Electronic Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Modeling, Design and Synthesis of new heteroaryl ethylenes active against MCF-7 breast cancer cell-line.

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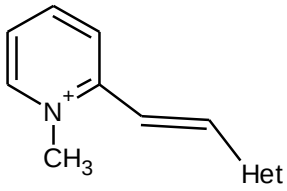
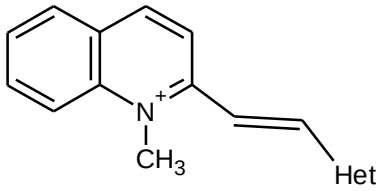
Table of Contents:

1. Structures of 50 compounds used to generate QSAR model

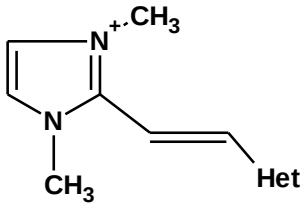
Scheme S1. Structures of 50 compounds used to generate QSAR model



compound	Het	SMILES
1	Furan-2-yl	c1(cccc[n+]1C)/C=C/c1occc1
2	5-Bromofuran-2-yl	c1(cccc[n+]1C)/C=C/c1oc(cc1)Br
3	5-(4-Bromophenyl)-furan-2-yl	c1(cccc[n+]1C)/C=C/c1oc(cc1)c1ccc(cc1)Br
4	5-(2-Chlorophenyl)-furan-2-yl	c1(cccc[n+]1C)/C=C/c1oc(cc1)c1c(cccc1)Cl
5	1-Methylpyrrol-2-yl	c1(cccc[n+]1C)/C=C/c1n(ccc1)C
6	Indol-5-yl	c1ccc([n+](c1)C)/C=C/c1cc2cc[nH]c2cc1
7	Indol-3-yl	c1ccc([n+](c1)C)/C=C/c1c[nH]c2ccccc12
8	5-Bromo-indol-3-yl	c1ccc([n+](c1)C)/C=C/c1c[nH]c2ccc(cc12)Br
9	1-Methyl-indol-3-yl	c1ccc([n+](c1)C)/C=C/c1cn(c2ccccc12)C
10	Indol-4-yl	c1ccc([n+](c1)C)/C=C/c1c2c(ccc1)[nH]cc2
11	5-(4-Chlorophenyl)-furan-2-yl	c1c(oc(c1)c1ccc(cc1)Cl)/C=C/c1cccc[n+]1C
12	5-(3-Chlorophenyl)-furan-2-yl	c1c(oc(c1)c1cc(ccc1)Cl)/C=C/c1ccc2c([n+]1C)cccc2
13	5-(2,5-dichlorophenyl)-furan-2-yl	c1c(oc(c1)c1c(ccc(c1)Cl)Cl)/C=C/c1cccc[n+]1C
14	5-(2,4-dichlorophenyl)-furan-2-yl	c1c(oc(c1)c1c(cc(cc1)Cl)Cl)/C=C/c1cccc[n+]1C
15	5-(3,4-dichlorophenyl)-furan-2-yl	c1c(oc(c1)c1cc(c(cc1)Cl)Cl)/C=C/c1cccc[n+]1C
16	5-(3-chloro-4-methoxy-phenyl)-furan-2-yl	c1c(oc(c1)c1cc(c(cc1)OC)Cl)/C=C/c1cccc[n+]1C
17	5-(2-chloro-5-trifluoromethyl-phenyl)-furan-2-yl	c(oc(c1)c1c(ccc(c1)C(F)(F)F)Cl)/C=C/c1ccc2ccccc2[n+]1C
18	3-phenyl-thiophen-2-yl	c1c(scc1c1ccccc1)/C=C/c1cccc[n+]1C
19	2-phenyl-thiophen-2-yl	c1(cccc[n+]1C)/C=C/c1ccc(s1)c1ccccc1
20	5-thiophen-thiophen-2-yl	c1(/C=C/c2ccc(s2)c2sccc2)cccc[n+]1C

