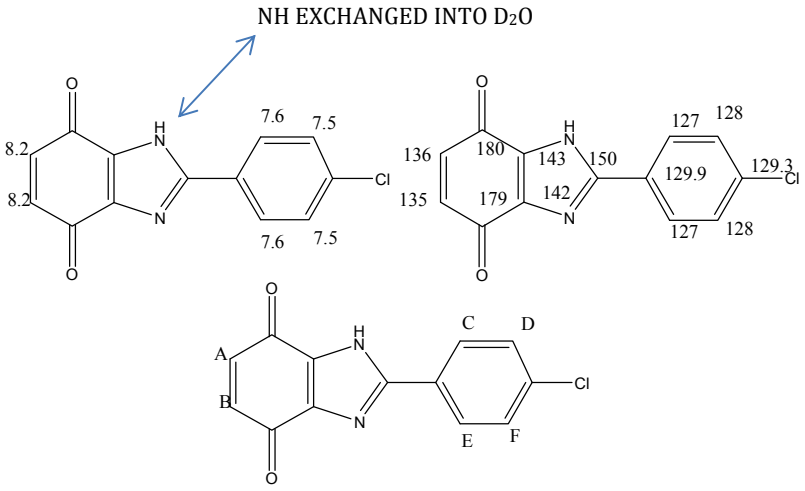
Supplementary Materials for Synthesis, Anticancer Activity and UPLC Analysis of the Stability of Some New Benzimidazole-4,7-dione Derivatives

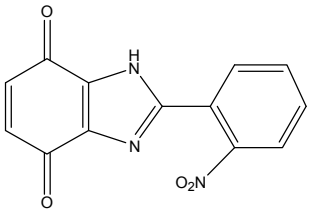
2-(4-Chlorophenyl)-1*H*-benzimidazol-4,7-dione (5a)

IR (KBr) υ/cm^{-1}: 3615 (NH), 1690 (C=O), 1485 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 14.5 (s, 1H, NH), 8.2 (d, 2H, CH, J = 7.9 Hz, 7.6 (d, 2H, CH, J = 2.0 Hz), 7.5 (d, 2H, CH, J = 2.0 Hz); ^{13}C-NMR (DMSO-d_6) δ: 180.0, 179.1, 150.2, 143.4, 142.8, 136.8, 135.7, 129.9, 129.3, 128.7, 127.9, ; MS *m/z* [M+1, M−1]: 259, 257.

