

Electronic Supplementary Information

Bisbenzimidazoles with Benzenediyl Spacer: An Efficient Fluorophore for Dihydrogenphosphate Sensing and Targeted Living Cell Imaging

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1.

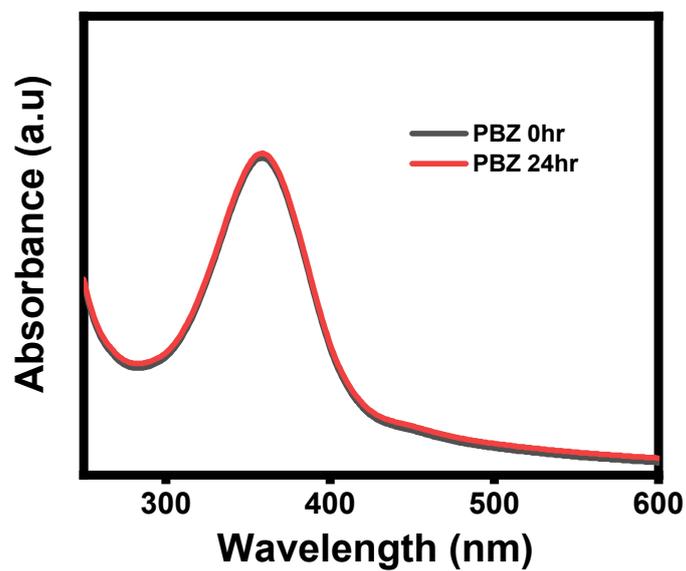


Fig. S1. UV-VIS Stability of PBZ for 24 h.

2.

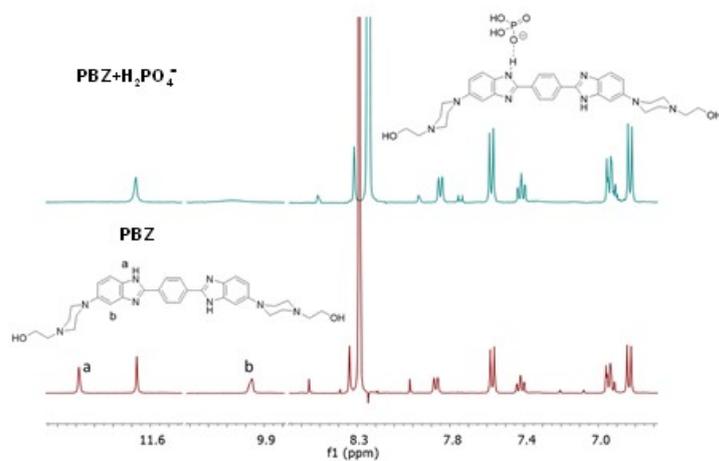


Fig. S2. ¹H NMR spectrum of PBZ in DMSO-D₆/CDCl₃ solvent mixture with and without H₂PO₄⁻.

3.