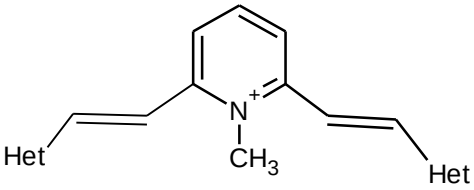


compound	Het	SMILES
21	Indol-5-yl	c1cc([n+](c2c1cccc2)C)/C=C/c1ccc2c(c1)cc[nH]2
22	Indol-3-yl	c1cc([n+](c2c1cccc2)C)/C=C/c1c2c([nH]c1)cccc2
23	1-Methyl-indol-3-yl	c1cc([n+](c2c1cccc2)C)/C=C/c1c2c(n(c1)C)cccc2
24	Indol-4-yl	c1cc([n+](c2c1cccc2)C)/C=C/c1c2cc[nH]c2ccc1
25	5-Bromofuran-2-yl	c1c(oc(c1)Br)/C=C/c1ccc2c([n+]1C)cccc2
26	5-(4-Chlorophenyl)-furan-2-yl	c1c(oc(c1)c1ccc(cc1)Cl)/C=C/c1ccc2c([n+]1C)cccc2
27	5-(3-Chlorophenyl)-furan-2-yl	c1c(oc(c1)c1cc(ccc1)Cl)/C=C/c1ccc2c([n+]1C)cccc2
28	5-(3-chloro-4-methoxy-phenyl)-furan-2-yl	c1c(oc(c1)c1cc(c(cc1)OC)Cl)/C=C/c1ccc2ccccc2[n+]1C
29	5-(2-nitro-4-chloro-phenyl)-furan-2-yl	c1c(oc(c1)c1c(cc(cc1)Cl)[N+](=O)[O-])/C=C/c1ccc2ccccc2[n+]1C
30	5-chloro-thiophen-2-yl	c1c(sc(c1)Cl)/C=C/c1ccc2c([n+]1C)cccc2
31	3-phenyl-thiophen-2-yl	c1c(scc1c1ccccc1)/C=C/c1[n+](c2ccccc2cc1)C
32	2-phenyl-thiophen-2-yl	c1(/C=C/c2ccc(s2)c2ccccc2)ccc2c([n+]1C)cccc2
33	5-thiophen-thiophen-2-yl	c1(/C=C/c2ccc(s2)c2sccc2)ccc2c([n+]1C)cccc2



compound	Het	SMILES
34	Furan-2-yl	o1cccc1/C=C/c1[n+](ccn1C)C
35	5-methyl-furan-2-yl	o1c(ccc1/C=C/c1[n+](ccn1C)C)C
36	5-Bromofuran-2-yl	o1c(ccc1/C=C/c1[n+](ccn1C)C)Br
37	5-(2-Chlorophenyl)-furan-2-yl	o1c(ccc1/C=C/c1[n+](ccn1C)C)c1ccccc1Cl
38	5-(4-bromophenyl)-furan-2-yl	o1c(ccc1/C=C/c1[n+](ccn1C)C)c1ccc(cc1)Br

39	5-bromo-thiophen-2-yl	C(=C\c1[n+](ccn1C)C)/c1sc(cc1)Br
40	N-methylpyrrol-2-yl	C(=C\c1[n+](ccn1C)C)/c1n(ccc1)C
41	5-(4-chlorophenyl)-furan-2-yl	c1c(oc(c1)c1ccc(cc1)Cl)/C=C/c1[n+](ccn1C)C
42	5-(3-chlorophenyl)-furan-2-yl	c1c(oc(c1)c1cc(ccc1)Cl)/C=C/c1[n+](ccn1C)C
43	5-(2,5-dichlorophenyl)-furan-2-yl	c1c(oc(c1)c1c(ccc(c1)Cl)Cl)/C=C/c1[n+](ccn1C)C
44	5-(3-chloro-4-methoxy-phenyl)-furan-2-yl	c1c(oc(c1)c1cc(c(cc1)OC)Cl)/C=C/c1[n+](cc[nH]1)C
45	3-phenyl-thiophen-2-yl	c1c(scc1c1ccccc1)/C=C/c1[n+](ccn1C)C
46	2-phenyl-thiophen-2-yl	[n+]1(c(n(cc1)C)/C=C/c1ccc(s1)c1ccccc1)C
47	5-thiophen-thiophen-2-yl	c1(/C=C/c2ccc(s2)c2sccc2)[n+](ccn1C)C



compound	Het	SMILES
48	furan-2-yl	[n+]1(c(cccc1/C=C/c1ccco1)/C=C/c1ccco1)C
49	thiazol-2-yl	[n+]1(c(cccc1/C=C/c1nccs1)/C=C/c1nccs1)C
50	1-methyl-pyrrol-2-yl	[n+]1(c(cccc1/C=C/c1cccn1C)/C=C/c1cccn1C)C

2. In vitro activity values against MCF-7 for the 50 compounds

Table S1. *In vitro* activity values, expressed as $logGI_{50}$, against MCF-7 for the 50 compounds

Compounds	MCF-7	Compounds	MCF-7
1	-5	**28**	-6,9
2	-5,1	**29**	-6,87
3	-7,75	**30**	-5,76
4	-5,65	**31**	-7,24
5	-4,82	**32**	-7,42
6	-4	**33**	-6,72
7	-5	**34**	-6,9
8	-5,4	**35**	-6,42
9	-5	**36**	-7,18
10	-4,7	**37**	-6,65
11	-5,6	**38**	-6,72
12	-5,76	**39**	-5,76
13	-6,38	**40**	-6,48
14	-5	**41**	-5,68
15	-4	**42**	-6,28
16	-4,48	**43**	-6
17	-4,4	**44**	-6,3
18	-5,86	**45**	-5,08
19	-6,21	**46**	-5,84
20	-4,84	**47**	-6,25
21	-4	**48**	-5,76
22	-6,75	**49**	-5,33
23	-5,2	**50**	-5,85
24	-6,75		
25	-5,5		
26	-6,66		
27	-6,2		