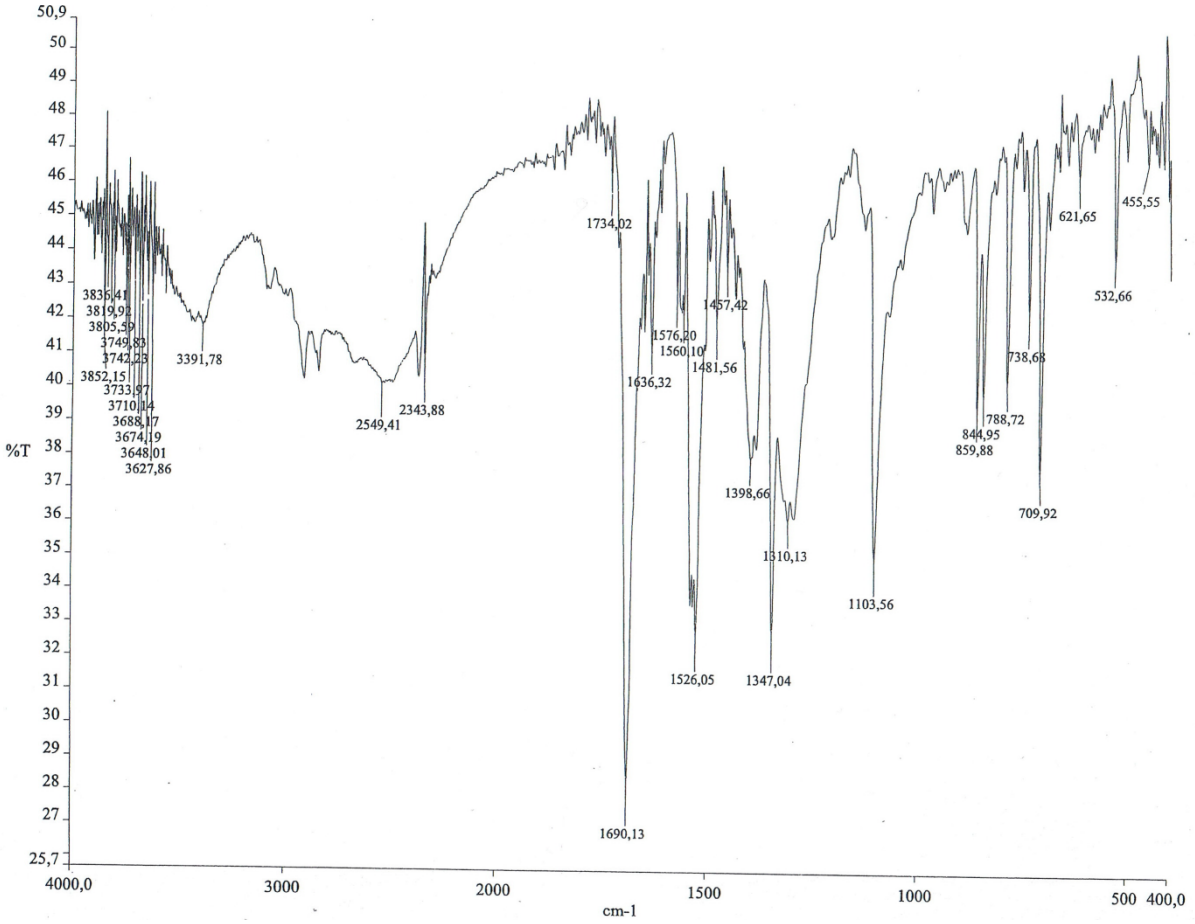


J_{AB} = 7.9; $J_{CE,DF}$ = 2.0

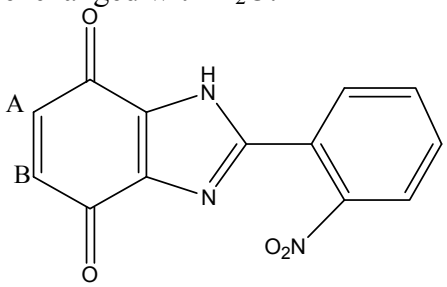
2-(2-Nitrophenyl)-1*H*-benzimidazol-4,7-dione (5b)



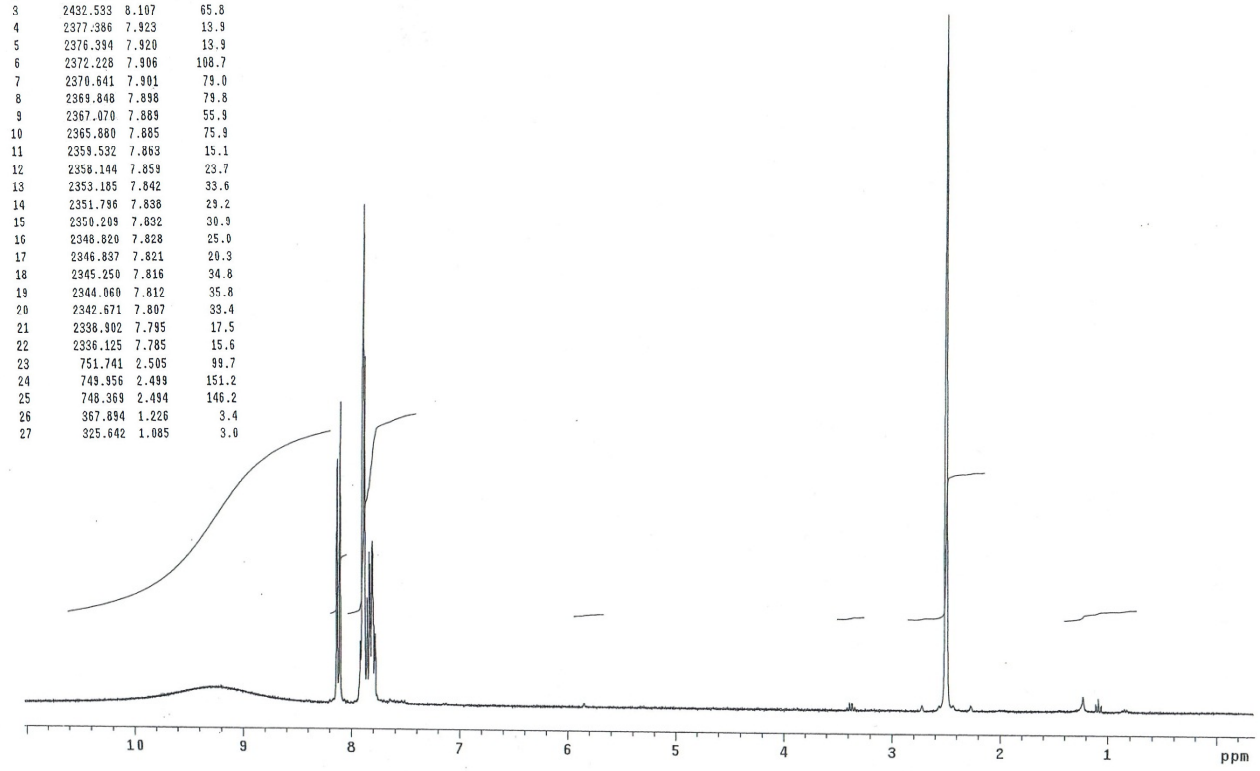
IR (KBr) υ/cm^{-1}: 3391 (NH), 1690 (C=O), 1526 (NO_2asym), 1347 (NO_2sym), 1481 (C=N).



