uM
11	-10.20	111.62 uM
12	-10.62	16.40 uM
13	-9.36	87.68 uM
14	-10.00	105.40 uM
15	-10.88	67.83 uM
16	-10.05	103.51 uM
17	-9.78	83.38 uM
18	-10.06	205.52 uM
19	-9.54	3.47 uM
20	-10.53	5.76 uM
21	-10.25	453.72 nM
22	-11.88	16.40 nM
23	-11.20	25.62 nM
24	-10.37	67.83 nM
25	-10.17	87.68 nM
26	-10.00	84.38 nM
27	-9.89	1.50 uM

Design, Synthesis, *In-silico* and *In-vitro* Evaluation of Di-Cationic Pyridinium Ionic Liquids as Potential Anticancer Scaffolds

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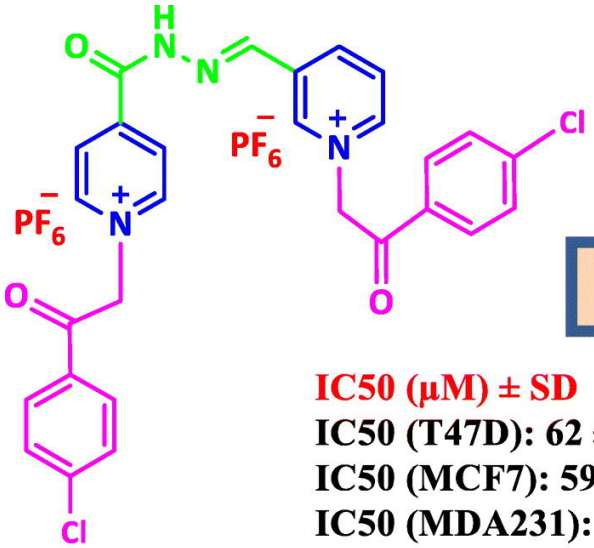
Highlights

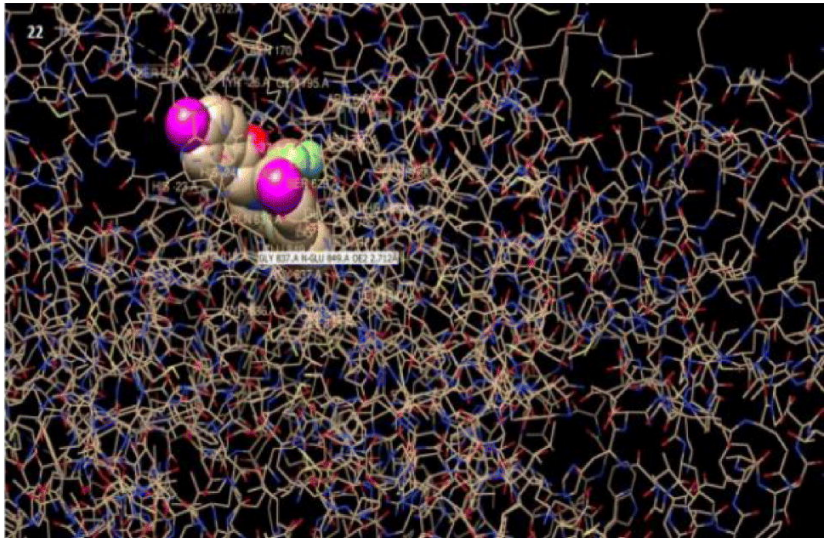
- Novel specific task fluorinated pyridinium ionic liquids (TsILs) were designed, synthesized and characterized.
- *In vitro* anticancer activity of synthesized ILs were carried out.
- *In silico* studies were investigated supporting the anticancer activity.



