

Synthesis, spectroscopic characterization and *in vitro* antitumor activity of new *trans* 1-heteroaryl-2-(1-methylpyridinium-2-yl) ethylenes

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Abstract

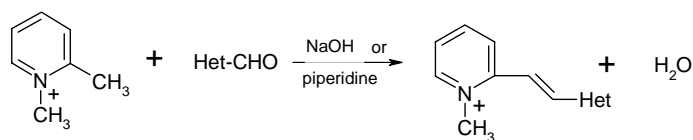
The synthesis and spectroscopic characterization of *trans* 1-heteroaryl-2-(1-methylpyridinium-2-yl) ethylene iodides is reported. The 5-(4-bromophenyl)furan derivative exhibited the highest *in vitro* antitumor activity against MCF7 (breast) and LNCap (prostate) carcinoma cells. Solvatochromic and NMR shifts provided evidence for modulation of electronic parameters which could be used to optimize frequency emission and conversion efficiency in such push-pull conjugated systems, which are potential components of non linear optics materials.

Keywords: Aromatic heterocycles, bio-organic, cytotoxicity, pharmaceuticals, pyridine

Introduction

We have recently reported the synthesis of 2,6-di-[2-(heteroaryl)vinyl]pyridines¹ and pyridiniums,² where three heteroaromatic rings are linked by two ethylenic double bonds exerting a "spacing" function. Antitumor *in vitro* tests against the 60 National Cancer Institute (NCI) tumor cell lines panel pointed out antiproliferative effects particularly evident for MCF7 mammary adenocarcinoma cells and multivariate analysis of DNA microarray data for responsive tumor cell lines suggests a mechanistic pathway involving polyamine biosynthesis and prolactin signal transduction.³

We here report the synthesis and spectroscopic characterization of water soluble *trans* 1-heteroaryl-2-(1-methylpyridinium-2-yl) ethylenes **1-12** as well as *in vitro* antitumour tests against selected cell lines.

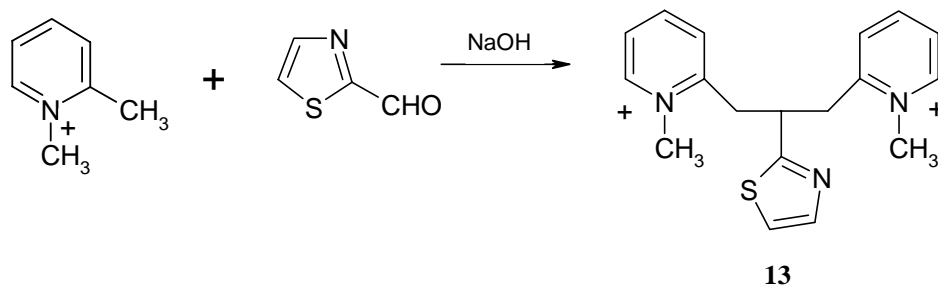


	Het
1	Furan-2-yl
2	5-Methylfuran-2-yl
3	5-Bromofuran-2-yl
4	5-(4-Bromophenyl)-furan-2-yl
5	5-(2-Chlorophenyl)-furan-2-yl
6	Thiophen-2-yl
7	5-Methyl thiophen-2-yl
8	3-Methyl thiophen-2-yl
9	5-Bromo thiophen-2-yl
10	4-Bromo thiophen-2-yl
11	1-Methylpyrrol-2-yl
12	Thiazol-2-yl

Compounds **1** – **12**, possessing heteroaromatic moieties with different electron donating abilities linked by a vinyl to the strong electron withdrawing pyridinium ring, belong to the class of the so called push-pull (donor-acceptor, D-A) molecules. Such push-pull conjugated systems are possible candidate components of non linear optics, which are already well documented for push-pull ethenes for example 4-dimethylamino-4'-nitrostilbene (DANS)⁴ and for heterocyclic analogues with π -excessive and π -deficient heteroaromatics (D-A systems).⁵ It was recently shown⁶ that methyl substituted 1-methylpyrroles **11** are able to efficiently convert the emission of a cheap IR laser into that of a valuable visible laser. In this context we here also report solvatochromic and NMR shifts providing evidence for a wide modulation of electronic parameters which could be used to optimize frequency emission and conversion efficiency.

Results and Discussion

The synthesis of *trans* 1-heteroaryl-2-(1-methylpyridinium-2-yl) ethylenes **1-12** is straightforward and can be easily achieved by condensation of (1,2,-dimethyl)pyridinium iodide with heteroaromatic aldehydes (see Experimental section). As expected, the pyridinium α methyls are quite reactive towards base due to the strong electron withdrawing effect of the positively charged ring nitrogen.