3. Computational methods

VolSurf+ descriptors

The interaction of molecules with biological membranes is mediated by surface properties such as shape, electrostatic forces, H-bonds and hydrophobicity. Therefore, the GRID[1] force field was chosen to characterize potential polar and hydrophobic interaction sites around target molecules by the water (OH_2), the hydrophobic (DRY), and the carbonyl oxygen (O) and amide nitrogen (N1) probe. The information contained in the MIF is transformed into a quantitative scale by calculating the volume or the surface of the interaction contours. The VolSurf+ procedure is as follows: i) in the first step, the 3D molecular field is generated from the interactions of the OH_2, the DRY, O and N1 probe around a target molecule; ii) the second step consists in the calculation of descriptors from the 3D maps obtained in the first step. The molecular descriptors obtained, called VolSurf+ descriptors, refer to molecular size and shape, to hydrophilic and hydrophobic regions and to the balance between them, to molecular diffusion, LogP, LogD, to the "*charge state"* descriptors, to the new 3D pharmacophoric descriptors and to some descriptors on some rilevant ADME properties. The definition of all 128 VolSurf+ descriptors is given in[2-6] (case studies with the old versions of VolSurf) and reported in detail in Table 5; iii) finally, chemometric tools (PCA[7], PLS[8]) are used to create relationships of the VolSurf+ descriptor matrix with ADME properties. The scheme of the VolSurf+ programme steps and a detailed definition of Volsurf+ descriptors have recently been reported[9].

4. Synthesis and spectroscopic characterization of new synthesized compounds

Materials and equipment: Solvents, and reagents used were of analytical grade and were purchased from Aldrich Chemical Company. 1,2,3-trimethyl imidazolium, 1,2-dimethyl quinolinium and 1,2-dimethyl pyridinium iodide salts were synthesized following previously published method[10]. Reactions were monitored by TLC using precoated silica gel aluminum plates containing a fluorescent indicator (Macherey-Nagel). ^{1}H and ^{13}C NMR spectra were recorded at 27°C using a Varian Inova 500 spectrometer. Chemical shifts (δ) are expressed in ppm and referenced to residual undeuterated solvent. NMR data were processed using MestReC software[11]. Two-dimensional (2D) NMR experiments (gCOSY; gHSQCAD; gHMBCAD) were carried out on all new compounds using the pulse sequences from the Varian user library. On the basis of 2DNMR analyses, complete assignments of ^{1}H and ^{13}C signals were obtained (see Electronic Supplementary Material).

The synthesis of compounds **5** and **7-9** has been already published[12].

*4.1.1. General procedure for preparation of compounds **1-4 ,6**.*

The heteroaromatic aldehyde (0.5 mmol) was solubilized in ethanol (3 ml); the appropriate heteroaromatic salts and few drops of piperidine were added. The mixture was kept under stirring at 50 °C for 6 h. At the end of the reaction, the solvent was evaporated under vacuum. The residue was recrystallized from methanol to give the pure compounds.

1,3-dimethyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-1H-imidazolium iodide **(1)**

Following the general procedure, the reaction of 1,2,3-trimethyl imidazolium iodide (112 mg, 0.48 mmol) gave product **1** as white needles (146 mg, 76%): ^{1}H NMR (DMSO-d6, 500 MHz) δ 9.24 (s, 2H, H_4-H_6), 9.23 (s, 1H, H_2), 7.99 (s, 4H, H_8-H_9, H_{11}-H_{12}), 7.76 (s, 2H, H_{17}-H_{18}), 7.63 (d, J=16.8 Hz, 1H, H_{14}), 7.40 (d, J=16.8 Hz, 1H, H_{13}), 3.97 (s, 6H, H_{21}-H_{20}) ppm. ^{13}C NMR (DMSO-d6, 125 MHz) δ 157.49, 154.67, 141.76, 141.29, 135.30, 135.08, 132.20, 128.75, 127.24, 123.50, 108.76, 36.06 ppm.

1-methyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-quinolinium iodide **(2)**

Following the general procedure, the reaction of 1,2-dimethyl quinolinium iodide (128 mg, 0.47 mmol) gave product **2** as brown needles (185 mg, 86%): ^{1}H NMR (DMSO-d6, 500 MHz) δ 9.28 (s, 2H, H_4-H_6), 9.26 (s, 1H, H_2), 9.13 (d, J=8.9 Hz, 1H, H_{20}), 8.61 (d, J=8.6 Hz, 1H, H_{17}), 8.59 (d, J=8.6 Hz, 1H, H_{16}), 8.39 (d, J=7.9 Hz, 1H, H_{23}), 8.27 (d, J=15.9 Hz, 1H, H_{14}), 8.22 (t, J=7.6 Hz, 1H, H_{21}), 8.16 (d, J=7.8 Hz, 2H, H_9-H_{11}), 8.07 (d, J=15.9 Hz, 1H, H_{13}), 8.04 (d, J=7.8 Hz, 2H, H_8-H_{12}), 7.98 (d, J=7.6 Hz, 1H, H_{22}), 4.61 (s, 3H, H_{25}) ppm. ^{13}C NMR (DMSO-d6, 125 MHz) δ 157.61, 155.91, 154.78, 145.72, 144.32, 139.16, 136.18, 135.24, 134.96, 132.11, 130.02, 129.88, 129.07, 127.89, 127.39, 121.17, 120.18, 119.32, 39.10 ppm.