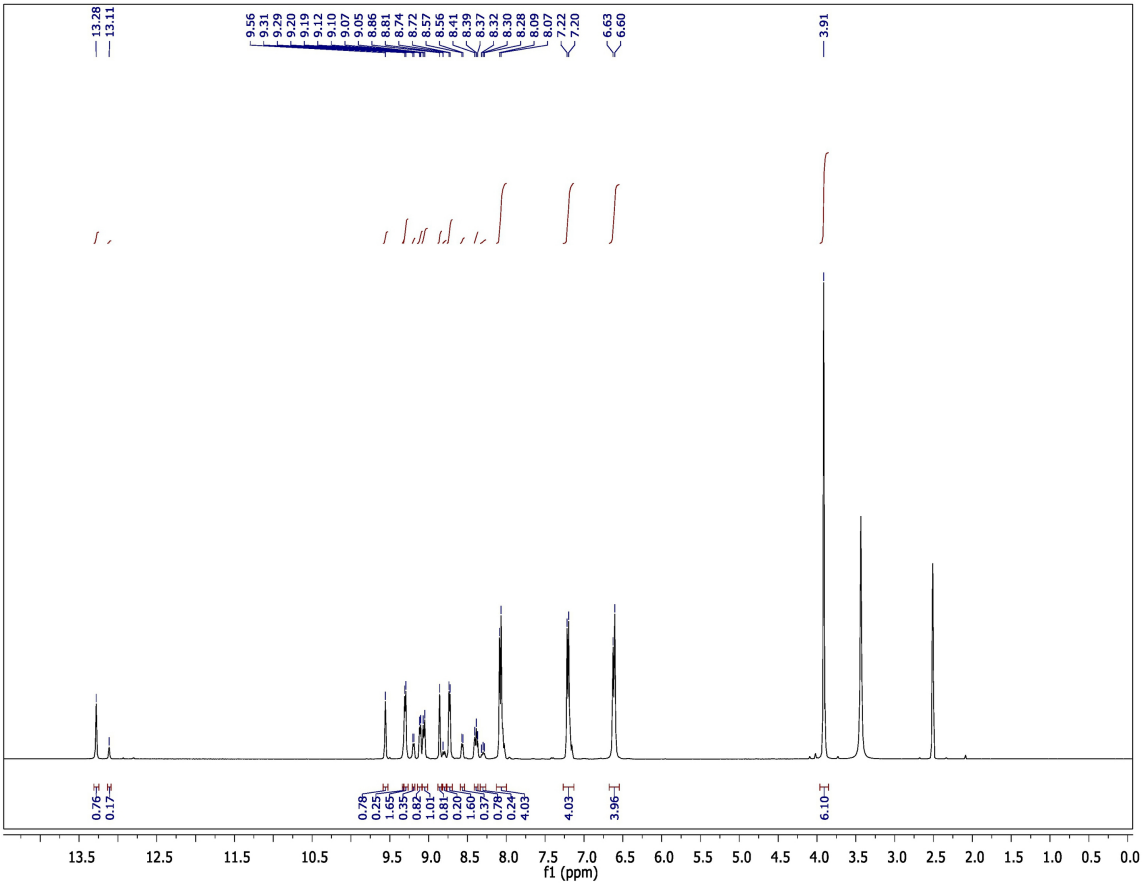
22

IC50 (µM) ± SD
IC50 (T47D): 62 ± 6
IC50 (MCF7): 59 ± 2
IC50 (MDA231): 59 ± 4
IC50 (Caco-2): 64 ± 3

In-silico



Graphics Abstract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8.09
8.07
7.22
7.20
6.63
6.60
3.91
0.76
0.17
0.78
0.25
1.65
0.35
0.82
1.01
0.81
0.20
1.60
0.37
0.78
0.24
4.03
4.03
3.96
6.10
13.5
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure 1