Under appropriate experimental conditions, outlined in the experimental section, pure *trans* isomers **1** – **11** were obtained, as evidenced by the ethylenic protons J coupling constants in the NMR spectra (see Experimental). Reaction of 2-thiazolaldehyde with 1-methylpicolinium iodide in the presence of one drop 5% NaOH afforded a mixture of the expected ethylene **12** and of bis cation **13** (1:5 ratio) as determined by NMR signals. On increasing the NaOH concentration up to 10%, pure bis cation **13** was obtained in 49 % yield.

Compounds **1-11** possess electron donating (furan, thiophene and pyrrol) moieties linked by a vinyl linker to the pyridinium ring, while the thiazole derivative **12** exerts a weak electronwithdrawing effect. Accordingly, all H_a ethylenic NMR shifts resonate at lower fields with respect to H_b, and, among them, that of **12** (8.13 ppm) is the most downfield shifted (see Experimental).

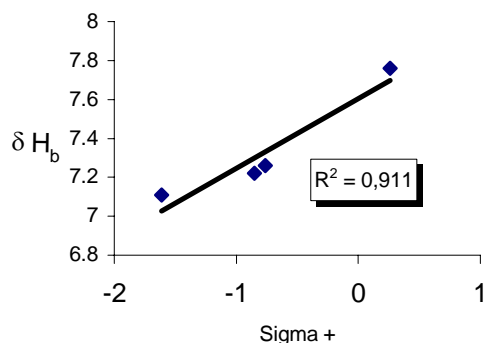


Figure 1. Correlation between δH_b and σ^+ constants from solvolysis reactions (-0.85 for furan-2-yl, -0.76 for thiophen-2-yl, -1.61 for pyrrol-2-yl and 0.26 for thiazol-2-yl).

Figure 1 shows a good correlation ($R^2 = 0.911$), in a wide range of σ^+ (σ^+ constants derived from solvolysis reactions.^{7,8}), between H_b ethylenic proton shifts for unsubstituted derivatives **1**, **6**, **11** and **12**. Derivatives with extended conjugation are expected to exhibit significant solvatochromic shifts. The absorption maxima of cations **1** - **11** in protic and aprotic solvent of various polarity are reported in Table 1.

Water-chloroform solvatochromic shifts above 20 nm are observed for cations **2**, **5**, **7**, and **11**, in agreement with the high donating ability of the five membered moieties in such push-pull systems. Solvatochromic shifts available for unsubstituted derivatives **1**, **6** and **11** follow closely the order of σ^+ constants for furan, thiophene and pyrrole moieties derived from solvolysis reactions,^{7,8} although the low number of points and their distribution suggest great caution in using the term correlation ($R^2 = 0.963$).

Solvatochromic and NMR shifts reported in the present paper span a wide range of variation (up to 1.87 σ^+ units) in the electronic effects of such push-pull conjugated molecules, providing the possibility to widely modulate electronic parameters, known to be essential in optimizing and

tuning frequency emission and conversion efficiency.⁶ The above finding indicates pointing out cations **1-12** as new possible candidate components for non linear optics.

Another purpose of the present work is to test the *in vitro* antitumor activity of the title *trans* 1-heteroaryl-2-(1-methylpyridinium-2-yl)ethylenes and to compare the results with those of previous studies on the activity of 2,6-di-[2-(heteroaryl)vinyl]pyridines⁹ and pyridiniums³ where three heteroaromatic rings are linked by two ethylenic double bonds.

Table 1. Absorption maxima for 1-heteroaryl-2-(1-methylpyridinium-2-yl) ethylenes **1-11**^a in different solvents

Compounds \ Solvents	CHCl ₃	EtOH	MeOH	H ₂ O
1	399.08 (3.94)	382.27 (3.79)	381.95 (3.79)	381.06 (3.91)
2	402.34 (3.95)	384.79 (3.69)	384.53 (3.81)	382.03 (3.87)
3	393.91 (3.74)	377.92 (3.81)	378.59 (3.83)	375.14 (3.83)
4	438.33 (4.32)	425.59 (4.23)	420.80 (4.14)	420.42 (4.25)
5	428.72 (4.41)	-	410.85 (4.42)	403.59 (4.36)
6	380.70 (4.09)	374.11 (4.11)	372.12 (4.14)	366.24 (4.18)
7	387.82 (4.01)	386.84 (4.11)	381.21 (4.10)	363.94 (4.06)
8	387.27 (4.02)	371.62 (3.85)	374.645 (3.70)	371.81 (3.94)
9	376.90 (3.81)	365.45 (3.83)	372.76 (3.74)	364.90 (3.85)
10	387.89 (4.36)	374.26 (4.33)	374.00 (4.37)	372.09 (4.33)
11	438.33 (4.19)	428.23 (4.18)	424.87 (4.16)	411.72 (4.16)

^alog_e in parentheses.

