Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

ELECTRONIC SUPPORTING INFORMATION

Biological and computational evaluation of an oxadiazole derivative (MD77) as a new lead for direct STAT3 inhibitors

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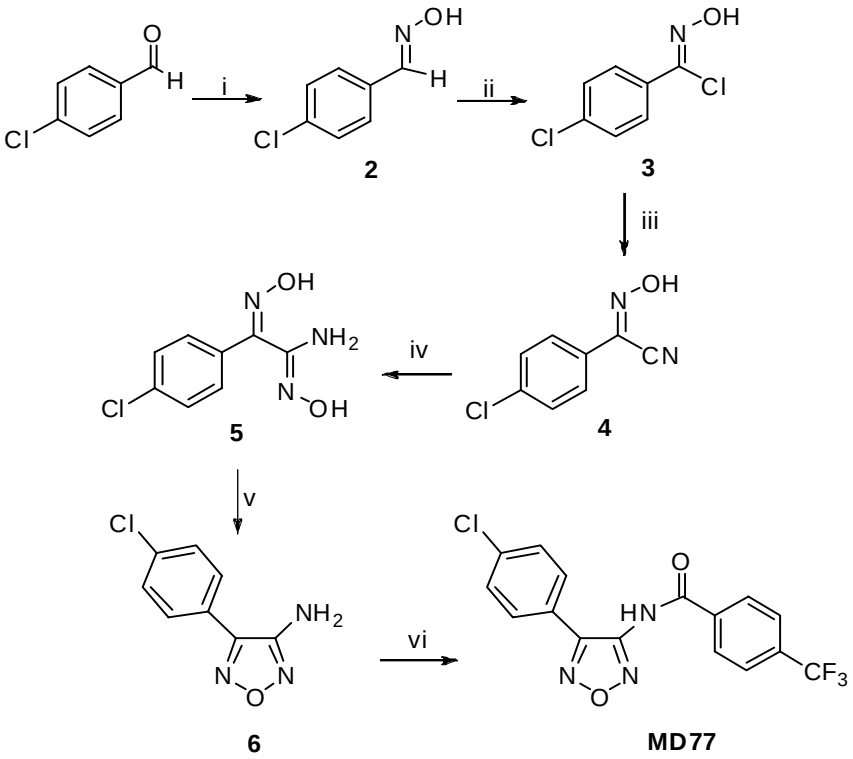
Synthetic scheme for the preparation of **MD77**



Reagents and conditions: i) NH_2OH HCl, $NaHCO_3$, MeOH, reflux; ii) NCS, DMF, rt; iii) KCN, $(CH_3CH_2)_2O$, H_2O, 0°C; iv) NH_2OH HCl, $NaHCO_3$, MeOH, reflux; v) 2N NaOH, reflux; vi) *p*CF_3-Ph-COCl, Py,rt.

Chemistry: experimental part

Materials and Methods. Reagents [4-chloro benzaldehyde, 4-(trifluoromethyl) benzoyl chloride] were purchased from Sigma-Aldrich (Milan, Italy) and were used without any further purification. Melting points were determined in open capillary tubes on a Büchi Melting Point B-540. ^{1}H and ^{13}C NMR spectra were acquired at ambient temperature on a Varian 300 MHz Oxford instrument. Chemical shifts are expressed in ppm from tetramethylsilane resonance in the indicated solvent (TMS: 0.0 ppm) and coupling constants (J-values) are given in Hertz (Hz). ^{1}H NMR data are reported in the following order: ppm, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), and number of protons. The course of the reaction was monitored by thin layer chromatography (TLC) on aluminum-backed Silica Gel 60 plates (0.2 mm, Merck). Intermediates and final compounds were purified by flash chromatography using Merck Silica Gel 60 (70-230 mesh). The purity of final compounds were determinated by HPLC analysis and were ≥95%.

4-chloro benzaldehyde oxime (2). To 4-chloro benzaldehyde (38 mmol) in methanol (100 mL) were added $NH_2OH{\cdot}HCl$ (50 mmol) and $NaHCO_3$ (50 mmol). The mixture was refluxed under

stirring for 2 h, subsequently water (100 mL) was added and the solvent was evaporated under vacuum. The aqueous phase was extracted with ethyl acetate (3 x 30mL), the organic solvent dried over Na_2SO_4 and evaporated under reduced pressure to obtain the oxime intermediate (**2**): yield 98%. ^{1}H NMR ($CDCl_3$) 7.42-7.47 (d, 2H, J= 9.6 Hz, ArH), 7.50-7.56 (d, 2H, J= 9.6 Hz, ArH), 8.10 (s, 1H, CH), 10.00 (s, 1H, NH).

4-chloro benzoyl chloride oxime (3). The intermediate (**2**) (38 mmol) and NCS (46 mmol) were dissolved in DMF (150 mL) and the solution was stirred for 12 h at rt. After addition of water (100 mL) to the reaction mixture, the aqueous solution was extracted by ethyl acetate (3x50 mL). The organic phases were collected, dried over Na_2SO_4 and evaporated under vacuum to give the crude hydroxylimino derivative (**3**) that was directly used without further purification in the next reaction.

