Synthesis, computational studies, and antiproliferative of thienylpicolinamidines

Mohamed A. Ismail [a,*], Ghada A. Abdelwahab [a], Mohamed H. Abdel-Rhman [a], Wafaa S. Hamama [a], Heba M. El-Shafeai[a], Wael M. El-Sayed [b,*]

[a]Department of Chemistry, Faculty of Science, Mansoura University, Mansoura 35516, Egypt.

[b]University of Ain Shams, Faculty of Science, Department of Zoology, Abbassia 11566, Cairo, Egypt.

Supplementary Material

Contents:

I. Tables

II. Figures

Table S1: Sequences of primers used for the Real-time PCR analysis

Gene	Forward primer	Reverse primer
p53	CTGCCCTCAACAAGATGTTTTG	CTATCTGAGCAGCGCTCATGG
Cdk1	CCGAAATCTGCCAGTTTGAT	CTGGCCAGTTCATGGATTCT
Caspase-3	TTAATAAAGGTATCCATGGAGAACACT	TAGTGATAAAAATAGAGTTCTTTTGTGAG
Topo II	TGCCTGTTTAGTCGCTTTC	TGAGGTGGTCTTAAGAAT
Txnrd1	GTTACTTGGGCATCCCTGGTGA	CGCACTCCAAAGCGACATAGGA
GAPDH	TCAAGAAGGTGGTGAAGCAG	AGGTGGAAGAATGGGAGTTG

Table S2. The DFT bond length for picolinamidines **4a-c** in comparison with x-ray values

4a		4b		4c		X-ray
Bond	Å	Bond	Å	Bond	Å	Å
N(20)-H(35)	1.01	N(20)-H(35)	1.01	N(20)-H(37)	1.01	0.875-0.909
N(20)-H(34)	1.01	N(20)-H(34)	1.01	N(20)-H(36)	1.01	0.875-0.909
N(19)-H(33)	1.02	N(19)-H(33)	1.02	N(19)-H(35)	1.02	0.859-0.957
N(19)-H(32)	1.01	N(19)-H(32)	1.01	N(19)-H(34)	1.01	0.859-0.957
C(18)-N(20)	1.34	C(18)-N(20)	1.33	C(18)-N(20)	1.33	1.31
C(18)-N(19)	1.33	C(18)-N(19)	1.32	C(18)-N(19)	1.33	1.31
C(15)-C(18)	1.46	C(15)-C(18)	1.46	C(15)-C(18)	1.46	
N(16)-C(17)	1.31	N(16)-C(17)	1.32	N(16)-C(17)	1.31	1.357-1.372
C(15)-N(16)	1.35	C(15)-N(16)	1.35	C(15)-N(16)	1.35	1.342-1.393
C(14)-C(15)	1.40	C(14)-C(15)	1.40	C(14)-C(15)	1.40	1.342-1.393
C(13)-C(14)	1.38	C(13)-C(14)	1.38	C(13)-C(14)	1.38	1.342-1.393
C(7)-C(17)	1.43	C(7)-C(17)	1.43	C(7)-C(17)	1.43	1.342-1.393
C(7)-C(13)	1.41	C(7)-C(13)	1.41	C(7)-C(13)	1.41	1.342-1.393
C(11)-C(12)	1.39	C(11)-C(12)	1.39	C(11)-C(12)	1.39	1.37-1.392
C(10)-C(11)	1.40	C(10)-C(11)	1.40	C(10)-C(11)	1.42	1.37-1.392
C(9)-C(10)	1.41	C(9)-C(10)	1.41	C(9)-C(10)	1.41	1.37-1.392
C(8)-C(9)	1.38	C(8)-C(9)	1.38	C(8)-C(9)	1.40	1.37-1.392
C(6)-C(12)	1.41	C(6)-C(12)	1.40	C(6)-C(12)	1.41	1.37-1.392
C(6)-C(8)	1.41	C(6)-C(8)	1.41	C(6)-C(8)	1.40	1.37-1.392
C(1)-C(6)	1.45	C(4)-C(6)	1.46	C(1)-C(6)	1.46	1.416-1.472
C(4)-C(7)	1.44	C(2)-C(7)	1.44	C(4)-C(7)	1.44	1.423-1.452
C(1)-S(5)	1.75	S(3)-C(4)	1.75	C(1)-S(5)	1.74	1.718-1.732
C(4)-S(5)	1.76	C(2)-S(3)	1.76	C(4)-S(5)	1.76	1.718-1.732
C(3)-C(4)	1.39	C(4)-C(5)	1.39	C(3)-C(4)	1.39	1.363-1.436
C(2)-C(3)	1.40	C(1)-C(5)	1.40	C(2)-C(3)	1.40	1.363-1.436

C(1)-C(2)	1.39	C(1)-C(2)	1.39	C(1)-C(2)	1.39	1.363-1.436
C(10)-O(21)	1.35	C(10)-O(22)	1.34	C(11)-O(21)	1.36	1.364
				C(10)-O(23)	1.36	1.364
				C(9)-O(25)	1.36	1.364
				O(25)-C(26)	1.44	1.415
O(21)-C(22)	1.43	O(22)-C(23)	1.43	O(23)-C(24)	1.44	1.415
				O(21)-C(22)	1.43	1.415
		C(9)-Cl(21)	1.74			1.74

Table S3. The dihedral angle data for the new picolinamidines **4a-c**.

4a		**4b**		**4c**	
Dihedral angle	**°**	**Dihedral angle**	**°**	**Dihedral angle**	**°**
C(15)-C(18)-N(20)-H(34)	179.57	C(15)-C(18)-N(20)-H(34)	-179.94	C(15)-C(18)-N(20)-H(36)	-179.95
C(15)-C(18)-N(20)-H(35)	-1.21	C(15)-C(18)-N(20)-H(35)	0.09	C(15)-C(18)-N(20)-H(37)	-0.21
C(14)-C(15)-C(18)-N(20)	-1.28	C(14)-C(15)-C(18)-N(20)	0.32	C(14)-C(15)-C(18)-N(20)	-0.15
N(16)-C(15)-C(18)-N(20)	178.79	N(16)-C(15)-C(18)-N(20)	-179.75	N(16)-C(15)-C(18)-N(20)	179.86
C(15)-C(18)-N(19)-H(32)	179.11	C(15)-C(18)-N(19)-H(32)	-179.95	C(15)-C(18)-N(19)-H(34)	179.96
C(15)-C(18)-N(19)-H(33)	0.41	C(15)-C(18)-N(19)-H(33)	0.01	C(15)-C(18)-N(19)-H(35)	0.01
C(14)-C(15)-C(18)-N(19)	178.63	C(14)-C(15)-C(18)-N(19)	-179.72	C(14)-C(15)-C(18)-N(19)	179.86
N(16)-C(15)-C(18)-N(19)	-1.30	N(16)-C(15)-C(18)-N(19)	0.20	N(16)-C(15)-C(18)-N(19)	-0.14
N(19)-C(18)-N(20)-H(34)	-0.34	N(19)-C(18)-N(20)-H(34)	0.11	N(19)-C(18)-N(20)-H(36)	0.05
N(19)-C(18)-N(20)-H(35)	178.88	N(19)-C(18)-N(20)-H(35)	-179.87	N(19)-C(18)-N(20)-H(37)	179.79
N(20)-C(18)-N(19)-H(32)	-0.98	N(20)-C(18)-N(19)-H(32)	0.01	N(20)-C(18)-N(19)-H(34)	-0.03
N(20)-C(18)-N(19)-H(33)	-179.67	N(20)-C(18)-N(19)-H(33)	179.96	N(20)-C(18)-N(19)-H(35)	-179.99
C(18)-C(15)-N(16)-C(17)	-179.85	C(18)-C(15)-N(16)-C(17)	179.90	C(18)-C(15)-N(16)-C(17)	-179.98
C(13)-C(14)-C(15)-C(18)	179.96	C(13)-C(14)-C(15)-C(18)	-179.78	C(13)-C(14)-C(15)-C(18)	179.86
S(5)-C(4)-C(7)-C(13)	3.01	S(3)-C(2)-C(7)-C(13)	4.37	S(5)-C(4)-C(7)-C(13)	-2.93
S(5)-C(4)-C(7)-C(17)	-176.88	S(3)-C(2)-C(7)-C(17)	-175.57	S(5)-C(4)-C(7)-C(17)	176.90
C(3)-C(4)-C(7)-C(13)	-177.00	C(1)-C(2)-C(7)-C(13)	-175.37	C(3)-C(4)-C(7)-C(13)	177.31
C(3)-C(4)-C(7)-C(17)	3.11	C(1)-C(2)-C(7)-C(17)	4.69	C(3)-C(4)-C(7)-C(17)	-2.86
C(4)-C(7)-C(17)-N(16)	179.92	C(2)-C(7)-C(17)-N(16)	-179.59	C(4)-C(7)-C(17)-N(16)	179.95
C(4)-C(7)-C(13)-C(14)	-179.81	C(2)-C(7)-C(13)-C(14)	179.73	C(4)-C(7)-C(13)-C(14)	179.92
C(7)-C(4)-S(5)-C(1)	-179.33	C(7)-C(2)-S(3)-C(4)	-179.05	C(7)-C(4)-S(5)-C(1)	179.41
C(2)-C(3)-C(4)-C(7)	179.48	C(5)-C(1)-C(2)-C(7)	179.15	C(2)-C(3)-C(4)-C(7)	-179.66
S(5)-C(1)-C(6)-C(8)	-20.06	S(3)-C(4)-C(6)-C(8)	-20.57	S(5)-C(1)-C(6)-C(8)	24.72
S(5)-C(1)-C(6)-C(12)	159.97	S(3)-C(4)-C(6)-C(12)	159.49	S(5)-C(1)-C(6)-C(12)	-155.68
C(1)-C(6)-C(8)-C(9)	-179.88	C(4)-C(6)-C(8)-C(9)	-179.87	C(1)-C(6)-C(8)-C(9)	179.35
C(1)-C(6)-C(12)-C(11)	179.86	C(4)-C(6)-C(12)-C(11)	179.80	C(1)-C(6)-C(12)-C(11)	179.69
C(6)-C(1)-S(5)-C(4)	179.92	C(2)-S(3)-C(4)-C(6)	179.88	C(6)-C(1)-S(5)-C(4)	-179.57
C(6)-C(1)-C(2)-C(3)	179.84	C(6)-C(4)-C(5)-C(1)	179.85	C(6)-C(1)-C(2)-C(3)	179.77
C(2)-C(1)-C(6)-C(8)	160.63	C(5)-C(4)-C(6)-C(8)	160.09	C(2)-C(1)-C(6)-C(8)	-155.76
C(2)-C(1)-C(6)-C(12)	-19.34	C(5)-C(4)-C(6)-C(12)	-19.85	C(2)-C(1)-C(6)-C(12)	23.85