The *in vitro* antitumor activity of water soluble compounds **1**, **3**, **4**, **5**, and **11** are recorded in Table 2.

Table 2. *In vitro* antitumor activities, expressed as log GI₅₀, for MCF7 and LNCap cell lines

Cell line/Compounds	1	3	4	5	11	PF ₂
MCF7 (breast)	-5.00	-5.10	-7.75	-5.65	-4.82	-7.23
LNCap (prostate)	-4.00	-4.50	-6.00	-5.00	-4.00	-5.38

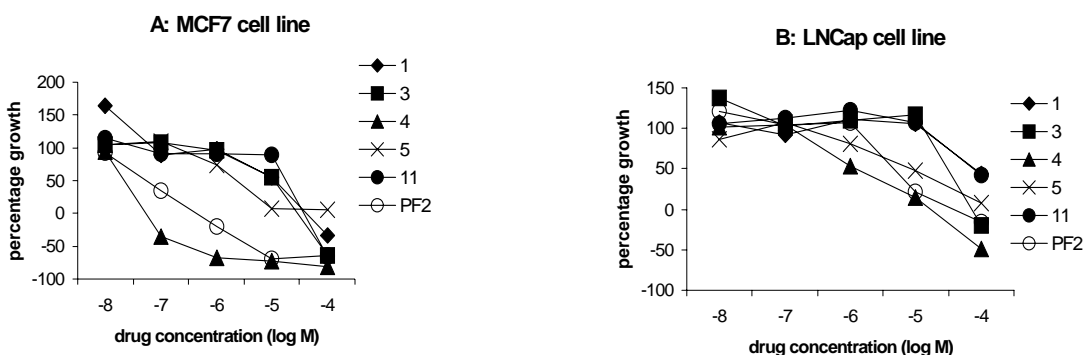


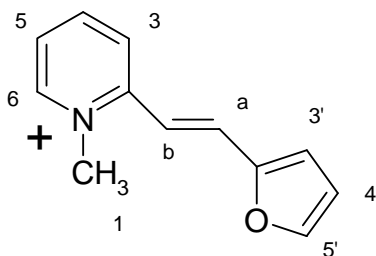
Figure 2. Dose – response curves of the antiproliferative activities of compounds **1**(♦), **3**(■), **4**(▲), **5**(×), **11**(●) and PF₂(○) on MCF7 (A) and LNCap (B) cell lines.

The anti-proliferative activity of typical derivatives was tested against two tumor cell lines, breast carcinoma (MCF7) and prostate carcinoma (LNCap). The percent of growth and the inhibition exerted by different doses (0.01-100 μM) are shown in figure 2 and log GI₅₀ values, calculated as described in the experimental section are recorded in Table 2. All compounds in Table 2 are more active towards MCF-7 than LNCap cell lines. Data relative to 2,6-di-[2-(furan-2-yl)vinyl]pyridinium iodide (PF₂), which was the most active in previous *in vitro* tests,³ are also reported for comparisons. Towards MCF-7 and LNCap carcinoma cells (Table 2) the 1-[5-(4-Bromophenyl)-furan-2-yl]-2-(1-methyl-pyridinium-2-yl) ethylene (ligand **4**) exhibits highest activity but slightly better than the PF₂. Figure 2 shows that, in addition to the antiproliferative effects (log GI₅₀), the bromophenyl derivative **4** exhibits a significant cytotoxic activity. In fact only 25% MCF7 carcinoma cells survive at 10⁻⁶ M concentration. This finding points to **4** as a possible lead compound for future antitumor studies. In conclusion, 1-heteroaryl-2-(1-methylpyridinium-2-yl)ethylenes **1-11** reported in the present work exhibit interesting potential applications both as candidates for more detailed *in vivo* antitumor studies and as components for non linear optics due to a wide modulation of the donor heteroaromatic moiety capabilities.