2-(4-chlorophenyl)-2-(hydroxyimino)acetonitrile (4). The derivative (**3**) (38 mmol) was dissolved in diethyl ether (100 mL) and cooled to 0°C. A solution of KCN (76 mmol) dissolved in water (100 mL) was added and the reaction mixture was stirred at rt for 5 h. Subsequently water (100 mL) was added and the aqueous phase was extracted by ethyl acetate (3 x 30 mL). The organic solvent was dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by flash chromatography (eluent: petroleum ether/ethyl acetate 9:1) to obtain (**4**) in 90% yield. ^{1}H NMR ($CDCl_3$) 7.55-7.63 (d, 2H, J= 9.6 Hz, ArH), 7.65-7.73 (d, 2H, J= 9.6 Hz, ArH), 8.95 (br s, 1H, OH).

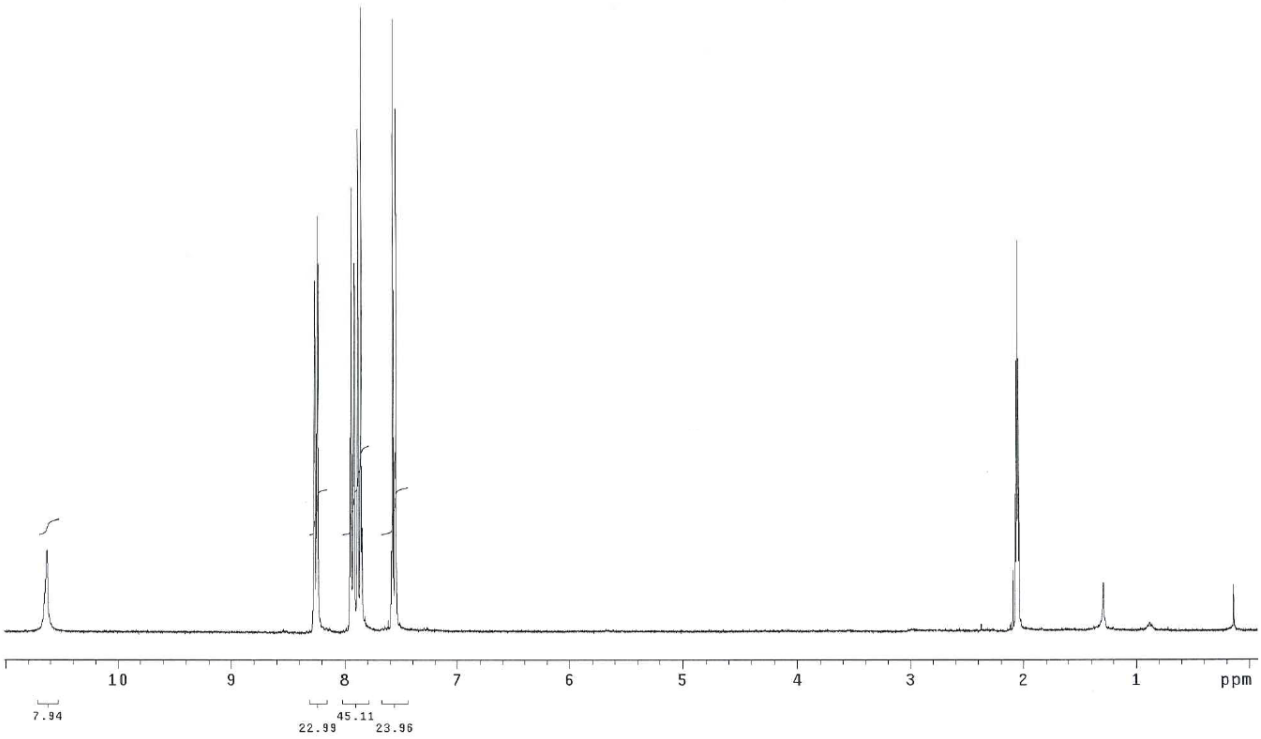
2-(4-chlorophenyl)-*N'*-hydroxy-2-(hydroxyimino)acetamidine (5). To the intermediate (**4**) (38 mmol) in methanol (150 mL) were added $NH_2OH{\cdot}HCl$ (57 mmol) and $NaHCO_3$ (57 mmol). The mixture was refluxed under stirring for 12 h. After addition of water (100 mL), the solvent was removed under vacuum. The aqueous phase was extracted by ethyl acetate (3 x 30 mL). The organic solvent dried over Na_2SO_4 and evaporated under reduced pressure to give the crude acetamidine intermediate (**7**) that was directly used without further purification in the next reaction.

4-(4-chlorophenyl)-1,2,5-oxadiazol-3-amine (6). The derivative (**5**) (38 mmol) was dissolved in 2N NaOH (100 mL) and the solution was refluxed under stirring for 12 h. The mixture was cooled to rt and the so formed precipitate was collected by filtration and washed with water. Yield 44%. ^{1}H NMR ($CDCl_3$) 4.20 (br s, 2H, NH_2), 7.50-7.55 (d, 2H, J=9.6 Hz, ArH), 7.62-7.67 (d, 2H, J=9.6 Hz, ArH).