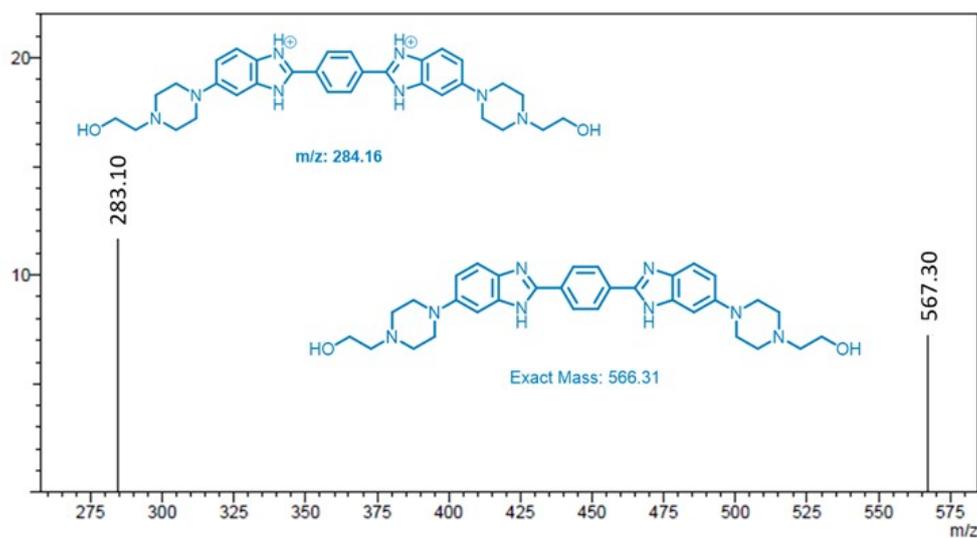


Fig. S3. LC-MS data of of PBZ

5.

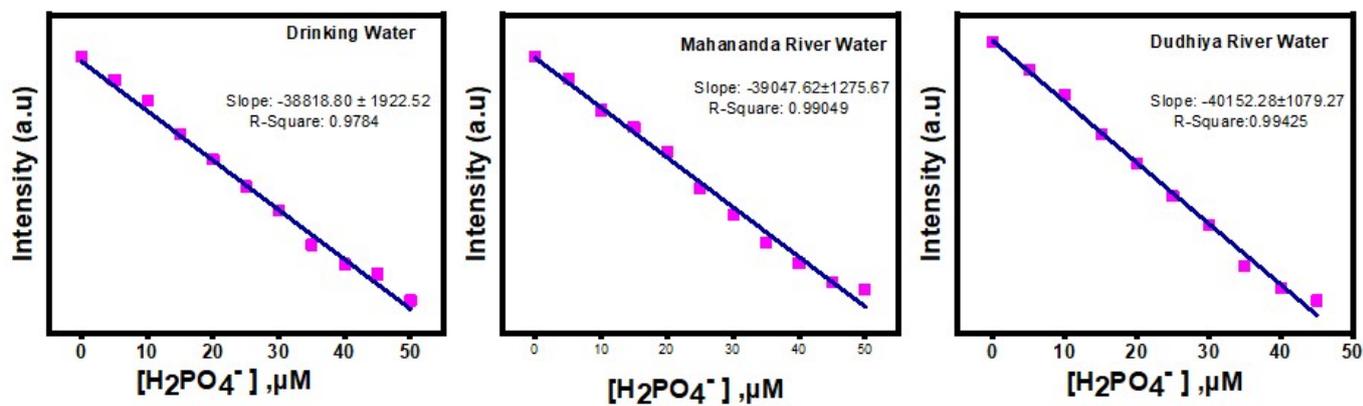


Fig. S4. Plot depicting the Calibration curve of real samples.

6.

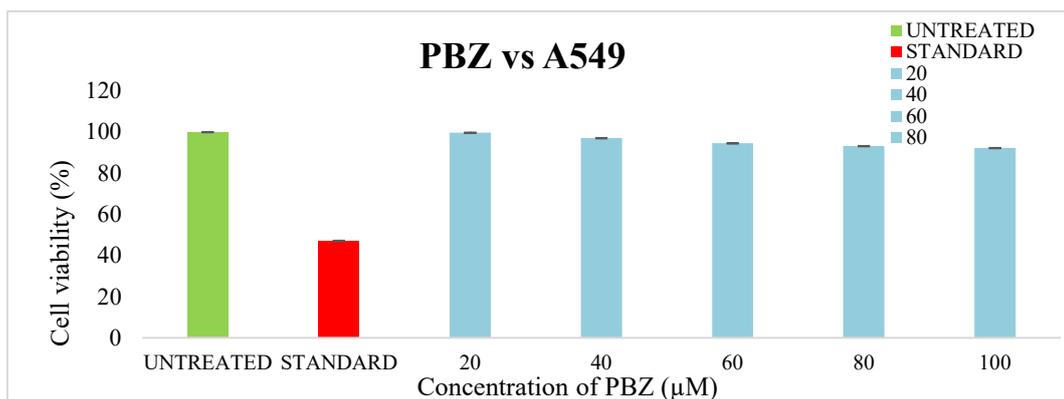


Fig S5: Plot depicting the cytotoxic effect of the samples (PBZ) compared to the untreated.