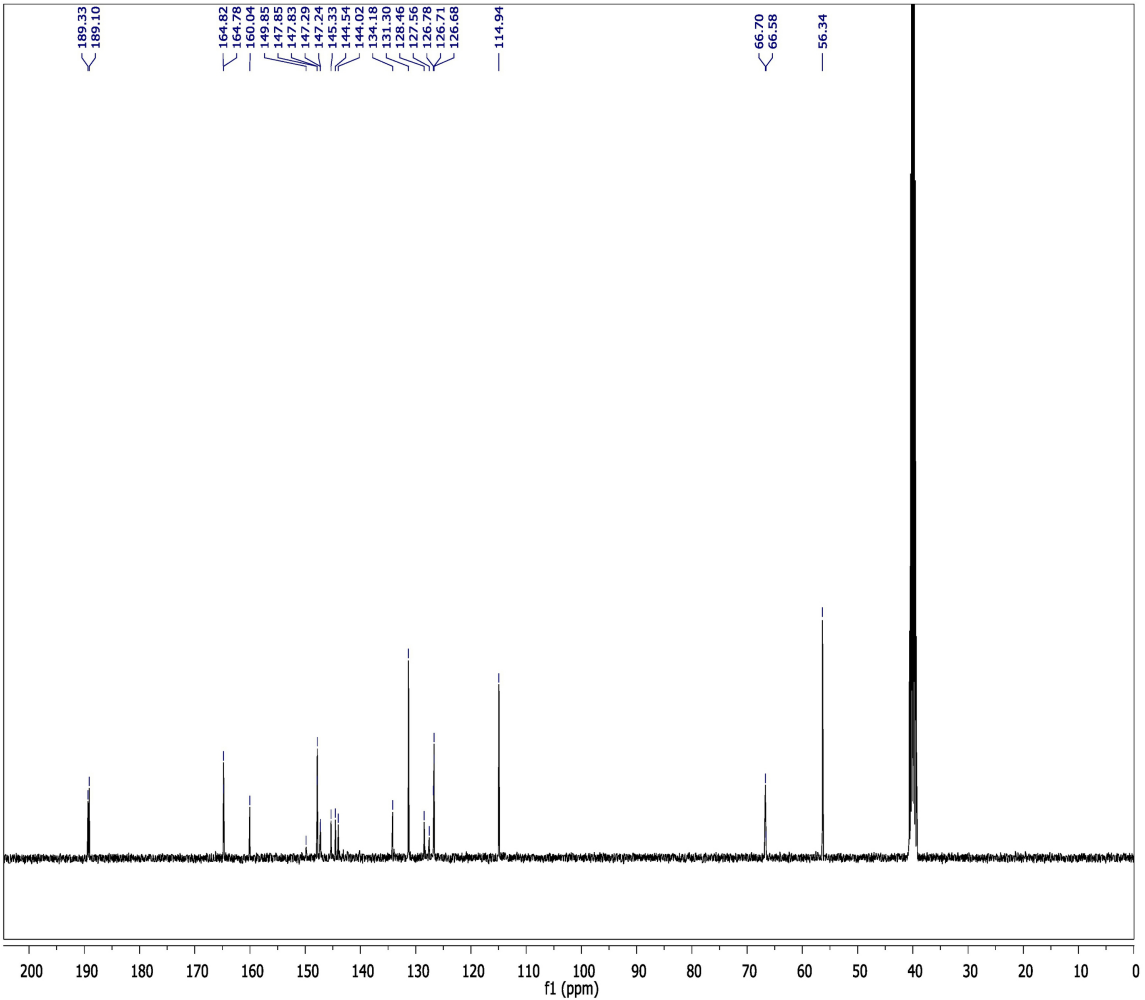
189.33
189.10
164.82
164.78
160.04
149.85
147.85
147.83
147.29
147.24
145.33
144.54
144.02
134.18
131.30
128.46
127.56
126.78
126.71
126.68
114.94
66.70
66.58
56.34
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Figure 2