C(14)-C(15)-N(16)-C(17)	0.22	C(14)-C(15)-N(16)-C(17)	-0.18	C(14)-C(15)-N(16)-C(17)	0.02
C(13)-C(7)-C(17)-N(16)	0.03	C(13)-C(7)-C(17)-N(16)	0.47	C(13)-C(7)-C(17)-N(16)	-0.21
C(13)-C(14)-C(15)-N(16)	-0.12	C(13)-C(14)-C(15)-N(16)	0.30	C(13)-C(14)-C(15)-N(16)	-0.14
C(15)-N(16)-C(17)-C(7)	-0.18	C(15)-N(16)-C(17)-C(7)	-0.23	C(15)-N(16)-C(17)-C(7)	0.17
C(7)-C(13)-C(14)-C(15)	-0.04	C(7)-C(13)-C(14)-C(15)	-0.03	C(7)-C(13)-C(14)-C(15)	0.09
C(17)-C(7)-C(13)-C(14)	0.08	C(17)-C(7)-C(13)-C(14)	-0.33	C(17)-C(7)-C(13)-C(14)	0.08
S(5)-C(1)-C(2)-C(3)	0.47	S(3)-C(4)-C(5)-C(1)	0.45	S(5)-C(1)-C(2)-C(3)	-0.67
C(2)-C(1)-S(5)-C(4)	-0.65	C(2)-S(3)-C(4)-C(5)	-0.67	C(2)-C(1)-S(5)-C(4)	0.83
C(3)-C(4)-S(5)-C(1)	0.67	C(1)-C(2)-S(3)-C(4)	0.73	C(3)-C(4)-S(5)-C(1)	-0.79
C(1)-C(2)-C(3)-C(4)	0.04	C(2)-C(1)-C(5)-C(4)	0.11	C(1)-C(2)-C(3)-C(4)	0.07
C(2)-C(3)-C(4)-S(5)	-0.53	C(5)-C(1)-C(2)-S(3)	-0.62	C(2)-C(3)-C(4)-S(5)	0.56
C(8)-C(6)-C(12)-C(11)	-0.11	C(8)-C(6)-C(12)-C(11)	-0.15	C(8)-C(6)-C(12)-C(11)	-0.70
C(12)-C(6)-C(8)-C(9)	0.09	C(12)-C(6)-C(8)-C(9)	0.07	C(12)-C(6)-C(8)-C(9)	-0.26
C(6)-C(8)-C(9)-C(10)	-0.02	C(6)-C(8)-C(9)-C(10)	0.08	C(6)-C(8)-C(9)-C(10)	0.56
C(8)-C(9)-C(10)-C(11)	-0.04	C(8)-C(9)-C(10)-C(11)	-0.15	C(8)-C(9)-C(10)-C(11)	0.09
C(9)-C(10)-C(11)-C(12)	0.02	C(9)-C(10)-C(11)-C(12)	0.07	C(9)-C(10)-C(11)-C(12)	-1.05
C(10)-C(11)-C(12)-C(6)	0.05	C(10)-C(11)-C(12)-C(6)	0.07	C(10)-C(11)-C(12)-C(6)	1.35
C(9)-C(10)-O(21)-C(22)	-179.50	C(9)-C(10)-O(22)-C(23)	-179.38	C(9)-C(10)-O(23)-C(24)	126.29
C(11)-C(10)-O(21)-C(22)	0.53	C(11)-C(10)-O(22)-C(23)	0.68	C(11)-C(10)-O(23)-C(24)	-60.19
O(21)-C(10)-C(11)-C(12)	179.99	O(22)-C(10)-C(11)-C(12)	-179.99	O(23)-C(10)-C(11)-C(12)	-174.56
C(8)-C(9)-C(10)-O(21)	179.99	C(6)-C(8)-C(9)-Cl(21)	179.57	C(8)-C(9)-C(10)-O(23)	173.83
		C(8)-C(9)-C(10)-O(22)	179.91	C(9)-C(10)-C(11)-O(21)	176.61
		Cl(21)-C(9)-C(10)-C(11)	-179.65	O(23)-C(10)-C(11)-O(21)	3.09
		Cl(21)-C(9)-C(10)-O(22)	0.41	C(8)-C(9)-O(25)-C(26)	145.14
				C(10)-C(9)-O(25)-C(26)	-37.82
				O(25)-C(9)-C(10)-C(11)	-176.83
				O(25)-C(9)-C(10)-O(23)	-3.09
				C(6)-C(8)-C(9)-O(25)	177.75
				O(21)-C(11)-C(12)-C(6)	-176.08
				C(10)-C(11)-O(21)-C(22)	-176.06
				C(12)-C(11)-O(21)-C(22)	1.50

Table S4. The bond angle data for the new picolinamidines **4a-c**.

4a		**4b**		**4c**		**x-ray value**
Angle	°	**Angle**	°	**Angle**	°	°
H(35)-N(20)-H(34)	116.89	H(35)-N(20)-H(34)	116.87	H(37)-N(20)-H(36)	116.89	120.7
H(35)-N(20)-C(18)	121.12	H(35)-N(20)-C(18)	121.13	H(37)-N(20)-C(18)	121.11	119.1-120.2
H(34)-N(20)-C(18)	121.99	H(34)-N(20)-C(18)	122.00	H(36)-N(20)-C(18)	122.00	119.1-120.2
H(33)-N(19)-H(32)	120.40	H(33)-N(19)-H(32)	120.38	H(35)-N(19)-H(34)	120.42	116.6
H(33)-N(19)-C(18)	116.03	H(33)-N(19)-C(18)	116.04	H(35)-N(19)-C(18)	116.02	119.5-122.4
H(32)-N(19)-C(18)	123.56	H(32)-N(19)-C(18)	123.58	H(34)-N(19)-C(18)	123.57	119.5-122.4
N(20)-C(18)-N(19)	120.65	N(20)-C(18)-N(19)	120.73	N(20)-C(18)-N(19)	120.67	118.8
N(20)-C(18)-C(15)	122.00	N(20)-C(18)-C(15)	121.96	N(20)-C(18)-C(15)	121.98	120.2

N(19)-C(18)-C(15)	117.35	N(19)-C(18)-C(15)	117.31	N(19)-C(18)-C(15)	117.35	120.2
C(18)-C(15)-N(16)	113.99	C(18)-C(15)-N(16)	113.95	C(18)-C(15)-N(16)	113.96	
C(18)-C(15)-C(14)	124.35	C(18)-C(15)-C(14)	124.32	C(18)-C(15)-C(14)	124.35	
C(17)-C(7)-C(4)	121.18	C(17)-C(7)-C(2)	121.11	C(17)-C(7)-C(4)	121.16	
C(13)-C(7)-C(4)	123.19	C(13)-C(7)-C(2)	123.18	C(13)-C(7)-C(4)	123.17	123.2
C(7)-C(4)-S(5)	121.83	C(7)-C(2)-S(3)	121.77	C(7)-C(4)-S(5)	121.79	118.5-121.8
C(7)-C(4)-C(3)	128.64	C(7)-C(2)-C(1)	128.63	C(7)-C(4)-C(3)	128.58	125.4-127.8
C(12)-C(6)-C(1)	120.47	C(12)-C(6)-C(4)	120.70	C(12)-C(6)-C(1)	119.66	121.1
C(8)-C(6)-C(1)	121.91	C(8)-C(6)-C(4)	121.43	C(8)-C(6)-C(1)	121.08	121.1
C(6)-C(1)-C(2)	128.55	C(6)-C(4)-C(5)	128.47	C(6)-C(1)-C(2)	128.59	129.5-131.2
C(6)-C(1)-S(5)	121.47	C(6)-C(4)-S(3)	121.45	C(6)-C(1)-S(5)	121.25	118.5-121.7
C(15)-C(14)-C(13)	118.95	C(15)-C(14)-C(13)	118.90	C(15)-C(14)-C(13)	118.93	118.3-122.1
N(16)-C(15)-C(14)	121.66	N(16)-C(15)-C(14)	121.73	N(16)-C(15)-C(14)	121.69	122.2
C(14)-C(13)-C(7)	120.41	C(14)-C(13)-C(7)	120.38	C(14)-C(13)-C(7)	120.39	118.3-122.1
C(17)-C(7)-C(13)	115.63	C(17)-C(7)-C(13)	115.71	C(17)-C(7)-C(13)	115.67	118.3-122.1
N(16)-C(17)-C(7)	124.01	N(16)-C(17)-C(7)	123.97	N(16)-C(17)-C(7)	124.00	122.2
C(17)-N(16)-C(15)	119.34	C(17)-N(16)-C(15)	119.31	C(17)-N(16)-C(15)	119.32	118.5
S(5)-C(4)-C(3)	109.53	S(3)-C(2)-C(1)	109.60	S(5)-C(4)-C(3)	109.63	110.2-110.9
S(5)-C(1)-C(2)	109.98	C(5)-C(4)-S(3)	110.08	S(5)-C(1)-C(2)	110.16	110.2-110.9
C(4)-S(5)-C(1)	92.48	C(4)-S(3)-C(2)	92.44	C(4)-S(5)-C(1)	92.40	92.8
C(4)-C(3)-C(2)	114.06	C(5)-C(1)-C(2)	114.00	C(4)-C(3)-C(2)	113.94	112.6-113.4
C(3)-C(2)-C(1)	113.95	C(4)-C(5)-C(1)	113.88	C(3)-C(2)-C(1)	113.87	112.6-113.4
C(9)-C(8)-C(6)	121.32	C(9)-C(8)-C(6)	121.13	C(9)-C(8)-C(6)	121.15	120.0-119.9
C(10)-C(9)-C(8)	120.28	C(10)-C(9)-C(8)	120.87	C(10)-C(9)-C(8)	119.97	120.0-119.9
C(11)-C(10)-C(9)	119.37	C(11)-C(10)-C(9)	118.11	C(11)-C(10)-C(9)	118.75	120.0-119.9
C(12)-C(11)-C(10)	119.78	C(12)-C(11)-C(10)	120.83	C(12)-C(11)-C(10)	120.76	120.0-119.9
C(11)-C(12)-C(6)	121.63	C(11)-C(12)-C(6)	121.20	C(11)-C(12)-C(6)	120.11	120.0-119.9
C(12)-C(6)-C(8)	117.63	C(12)-C(6)-C(8)	117.87	C(12)-C(6)-C(8)	119.25	120.0-119.9
C(22)-O(21)-C(10)	119.41	C(23)-O(22)-C(10)	119.51			117.7
O(21)-C(10)-C(11)	124.79	O(22)-C(10)-C(11)	124.89			125.2
O(21)-C(10)-C(9)	115.84	O(22)-C(10)-C(9)	117.00			115.4
		Cl(21)-C(9)-C(10)	119.43			118.369-119.535
		Cl(21)-C(9)-C(8)	119.70			118.369-119.535
				O(21)-C(11)-C(12)	123.98	125.2
				O(21)-C(11)-C(10)	115.22	115.4
				C(26)-O(25)-C(9)	121.30	117.7
				C(24)-O(23)-C(10)	119.24	117.7
				C(22)-O(21)-C(11)	119.12	117.7
				O(23)-C(10)-C(11)	122.18	125.2
				O(23)-C(10)-C(9)	118.76	115.4
				O(25)-C(9)-C(10)	124.67	125.2
				O(25)-C(9)-C(8)	115.30	115.4