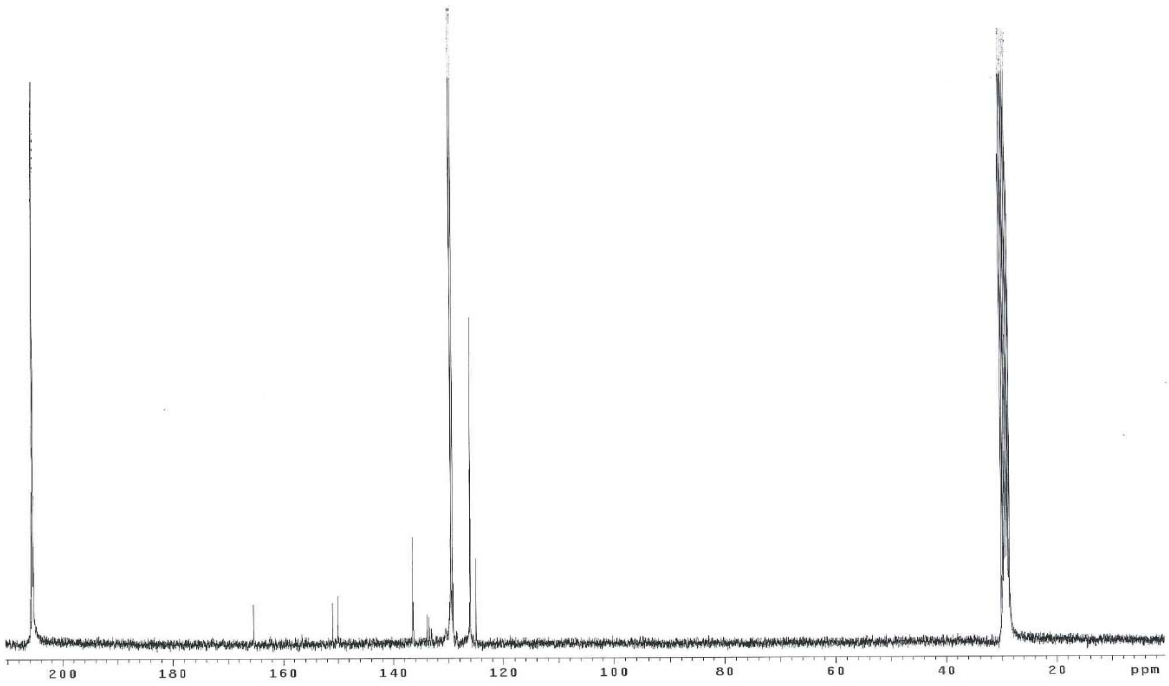
N-[4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]-4-(trifluoromethyl)benzamide (MD77). To a stirred and ice cooled solution of (**6**) (1 mmol) in a mixture of toluene (2 mL) and diethyl ether (0.5 mL), pyridine (0.08 mL, 1 mmol) and then 4-(trifluoromethyl)benzoyl chloride (0.15mL, 1 mmol) were added dropwise. The reaction mixture was kept under stirring at 0°C for 30 min and then at room temperature for 3h. The reaction mixture was evaporated under vacuum and the residue purified by flash chromatography (eluent: petroleum ether/ethyl acetate 9:1) to give **MD77** in 50% yield. M.p.

111-114°C, ^{1}H NMR (acetone-d_6) 7.54-7.59 (d, 2H, ArH), 7.85-7.89 (m, 2H, ArH), 7.93 (d, 2H, J=8.1 Hz, ArH), 8.25 (d, 2H, J=8.1 Hz, ArH) ; ^{13}C NMR (acetone-d_6) 124.9, 125.9, 126.0, 128.3, 128.51, 133.5, 133.9, 136.4, 136.5, 150.04, 151.0, 165.4. HRMS (ESI) $C_{16}H_9ClF_3N_3O_2$ $[M+H]^+$: 368.25 (requires 368.03); Elemental analysis: found C: 52.11, H: 2.59, Cl: 9.72, F:15.25, N: 11.54 (calculated C: 52.26, H: 2.47, Cl: 9.64, F: 15.50, N: 11.43).

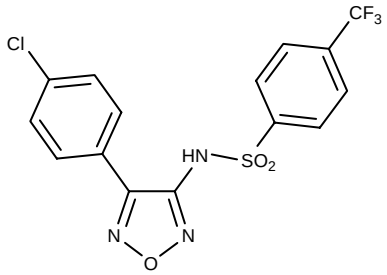
^{1}H NMR (acetone-d_6) of **MD77**



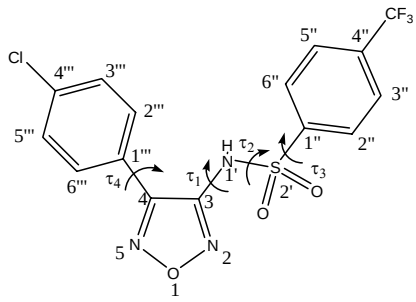
^{13}C NMR (acetone-d_6) of **MD77**



Chemical structure of compound **1**.