1-methyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-pyridinium iodide **(3)**

Following the general procedure, the reaction of 1,2-dimethyl pyridinium iodide (110 mg, 0.47 mmol) gave product **3** as yellow needles (149 mg, 79%): %): ^{1}H NMR (DMSO-d6, 500 MHz) δ 9.25 (s, 2H, H_4-H_6), 9.23 (s, 1H, H_2), 8.92 (d, J=8.3 Hz, 1H, H_{19}), 8.53 (m, 2H, H_{16}-H_{17}), 8.03 (d, J=16.0 Hz, 1H,

H_{14}), 8.01 (m, 4H, H_9-H_{11}, H_8-H_{12}), 7.93 (m, 1H, H_{18}), 7.72 (d, J=16.0 Hz, 1H, H_{13}), 4.40 (s, 3H, H_{21}) ppm. ^{13}C NMR (DMSO-d6, 125 MHz) δ 157.53, 154.71, 152.10, 146.11, 144.30, 141.86, 135.57, 135.27, 132.18, 129.29, 127.33, 125.26, 124.94, 118.30, 46.03 ppm.

(E)-2-(4-(dimethylamino)styryl)-1-methylpyridinium iodide **(4)**

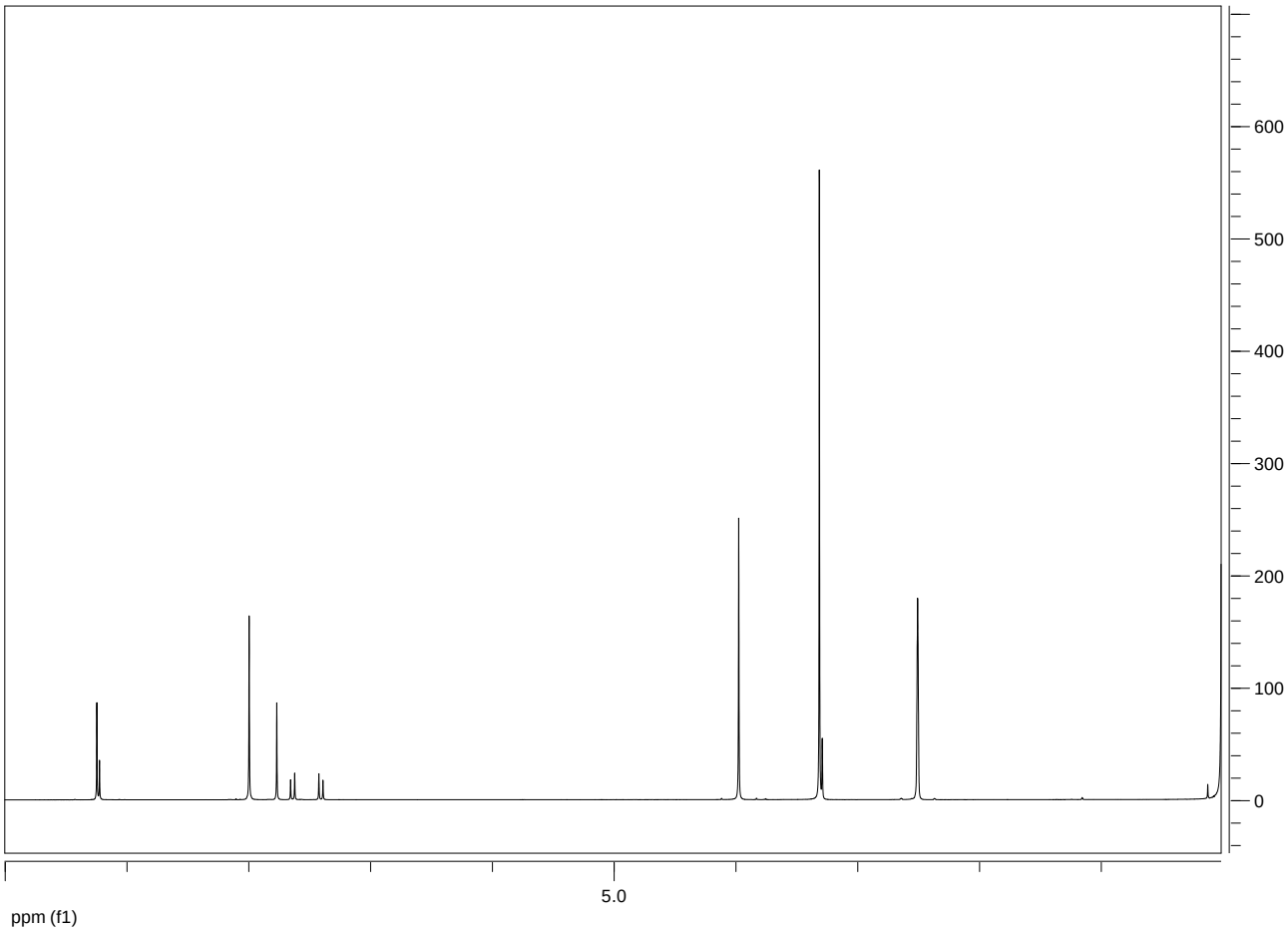
4-(dimethylamino)benzaldehyde and 2,2'-bithiophene-5-carbaldehyde were Aldrich commercial products. Compound **4** was prepared by a modification of the literature procedure[13].