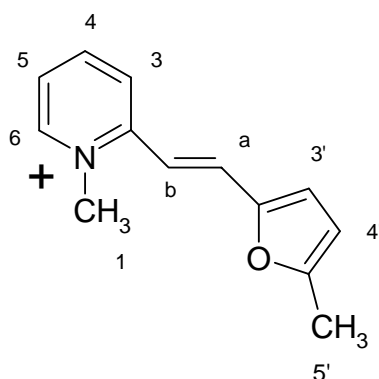
Experimental Section

General Procedures. Heteroaromatic carboxaldehydes, Aldrich commercial products, were used without further purification. UV-Vis spectra were recorded on a Perkin Elmer Lambda 2S spectrometer. ^1H NMR spectra were recorded on a Varian Unity Inova spectrometer operating at 500 MHz, at 25°C in $(\text{CD}_3)_2\text{SO}$ using TMS as internal standards. The spectral width was set to 5,000 Hz, with an excitation pulse of 60 degrees, an acquisition time of 3.5 s and a digital resolution after zero-filling of 0.15 Hz/pt. Fast Atom Bombardment (FAB) mass spectra were recorded on a double focusing Kratos MS 50 mass spectrometer equipped with the standard FAB source and the Maspec2 data acquisition and processing system (Mass Spectrometry Services Ltd). Mass resolution was 3.000 and the matrix 3-nitro-benzyl alcohol. Mass spectra ESI were recorded on a LCQ Deca Finnigan system with a flow of 5 $\mu\text{l}/\text{min}$. Compounds 1-13, all iodide salts, were obtained by reacting in equimolar amounts 1,2 dimethylpyridinium iodide with the appropriate heteroaromatic aldehyde in the presence of a base. Details on the synthetic conditions (base, solvent, reaction time and temperature) and products characterization are reported below:

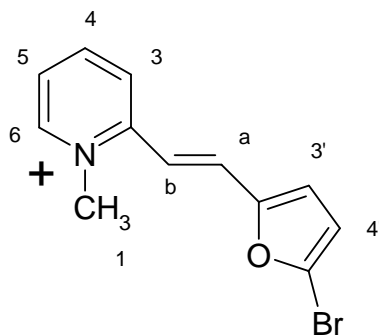
1-(Furan-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (1). Piperidine, EtOH, 8 hours, 25 °C. Yield 23%; (Green prisms from ethanol); mp 218-219 °C (lit.,¹⁰ 204-206°C, no NMR available); ^1H NMR, (DMSO- d_6): δ = 7.86 (d, J =15.5 Hz, 1H, a-H), 7.22 (d, J =16 Hz, 1H, b-H), 4.32 (s, 3H, N^+Me), 8.47 (m, 1H, 3-H), 8.45 (m, 1H, 4-H), 7.86 (m, 1H, 5-H), 8.88 (d, J =6.5 Hz, 1H, 6-H), 7.05 (d, J =3.5 Hz, 1H, 3'-H), 6.73 (dd, J =2 Hz, 1H, 4'-H), 7.97 (d, J =1.5 Hz, 1H, 5'-H). MS: positive FAB: (M^+ =185.9); Anal. Calcd for $\text{C}_{12}\text{H}_{12}\text{INO}$: C, 46.03; H, 3.86; N, 4.47. Found: C, 46.15; H, 3.78; N, 4.50.



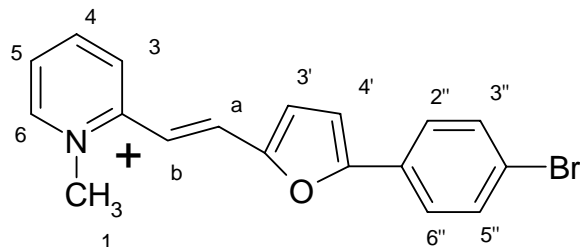
1-(5-Methylfuran-2-yl) 2-(1-methylpyridinium-2-yl) ethylene iodide (2). 5% NaOH, EtOH, 5 min, reflux. Yield 54%; (Yellow prisms from ethanol); mp 260-261 °C; ^1H NMR (DMSO- d_6), δ = 7.78 (d, J =16 Hz, 1H, a-H), 7.06 (d, J =15.5 Hz, 1H, b-H), 4.30 (s, 3H, N^+Me), 8.43 (m, 1H, 3-H), 8.41 (m, 1H, 4-H), 7.82 (m, 1H, 5-H), 8.85 (d, J =6.5 Hz, 1H, 6-H), 6.96 (d, J =3.5 Hz, 1H, 3'-H), 6.61 (d, J =3 Hz, 1H, 4'-H), 2.40 (s, 3H, 5'-Me). MS: ESI (M^+ = 200); Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{INO}$: C, 38.36; H, 3.47; N, 3.44. Found: C, 37.40; H, 3.51; N, 3.50.