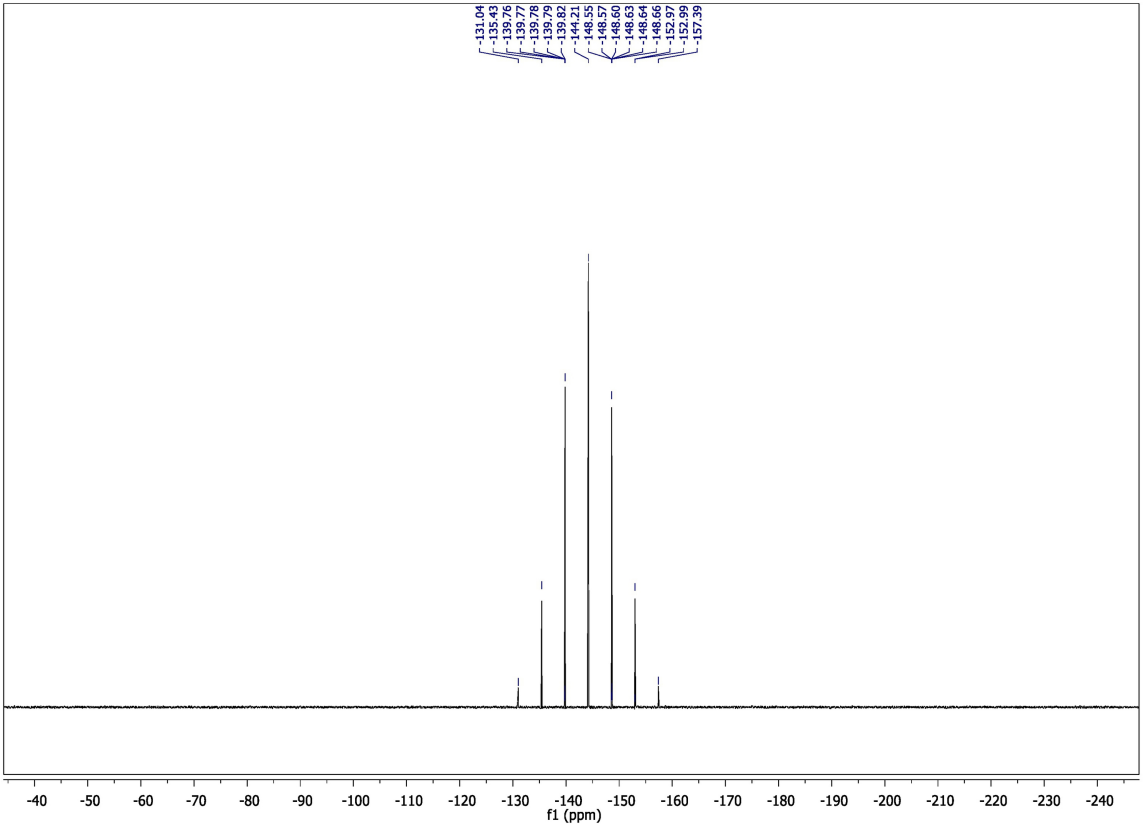
-131.04
-135.43
-139.76
-139.77
-139.78
-139.79
-139.82
-144.21
-148.55
-148.57
-148.60
-148.63
-148.64
-148.66
-152.97
-152.99
-157.39
-40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240
f1 (ppm)