Mulliken's charges

The Mulliken's atomic charge was calculated at DFT/B3LYP level (**Table S5**) where it has an important role in describing the processes of electronegativity and charge transfer [1]. The data showed that the pyridyl nitrogen atoms have positive charges indicating involvement of their lone pair in resonating structure of the pyridyl ring where the pyridyl carbon atom at position 6 has a negative charge. The pyridyl carbons at position 2 and 5 have a positive charge that may be attributed to the strong electron withdrawing effect of the cationic amidine group and thienyl ring, respectively. The thienyl sulfur atoms were negatively charged in range of -1.0609 for the trimethoxy derivative **4c** to -1.1693 for the chloro-methoxy derivative **4b**. Similarly, the thienyl carbon atoms at position 5, attached to the phenyl ring, displayed negative charge where the chloro-methoxy derivative **4b** was the highest one. On contrary, the phenyl carbon at position 1, attached to thienyl ring, has positive charge and decreases in order **4c** > **4a** > **4b**. Furthermore, in monosubstituted derivative **4a**, the methoxy oxygen atom at position 4 has a negative charge -0.1368 while in the trimethoxy **4c**, it became more negative, -0.1900, while the chloro substituted **4b** showed a strong reduction in its charge to -0.0832.

Table S5. The Mulliken's atomic charges of the investigated picolinamidines **4a-c**

atom	**4a**	**4b**	**4c**
C_{am}	0.0235	0.0305	-0.0452
N_{am}	-0.3820	-0.3832	-0.3752
N^{+}_{am}	-0.3696	-0.3682	-0.3710
N_{Py}	0.0821	0.0827	0.0826
Py^{2}	0.0862	0.0784	0.1884
Py^{3}	-0.4881	-0.5140	-0.5979
Py^{4}	0.3192	0.2588	-0.1399
Py^{5}	0.2376	0.2943	0.3478
Py^{6}	-0.6733	-0.6251	-0.2348
S_{Th}	-1.1444	-1.1693	-1.0609
Th^{2}	0.5791	0.5685	0.6153
Th^{3}	0.1443	0.1753	0.2306
Th^{4}	0.3187	0.3352	-0.0826

Th[5]	-0.4004	-0.5518	-0.4518
Ph[1]	0.9091	0.5804	1.3124
Ph[2]	-0.0276	-0.2501	-0.3423
Ph[3]	-0.3887	0.6466	-0.1481
Ph[4]	-0.6026	-1.1256	0.1450
Ph[5]	-0.1150	-0.2656	-0.9745
Ph[6]	-0.0540	-0.1483	0.3326
MeO[3]			-0.3043
OMe[3]			-0.1539
MeO[4]	-0.3191	-0.2888	-0.2840
OMe[4]	-0.1368	-0.0832	-0.1900
MeO[5]			-0.3030
OMe[5]			-0.1562
Cl[3]		0.4667	

Fukui's and relative nucleophilicity indices

The Fukui indices toward nucleophilic and electrophilic attacks (f^{+}_{k} and f^{-}_{k}) were calculated to explore the reactivity of each atom in the investigated derivatives by the following equations [2-4], where $q_k(N)$, $q_k(N+1)$ and $q_k(N-1)$ are the atomic charges of the systems with N, N+1 and N-1 electrons, respectively [5].

$$f^{+}_{k} = q_k(N+1) - q_k(N)$$

$$f^{-}_{k} = q_k(N) - q_k(N-1)$$

According to the Fukui's indices (f^{+}_{k}), the picolinamidine derivatives **4a-c** exhibited close trend where the most susceptible for nucleophilic attack was the amidinic carbon C_{am} while the other four top atoms were Py[4], S_{Th}, N_{am} and N^{+}_{am} (**Table S6**). On the other hand, the Fukui's indices (f^{-}_{k}) of the 4-methoxyphenyl derivative **4a**, indicated that the most susceptible atoms for electrophilic attack was OMe[4] while 3-chloro-4-methoxyphenyl

derivative **4b** showed that the most susceptible atom for electrophilic attack was Cl and 3,4,5-trimethoxyphenyl derivative **4c**, indicated that the most susceptible atom for electrophilic attack was C-4 of 3,4,5-trimethoxyphenyl moiety.

The local relative electrophilicity and nucleophilicity descriptors, s_k^-/s_k^+ and s_k^+/s_k^-, respectively, were calculated using the following equations, to overcome the Fukui's indices unreliability in some cases, generate improved intramolecular reactivity trends and detect the preferable site for nucleophilic and electrophilic attack, respectively, where δ is global softness [6-8].

$s_k^+ = f_k^+ \times \delta$ $\qquad$ $s_k^- = f_k^- \times \delta$

The relative electrophilicity descriptors data, s_k^-/s_k^+, showed that the most susceptible atom for nucleophilic attack was thienyl carbon atom Th-4 in case of **4a** derivative while it became in the second position after the phenyl carbon atom Ph-3 and oxygen of methoxy group OMe-4 in both **4b** and **4c**, respectively (**Table S6**). On the other hand, the relative nucleophilicity descriptors data, s_k^+/s_k^-, showed that the top five atoms susceptible for electrophilic attack were $Th^3 > C_{am} > Py^3 > N^+_{am} > N_{am}$ in both **4a** and **4b** derivatives while in **4c** derivative, the pyridyl carbon atom Py^4 was the most susceptible atom.

[1] J.B. Bhagyasree, H.T. Varghese, C.Y. Panicker, J. Samuel, C. Van Alsenoy, K. Bolelli, I. Yildiz, E. Aki, Vibrational spectroscopic (FT-IR, FT-Raman, ^{1}H NMR and UV) investigations and computational study of 5-nitro-2-(4-nitrobenzyl) benzoxazole, Spectrochim. Acta. A, 102 (2013) 99-113.

[2] L.O. Olasunkanmi, I.B. Obot, E.E. Ebenso, Adsorption and corrosion inhibition properties of N-{n-[1-R-5-(quinoxalin-6-yl)-4, 5-dihydropyrazol-3-yl] phenyl} methanesulfonamides on mild steel in 1 M HCl: experimental and theoretical studies, RSC Advances, 6 (2016) 86782-86797.

[3] Z. El Adnani, M. Mcharfi, M. Sfaira, M. Benzakour, A. Benjelloun, M.E. Touhami, DFT theoretical study of 7-R-3-methylquinoxalin-2 (1H)-thiones (R; H; CH_3; Cl) as corrosion inhibitors in hydrochloric acid, Corros. Sci. 68 (2013) 223-230.

[4] H. Mi, G. Xiao, X. Chen, Theoretical evaluation of corrosion inhibition performance of three antipyrine compounds, Comput. Theor. Chem. 1072 (2015) 7-14.

[5] M. Messali, M. Larouj, H. Lgaz, N. Rezki, F. Al-Blewi, M. Aouad, A. Chaouiki, R. Salghi, I.-M. Chung, A new schiff base derivative as an effective corrosion inhibitor for mild steel in acidic media: Experimental and computer simulations studies, J. Mol. Struc. 1168 (2018) 39-48.

[6] R. Roy, S. Krishnamurti, P. Geerlings, S. Pal, Local softness and hardness based reactivity descriptors for predicting intra-and intermolecular reactivity sequences: carbonyl compounds, J. Phys. Chem. A, 102 (1998) 3746-3755.

[7] R. Roy, F.d. de Proft, P. Geerlings, Site of protonation in aniline and substituted anilines in the gas phase: a study via the local hard and soft acids and bases concept, J. Phys. Chem. A, 102 (1998) 7035-7040.

[8] R.K. Roy, S. Pal, K. Hirao, On non-negativity of Fukui function indices, J. Chem. Phys. 110 (1999) 8236-8245.

Table S6. The atomic Fukui's indices, relative nucleophilicity and electrophilicity descriptors of the investigated picolinamidines **4a-c**

Atom	4a				4b				4c			
	f_k^-	f_k^+	s_k^-/s_k^+	s_k^+/s_k^-	f_k^-	f_k^+	s_k^-/s_k^+	s_k^+/s_k^-	f_k^-	f_k^+	s_k^-/s_k^+	s_k^+/s_k^-
C_{am}	0.021	0.081	0.259	3.857	0.020	0.081	0.247	4.050	0.020	0.081	0.247	4.050
N_{am}	0.025	0.054	0.463	2.160	0.024	0.055	0.436	2.292	0.023	0.054	0.426	2.348
N^+_{am}	0.022	0.048	0.458	2.182	0.021	0.049	0.429	2.333	0.020	0.048	0.417	2.400
N_{Py}	0.027	0.009	3.000	0.333	0.025	0.008	3.125	0.320	0.023	0.008	2.875	0.348
Py^2	0.018	0.041	0.439	2.278	0.017	0.041	0.415	2.412	0.016	0.041	0.390	2.563
Py^3	0.019	0.010	1.900	0.526	0.018	0.010	1.800	0.556	0.015	0.010	1.500	0.667
Py^4	-0.002	0.055	-0.036	-27.500	-0.002	0.056	-0.036	-28.000	0.001	0.056	0.018	56.000
Py^5	0.024	0.008	3.000	0.333	0.022	0.008	2.750	0.364	0.020	0.008	2.500	0.400
Py^6	0.011	0.039	0.282	3.545	0.011	0.039	0.282	3.545	0.010	0.038	0.263	3.800
S_{Th}	0.060	0.056	1.071	0.933	0.055	0.055	1.000	1.000	0.049	0.055	0.891	1.122
Th^2	0.036	-0.013	-2.769	-0.361	0.034	-0.014	-2.429	-0.412	0.026	-0.013	-2.000	-0.500
Th^3	0.006	0.041	0.146	6.833	0.005	0.041	0.122	8.200	0.004	0.041	0.098	10.250
Th^4	0.039	0.008	4.875	0.205	0.036	0.007	5.143	0.194	0.031	0.008	3.875	0.258
Th^5	-0.007	0.039	-0.179	-5.571	-0.008	0.040	-0.200	-5.000	-0.014	0.039	-0.359	-2.786
Ph^1	0.045	-0.007	-6.429	-0.156	0.041	-0.007	-5.857	-0.171	0.039	-0.006	-6.500	-0.154
Ph^2	0.011	0.012	0.917	1.091	0.012	0.013	0.923	1.083	0.018	0.015	1.200	0.833