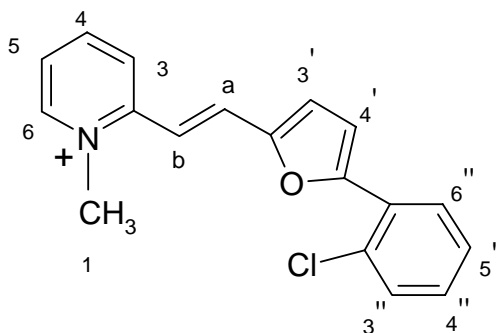
1-(5-Bromofuran-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (3). 20% NaOH, EtOH, 2 hours, reflux. Yield 49%; (Yellow needles from ethanol); mp > 200 °C; $^1\text{H NMR}$ (DMSO- d_6): $\delta=7.78$ (d, $J=16$ Hz, 1H, a-H), 7.21 (d, $J=16$ Hz, 1H, b-H), 4.34 (s, 3H, N^+Me), 8.91 (d, $J=6$ Hz, 1H, 6-H), 8.48 (t, $J=8$ Hz, 1H, 4-H), 8.44 (d, $J=8.5$ Hz, 1H, 3-H), 7.89 (m, $J=6.25$ Hz, 1H, 5-H), 7.09 (d, $J=4$ Hz, 1H, 3'-H), 6.87 (d, $J=3.5$ Hz, 1H, 4'-H). MS: positive FAB ($\text{M}^+ \text{Br}_{79} = 264$, $\text{M}^+ \text{Br}_{81} = 266$); Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{BrINO}$: C, 36.77; H, 3.86; N, 3.57. Found: C, 36.82; H, 3.74; N, 3.63.



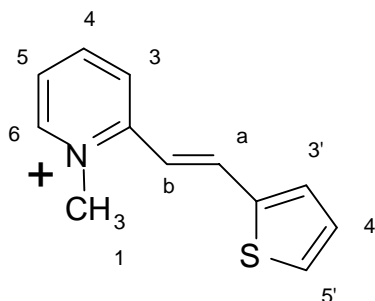
1-[5-(4-Bromophenyl)-furan-2-yl]-2-(1-methylpyridinium-2-yl) ethylene iodide (4). 10% NaOH, EtOH, 10 min, reflux. Yield 42%; (Orange needles from aqueous-ethanol); mp 260-263 °C; $^1\text{H NMR}$ (DMSO- d_6), $\delta=7.88$ (d, $J=16$ Hz, 1H, a-H), 7.37 (d, $J=16$ Hz, 1H, b-H), 4.37 (s, 3H, N^+Me), 8.48 (d, 1H, 3-H), 8.46 (dd, $J=8.5$ Hz, 1H, 4-H), 7.87 (m, 1H, 5-H), 8.87 (d, $J=6.5$ Hz, 1H, 6-H), 7.31 (d, $J=4$ Hz, 1H, 3'-H), 7.16 (d, $J=4$ Hz, 1H, 4'-H), 7.07 (m, 2H, 3''-H, 5''-H), 7.70 (d, $J=8.5$ Hz, 2H, 2''-H, 6''-H). MS: positive FAB ($\text{M}^+ \text{Br}_{79} = 339.7$; $\text{M}^+ \text{Br}_{81} = 341.7$). Anal. Calcd for $\text{C}_{18}\text{H}_{15}\text{BrINO}$: C, 46.18; H, 3.23; N, 3.00. Found: C, 46.24; H, 3.19; N, 3.12.



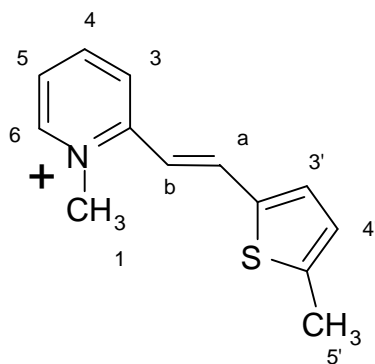
1-[5-(2-Chlorophenyl)-furan-2-yl]-2-(1-methylpyridinium-2-yl) ethylene iodide (5). 20% NaOH, EtOH, 10 min, reflux. Yield 34%; (Orange needles from ethanol); mp 190-193 °C; ^1H NMR (DMSO- d_6), δ =7.90 (d, J =15.5 Hz, 1H, a-H), 7.40 (d, J =15.5 Hz, 1H, b-H), 4.38 (s, 3H, N^+Me), 8.48 (d, 1H, 3-H), 8.50 (dd, J =7.5 Hz, 1H, 4-H), 7.88 (m, 1H, 5-H), 8.90 (d, J =6 Hz, 1H, 6-H), 6.17 (d, J =3.5 Hz, 1H, 3'-H), 6.34 (d, J =4 Hz, 1H, 4'-H), 7.07 (dd, J =8 Hz, 1H, 3''-H), 6.39 (m, J =8 Hz, 1H, 4''-H), 6.47 (m, J =8 Hz, 1H, 5''-H), 6.58 (dd, J =8 Hz, 1H, 6''-H). MS: positive FAB ($\text{M}^+ \text{Cl}_{35}$ =296.1 ; $\text{M}^+ \text{Cl}_{37}$ =298.1). Anal. Calcd for $\text{C}_{18}\text{H}_{15}\text{ClINO}$: C, 51.03; H, 3.57; N, 3.30. Found: C, 50.09; H, 3.62; N, 3.38.