Figure 3

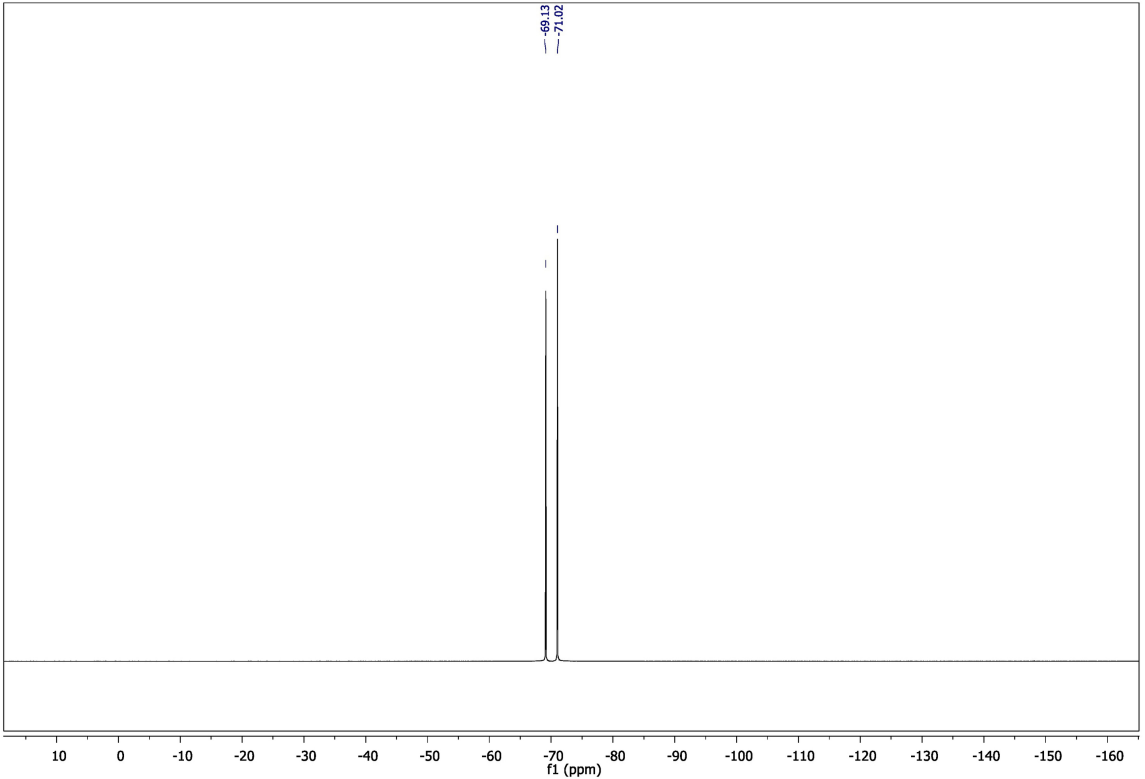
-69.13
-71.02
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
f1 (ppm)

Figure 4

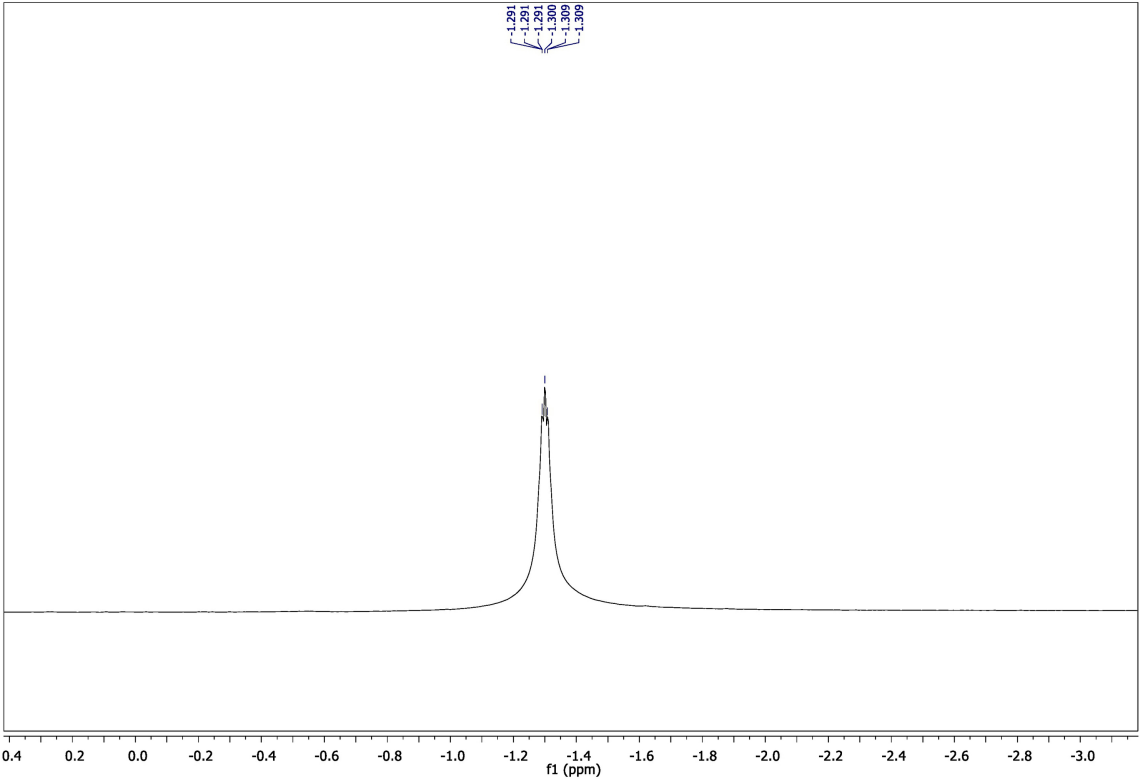
-1.291
-1.291
-1.291
-1.300
-1.309
-1.309
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
-2.2
-2.4
-2.6
-2.8
-3.0
f1 (ppm)