Ph^3	0.036	0.011	3.273	0.306	0.018	0.002	9.000	0.111	0.029	0.010	2.900	0.345
Ph^4	0.034	0.026	1.308	0.765	0.040	0.027	1.481	0.675	0.068	0.034	2.000	0.500
Ph^5	0.032	0.009	3.556	0.281	0.026	0.007	3.714	0.269	0.031	0.011	2.818	0.355
Ph^6	0.019	0.016	1.188	0.842	0.025	0.015	1.667	0.600	0.017	0.013	1.308	0.765
MeO^4	-0.030	-0.015	2.000	0.500	-0.030	-0.014	2.143	0.467	-0.028	-0.013	2.154	0.464
OMe^4	0.067	0.023	2.913	0.343	0.064	0.021	3.048	0.328	0.058	0.016	3.625	0.276
MeO^3									-0.024	-0.011	2.182	0.458
OMe^3									0.039	0.008	4.875	0.205
MeO^5									-0.022	-0.009	2.444	0.409
OMe^5									0.029	0.010	2.900	0.345
Cl					0.112	0.045	2.489	0.402				

Table S7. The DFT calculated and experimental ^{1}H-NMR data of the investigated picolinamidines **4a-c**

Proton	**4a**		**4b**		**4c**	
	Exp.	**DFT**	**Exp.**	**DFT**	**Exp.**	**DFT**
HN^+_{am}	9.59	7.05	9.59	6.12	9.6	7.10
HN^+_{am}	9.59	6.87	9.59	5.92	9.6	6.89
HN_{am}	9.39	8.53	9.38	8.38	9.41	8.56
HN_{am}	9.39	7.05	9.38	5.92	9.41	7.10
Ph^2	7.67	8.07	7.83	7.93	6.99	7.35
Ph^3	7.02	7.67	-	-	-	-
Ph^5	7.02	7.45	7.24	7.10	-	-
Ph^6	7.66	8.30	7.66	7.93	6.99	7.10
Py^3	8.37	8.30	8.36	7.93	8.38	8.29
Py^4	8.40	8.30	8.41	8.19	8.44	8.38
Py^6	9.11	9.42	9.12	9.46	9.14	9.45
Th^3	7.92	8.30	7.94	8.12	7.97	8.29
Th^4	7.56	7.77	7.66	7.43	7.7	7.71
MeO^3	-	-	-	-	3.86	5.35
MeO^3	-	-	-	-	3.86	4.52
MeO^3	-	-	-	-	3.86	4.32
MeO^4	3.80	4.62	3.90	4.33	3.69	5.48
MeO^4	3.80	4.41	3.90	3.95	3.69	4.06
MeO^4	3.80	4.41	3.90	3.90	3.69	4.57
MeO^5	-	-	-	-	3.86	4.32
MeO^5	-	-	-	-	3.86	4.78
MeO^5	-	-	-	-	3.86	4.46

Table S8. The DFT calculated and experimental ^{13}C-NMR data of the investigated picolinamidines **4a-c**

Atom	4a		4b		4c	
	Exp	**DFT**	**Exp**	**DFT**	**Exp**	**DFT**
C_{am}	161.43	163.65	161.31	164.67	161.37	163.74
Py^2	146.23	137.22	145.71	137.84	146.26	137.82
Py^3	123.77	126.73	123.73	127.78	123.76	126.78
Py^4	133.14	134.04	133.6	135.48	133.33	134.57
Py^5	133.77	141.31	136.45	143.03	133.69	141.26
Py^6	145.62	147.24	144.44	148.78	145.69	147.40
Th^2	141.6	146.74	141.85	147.85	137.89	148.56
Th^3	128.96	133.57	129	135.16	128.82	133.24
Th^4	125.58	125.94	126.68	127.88	128.67	127.49
Th^5	135.66	164.77	136.45	164.36	136.54	163.90
Ph^1	124.33	129.73	125.62	131.12	125.73	132.90
Ph^2	126.95	131.62	126.53	133.43	103.17	114.36
Ph^3	114.68	121.00	121.88	134.58	153.37	158.10
Ph^4	159.54	167.13	154.58	162.44	141.82	144.40
Ph^5	114.68	111.86	113.44	115.23	153.37	161.42
Ph^6	126.95	130.79	125.37	130.76	103.17	102.64
MeO^3	-	-	-	-	56.09	62.09
MeO^4	55.32	55.83	56.35	57.24	60.16	61.24
MeO^5	-	-	-	-	56.09	55.10

Table S9. *In vitro* anti-proliferative activity of the new picolinamidines **4a-c** against a panel of 60 cell lines at five dose level [a,b]

Cancer type/cell line	4a	4b	4c	Cancer type/cell line	4a	4b	4c
Leukemia				**CNS cancer**			
CCRF-CEM	1.43	1.18	2.49	SF-268	1.76	1.81	14.6
HL-60(TB)	1.78	1.66	1.87	SF-295	1.71	1.80	NT
K-562	1.09	0.90	1.39	SF-539	1.73	1.74	1.61
MOLT-4	1.90	1.72	2.53	SNB-19	1.72	1.70	4.73
RPMI-8226	2.13	2.25	1.67	SNB-75	1.55	1.43	1.50
SR	0.34	0.58	2.48	U251	1.81	1.82	1.74
Colon cancer				**Ovarian cancer**			
COLO 205	1.89	1.94	1.60	IGROV-1	1.84	1.78	2.63
HCC-2998	1.82	1.86	2.00	OVCAR-3	1.82	1.83	2.60
HCT-116	1.62	1.82	1.47	OVCAR-4	1.79	1.74	2.69

HCT-15	1.27	1.57	2.18	OVCAR-5	1.67	1.86	2.10
HT29	0.88	1.35	1.37	OVCAR-8	2.12	2.60	2.30
KM12	1.81	1.81	2.78	NCI/ADR-RES	1.94	2.01	5.20
SW-620	0.43	1.79	2.09	SK-OV-3	1.70	1.75	13.1
Melanoma				**NSCLC**			
LOX IMVI	1.88	1.87	1.87	A549/ATCC	1.84	2.01	2.06
MALME-3M	1.84	1.78	1.53	EKVX	1.70	1.79	5.19
M14	1.80	1.73	1.66	HOP-62	1.80	1.90	2.79
MDA-MB-435	1.73	1.73	1.62	HOP-92	1.50	1.63	1.91
SK-MEL-2	2.05	2.15	2.09	NCI-H226	1.81	1.92	10.8
SK-MEL-28	1.75	1.73	1.62	NCI-H23	1.84	1.80	6.40
SK-MEL-5	1.79	1.82	1.67	NCI-H322M	1.59	1.67	3.23
UACC-257	1.80	1.88	2.15	NCI-H460	0.52	1.80	2.10
UACC-62	1.81	1.76	1.73	NCI-H522	1.90	2.10	1.80
Renal Cancer				**Breast Cancer**			
786-0	1.88	2.00	2.12	MCF-7	1.74	1.75	1.30
A498	NT	NT	4.91	MDA-MB-231/ATCC	1.75	1.70	1.85
ACHN	1.84	1.77	1.73	HS-578T	1.82	1.85	2.59
CAKI-1	1.64	1.74	1.59	BT-549	1.70	1.72	1.67
RXF 393	1.77	1.76	2.19	T-47D	1.92	1.99	NT
SN12C	1.83	1.79	2.05	MDA-MB-468	1.63	1.74	1.74
TK-10	1.89	1.90	3.19	**Prostate cancer**			
UO-31	1.64	1.55	1.88	PC-3	1.68	1.78	3.11
				DU-145	1.77	1.77	3.45

[a] data represent GI_{50} in μM against the tested cell lines.

[b] NT: not tested.

II. Figures

- Optimized structures of thienylpicolinamidine derivatives **4a-c**

(A)

(B)

(C)