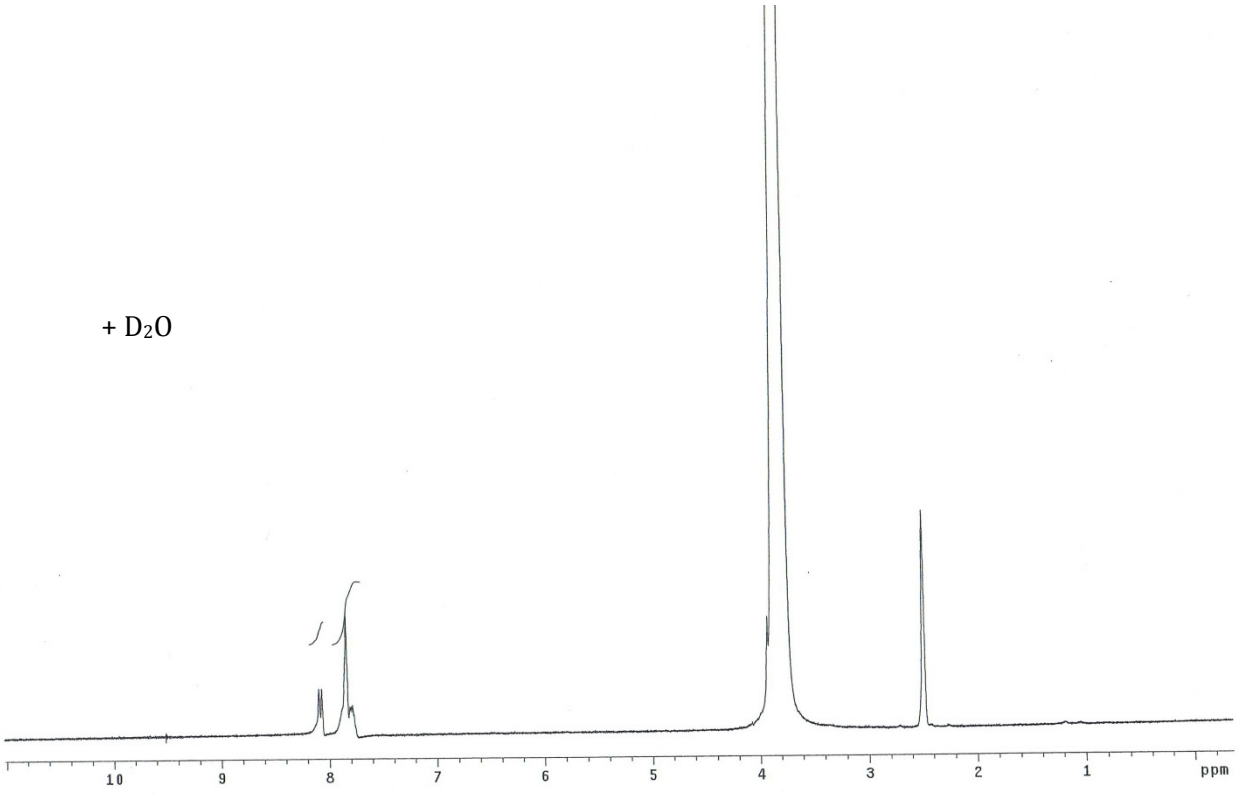
^{1}H-NMR (DMSO-d_6) δ: 9. 2 (s, 1H, NH), 8.1 (d, 2H, CH, J = 7.9 Hz), 7.8 (m, 4H, CH); 8.1 (d, 2H, CH, J = 7.9 Hz) = J_{AB}; NH signal exchanged with D_2O.



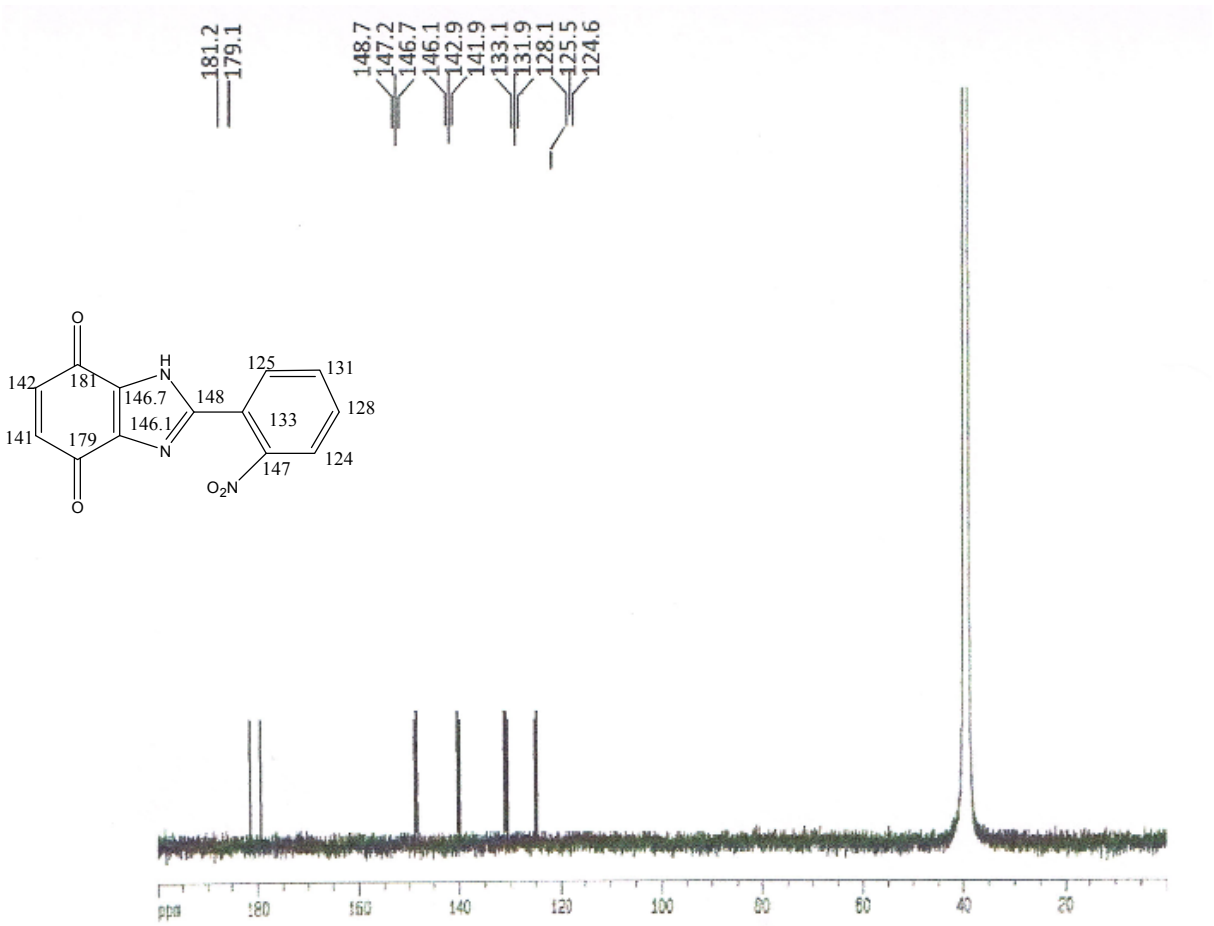
INDEX	FREQUENCY	PPM	HEIGHT
1	2762.621	9.207	4.1
2	2440.467	8.133	53.4
3	2432.533	8.107	65.8
4	2377.386	7.923	13.9
5	2376.394	7.920	13.9
6	2372.228	7.906	108.7
7	2370.641	7.901	79.0
8	2369.848	7.898	79.8
9	2367.070	7.889	55.9
10	2365.880	7.885	75.9
11	2359.532	7.863	15.1
12	2358.144	7.859	23.7
13	2353.185	7.842	33.6
14	2351.796	7.838	29.2
15	2350.209	7.832	30.9
16	2348.820	7.828	25.0
17	2346.837	7.821	20.3
18	2345.250	7.816	34.8
19	2344.060	7.812	35.8
20	2342.671	7.807	33.4
21	2338.902	7.795	17.5
22	2336.125	7.785	15.6
23	751.741	2.505	99.7
24	749.956	2.499	151.2
25	748.369	2.494	146.2
26	367.894	1.226	3.4
27	325.642	1.085	3.0