7.

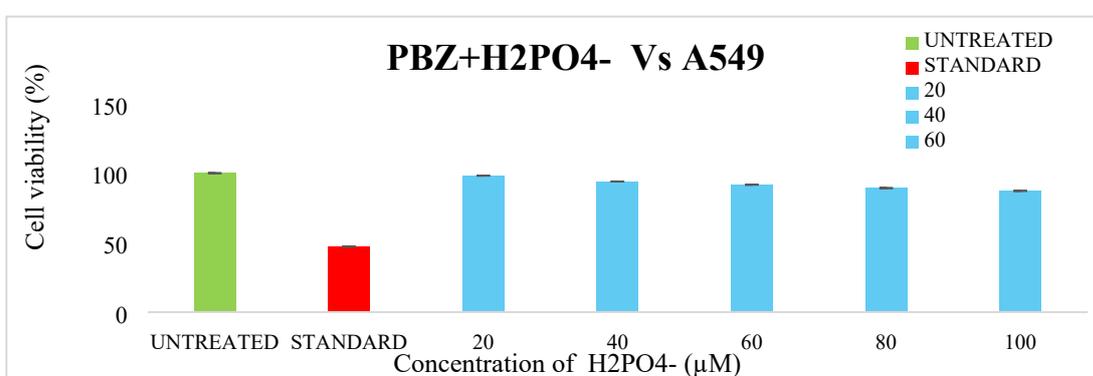


Fig. S6. Plot depicting the cytotoxic effect of the samples (PBZ+H₂PO₄⁻) compared to the untreated.

Study of Cytotoxic Effect and Living Cell Imaging

Table S1: Test Samples Details

Sl. No.	Sample name/ code	Concentrations	Cell line
1	PBZ	5 (20, 40, 60, 80 and 100 μM)	A549
2	PBZ+H ₂ PO ₄ ⁻	5 (20, 40, 60, 80 and 100 μM)	A549

Background of the Study

MTT assay is a colorimetric assay used to determine cell proliferation and cytotoxicity by reducing the yellow-coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which exhibit a purple colour upon dissolution into an appropriate solvent. The intensity of this colour is proportional to the number of viable cells and can be measured spectrophotometrically at 570nm

This study evaluated the samples to analyse the cytotoxicity effect on the A549 cell line. The concentrations of the test compounds used to treat the cells are as follows:

Table S2: Details of the test compound concentrations

Sl. No	Test compounds	Cell line	Concentration treated on cells
1	Untreated	A549	No treatment
2	Standard (Doxorubicin)	A549	~9.4 μ M – A549
4	PBZ	A549	5 (20, 40, 60, 80 and 100 μ M)
5	PBZ+H ₂ PO ₄ ⁻	A549	5 (20, 40, 60, 80 and 100 μ M)

Coordinates of the PBZ molecule:

Atoms	Coordinates (Angstroms)		
	X	Y	Z
C	0.364105	-1.21081	-0.18609
C	1.346021	-0.20491	-0.09534
C	-0.4283	1.458815	-0.01847
C	-1.41007	0.45277	-0.10816
C	-0.98702	-0.8872	-0.19222
C	2.776141	-0.50055	-0.09171
C	-2.84	0.748552	-0.1228
N	-3.37508	2.018953	0.030434
C	-4.76088	1.908524	-0.03811
C	-5.01423	0.532254	-0.24105
N	-3.80388	-0.16912	-0.28841
N	3.307694	-1.78109	-0.12873
C	4.694745	-1.66705	-0.11956
C	4.952999	-0.27773	-0.07201
N	3.74399	0.427279	-0.05613
C	-5.80606	2.832963	0.049222