Figure 5

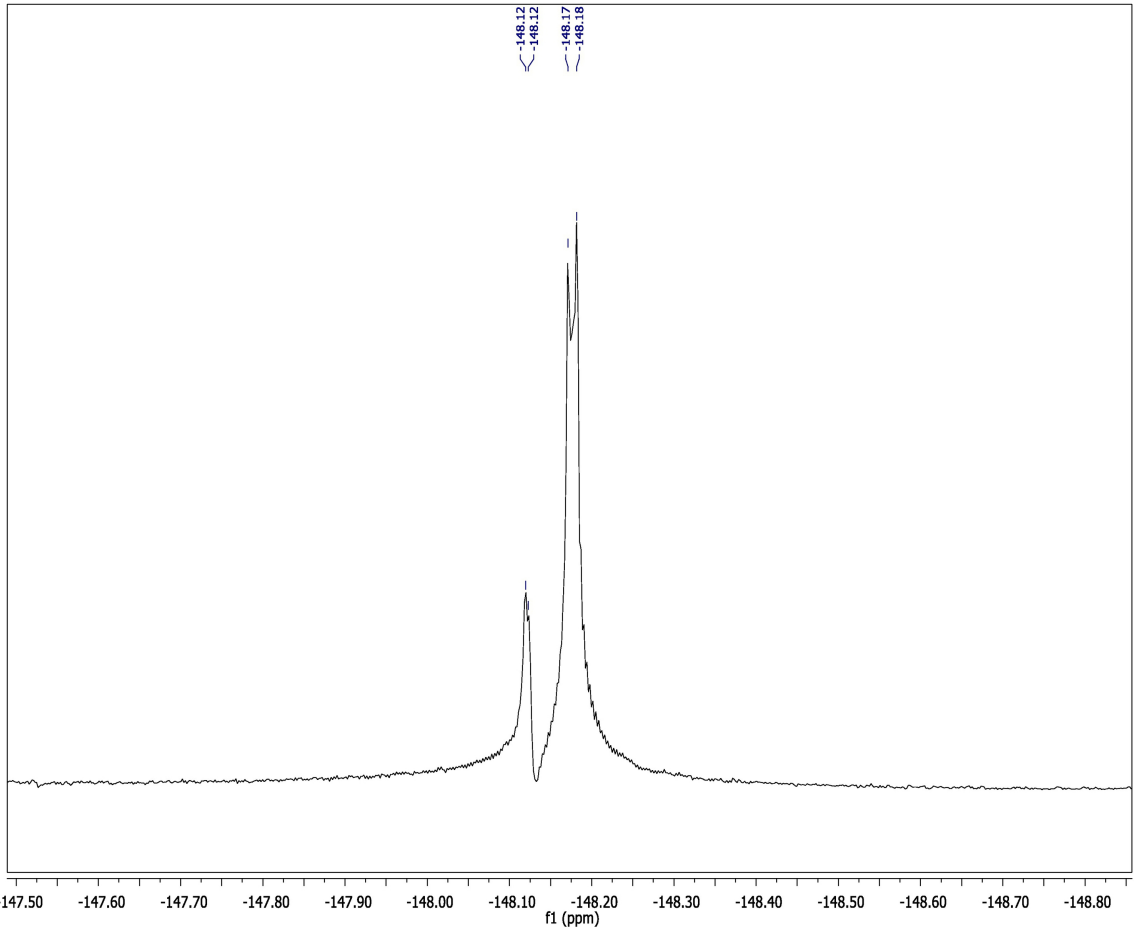
-148.12
-148.12
-148.17
-148.18
-147.50
-147.60
-147.70
-147.80
-147.90
-148.00
-148.10
-148.20
-148.30
-148.40
-148.50
-148.60
-148.70
-148.80
f1 (ppm)