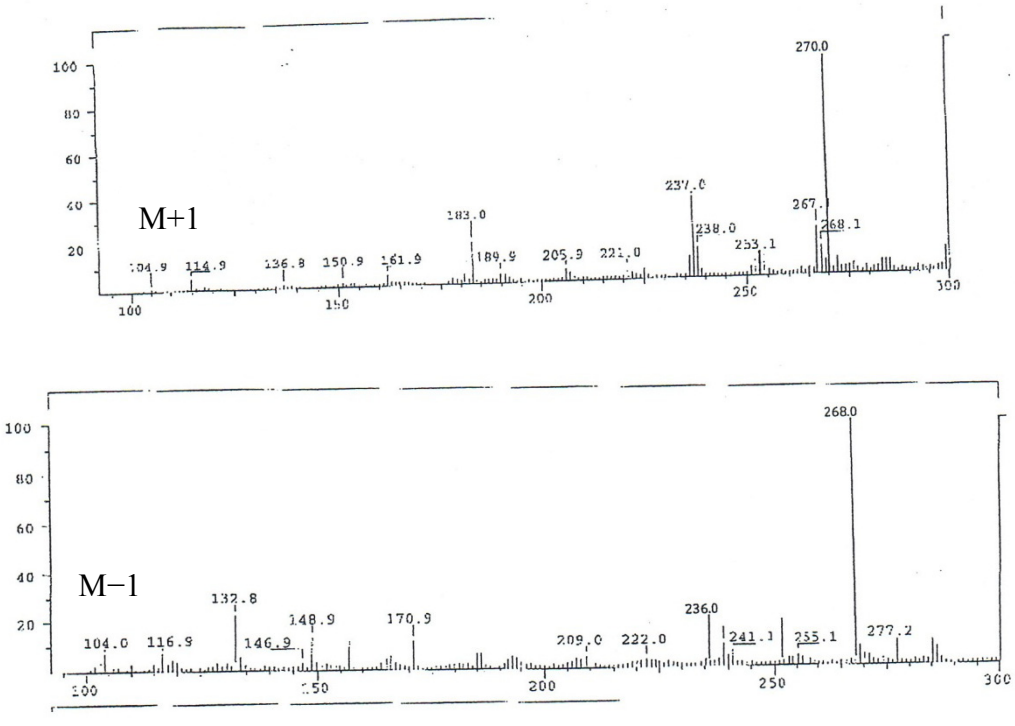
+ D_2O



^{13}C-NMR (DMSO-d_6) δ: 181.2, 179.1, 148.7, 147.2, 146.7, 146.1, 142.9, 141.9, 133.1, 131.9, 128.1, 125.5, 124.6.

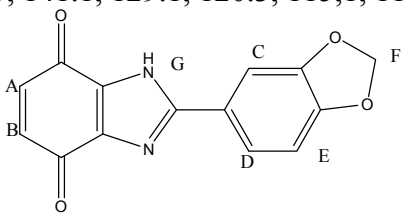


MS *m/z* [M+1, M−1]: 270, 268.



2-Benzo[1,3]dioxol-1*H*-benzimidazol-4,7-dione (5c)

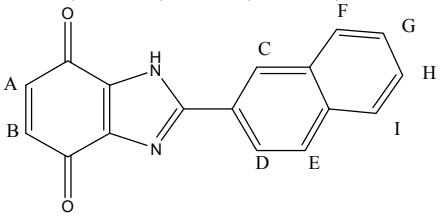
IR (KBr) υ/cm^{-1}: 3324 (NH), 2960 (CH_2), 1701 (C=O), 1503 (C=N), 1264 (C-O-Csym), 1036 (C-O-Casym); ^{1}H-NMR (DMSO-d_6) δ: 4.0 (s, 1H, NH), 7.9 (d, 2H, CH, J = 1.98 Hz), 7.2 (d, 1H, CH, J = 7.9 Hz), 7.0 (d, 2H, CH, J = 8.0 Hz), 6.2 (s, 2H, CH_2); ^{13}C-NMR (DMSO-d_6) δ: 179.5, 179.0, 149.1, 148.6, 147.6, 142.1, 141.5, 141.3, 141.1, 129.1, 120.3, 115,1, 113.2, 91.