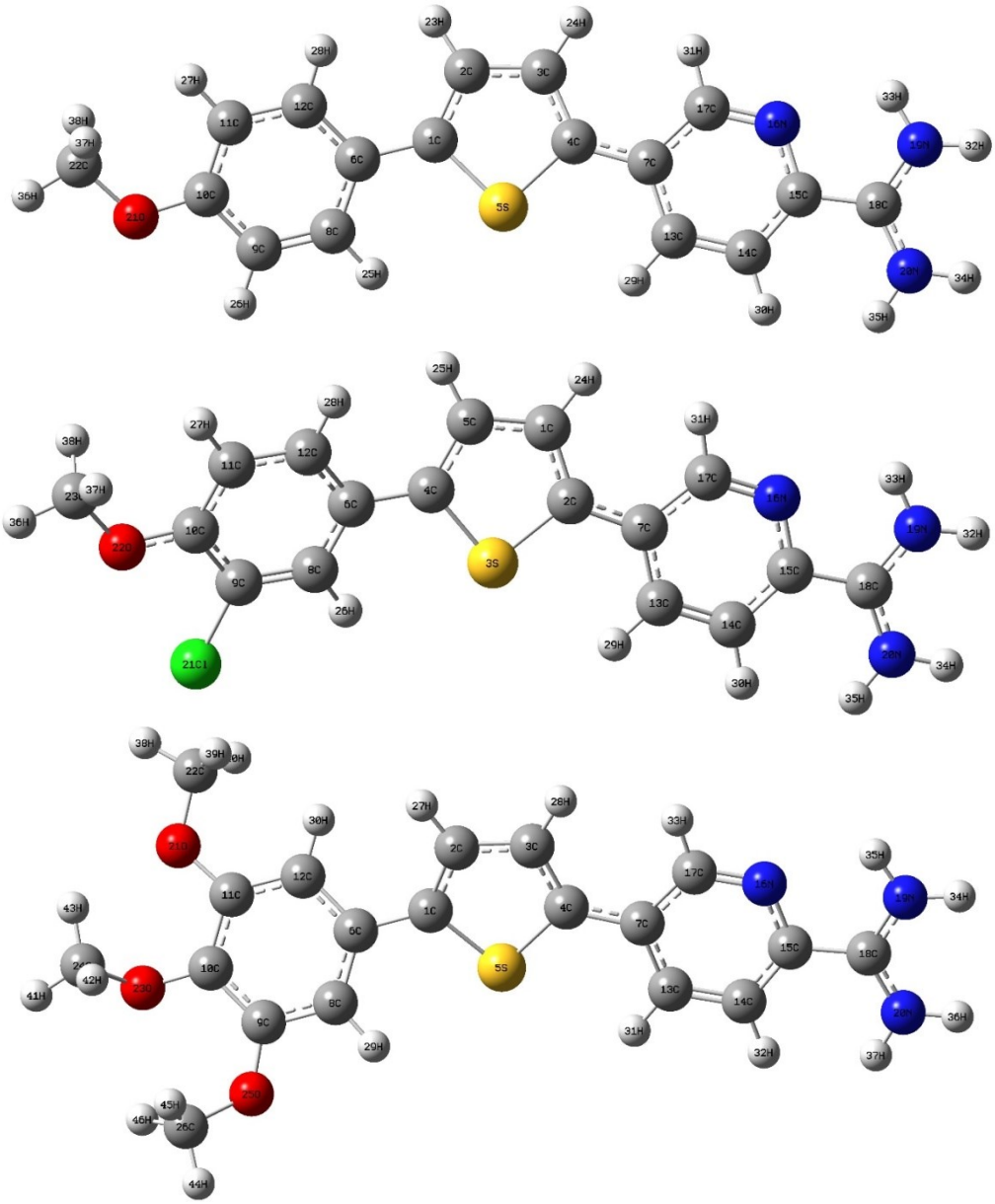


Figure S1. Optimized structure of 4-methoxyphenyl derivative **4a (A), 4b (B)** and **4c (C).**

- **Figures S2-S7 for NMR Spectra of the new picolinamidines 4a-c**
  - **Figure S2: Compound 4a** ^{1}H-NMR/JEOL 500 MHz

- **Figure S3: Compound 4a** ^{13}C-NMR/JEOL 125 MHz
- **Figure S4: Compound 4b** ^{1}H-NMR/ JEOL 500 MHz
- **Figure S5: Compound 4b** ^{13}C-NMR/JEOL 125 MHz
- **Figure S6: Compound 4c** ^{1}H-NMR/JEOL 500 MHz
- **Figure S7: Compound 4c** ^{13}C-NMR/JEOL 125 MHz

Figures S8-S10 for Mass Spectra of the new picolinamidines 4a-c

- **Figure S8: Compound 4a** Mass spectrum
- **Figure S9: Compound 4b** Mass spectrum
- **Figure S10: Compound 4c** Mass spectrum

Figures for NMR Spectra of the new picolinamidines 4a-c

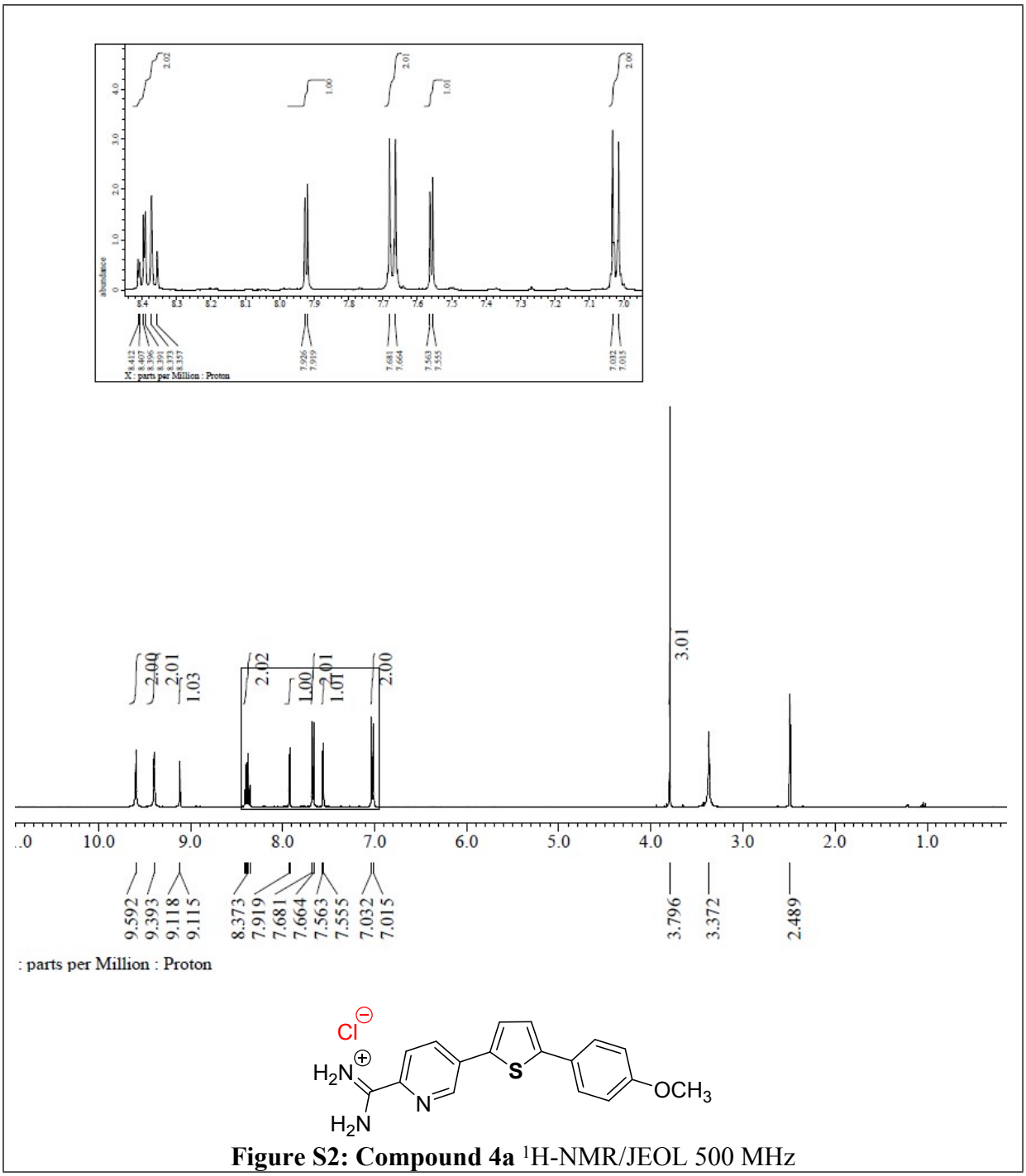


Figure S2: Compound 4a ^{1}H-NMR/JEOL 500 MHz

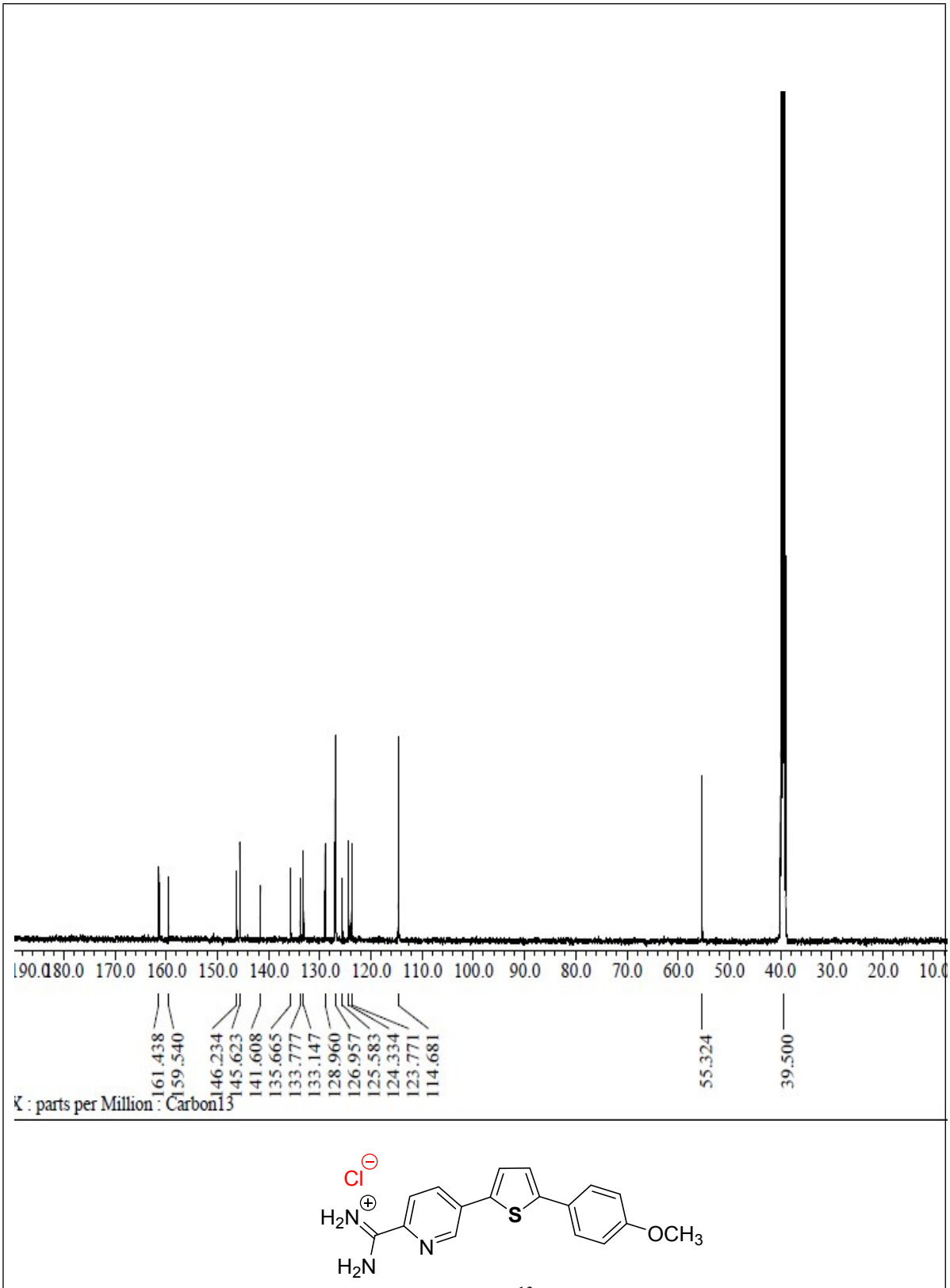
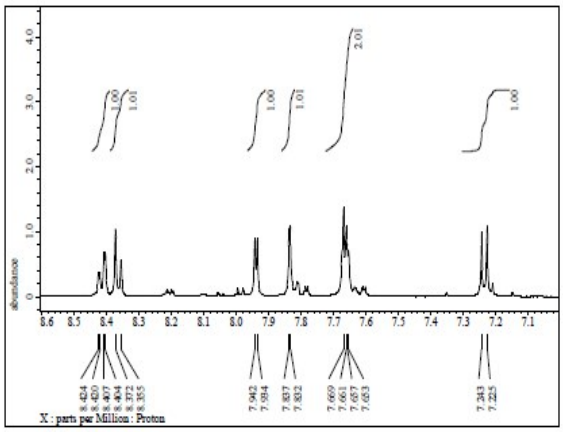
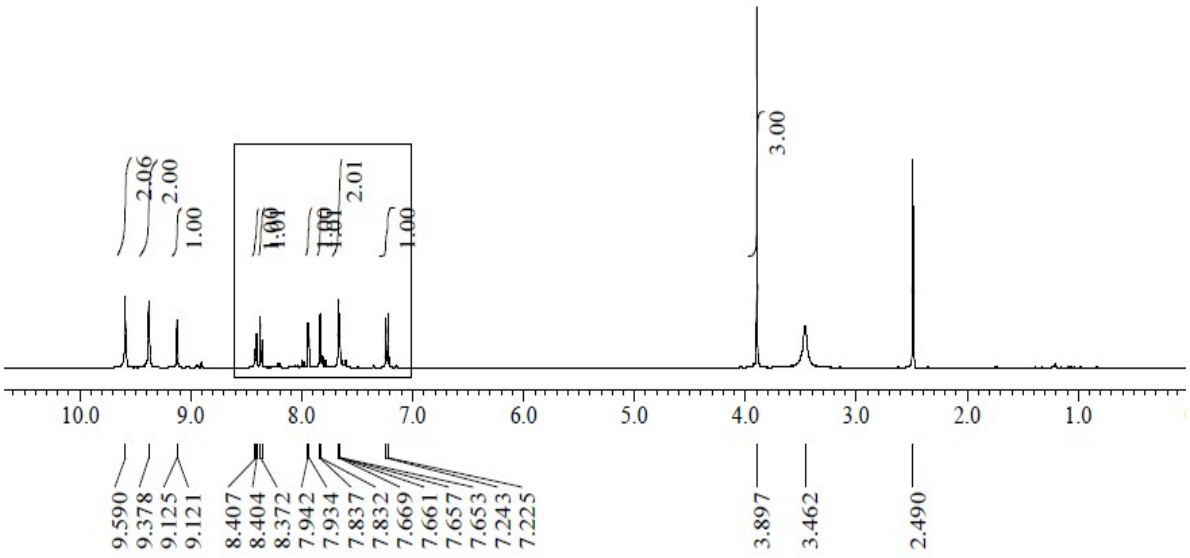