1,2-dimethylpyridinium iodide (0.470 g, 2.00 mmol) and 4-(dimethylamino)benzaldehyde (0.313 g, 2.10 mmol) were dissolved in warm absolute ethanol (5 mL). Piperidine (0.020 mL, 0.200 mmol) was added, and the solution obtained refluxed overnight, under nitrogen atmosphere. After cooling, the mixture was filtered to give needles, which were washed with diethyl ether and dried under vacuum. Yield: 0.292 g. 40%, red-purple needles, m.p.:271-272°C, ^{1}H NMR (DMSO-d_6): δ = 3.03 (s, 6 H, ArNCH_3), 4.29 (s, 3 H, PyN$^+$CH_3), 6.79 (d, $^3J_{H\text{-}H}$ = 9.0 Hz, 2 H, Ar*H*), 7.23 (d, $^3J_{H\text{-}H}$ = 15.5 Hz, 1 H, C*H*=), 7.70 (m, 3 H, Py*H*+Ar*H*), 7.90 (d, $^3J_{H\text{-}H}$ = 15.5 Hz, 1 H, C*H*=), 8.34 (t, $^3J_{H\text{-}H}$ = 8.0 Hz, 1 H, Py*H*), 8.43 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1 H, Py*H*), 8.73 (d, $^3J_{H\text{-}H}$ = 6.5 Hz, 1 H, Py*H*), MS (positive ESI): *m/z* = 240 [M-I]$^+$.

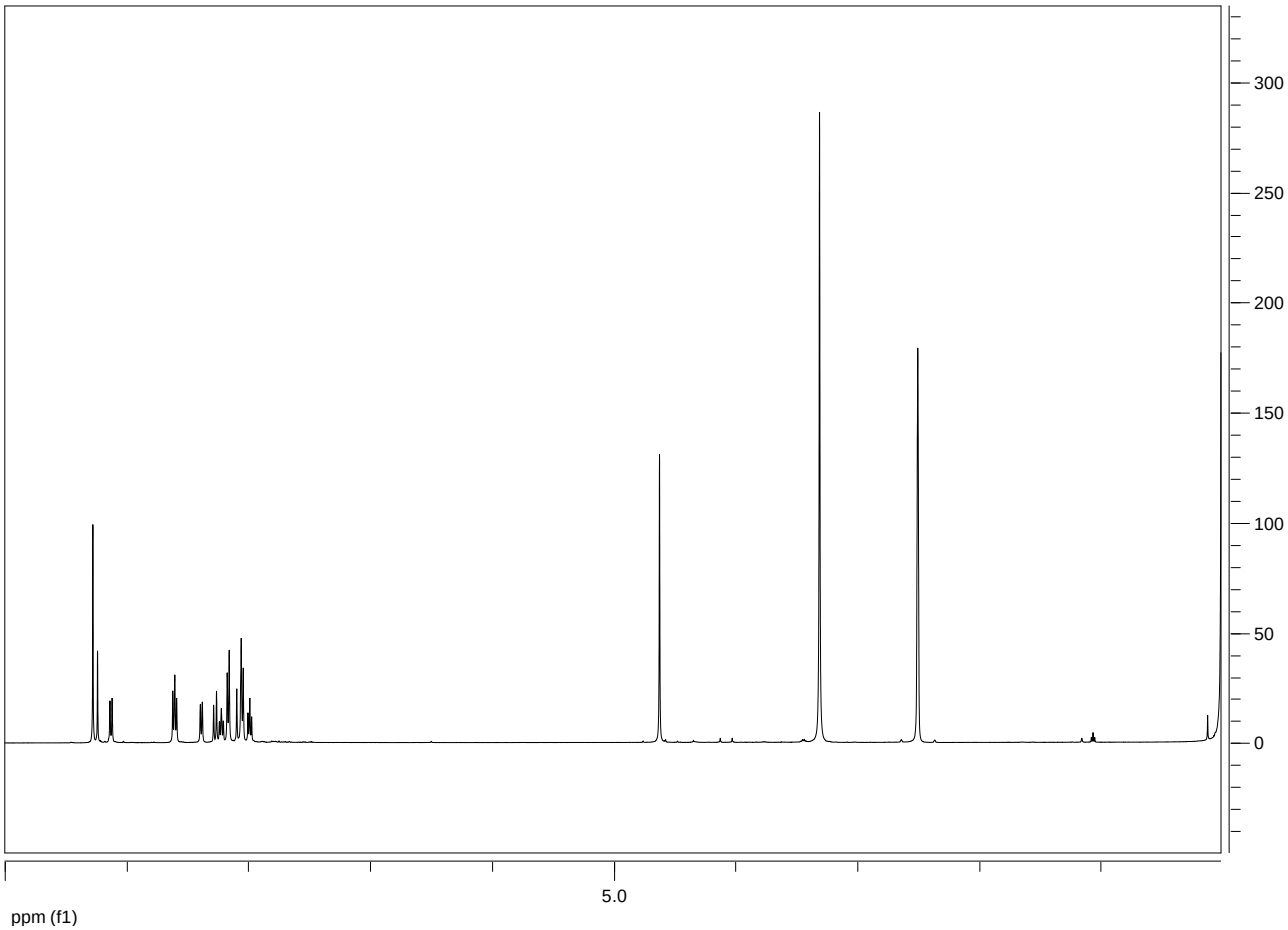
1-methyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-quinolinium iodide **(6)**