J_{AB} = 8.0, J_{CD} = 1.98, J_{DE} = 7.9

2-Naphthyl-1*H*-benzimidazol-4,7-dione (5d)

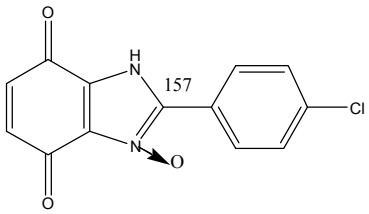
IR (KBr) υ/cm^{-1}: 3424 (NH), 3033 (ArH), 1677 (C=O); 1452 (C=N); ^{1}H-NMR (DMSO-d_6) δ: 13.0 (s, 1H, NH), 8.7 (s, 1H, CH) 8.3 (d, 2H, CH, J = 1.8 Hz), 8.1 (d, 2H, CH, J = 8.1Hz), 7.9 (d, 2H, CH, J = 1.8 Hz), 7.6 (d, 2H, CH, J = 1.8 Hz); ^{13}C-NMR (DMSO-d_6) δ: 179.9, 179.1, 150.2, 148.6, 148.5, 147.7, 147.3, 142.2, 141.2, 134.1, 133.8, 133.6, 128.5, 126.5, 124.3.



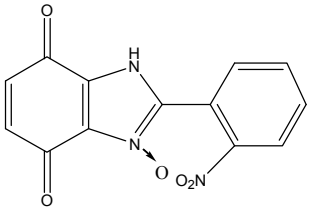
J_{AB} = 8.1; J_{CD} = $_{FH}$ =$_{IG}$ = 1.8

2-(4-Chlorophenyl)-1*H*-benzimidazol-4,7-dione N-oxide (6a)

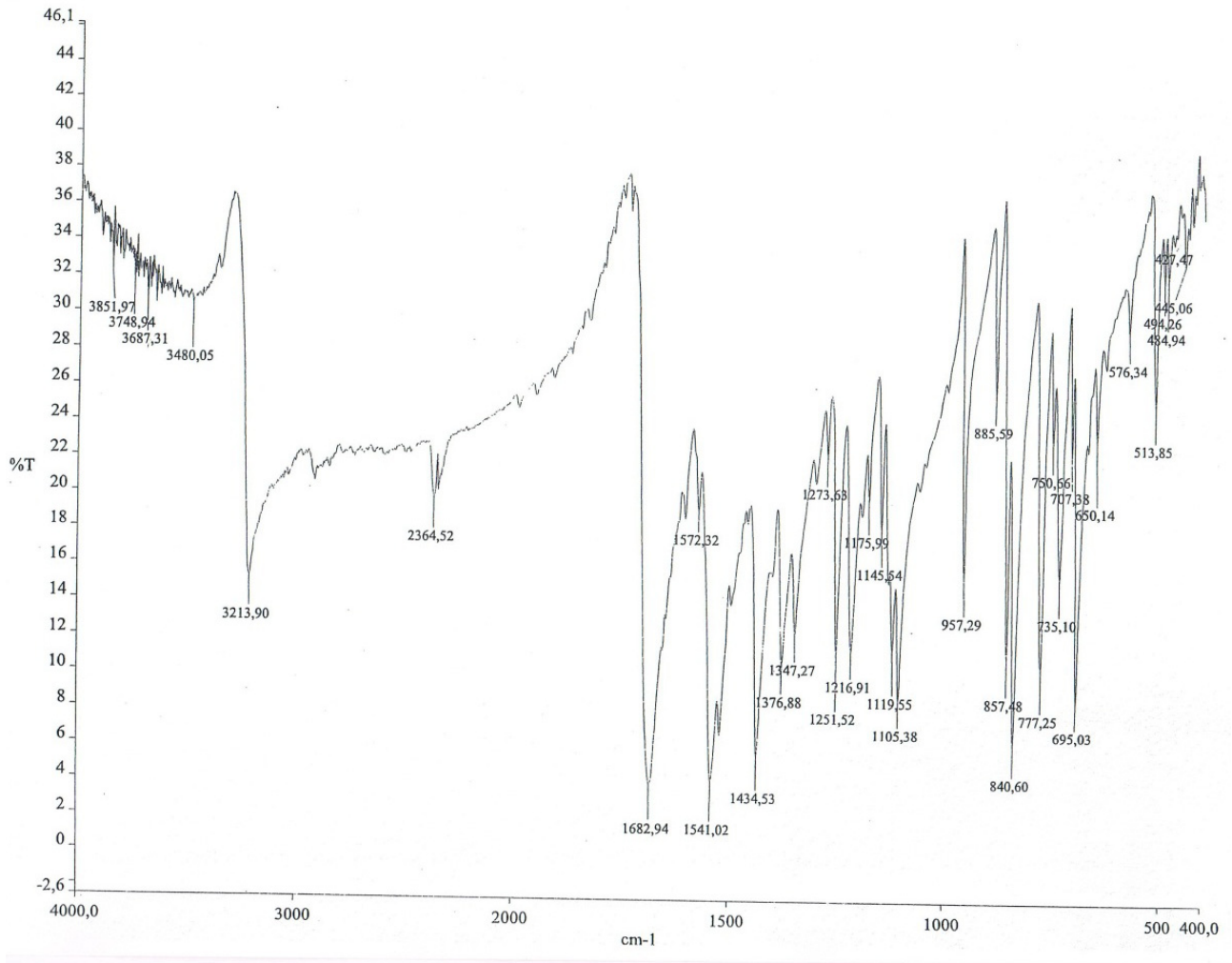
IR (KBr) υ/cm^{-1}: 3445 (NH), 1685 (C=O), 1661 (C=O), 1484 (C=N), 1282 (N-O); ^{1}H-NMR (DMSO-d_6) δ: 14.5 (s, 1H, NH), 8.1 (d, 2H, CH, J = 8.0 Hz), 7.6 (d, 2H, CH, J = 2.0 Hz), 7.5 (d, 2H, CH, J = 2.0 Hz), ^{13}C-NMR (DMSO-d_6) δ: 180.2, 179.3, 157.2, 143.1, 142.6, 136.5, 135.2, 129.8, 129.1, 128.6, 128.0; MS *m/z* [M+1, M−1]: 275, 273.