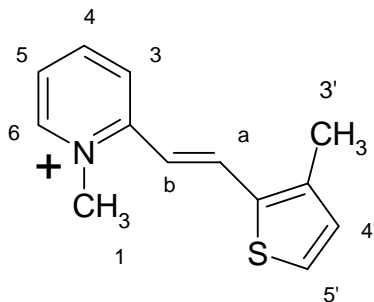
1-(Thiophen-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (6). 20% NaOH, EtOH, 1 hour, reflux. Yield 63%; (Brown needles from ethanol); mp 225-229°C (lit.,¹¹ 231-232°C, no NMR available); ^1H NMR, (DMSO- d_6) δ = 8.16 (d, J =15.5 Hz, 1H, a-H), 7.26 (d, J =15.5 Hz, 1H, b-H), 4.34 (s, 3H, N^+Me), 8.88 (d, J =6.5 Hz, 1H, 6-H), 7.24 (dd, 1H, 3-H), 8.47 (dd, 1H, 4-H), 6.70 (d, 1H, 5-H), 7.85-7.88 (m, 3H, 3'-H, 4'-H, 5'-H). MS: positive FAB (M^+ = 201.6). Anal. Calcd for $\text{C}_{12}\text{H}_{12}\text{INS}$: C, 43.78; H, 3.67; N, 4.25; S, 9.74. Found: C, 43.60; H, 3.74; N, 4.34; S, 9.70.



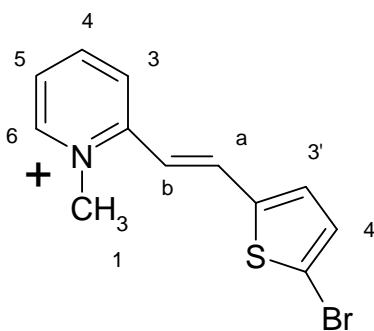
1-(5-Methylthiophen-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (7). 5% NaOH, EtOH, 10 min, reflux. Yield 11%; (Brown prisms from ethanol); mp 271-273 °C ; ^1H NMR (DMSO- d_6) , δ = 8.09 (d, $J=16$ Hz, 1H, a-H), 7.16 (d, $J=16$ Hz, 1H, b-H), 4.30 (s, 3H, N^+Me), 8.45 (d, $J=8$ Hz, 1H, 3-H), 8.42 (t, $J=7.5$ Hz, 1H, 4-H), 7.82 (t, $J=6.5$ Hz, 1H, 5-H), 8.85 (d, $J=6.5$ Hz, 1H, 6-H), 7.46 (d, $J=4$ Hz, 1H, 3'-H), 6.94 (d, $J=3$ Hz, 1H, 4'-H). 2.52 (s, 3H, 5'-Me). MS: ESI ($\text{M}^+ = 216$). Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{INS}$: C, 45.23; H, 4.67; N, 4.06; S, 9.29. Found: C, 45.32; H, 4.00; N, 4.12; S, 9.20.



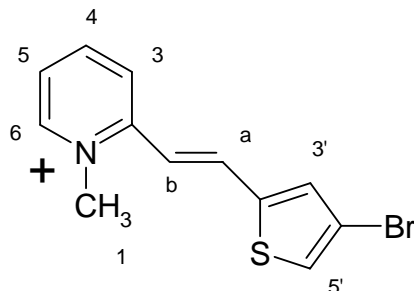
1-(3-Methylthiophen-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (8). 10% NaOH, EtOH, 10 min, reflux. Yield 45%; (Yellow needles from ethanol); mp 259-262 °C ; ^1H NMR (DMSO- d_6) , $\delta=8.08$ (d, $J=15.5$ Hz, 1H, a-H), 7.04 (d, $J=15.5$ Hz, 1H, b-H), 4.31 (s, 3H, N^+Me), 8.61 (d, $J=8$ Hz, 1H, 3-H), 8.45 (t, $J=8$ Hz, 1H, 4-H), 7.85 (t, $J=6.5$ Hz, 1H, 5-H), 8.85 (d, $J=6$ Hz, 1H, 6-H), 2.48 (s, 3H, 3'-Me), 7.06 (d, $J=5$ Hz, 1H, 4'-H), 7.73 (d, $J=4.5$ Hz, 1H, 5'-H). MS: ESI ($\text{M}^+ = 216$). Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{INS}$: C, 45.23; H, 4.67; N, 4.06; S, 9.29. Found: C, 45.41; H, 4.56; N, 4.12; S, 9.10.