N	-7.10256	2.348257	-0.07409
C	-7.38835	0.962014	-0.26808
C	-6.32578	0.049116	-0.34475
C	5.737375	-2.59819	-0.14569
C	7.036686	-2.10628	-0.12331
C	7.327273	-0.70787	-0.09113
C	6.266854	0.210458	-0.0702
H	0.642245	-2.25326	-0.25658
H	-0.70687	2.501842	0.04097
H	-1.73381	-1.66337	-0.26323
H	-2.86429	2.870753	0.180904
H	2.793823	-2.64387	-0.15205
H	-5.62475	3.88934	0.189812
H	-6.48887	-1.00945	-0.4707
H	5.55222	-3.66304	-0.16612
H	6.432471	1.276077	-0.06532
H	-7.92197	3.051493	-0.05323
H	7.856192	-2.80928	-0.09994
H	10.42733	1.277158	0.724493
H	8.973049	1.118928	0.272708
N	8.68347	-0.29008	-0.04294
C	9.62922	-0.85559	-1.03466
C	11.07172	-0.68763	-0.56133
N	11.33912	0.735699	-0.29289
H	10.54806	0.777446	1.69942
H	8.316089	1.442293	1.079108
H	9.417495	-1.91121	-1.18174
H	11.24006	-1.31335	0.325756
C	12.7449	1.148897	-0.18646
C	13.51753	0.742807	1.074895
H	13.27182	0.767187	-1.06648
H	12.77694	2.241618	-0.25284
H	14.49388	1.238995	1.068986
H	12.98858	1.045859	1.975977
O	13.6898	-0.70585	1.211427
H	14.17615	-1.05361	0.442851
C	0.92289	1.135173	-0.01175
H	1.669506	1.911842	0.055589
C	-11.1563	0.915365	0.015164
C	-9.73244	1.021845	0.580746
N	-8.74041	0.555182	-0.41948
C	-9.01597	-0.81337	-0.89551
C	-10.4541	-0.89827	-1.43546
N	-11.4782	-0.4429	-0.47475
H	-11.8783	1.205374	0.778295
H	-9.52831	2.055048	0.848403

H	-8.32366	-1.05439	-1.70149
H	-10.5235	-0.26616	-2.32499
C	-11.8851	-1.41045	0.564086
C	-13.3819	-1.31482	0.834066
O	-13.6814	-2.27296	1.898708
H	-11.6621	-2.41774	0.211203
H	-11.3478	-1.27696	1.511959
H	-13.9342	-1.55809	-0.0767
H	-13.6536	-0.30271	1.147593
H	-14.6302	-2.25362	2.109151
H	8.793682	1.770704	-0.59617
H	9.505137	-0.34748	-2.00285
H	11.75	-1.03024	-1.34457
H	10.64259	2.338061	0.864157
H	-9.64562	0.421688	1.499607
H	-11.258	1.613292	-0.81971
H	-10.6765	-1.92202	-1.73618
H	-8.86786	-1.55517	-0.09445

Coordinates of Adduct-I:

Atomic number	Coordinates (Angstroms)		
	X	Y	Z
C	0.609332	-1.59661	-0.88552
C	1.368684	-0.51602	-1.36847
C	-0.66917	0.56758	-2.09854
C	-1.43664	-0.42913	-1.47574
C	-0.7842	-1.55324	-0.93814
C	2.786681	-0.35829	-1.04455
C	-2.86219	-0.18072	-1.25509
N	-3.27773	1.092962	-0.90354
C	-4.64843	1.029452	-0.68923
C	-5.01184	-0.31771	-0.93661
N	-3.87181	-1.0605	-1.28399
N	3.254458	0.886951	-0.65699
C	4.600672	0.738067	-0.34832
C	4.897464	-0.62778	-0.58021
N	3.740164	-1.29786	-1.01083
C	-5.60487	1.975831	-0.30651
C	-6.92193	1.549749	-0.18465
C	-7.31221	0.198053	-0.42329
C	-6.34097	-0.74084	-0.79946
C	5.586326	1.624197	0.100138