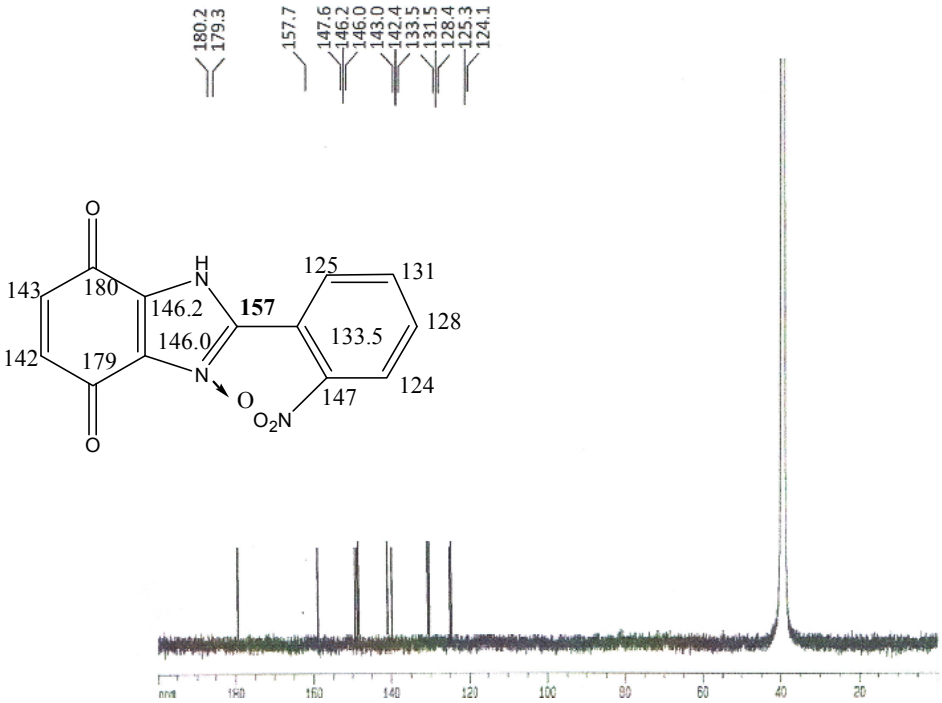
2-(2-nitrophenyl)-1*H*-benzimidazol-4,7-dione N-oxide (6b)



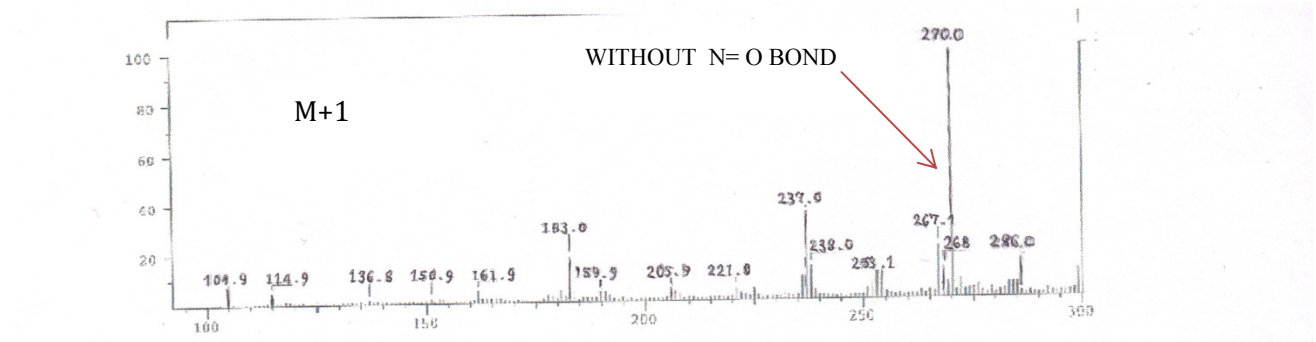
IR (KBr) υ/cm^{-1}: 3480 (NH), 1682 (C=O), 1541 (NO_2asym), 1347 (NO_2sym) 1434 (C=N), **1251** (**N-O**).