Compound **1**
N-[4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl]-4-(trifluoromethyl)benzenesulfonamide

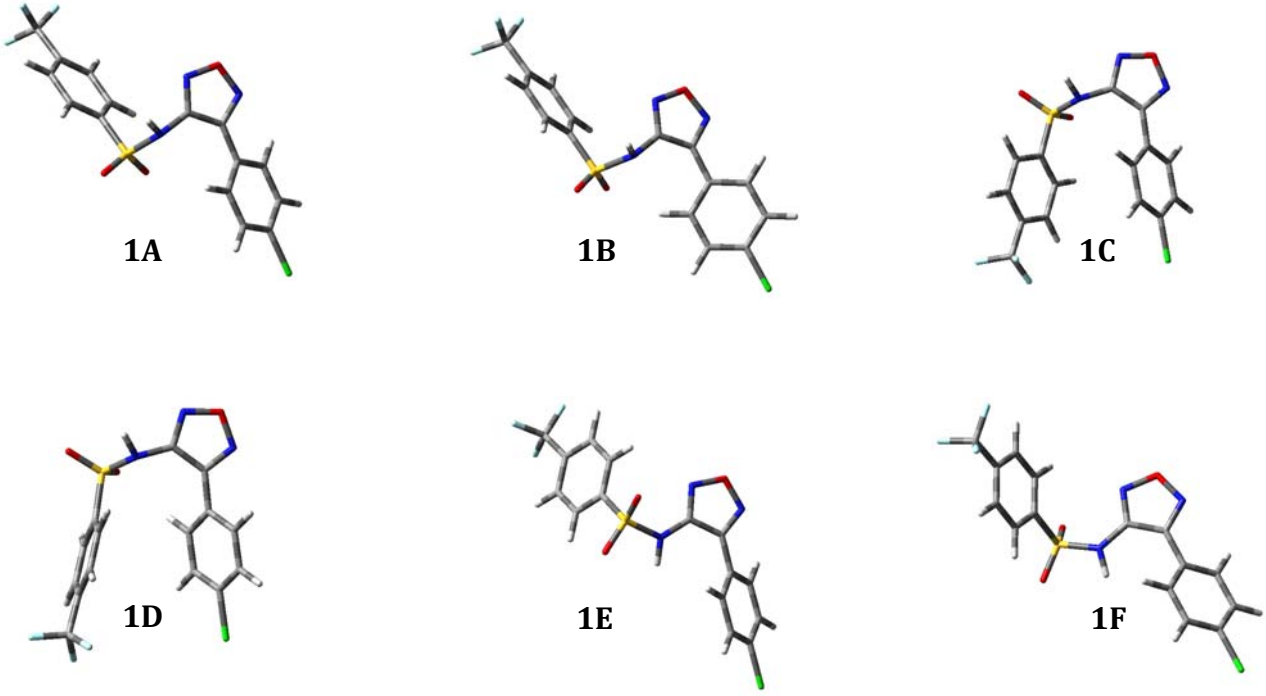


Relative energies (kcal/mol), equilibrium percentages (%) in the gas phase and in water, and significant torsional angles[a] (°) of the located conformations of compound **1**.