C	6.864802	1.118847	0.303222
C	7.186619	-0.2546	0.086766
C	6.185979	-1.13233	-0.35617
H	1.108845	-2.41943	-0.3933
H	-1.15916	1.409551	-2.56242
H	-1.36987	-2.34242	-0.48766
H	-2.63002	1.863152	-0.68336
H	2.643782	1.694793	-0.4723
H	-5.34074	3.008504	-0.12505
H	-6.58674	-1.7771	-0.97249
H	5.37481	2.671199	0.268084
H	6.377327	-2.18196	-0.51595
H	-7.6833	2.273541	0.068178
H	7.65131	1.795447	0.605041
C	10.46481	-2.07122	-0.33101
C	8.938834	-1.99444	-0.23006
N	8.529319	-0.6801	0.291101
C	9.169245	-0.36481	1.589663
C	10.68968	-0.44476	1.471613
N	11.07593	-1.77642	0.97335
H	10.79986	-1.37202	-1.11417
H	8.510419	-2.1302	-1.22234
H	8.881573	0.635037	1.903999
H	11.0466	0.359462	0.814187
C	12.48446	-2.16637	1.128895
C	13.51617	-1.495	0.213601
H	12.76438	-1.99271	2.172576
H	12.55275	-3.24692	0.963096
H	14.48593	-1.98497	0.351721
H	13.23546	-1.58875	-0.83313
O	13.64442	-0.05267	0.438657
H	13.91244	0.114665	1.359749
C	0.714226	0.525005	-2.04552
H	1.289481	1.334739	-2.46739
C	-10.9141	0.217783	0.726461
C	-9.39383	0.22629	0.944153
N	-8.68836	-0.14692	-0.30655
C	-9.13193	-1.44843	-0.8376
C	-10.6583	-1.43788	-1.03027
N	-11.4087	-1.06834	0.186933
H	-11.4227	0.435127	1.665697
H	-9.07964	1.217769	1.260547
H	-8.65498	-1.6169	-1.80288
H	-10.9002	-0.70863	-1.80855
C	-11.6112	-2.13622	1.187095
C	-13.0026	-2.03907	1.801291

O	-13.0931	-3.08818	2.816995
H	-11.5177	-3.10334	0.692264
H	-10.8682	-2.11581	1.99473
H	-13.7566	-2.18214	1.02348
H	-13.1542	-1.05643	2.257224
H	-13.9646	-3.06302	3.246863
H	8.580112	-2.81395	0.412117
H	8.828622	-1.07345	2.360292
H	11.13795	-0.29572	2.455592
H	10.75306	-3.07894	-0.63642
H	-9.12421	-0.47494	1.749821
H	-11.1746	1.00824	0.018125
H	-10.9957	-2.41582	-1.37346
H	-8.84134	-2.27821	-0.17283
O	-1.36737	3.011908	-0.24125
O	1.41756	2.913944	-0.09083
P	-0.00737	3.239051	0.551363
O	-0.21523	2.420438	2.049312
O	0.042966	4.856612	1.174615
H	0.38236	5.522156	0.550604
H	0.553783	2.447316	2.644913

Coordinates of Adduct-II:

Atoms	Coordinates (Angstroms)		
	X	Y	Z
C	1.044871	-0.59738	-0.32739
C	1.723978	0.63374	-0.22579
C	-0.43119	1.763759	-0.12432
C	-1.10693	0.534174	-0.23462
C	-0.3419	-0.64599	-0.3327
C	3.184517	0.64719	-0.23423
C	-2.56579	0.450325	-0.25282
N	-3.40449	1.542395	-0.13237
C	-4.7078	1.069139	-0.19774
C	-4.60228	-0.33332	-0.36342
N	-3.25426	-0.69906	-0.39338
N	3.957837	-0.44682	-0.18772
C	5.280795	0.015729	-0.23074
C	5.289453	1.430496	-0.30395
N	3.947303	1.803823	-0.30382
C	-5.9572	1.697405	-0.1366
C	-7.08693	0.896406	-0.24778
C	-7.0081	-0.52183	-0.40281
C	-5.74916	-1.13676	-0.45371
C	6.474212	-0.70827	-0.19454
C	7.696666	-0.01358	-0.2531
C	7.674599	1.410271	-0.3465
C	6.486545	2.141118	-0.36023
H	1.625383	-1.50455	-0.40535
H	-0.98236	2.689277	-0.03916
H	-0.85633	-1.59179	-0.41573
H	-3.14013	2.534837	-0.01862
H	-6.04902	2.768693	-0.02415
H	-5.63879	-2.20546	-0.55127
H	6.436952	-1.78265	-0.09934
H	6.509222	3.21994	-0.4275
H	-8.05959	1.366614	-0.24697
C	11.28075	-0.89238	0.5159
C	10.18747	0.018775	-0.05013
N	8.923817	-0.72188	-0.19712
C	9.07617	-1.96194	-0.99043
C	10.16955	-2.85268	-0.40256
N	11.42874	-2.09391	-0.31645
H	11.02493	-1.13889	1.559367
H	10.0394	0.847273	0.640101
H	8.137787	-2.50809	-1.00247
H	9.846695	-3.22803	0.578094
C	12.66773	-2.86078	-0.1285
C	12.93525	-3.45828	1.25849

H	12.67848	-3.66329	-0.87248
H	13.50877	-2.20112	-0.36791
H	13.94474	-3.88287	1.273939
H	12.87594	-2.69703	2.03334
O	11.95571	-4.47287	1.655514
H	11.95759	-5.20209	1.010327
C	0.960052	1.81021	-0.11913
H	1.435575	2.7764	-0.01945
C	-10.6345	-1.54031	-0.06211
C	-9.28044	-1.04972	0.470811
N	-8.21582	-1.26271	-0.54096
C	-8.12945	-2.66705	-0.98295
C	-9.50138	-3.13907	-1.49372
N	-10.5951	-2.94609	-0.52145
H	-11.3953	-1.43412	0.711035
H	-9.3512	0.007565	0.71238
H	-7.40702	-2.7388	-1.79562
H	-9.74721	-2.56918	-2.39403
C	-10.7226	-3.9637	0.540884
C	-12.1893	-4.2688	0.821328
O	-12.2183	-5.24798	1.908456
H	-10.2365	-4.88161	0.208769
H	-10.239	-3.66774	1.480764
H	-12.6577	-4.6723	-0.07957
H	-12.7225	-3.36006	1.115286
H	-13.1371	-5.47881	2.126465
H	10.52536	0.430665	-1.01327
H	9.335815	-1.71178	-2.03016
H	10.3222	-3.71321	-1.05616
H	12.22694	-0.34806	0.523902
H	-9.02488	-1.58134	1.400859
H	-10.93	-0.91231	-0.90648
H	-9.45121	-4.19232	-1.76979
H	-7.78359	-3.32586	-0.16983
O	-2.88502	4.149585	0.136598
O	-0.49134	5.514897	0.370203
P	-2.07636	5.475065	0.483181
O	-2.62359	5.923268	2.05296
O	-2.76794	6.816503	-0.3768
H	-2.48522	6.892609	-1.3053
H	-2.17495	6.69677	2.436061
H	3.601674	2.744193	-0.3753
H	8.602783	1.953708	-0.42452

Coordinates of the adduct-III:

Atom	Coordinates (Angstroms)		
	X	Y	Z
C	0.565032	-2.00989	-0.20291
C	1.526493	-0.97992	-0.20704
C	-0.28281	0.649523	-0.2775
C	-1.24305	-0.38204	-0.27493
C	-0.79285	-1.71547	-0.23623
C	2.962015	-1.24709	-0.17404
C	-2.6785	-0.10996	-0.31204
N	-3.22443	1.159175	-0.33793
C	-4.60429	1.012591	-0.37347
C	-4.84584	-0.38261	-0.3682
N	-3.62801	-1.06605	-0.32869
N	3.517487	-2.51858	-0.18186
C	4.902031	-2.37992	-0.14761
C	5.134568	-0.98571	-0.11539
N	3.913111	-0.30201	-0.1336
C	-5.66156	1.928773	-0.41498
C	-6.95386	1.420023	-0.45203
C	-7.22582	0.017161	-0.43173
C	-6.15506	-0.8872	-0.38278
C	5.960838	-3.29258	-0.13949
C	7.251075	-2.77747	-0.09792
C	7.515924	-1.37428	-0.07992
C	6.439471	-0.47456	-0.09395
H	0.864147	-3.04873	-0.16985
H	-0.58925	1.685483	-0.30612
H	-1.52322	-2.51053	-0.23172
H	-2.72825	2.066522	-0.33012
H	3.019648	-3.39011	-0.21734
H	-5.48713	2.995509	-0.43524
H	-6.30928	-1.95423	-0.34834
H	5.794765	-4.36079	-0.14827
H	6.586598	0.593838	-0.10081
H	-7.78111	2.110314	-0.52908
H	8.082469	-3.46505	-0.04747
C	10.56608	0.670535	0.776622
C	9.123707	0.484272	0.298022
N	8.864229	-0.93216	-0.01016
C	9.836252	-1.48789	-0.98137
C	11.26692	-1.29267	-0.48326
N	11.50599	0.136516	-0.21898
H	10.67638	0.180709	1.757849
H	8.445374	0.803877	1.088041

H	9.644595	-2.54786	-1.12503
H	11.42922	-1.91014	0.410741
C	12.90254	0.572786	-0.08887
C	13.66004	0.181467	1.186404
H	13.45122	0.198453	-0.95875
H	12.91769	1.665751	-0.15684
H	14.6265	0.6966	1.198101
H	13.10905	0.473528	2.077871
N	13.85853	-1.26368	1.326025
H	14.36521	-1.6016	0.566208
C	1.074672	0.353129	-0.24328
H	1.80279	1.150377	-0.2443
C	-10.9914	-0.03476	-0.07533
C	-9.55835	0.183734	0.431806
N	-8.57804	-0.42026	-0.50417
C	-8.83891	-1.8505	-0.75139
C	-10.2854	-2.04416	-1.23718
N	-11.2979	-1.45789	-0.33765
H	-11.7029	0.360576	0.649537
H	-9.3703	1.250109	0.523917
H	-8.15875	-2.20593	-1.52491
H	-10.3862	-1.56487	-2.21495
C	-11.6716	-2.25435	0.848087
C	-13.1684	-2.14917	1.115441
N	-13.4398	-2.92619	2.325292
H	-11.4265	-3.30028	0.660964
H	-11.1299	-1.95565	1.75474
H	-13.721	-2.54998	0.262223
H	-13.461	-1.10532	1.261632
H	-14.3872	-2.89068	2.540035
H	8.951645	1.125259	-0.58035
H	9.72106	-0.98826	-1.95519
H	11.96523	-1.62843	-1.25176
H	10.76116	1.735948	0.911552
H	-9.43943	-0.25638	1.434491
H	-11.1239	0.52138	-1.00689
H	-10.4931	-3.10656	-1.36436
H	-8.66077	-2.45512	0.152814
N	-2.11728	3.589804	-0.34801
N	0.515564	4.351349	0.019418
P	-1.03898	4.67347	0.094791
N	-1.52213	5.157525	1.675562
N	-1.37745	6.179231	-0.7018
H	-1.06141	6.233473	-1.62082
H	-0.91673	5.780432	2.113784