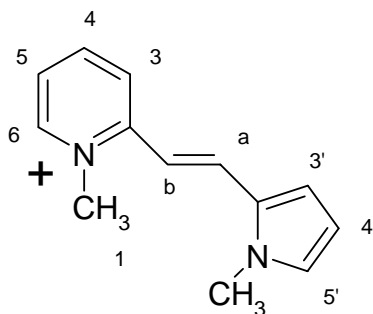
1-(5-Bromothiophen-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (9). 10% NaOH, EtOH, 5 min, reflux. Yield 73%; (Yellow prisms from ethanol); mp 241-242 °C; ^1H NMR (DMSO- d_6), δ = 8.06 (d, $J=16$ Hz, 1H, a-H), 7.22 (d, $J=16$ Hz, 1H, b-H), 4.31 (s, 3H, N^+Me), 8.43 (m, 2H, 3-H, 4-H), 7.87 (t, $J=6.5$ Hz, 1H, 5-H), 8.85 (d, $J=6$ Hz, 1H, 6-H), 7.37 (d, $J=4$ Hz, 1H, 3'-H), 7.47 (d, $J=4$ Hz, 1H 4'-H). MS: ESI ($\text{M}^+ = 281$). Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{BrINS}$: C, 35.32; H, 2.72; N, 3.43; S, 7.85. Found: C, 35.41; H, 2.65; N, 3.56; S, 7.81.



1-(4-Bromothiophen 2-yl)-2-(1-methylpyridinium 2-yl) ethylene iodide (10). 5% NaOH, EtOH, 10 min, reflux. Yield 84%; (Yellow needles from aqueous-ethanol); mp 262-265 °C ; ^1H NMR (DMSO- d_6), δ = 8.03 (d, $J=16$ Hz, 1H, a-H), 7.39 (d, $J=16$ Hz, 1H, b-H), 4.33 (s, N^+Me), 8.43 (d, $J=7.5$ Hz, 1H, 3-H), 8.47 (t, $J=8$ Hz, 1H, 4-H), 7.88 (t, $J=6$ Hz, 1H, 5-H), 8.89 (d, $J=6$ Hz, 1H, 6-H), 7.94 (s, 1H, 3'-H), 7.77 (s, 1H, 5'-H). MS: ESI ($\text{M}^+ = 281$). Anal. Calcd for $\text{C}_{12}\text{H}_{11}\text{BrINS}$: C, 35.32; H, 2.72; N, 3.43; S, 7.86. Found: C, 35.26; H, 2.78; N, 3.46; S, 7.80.



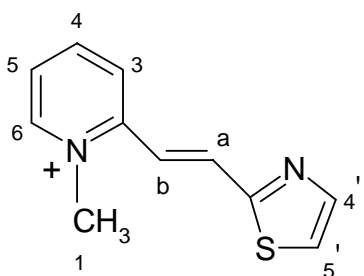
1-(1-Methylpyrrol-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (11). 10% NaOH, EtOH, 20 min, reflux. Yield 39%; (Red needles from ethanol); mp 226-228 °C (lit.,⁵ 187-188°C as CF₃SO₃⁻); ¹H NMR (DMSO-d₆, NOE on irradiation of N⁺Me provided unambiguous assignment for b-H and 6-H) δ = 7,88 (d, *J*=15.5 Hz, 1H, a-H), 7,11 (d, *J*=15.5 Hz, 1H, b-H), 4,25 (s, 3H, 1-H), 8,55 (d, *J*=9 Hz, 1H, 3-H), 8,33 (t, 1H, 4-H), 7,69 (t, 1H, 5-H), 8,72 (d, *J*=6.5 Hz, 1H, 6-H), 7,08 (m, 2H, 3'-H, 5'-H), 6,23 (m, 1H, 4'-H), 3,81 (s, 3H, N-Me). MS : positive FAB (M⁺ =198.8). Anal. Calcd for C₁₃H₁₅IN₂: C, 47.87; H, 4.63; N, 8.59. Found: C, 47.95; H, 4.60; N, 8.71.