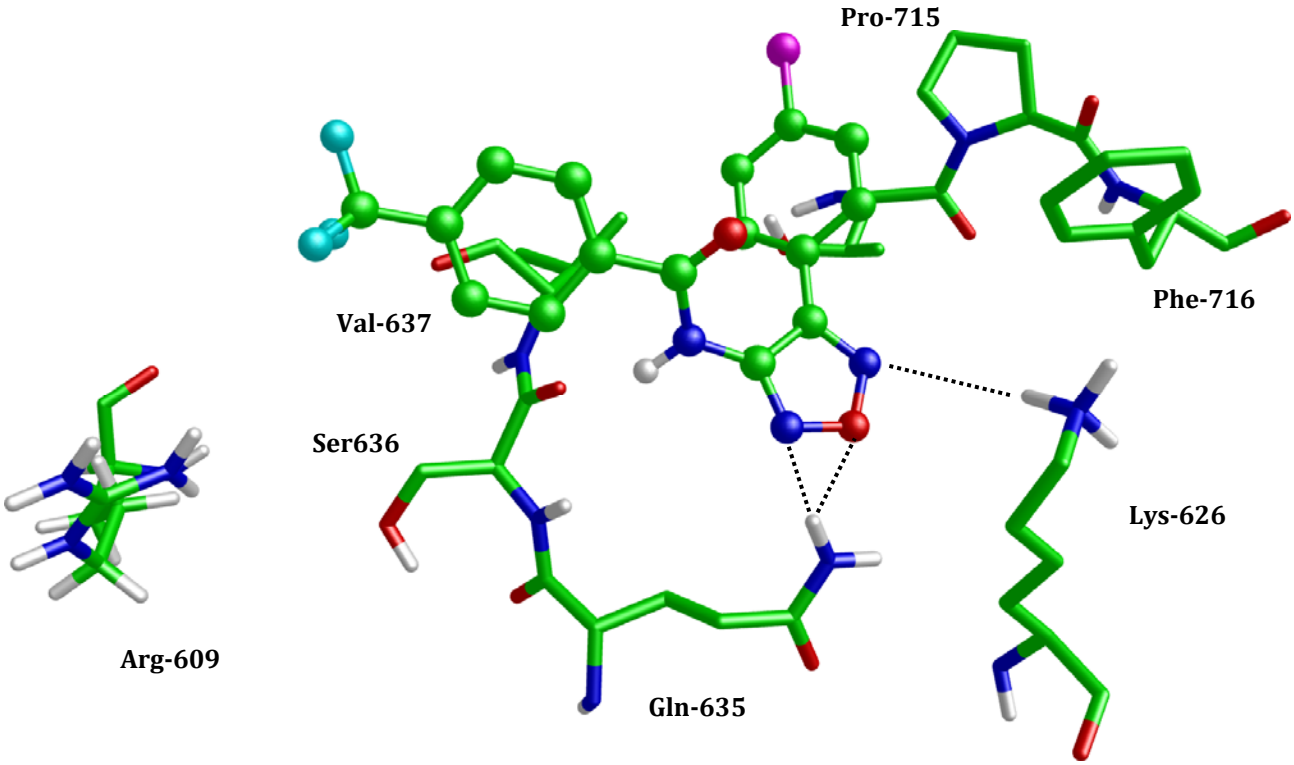
	E_{rel} vacuo (kcal/mol)	P vacuo (%)	E_{rel} water (kcal/mol)	P water (%)	τ_1 (°)	τ_2 (°)	τ_3 (°)	τ_4 (°)
1A	0.36	21.7	0.74	16.6	100	-66	92	-25
1B	0.57	15.4	1.47	4.8	84	-65	-87	35
1C	2.18	1.0	2.58	0.7	101	122	-104	-41
1D	4.33	0.0	4.63	0.0	112	113	-150	65
1E	0.00	40.0	0.00	57.4	-10	69	-91	-40
1F	0.36	21.9	0.61	20.5	24	-81	94	-42

[a] τ_1 : N2-C3–N1'–S2'; τ_2 : C3–N1'–S2'–C1"; τ_3 : N1'–S2'– C1"– C2"; τ_4 : C3–C4–C1'"–C2'".

3D plots of conformations **A-F** of compound **1**.



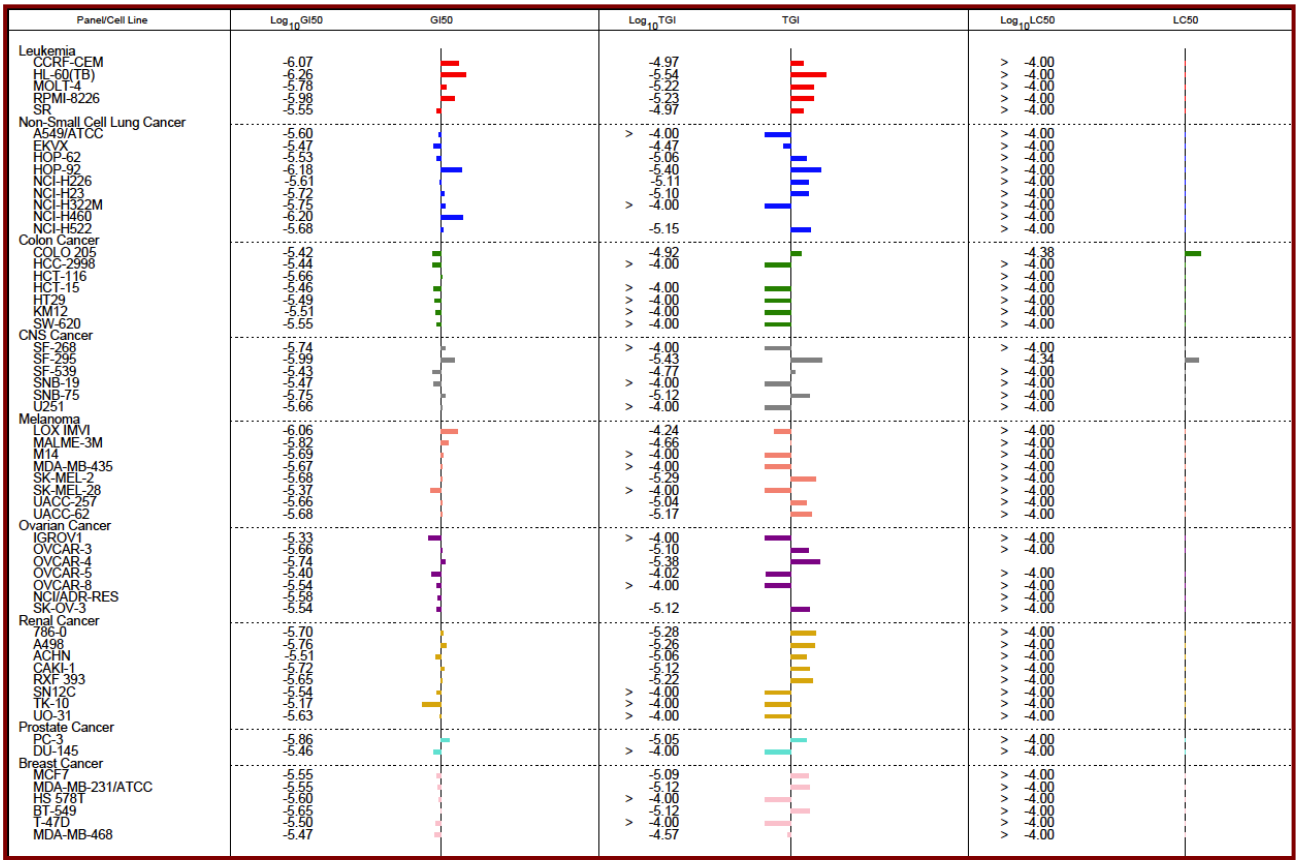
Docking pose of **MD77** conformation referable to **C-D** conformers. The ligand interaction energy is worse than the most stable complex shown in **Figure 6** and the binding mode is much different, because **MD77** is partially inserted in the *p*Tyr-705 pocket.