Following the general procedure, the reaction of 1,2-dimethyl quinolinium iodide (128 mg, 0.47 mmol) gave product **6** as black powder (203 mg, 76%): ^{1}H NMR (DMSO-d6, 500 MHz) δ 8.66 (d, J=9.4 Hz, 1H, H_{24}), 8.58 (d, J=15.1 Hz, 1H, H_{17}), 8.43 (d, J=8.0 Hz, 1H, H_{21}), 8.09 (d, J=8.9 Hz, 1H, H_{27}), 7.89 (t, J=8.9 Hz, 1H, H_{25}), 7.83 (d, J=8.2 Hz, 1H, H_{20}), 7.74 (d, J=4.0 Hz, 1H, H_4), 7.58 (t, J=7.4 Hz, 1H, H_{26}), 7.05 (s 1H, H_{15}), 7.03 (d, J=15.1 Hz, 1H, H_{18}), 6.65 (s, 1H, H_{16}), 5.98 (d, J=4.0 Hz, 1H, H_3), 4.54, (s, 3H, H_{29}), 3.25 (m, 4H, $H_{7\text{-}11}$), 1.76 (m, 4H, $H_{9\text{-}10}$), 1.65 (m, 2H, H_8) ppm; ^{13}C NMR (DMSO-d6, 125 MHz) δ162.30, 155.51, 148.26, 142.83, 141.89, 141.86, 139.22, 135.93, 134.26, 129.87, 128.23, 127.85, 126.95, 122.10, 121.46, 120.16, 117.988, 111.91, 110.07, 108.22, 104.85, 51.57, 40.50, 25.04, 23.60 ppm.