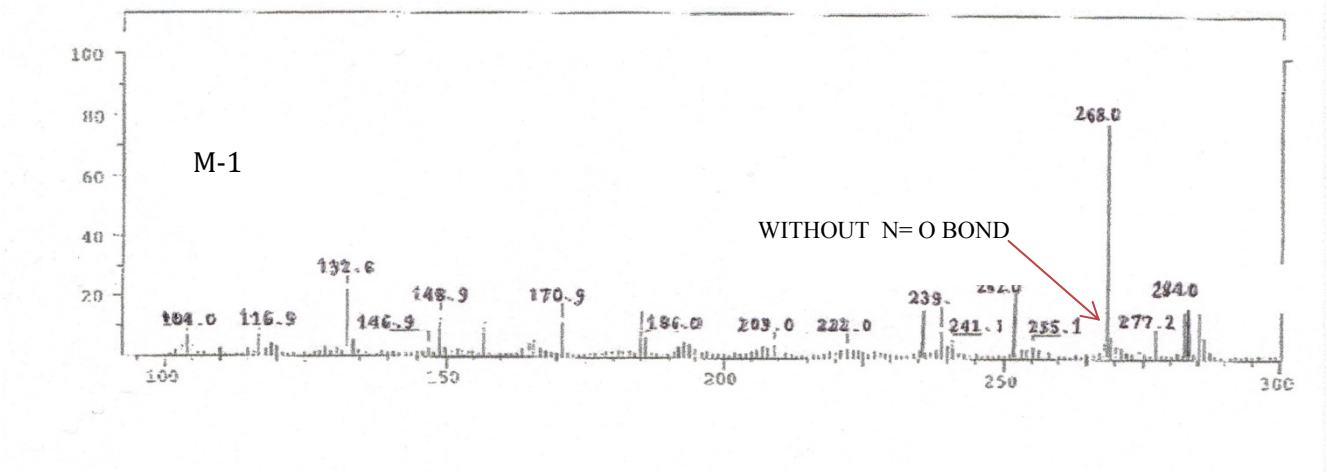


^{13}C-NMR (DMSO-d_6) δ: 180.2, 179.3, 157.7, 147.6, 146.2, 146.0, 143.0, 142.4, 133.5, 131.5, 128.4, 125.3, 124.1.



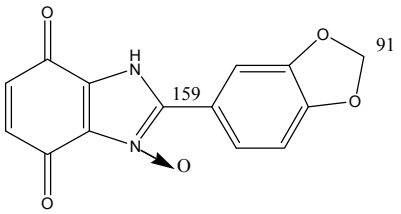
MS *m/z* [M+1, M−1]: 286, 284.





2-Benzo[1,3]dioxol-1*H*-benzimidazol-4,7-dione *N*-oxide (6c)

IR (KBr) υ/cm^{-1}: 3346 (NH), 2961 (CH_2), 1680 (C=O), 1470 (C=N), 1255 (C-O-Csym), 1045 (C-O-Casym), 1249 (N-O); ^{1}H-NMR (DMSO-d_6) δ: 10.0 (s,1H,NH), 7.8 (d, 2H, CH, J = 8.1 Hz), 7.7 (d, 1H, CH, J = 8.0 Hz), 7.6 (d, 2H, CH, J = 2.0 Hz), 6.0 (s, 2H, CH_2); ^{13}C-NMR (DMSO-d_6) δ: 18025, 179.8, 159.1, 148.2, 147.1, 142.0, 141.7, 141.5, 141.0, 129.3, 120.1, 115.5, 113.3, 91.2.



2-Naphthyl-1*H*-benzimidazol-4,7-dione *N*-oxide (6d)

IR (KBr) υ/cm^{-1}: 3300 (NH), 3031 (ArH), 1670 (C=O), 1465 (C=N), 1261 (N-O); ^{1}H-NMR (DMSO-d_6) δ: 13.1 (s, 1H, NH), 8.8 (s, 1H, CH), 8.3 (d, 2H, CH, J = 2.0 Hz) 8.0 (d, 2H, CH, J = 7.9 Hz), 7.6 (d, 2H, CH, J = 2.0 Hz), 7.4 (d, 2H, CH, J = 2.0 Hz); ^{13}C-NMR (DMSO-d_6) δ: 179.5, 179.2, 160.1, 147.7, 147.5, 142.6, 142.1, 141.0, 140.2, 134.3, 133.7, 133.5, 128.1, 126.2, 124.7.

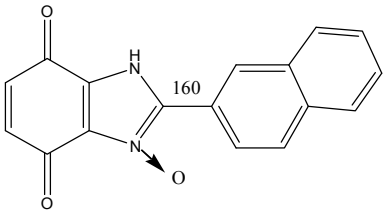


Figure S1. Chromatograms and UV spectrums of analysed compounds. **A** and **B**, Chromatogram and UV spectrum of **5b**; **C** and **D**, Chromatogram and UV spectrum of **6b**.