Mean optical densities, percent growth, GI_{50}, TGI and LC_{50} of **MD77** evaluated on a panel of 58 human tumor cell lines (data obtained from NCI).

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.516	1.667	1.641	1.583	1.052	0.520	0.453	98	93	47	.	-12	8.42E-7	1.07E-5	> 1.00E-4
HL-60(TB)	0.896	2.698	2.551	2.597	1.512	0.530	0.508	92	94	34	-41	-43	5.46E-7	2.85E-6	> 1.00E-4
MOLT-4	0.792	2.313	2.252	2.182	1.856	0.638	0.518	96	91	70	-20	-35	1.67E-6	6.05E-6	> 1.00E-4
RPMI-8226	0.882	2.526	2.422	2.319	1.725	0.747	0.667	94	87	51	-15	-24	1.05E-6	5.89E-6	> 1.00E-4
SR	0.482	1.949	1.759	1.826	1.815	0.497	0.306	87	92	91	1	-37	2.85E-6	1.06E-5	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.323	1.791	1.764	1.647	1.506	0.391	0.373	98	90	81	5	3	2.53E-6	> 1.00E-4	> 1.00E-4
EKVX	0.795	1.899	1.953	1.846	1.816	0.935	0.705	105	95	92	13	-11	3.40E-6	3.36E-5	> 1.00E-4
HOP-62	0.453	1.182	1.134	1.122	1.176	0.425	0.357	93	92	99	-6	-21	2.93E-6	8.74E-6	> 1.00E-4
HOP-92	1.283	1.815	1.760	1.751	1.506	0.922	0.816	90	88	42	-28	-36	6.68E-7	3.96E-6	> 1.00E-4
NCI-H226	0.762	1.666	1.635	1.656	1.570	0.682	0.724	97	99	89	-10	-5	2.48E-6	7.85E-6	> 1.00E-4
NCI-H23	0.591	2.066	1.962	1.919	1.656	0.544	0.562	93	90	72	-8	-5	1.89E-6	7.94E-6	> 1.00E-4
NCI-H322M	0.790	1.913	1.716	1.706	1.507	0.888	0.808	82	82	64	9	2	1.78E-6	> 1.00E-4	> 1.00E-4
NCI-H460	0.250	2.519	2.561	2.629	1.071	0.212	0.378	102	105	36	-15	6	6.29E-7	.	> 1.00E-4
NCI-H522	0.827	2.251	2.107	2.076	1.959	0.713	0.700	90	88	79	-14	-15	2.07E-6	7.12E-6	> 1.00E-4
Colon Cancer															
COLO 205	0.318	1.053	1.078	1.039	1.116	0.373	0.045	103	98	109	7	-86	3.80E-6	1.20E-5	4.13E-5
HCC-2998	0.405	1.471	1.416	1.407	1.379	0.592	0.529	95	94	91	17	12	3.63E-6	> 1.00E-4	> 1.00E-4
HCT-116	0.169	1.368	1.324	1.308	1.091	0.164	0.211	96	95	77	-3	3	2.17E-6	.	> 1.00E-4
HCT-15	0.462	2.285	2.193	2.239	2.342	0.564	0.501	95	97	103	6	2	3.50E-6	> 1.00E-4	> 1.00E-4
HT29	0.187	1.162	1.160	1.122	1.167	0.201	0.221	100	96	100	1	3	3.23E-6	> 1.00E-4	> 1.00E-4
KM12	0.437	2.310	2.302	2.220	2.168	0.534	0.557	100	95	92	5	6	3.06E-6	> 1.00E-4	> 1.00E-4
SW-620	0.255	1.810	1.808	1.745	1.574	0.374	0.568	100	96	85	8	20	2.82E-6	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.430	1.384	1.302	1.328	1.052	0.487	0.533	91	94	65	6	11	1.81E-6	> 1.00E-4	> 1.00E-4
SF-295	0.662	2.396	2.298	2.223	1.545	0.409	0.291	94	90	51	-38	-56	1.02E-6	3.72E-6	4.54E-5
SF-539	0.870	2.107	2.043	2.045	2.172	0.965	0.652	95	95	105	8	-25	3.68E-6	1.71E-5	> 1.00E-4
SNB-19	0.506	1.630	1.640	1.661	1.570	0.622	0.586	101	103	95	10	7	3.38E-6	> 1.00E-4	> 1.00E-4
SNB-75	1.084	1.617	1.520	1.595	1.459	0.978	0.777	82	96	70	-10	-28	1.79E-6	7.54E-6	> 1.00E-4
U251	0.270	1.413	1.330	1.336	1.100	0.339	0.298	93	93	73	6	2	2.19E-6	> 1.00E-4	> 1.00E-4
Melanoma															
LOX IMVI	0.169	1.395	1.361	1.332	0.748	0.293	0.164	97	95	47	10	-3	8.72E-7	5.70E-5	> 1.00E-4