Figure 6

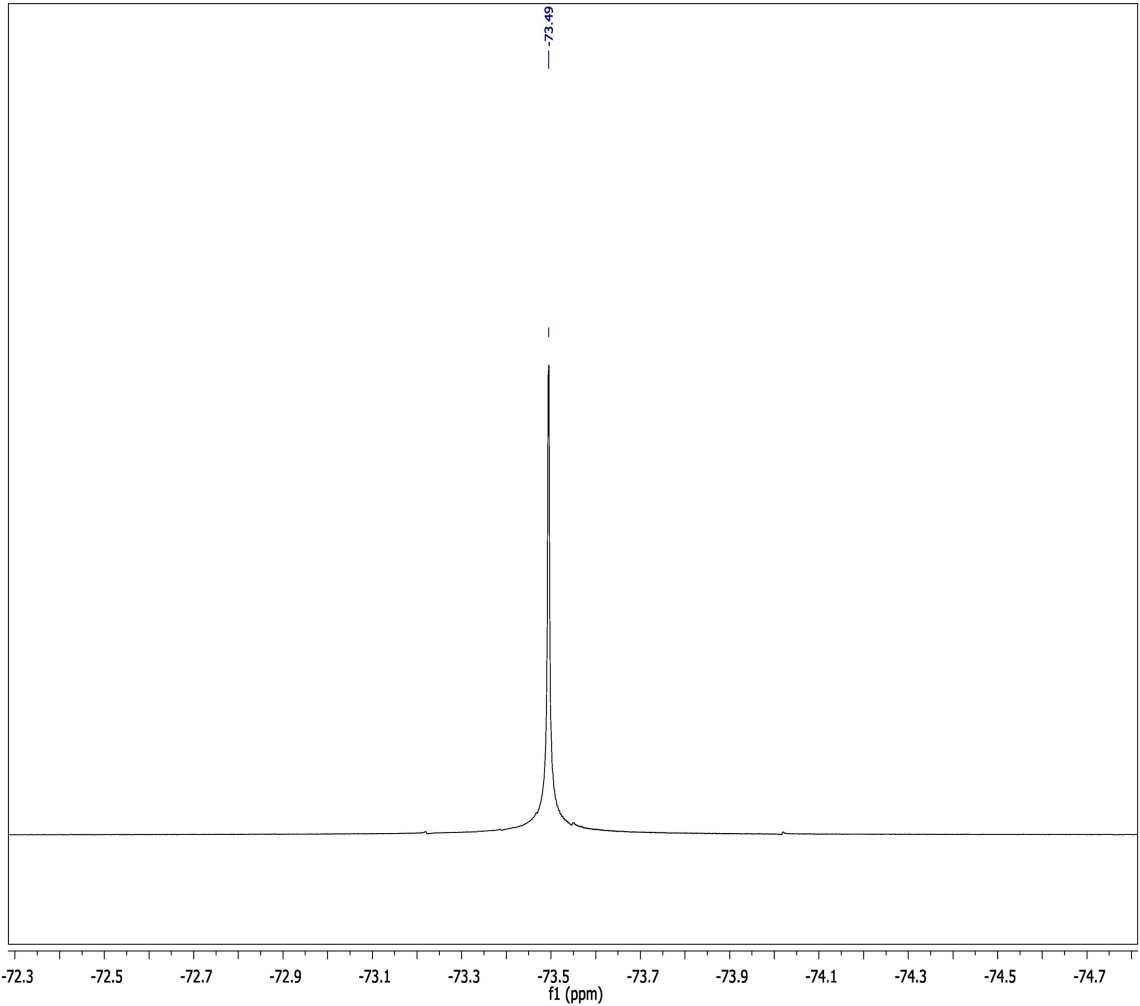