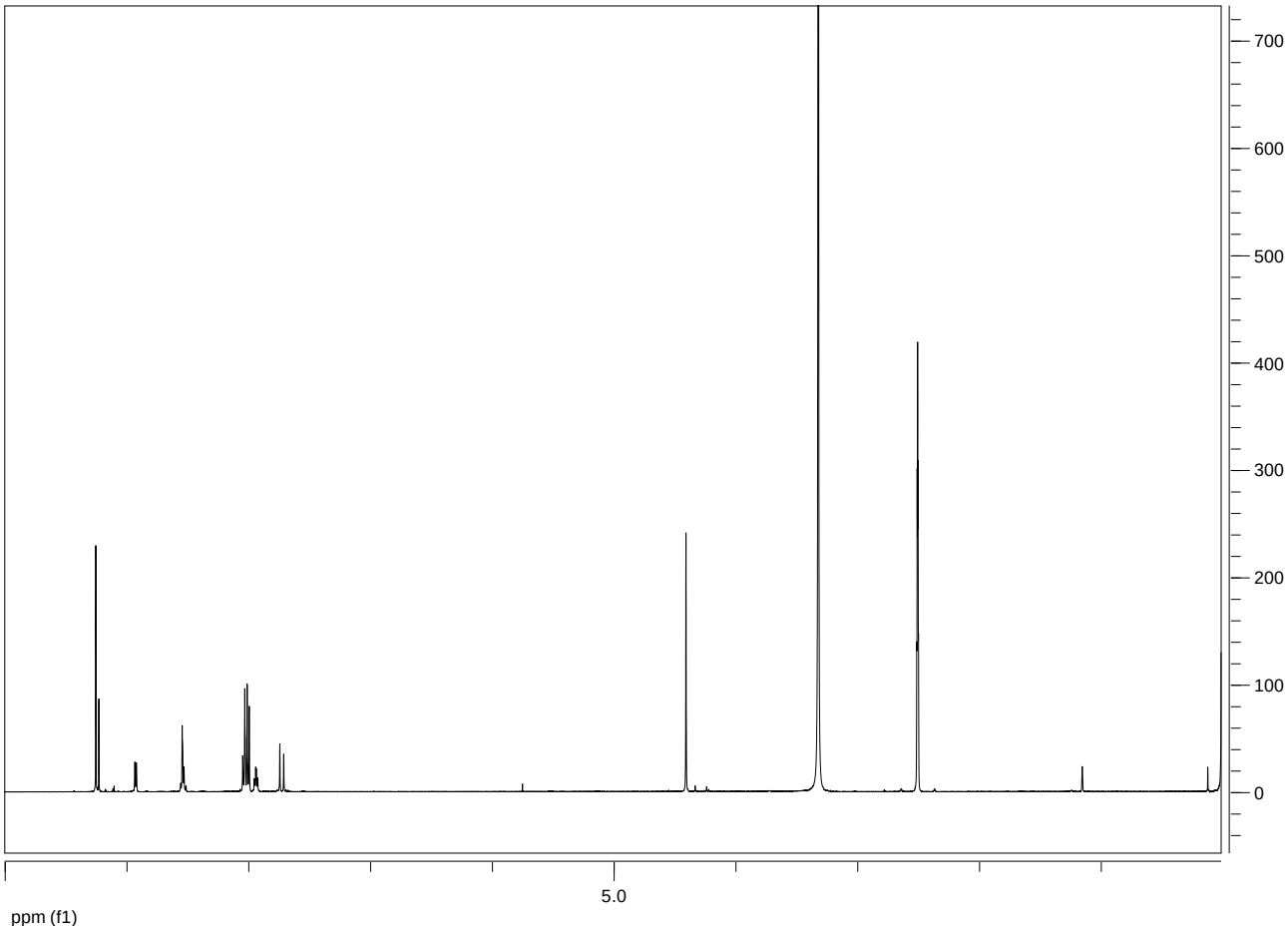
5. ^{1}H-NMR spectra



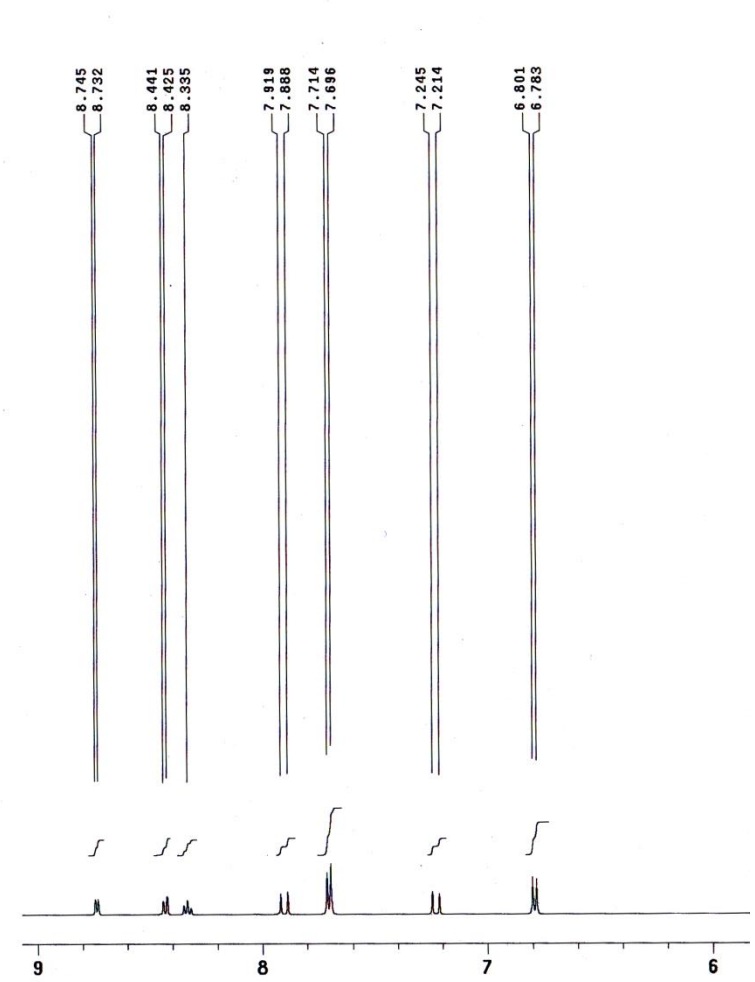
^{1}H NMR spectrum of 1,3-dimethyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-1H-imidazolium iodide **(1)**