MALME-3M	0.631	1.351	1.245	1.277	1.066	0.654	0.592	85	90	60	3	-6	1.52E-6	2.18E-5	> 1.00E-4
M14	0.367	1.353	1.315	1.317	1.049	0.438	0.436	96	96	69	7	7	2.04E-6	> 1.00E-4	> 1.00E-4
MDA-MB-435	0.391	1.878	1.808	1.755	1.477	0.446	0.540	95	92	73	4	10	2.15E-6	> 1.00E-4	> 1.00E-4
SK-MEL-2	0.684	1.261	1.219	1.222	1.214	0.426	0.364	93	93	92	-38	-47	2.10E-6	5.11E-6	> 1.00E-4
SK-MEL-28	0.519	1.283	1.295	1.252	1.223	0.710	0.660	102	96	92	25	18	4.25E-6	> 1.00E-4	> 1.00E-4
UACC-257	0.760	1.961	1.921	1.877	1.686	0.733	0.702	97	93	77	-4	-8	2.17E-6	9.02E-6	> 1.00E-4
UACC-62	0.695	2.450	2.392	2.372	2.128	0.583	0.686	97	96	82	-16	-1	2.11E-6	6.83E-6	> 1.00E-4
Ovarian Cancer															
IGROV1	0.623	2.102	2.103	2.061	2.100	1.005	0.935	100	97	100	26	21	4.72E-6	> 1.00E-4	> 1.00E-4
OVCAR-3	0.475	1.523	1.480	1.461	1.315	0.432	0.468	96	94	80	-9	-2	2.18E-6	7.90E-6	> 1.00E-4
OVCAR-4	0.576	1.062	1.051	1.058	0.996	0.277	0.462	98	99	86	-52	-20	1.83E-6	4.21E-6	.
OVCAR-5	0.565	1.219	1.167	1.162	1.227	0.669	0.564	92	91	101	16	.	3.98E-6	9.63E-5	> 1.00E-4
OVCAR-8	0.349	1.880	1.838	1.872	1.635	0.497	0.445	97	99	84	10	6	2.87E-6	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.504	1.865	1.923	1.861	1.727	0.484	0.527	104	100	90	-4	2	2.66E-6	.	> 1.00E-4
SK-OV-3	0.507	1.026	1.027	1.012	1.057	0.435	0.394	100	97	106	-14	-22	2.92E-6	7.60E-6	> 1.00E-4
Renal Cancer															
786-0	0.638	2.146	2.132	2.121	1.928	0.421	0.421	99	98	86	-34	-34	1.98E-6	5.19E-6	> 1.00E-4
A498	1.240	2.029	1.895	1.810	1.822	0.915	0.893	83	72	74	-26	-28	1.73E-6	5.46E-6	> 1.00E-4
ACHN	0.491	1.697	1.690	1.597	1.752	0.459	0.429	99	92	105	-7	-13	3.10E-6	8.74E-6	> 1.00E-4
CAKI-1	0.731	1.927	1.893	1.894	1.605	0.659	0.682	97	97	73	-10	-7	1.90E-6	7.59E-6	> 1.00E-4
RXF 393	0.695	1.084	1.102	1.107	1.043	0.523	0.441	104	106	89	-25	-37	2.21E-6	6.07E-6	> 1.00E-4
SN12C	0.595	2.074	2.078	2.026	1.931	0.629	0.648	100	97	90	2	4	2.87E-6	> 1.00E-4	> 1.00E-4
TK-10	0.640	1.377	1.307	1.290	1.332	0.942	0.715	91	88	94	41	10	6.75E-6	> 1.00E-4	> 1.00E-4
UO-31	0.572	1.715	1.499	1.487	1.468	0.601	0.626	81	80	78	2	5	2.37E-6	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.600	2.183	2.110	2.096	1.527	0.582	0.565	95	94	59	-3	-6	1.38E-6	8.91E-6	> 1.00E-4
DU-145	0.402	1.476	1.483	1.509	1.390	0.558	0.507	101	103	92	15	10	3.48E-6	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.309	1.543	1.537	1.507	1.536	0.279	0.235	100	97	99	-10	-24	2.84E-6	8.15E-6	> 1.00E-4
MDA-MB-231/ATCC	0.623	1.414	1.402	1.383	1.425	0.537	0.447	99	96	101	-14	-28	2.79E-6	7.59E-6	> 1.00E-4
HS 578T	0.765	1.761	1.697	1.686	1.518	0.886	0.933	94	92	76	12	17	2.53E-6	> 1.00E-4	> 1.00E-4
BT-549	0.793	1.708	1.633	1.660	1.546	0.708	0.619	92	95	82	-11	-22	2.22E-6	7.66E-6	> 1.00E-4
T-47D	0.610	1.424	1.427	1.392	1.384	0.643	0.631	100	96	95	4	3	3.13E-6	> 1.00E-4	> 1.00E-4
MDA-MB-468	0.514	0.863	0.862	0.850	0.816	0.574	0.398	100	96	87	17	-23	3.36E-6	2.70E-5	> 1.00E-4