Figure S3: Compound 4a ^{13}C-NMR/JEOL 125 MHz





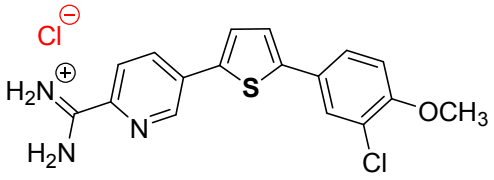


Figure S4: Compound 4b ^{1}H-NMR/JEOL 500 MHz

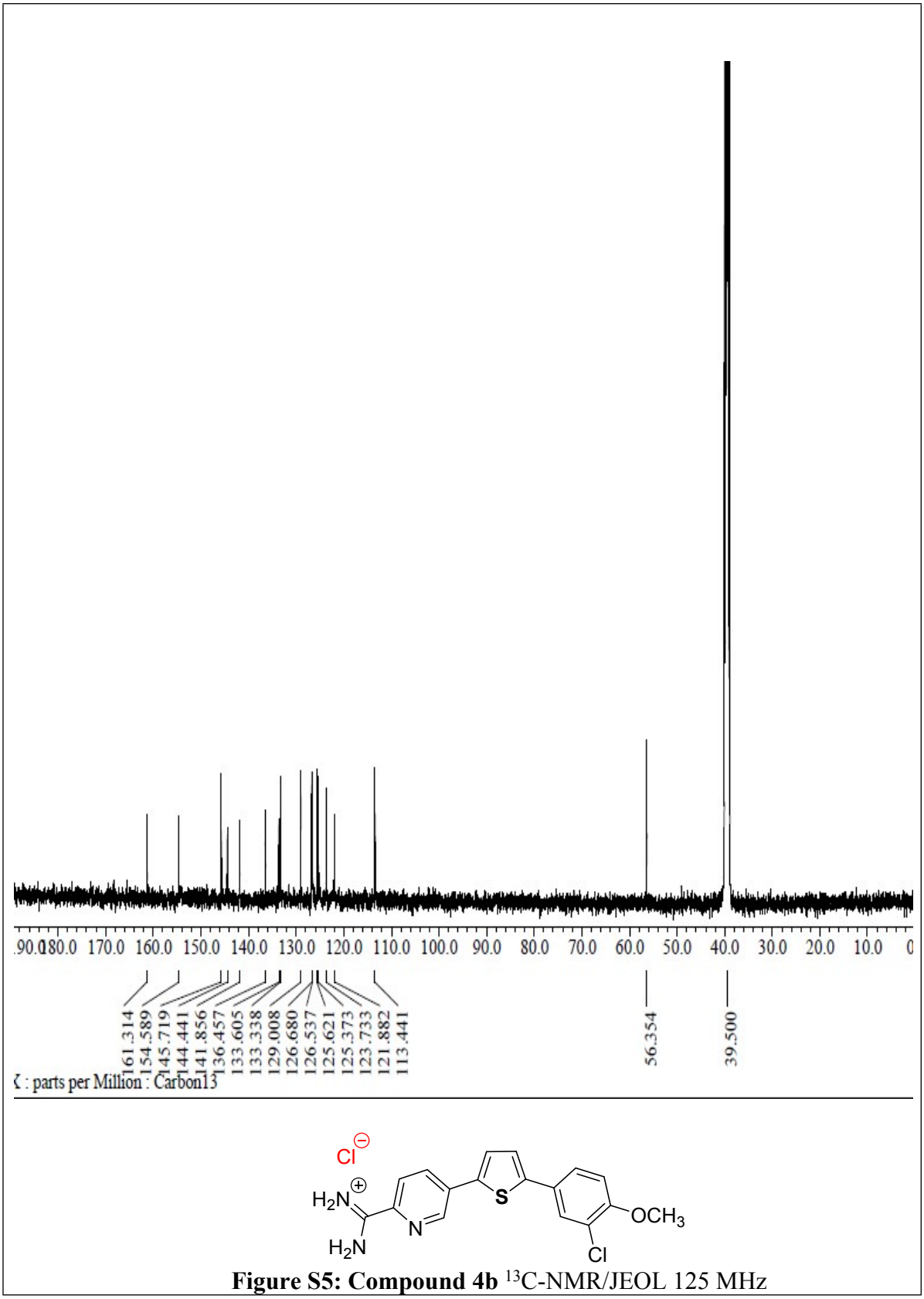


Figure S5: Compound 4b ^{13}C-NMR/JEOL 125 MHz

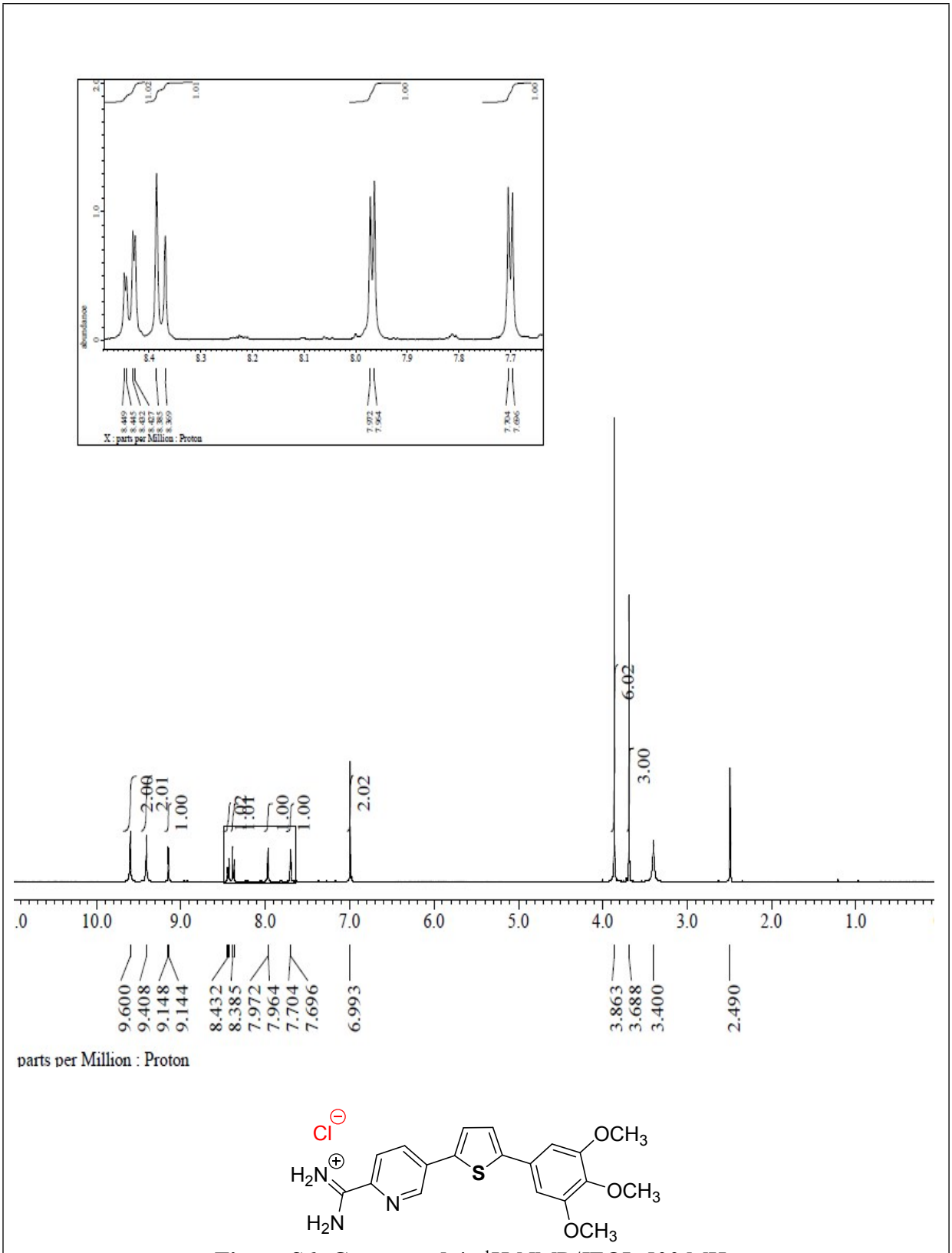


Figure S6: Compound 4c ^{1}H-NMR/JEOL 500 MHz

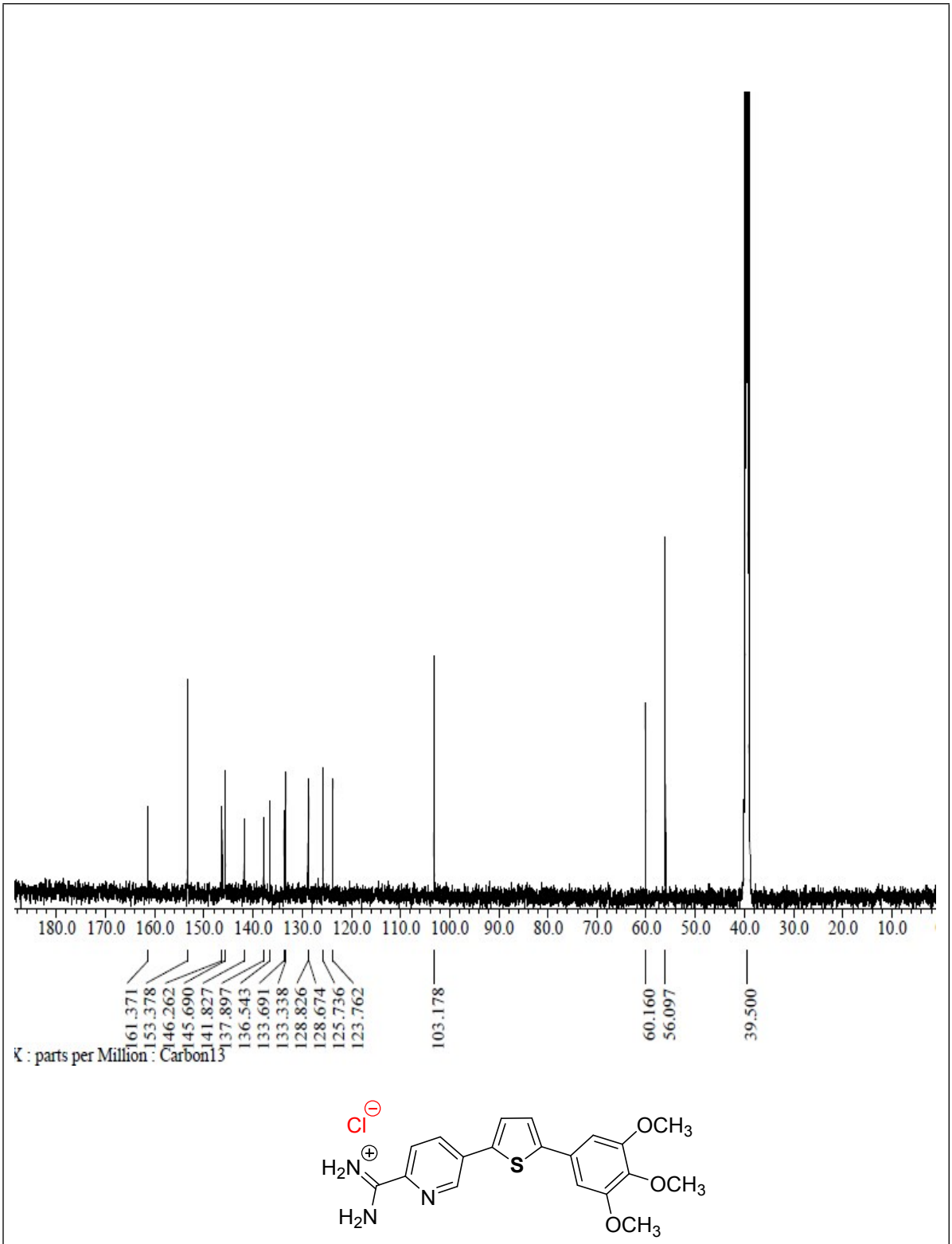
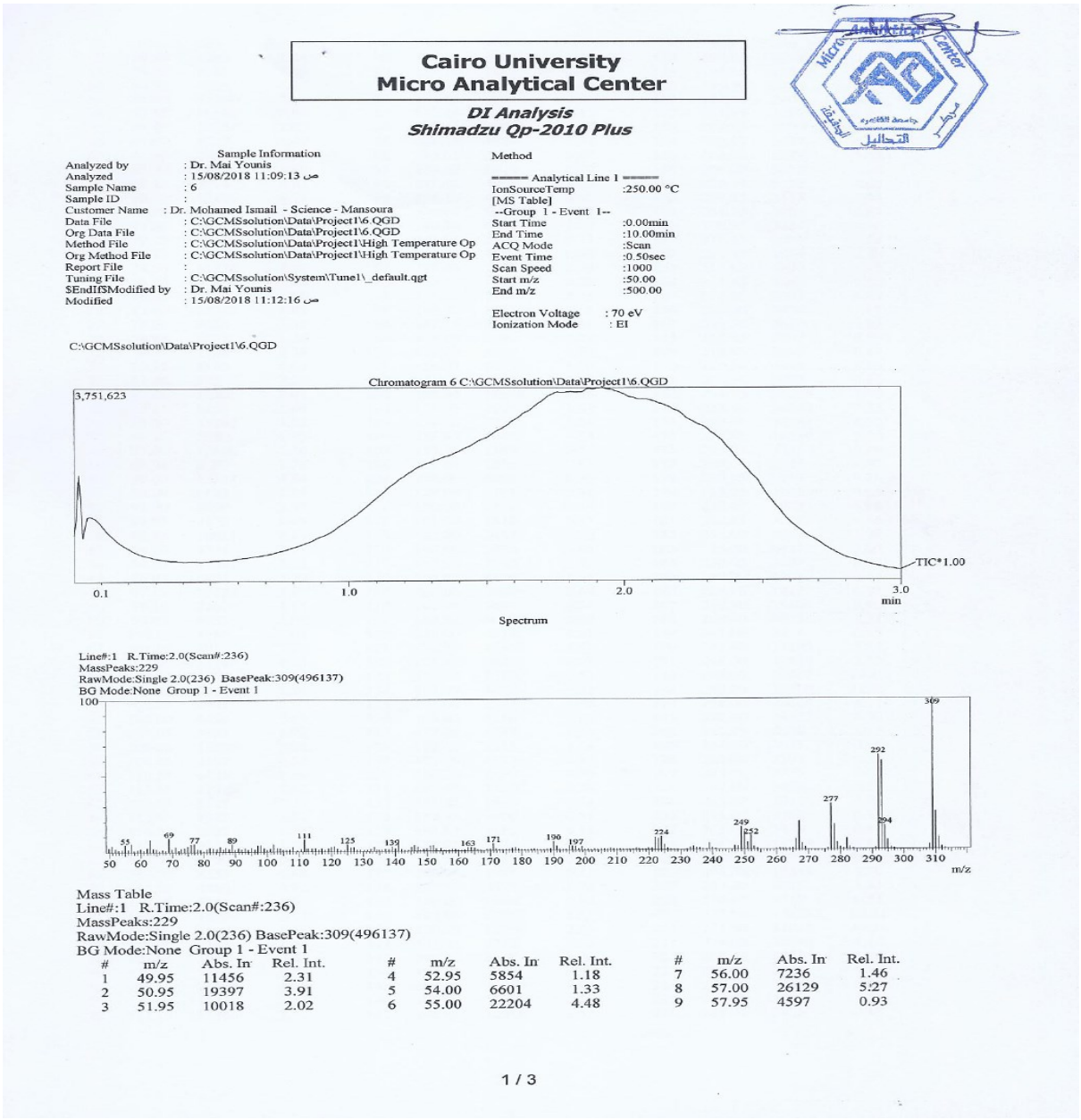


Figure S7: Compound 4c ^{13}C-NMR/JEOL 125 MHz

Figures for Mass Spectra of picolinamidines 4a-c



Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 15/08/2018 11:09:13 ص
Sample Name	: 6
Sample ID	:
Customer Name	: Dr. Mohamed Ismail - Science - Mansoura
Data File	: C:\GCMSsolution\Data\Project1\6.QGD
Org Data File	: C:\GCMSsolution\Data\Project1\6.QGD
Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1_default.qgt
SEndIfSModified by	: Dr. Mai Younis
Modified	: 15/08/2018 11:12:16 ص

Method

====== Analytical Line 1 ======	
IonSourceTemp	:250.00 °C
[MS Table]	
--Group 1 - Event 1--	
Start Time	:0.00min
End Time	:10.00min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:1000
Start m/z	:50.00
End m/z	:500.00
Electron Voltage	: 70 eV
Ionization Mode	: EI

C:\GCMSsolution\Data\Project1\6.QGD

Chromatogram 6 C:\GCMSsolution\Data\Project1\6.QGD

Spectrum

Line#:1 R.Time:2.0(Scan#:236)
MassPeaks:229
RawMode:Single 2.0(236) BasePeak:309(496137)
BG Mode:None Group 1 - Event 1

Mass Table
Line#:1 R.Time:2.0(Scan#:236)
MassPeaks:229
RawMode:Single 2.0(236) BasePeak:309(496137)
BG Mode:None Group 1 - Event 1

#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	49.95	11456	2.31	4	52.95	5854	1.18	7	56.00	7236	1.46
2	50.95	19397	3.91	5	54.00	6601	1.33	8	57.00	26129	5.27
3	51.95	10018	2.02	6	55.00	22204	4.48	9	57.95	4597	0.93