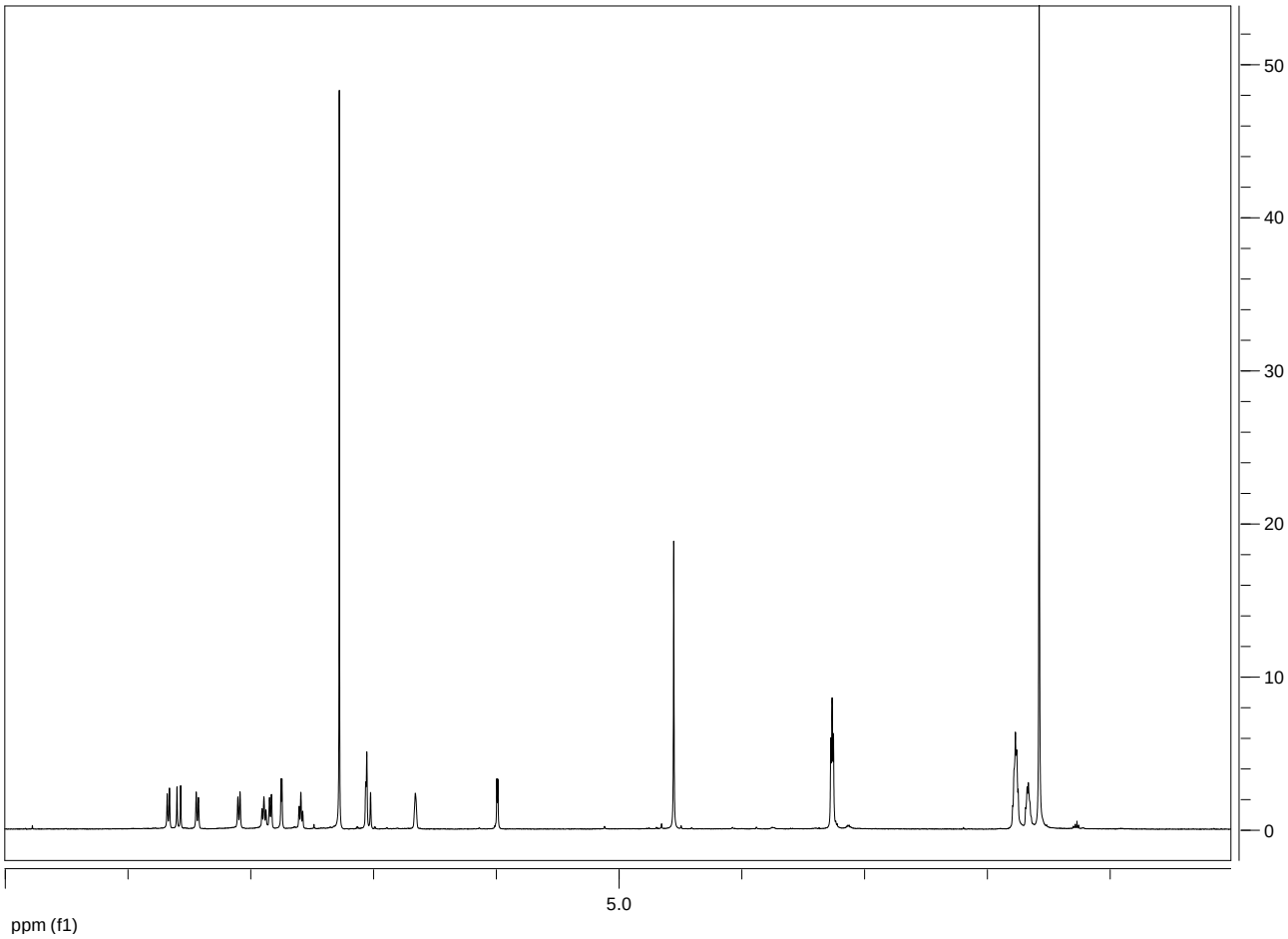
^{1}H NMR spectrum of 1-methyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-quinolinium iodide **(2)**



^{1}H NMR spectrum of 1-methyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-pyridinium iodide **(3)**



(E)-2-(4-(dimethylamino)styryl)-1-methylpyridinium iodide **(4)**



^{1}H NMR spectrum of 1-methyl-2-[(E)-2-(4-pyrimidin-5-ylphenyl)vinyl]-quinolinium iodide **(6)**

6. Biological essays

Human cell lines (MCF7). 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% CO2, 95% air. The culture medium was changed twice a week.

Treatment with antitumor agents and MTT colorimetric assay. Human cancer cell line (5x103 cells/0.33cm2) were plated in 96 well plates "Nunclon TM Microwell TM" (Nunc) and were incubated at 37 °C. After 24 h, cells were treated with the compounds (final concentration 0.01-100 µM). Untreated cells were used as controls. Microplates were incubated at 37 °C in humidified atmosphere

of 5% CO2, 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)[14]. 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 x (T - T0)/(C-T0) = 50 (T is the optical density of the test well after a 48-h period of exposure to test drug; T0 is the optical density at time zero; C is the control optical density).

7. Laser Scanning Confocal Microscopy

MCF-7 cell line (5 x 10^4cells/1.99 cm^2 growth area) was plated in Nunclon TM 24-well plates (Nunc, Roskilde,Denmark).

LSM observations were carried out by using an Olympus FV1000 confocal laser scanning microscope equipped with Ar multiline laser, HeNe lasers and spectral filtering system. Images were recorded by using an objective lens UPLAPO 20X (NA:0.70). Excitation wavelengths were set at 543 nm and 633, and emitted lights were detected at 598 nm and 647 nm, respectively. The detector gain was fixed at a constant value, and images were taken for all of the samples, at random locations throughout the area of the well.

8. LC_{50} values

Table S2. *In vitro* citotoxicity, expressed as log LC_{50}, for MCF7 cell line.

Cmpds/ Cell line	**5**	**6**	**7**	**9**
MCF-7	**-4.00**	**-4.00**	**-4.00**	**-4.00**

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