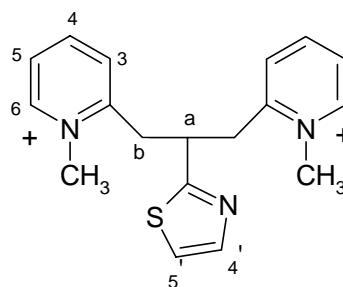
Formation of 1-(Tiazol-2-yl)-2-(1-methylpyridinium-2-yl) ethylene iodide (12) and of biscation (13). 1,2 Dimethylpyridinium iodide (6.4×10^{-4} mol) were dissolved in 2 mL hot ethanol. On addition of a drop 5% NaOH and an equimolar amount of 2-tiazolcarboxaldehyde a white precipitate was noticed. After filtration and recrystallization from ethanol, 0.06 g. yellow needles, were obtained. The solid was a mixture of the expected monocation **12** and of biscation **13** (1:5 ratio), as evidenced by ¹H NMR. By subtraction of the ¹H NMR spectrum of pure biscation **13** (see below), it is possible to assign the follows signals to monocation **12**. ¹H NMR (DMSO-d₆), (compound **12**): δ=8.13 (d, *J*=15.5 Hz, 1H, a-H), 7.78 (d, *J*=17 Hz, 1H, b-H), 4.33 (s, 3H, N⁺Me), 8.58 (d, *J*=1 Hz, 1H, 3-H), 8.48 (t, 1H, 4-H), 7.69 (m, 3H, 5-H, 4'-H, 5'-H), 8.98 (d, *J*=4 Hz, 1H, 6-H). MS: ESI, FAB [M⁺ (**12**) = 203, M²⁺ (**13**) = 301.1].

Biscation **13** was then synthesized according to the following procedure: 1,2 Dimethylpyridinium iodide (6.6×10^{-4} mol) were dissolved in 2 mL refluxing ethanol. One drop 10% NaOH was added with stirring and after few minutes 0.03 mL 2-thiazolcarboxaldehyde

(3.28×10^{-4} mol) were added. The resulting white precipitate was filtered and identified as pure biscation 13. Yield 49%; (Yellow needles from ethanol); mp >200 °C; $^1\text{H NMR}$ (DMSO- d_6), δ = 7.60 (d, $J=3.3$ Hz, 1H, 5'-H), 7.76 (d, $J=3.3$ Hz, 1H, 4'-H), 4.56 (m, 1H, a-H), 3.80 (t, $J=1.2$ Hz, 4H, b-H), 4.34 (s, 6H, N^+Me), 9.00 (d, $J=6$ Hz, 2H, 6-H), 7.98 (t, $J=1$ Hz, 2H, 5-H), 8.45 (t, $J=7$ Hz, 2H, 4-H), 7.93 (d, $J=8$ Hz, 2H, 3-H), MS: positive FAB ($\text{M}^{2+} = 301.1$). Anal. Calcd for $\text{C}_{18}\text{H}_{21}\text{I}_2\text{N}_3\text{S}$: C, 38.25; H, 3.74; N, 7.43; S, 5.67. Found: C, 38.33; H, 3.82; N, 7.40; S, 5.68.



12



13

Biological assays

Human cell lines (LNCap and MCF7). Human prostate adenocarcinoma cells (LNCap) were grown in RPMI 1640. Human mammary adenocarcinoma (MCF7) were grown in Dulbecco's MEM (DMEM), 1.0 g/l D-glucose. Each medium was supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 2mM L-Alanyl-L-Glutamine, penicillin-streptomycin (50 units-50 μg for ml) and incubated at 37 °C in humidified atmosphere of 5% CO_2 , 95% air. The culture medium was changed twice a week.

Treatment with antitumor agents and MTT colorimetric assay. Each human cancer cell line (5×10^3 cells/ 0.33cm^2) were plated in 96 well plates "Nunc TM Microwell TM" (Nunc) and were incubated at 37 °C. After 24 h, cells were treated with the compounds 1, 3, 4, 5, 6 and 11 (final concentration 0.01-100 μM). Untreated cells were used as controls. Microplates were incubated at 37 °C in humidified atmosphere of 5% CO_2 , 95% air for 3 days and then cytotoxicity was measured with colorimetric assay based on the use of tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide).¹² The results were read on a multiwell scanning spectrophotometer (Multiscan reader), using a wavelength of 570 nm. Each value was the average of 8 wells (standard deviations were less than 20%). The GI_{50} value was calculated according to NCI: thus, GI_{50} is the concentration of test compound where $100 \times (\text{T} - \text{T}_0)/(\text{C} - \text{T}_0) = 50$ (T is the optical density of the test well after a 48-h period of exposure to test drug; T_0 is the optical density at time zero; C is the control optical density).

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