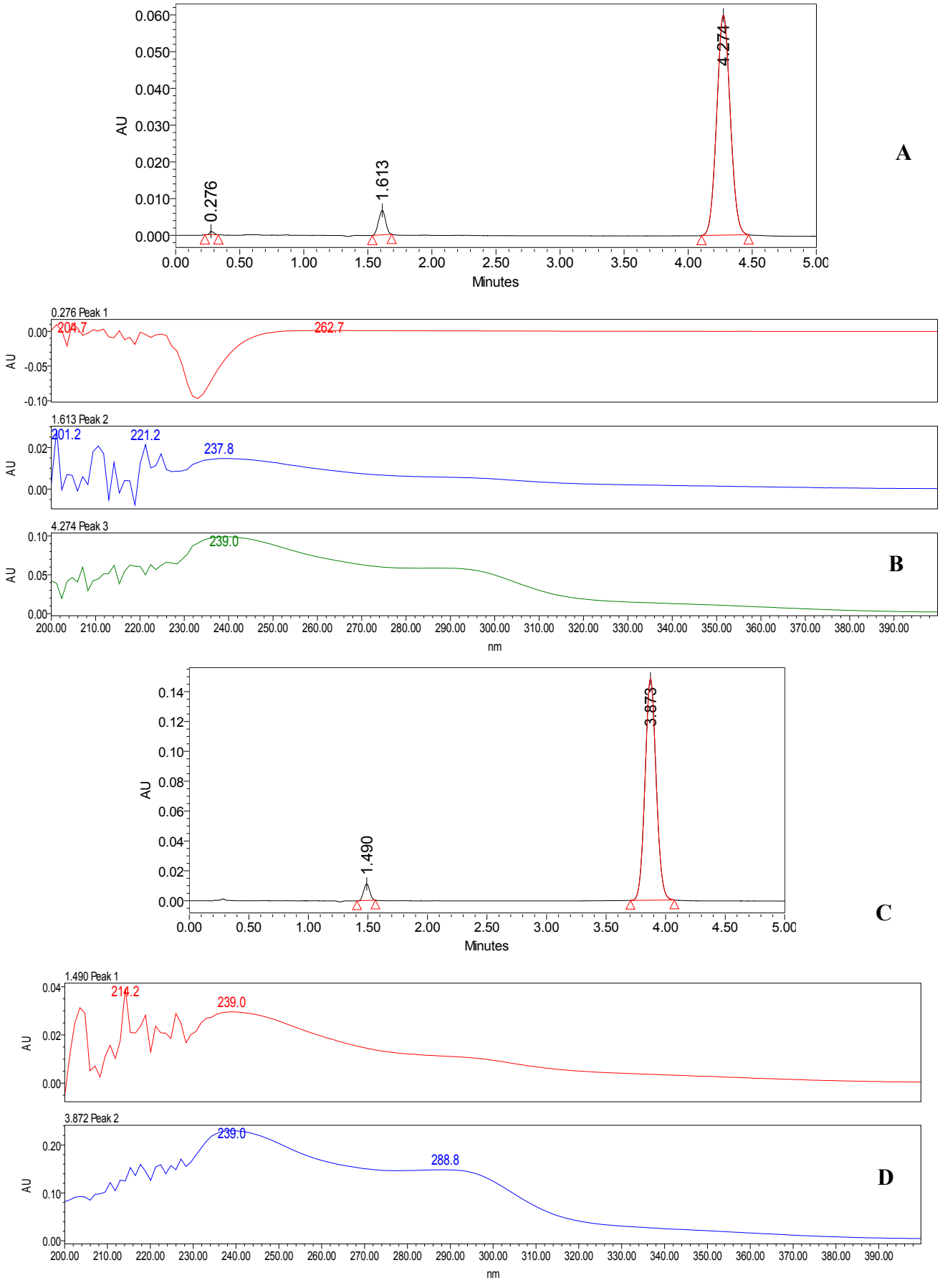


Table S1. Precision of the analytical method.

Conc. (mg/mL)	**Compound 5b**					
n = 3	25%	39%	52%	57%	78%	100%
$\bar{x}$	0.052	0.081	0.108	0.120	0.162	0.209
S	0.0006	0.0006	0.0010	0.0006	0.0012	0.0006
μ	0.052 ± 0.0012	0.081 ± 0.0012	0.108 ± 0.0020	0.120 ± 0.0012	0.162 ± 0.0023	0.209 ± 0.0012
RSD (%)	1.10	0.72	0.93	0.48	0.71	0.28
Conc. (mg/mL)	**Compound 6b**					
n = 3	25%	35%	51%	63%	84%	100%
$\bar{x}$	0.053	0.073	0.107	0.132	0.175	0.210
S	0.0006	0.0006	0.0006	0.0010	0.0006	0.0006
μ	0.053 ± 0.0012	0.073 ± 0.0012	0.107 ± 0.0012	0.132 ± 0.0020	0.175 ± 0.0012	0.210 ± 0.0012
RSD (%)	1.10	0.79	0.54	0.76	0.33	0.28

$\bar{x}$, arithmetic mean; s, standard deviation; μ, mean ± SD; RSD (%), relative standard deviation.

Table S2. Accuracy of the analytical method.

	Content of the determined substance **5b** in relation to the declared (mg/mL)							
	25%		**50%**		**75%**		**100%**	
Samples	**Real value**	**Determined value**	**Real value**	**Determined value**	**Real value**	**Determined value**	**Real value**	**Determined value**
1	0.05	0.05	0.104	0.104	0.157	0.157	0.209	0.209
2	0.055	0.055	0.111	0.111	0.166	0.166	0.222	0.221
3	0.026	0.025	0.052	0.053	0.078	0.078	0.104	0.104
	mean recovery (%) = 100.07; s (%) = 0.54; μ (%) = 100.07 ± 1.08; RSD (%) = 0.54							
	Content of the determined substance **6b** in relation to the declared (mg/mL)							
	25%		**50%**		**75%**		**100%**	
Samples	**Real value**	**Determined value**	**Real value**	**Determined value**	**Real value**	**Determined value**	**Real value**	**Determined value**
1	0.06	0.06	0.115	0.115	0.172	0.172	0.23	0.23
2	0.066	0.066	0.132	0.132	0.197	0.197	0.263	0.262
3	0.06	0.06	0.125	0.125	0.187	0.187	0.25	0.25
	mean recovery (%) = 98.99; s (%) = 0.82; μ (%) = 100.07 ± 1.64; RSD (%) = 0.83							

$\bar{x}$, arithmetic mean; s, standard deviation; μ, mean ± SD; RSD (%), relative standard deviation.

Figure S2. Cell viability of compounds **5a**–**b** and **6a**–**b** at normoxia and hypoxia conditions. Data is expressed as mean-SD, n = 3, 0—control, , T-tirapazamine.