Figure 7

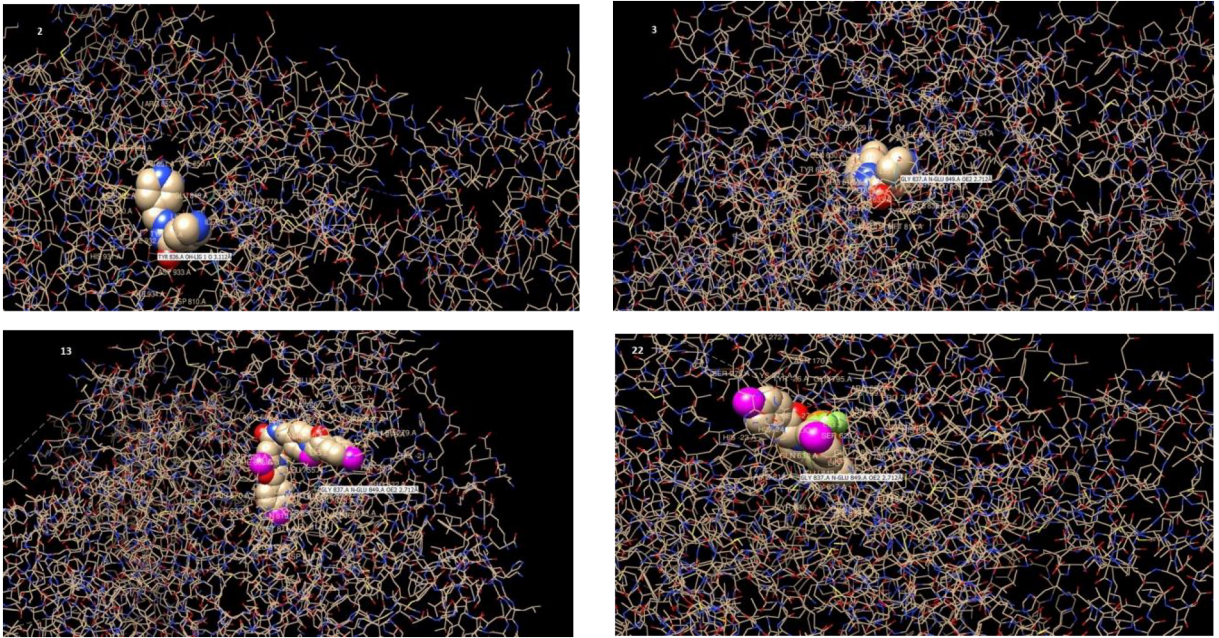

Figure 8