Mean graph generated from GI_{50}, TGI, or LC_{50} values of **MD77** (data obtained from NCI).



Panel/Cell Line	$Log_{10}GI50$	$Log_{10}TGI$	$Log_{10}LC50$
Leukemia			
CCRF-CEM	-6.07	-4.97	> -4.00
HL-60(TB)	-6.26	-5.54	> -4.00
MOLT-4	-5.78	-5.22	> -4.00
RPMI-8226	-5.98	-5.23	> -4.00
SR	-5.55	-4.97	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	-5.60	> -4.00	> -4.00
EKVX	-5.47	-4.47	> -4.00
HOP-62	-5.53	-5.06	> -4.00
HOP-92	-6.18	-5.40	> -4.00
NCI-H226	-5.61	-5.11	> -4.00
NCI-H23	-5.72	-5.10	> -4.00
NCI-H322M	-5.75	> -4.00	> -4.00
NCI-H460	-6.20		> -4.00
NCI-H522	-5.68	-5.15	> -4.00
Colon Cancer			
COLO 205	-5.42	-4.92	-4.38
HCC-2998	-5.44	> -4.00	> -4.00
HCT-116	-5.66		> -4.00
HCT-15	-5.46	> -4.00	> -4.00
HT29	-5.49	> -4.00	> -4.00
KM12	-5.51	> -4.00	> -4.00
SW-620	-5.55	> -4.00	> -4.00
CNS Cancer			
SF-268	-5.74	> -4.00	> -4.00
SF-295	-5.99	-5.43	-4.34
SF-539	-5.43	-4.77	> -4.00
SNB-19	-5.47	> -4.00	> -4.00
SNB-75	-5.75	-5.12	> -4.00
U251	-5.66	> -4.00	> -4.00
Melanoma			
LOX IMVI	-6.06	-4.24	> -4.00
MALME-3M	-5.82	-4.66	> -4.00
M14	-5.69	> -4.00	> -4.00
MDA-MB-435	-5.67	> -4.00	> -4.00
SK-MEL-2	-5.68	-5.29	> -4.00
SK-MEL-28	-5.37	> -4.00	> -4.00
UACC-257	-5.66	-5.04	> -4.00
UACC-62	-5.68	-5.17	> -4.00
Ovarian Cancer			
IGROV1	-5.33	> -4.00	> -4.00
OVCAR-3	-5.66	-5.10	> -4.00
OVCAR-4	-5.74	-5.38	
OVCAR-5	-5.40	-4.02	> -4.00
OVCAR-8	-5.54	> -4.00	> -4.00
NCI/ADR-RES	-5.58		> -4.00
SK-OV-3	-5.54	-5.12	> -4.00
Renal Cancer			
786-0	-5.70	-5.28	> -4.00
A498	-5.76	-5.26	> -4.00
ACHN	-5.51	-5.06	> -4.00
CAKI-1	-5.72	-5.12	> -4.00
RXF 393	-5.65	-5.22	> -4.00
SN12C	-5.54	> -4.00	> -4.00
TK-10	-5.17	> -4.00	> -4.00
UO-31	-5.63	> -4.00	> -4.00
Prostate Cancer			
PC-3	-5.86	-5.05	> -4.00
DU-145	-5.46	> -4.00	> -4.00
Breast Cancer			
MCF7	-5.55	-5.09	> -4.00
MDA-MB-231/ATCC	-5.55	-5.12	> -4.00
HS 578T	-5.60	> -4.00	> -4.00
BT-549	-5.65	-5.12	> -4.00
T-47D	-5.50	> -4.00	> -4.00
MDA-MB-468	-5.47	-4.57	> -4.00