1 / 3

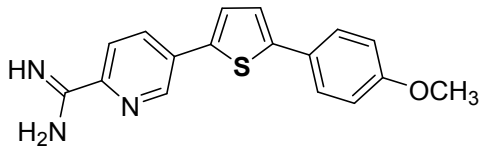
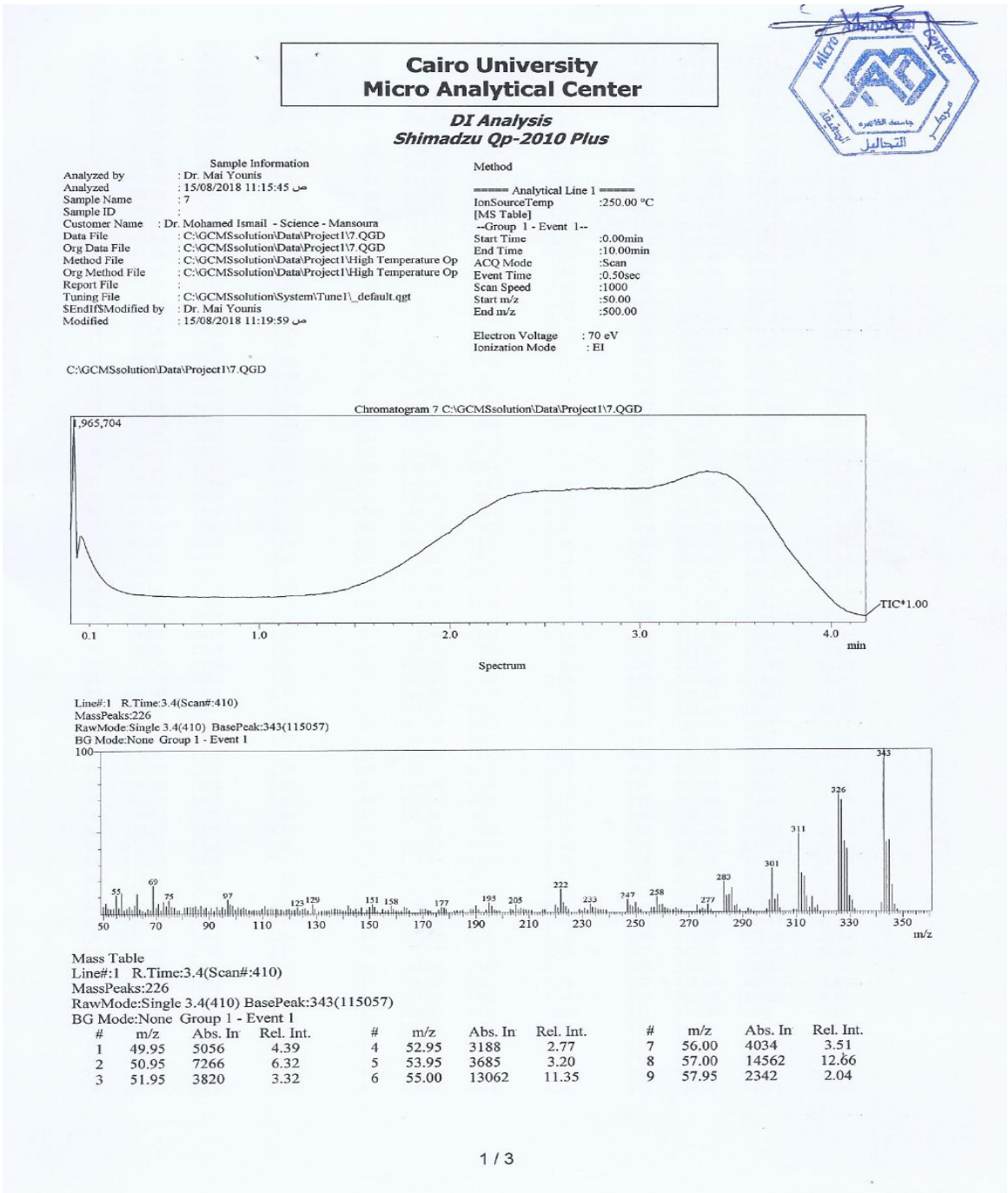


Figure S8: Mass spectrum of **compound 4a (free base)**



Cairo University
Micro Analytical Center

DI Analysis
Shimadzu Qp-2010 Plus

Sample Information

Analyzed by	: Dr. Mai Younis
Analyzed	: 15/08/2018 11:15:45 ص
Sample Name	: 7
Sample ID	:
Customer Name	: Dr. Mohamed Ismail - Science - Mansoura
Data File	: C:\GCMSsolution\Data\Project1\7.QGD
Org Data File	: C:\GCMSsolution\Data\Project1\7.QGD
Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Org Method File	: C:\GCMSsolution\Data\Project1\High Temperature Op
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1_default.qgt
$EndIf$Modified by	: Dr. Mai Younis
Modified	: 15/08/2018 11:19:59 ص

Method

===== Analytical Line 1 =====

IonSourceTemp	:250.00 °C
[MS Table]	
--Group 1 - Event 1--	
Start Time	:0.00min
End Time	:10.00min
ACQ Mode	:Scan
Event Time	:0.50sec
Scan Speed	:1000
Start m/z	:50.00
End m/z	:500.00
Electron Voltage	: 70 eV
Ionization Mode	: EI

C:\GCMSsolution\Data\Project1\7.QGD

Chromatogram 7 C:\GCMSsolution\Data\Project1\7.QGD

Spectrum

Line#:1 R.Time:3.4(Scan#:410)
MassPeaks:226
RawMode:Single 3.4(410) BasePeak:343(115057)
BG Mode:None Group 1 - Event 1

Mass Table

Line#:1 R.Time:3.4(Scan#:410)
MassPeaks:226
RawMode:Single 3.4(410) BasePeak:343(115057)
BG Mode:None Group 1 - Event 1

#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.	#	m/z	Abs. In	Rel. Int.
1	49.95	5056	4.39	4	52.95	3188	2.77	7	56.00	4034	3.51
2	50.95	7266	6.32	5	53.95	3685	3.20	8	57.00	14562	12.66
3	51.95	3820	3.32	6	55.00	13062	11.35	9	57.95	2342	2.04

1 / 3

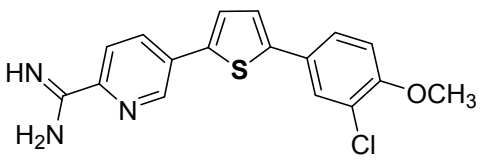
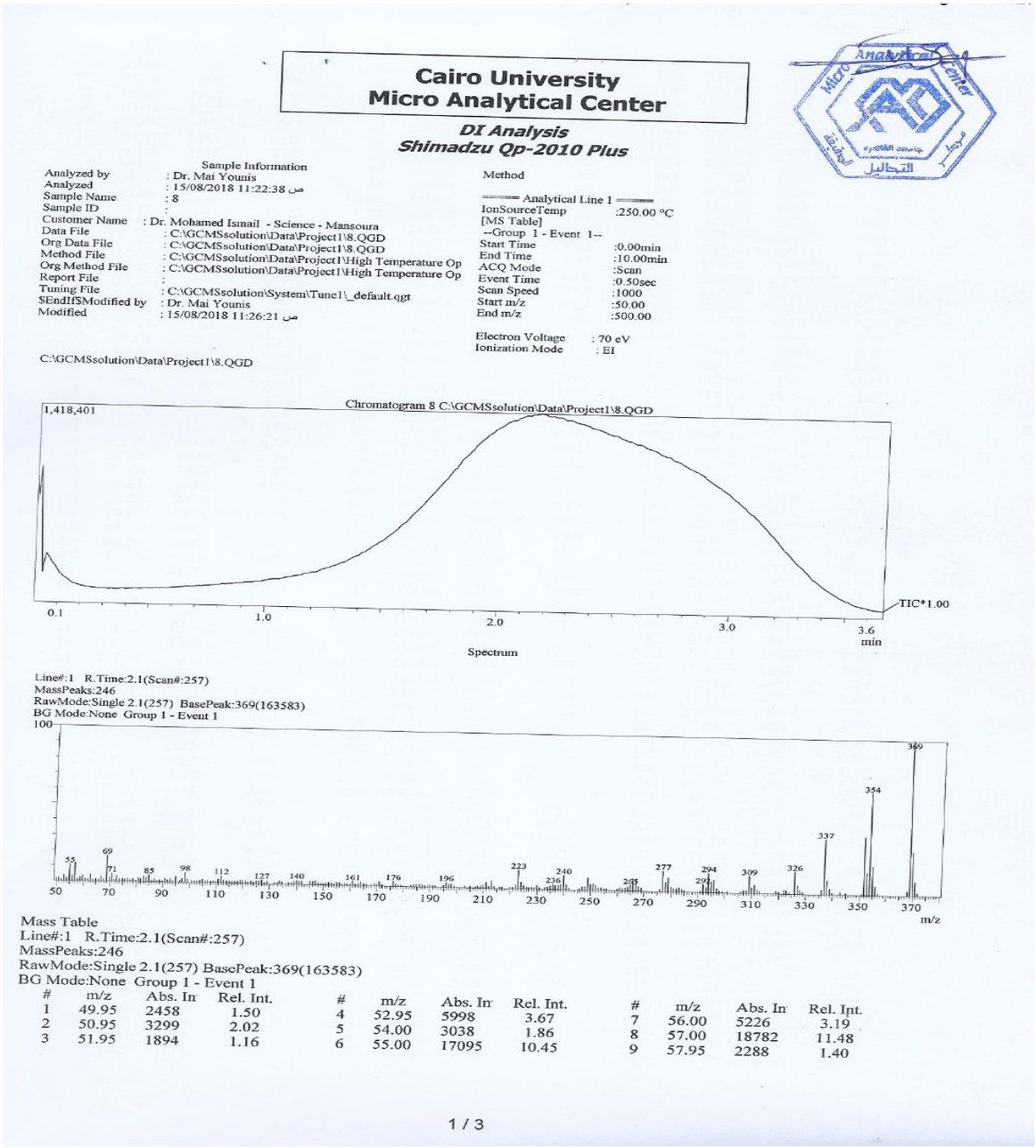


Figure S9: Mass spectrum of **compound 4b (free base)**



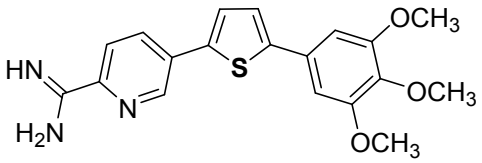


Figure S10: Mass spectrum of **compound 4c (free base)**