Supplementary Information (SI) for Physical Chemistry Chemical Physics.

Behind the glow: unveiling the nature of NanoLuc reactants and products

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SUPPLEMENTARY INFORMATION

DFT benchmark. With the aim to predict the spectroscopic properties of the NanoLuc-furimamide pairs, a preliminary investigation was done. The aim was to select, within the Density Functional Theory approach, which functional was able to provide the most reliable results among the following: the range separated CAM-B3LYP[1] , that provides a good description of charge transfer (CT) excitations, and the global hybrid ones B3LYP[2,3] and PBE0[4]. Because no experimental data are available for furimazine nor furimamide, calculation of the absorption spectra were performed on coelenterazine (Figure S1), a luciferin system presenting a chemical structure very similar to that of furimazine and for which experimental spectra are available[5]. Geometry optimization in methanol and absorption spectra prediction were carried out with each of the three aforementioned functionals and results are reported in Figure S2. Spectra analysis show that CAM-B3LYP best performs in reproducing the spectral shape, despite giving transition energies blue-shifted by ~0.3 eV, while PBE0 better predicts the energy of the transitions.

On the basis of these results, geometry optimizations of the investigated systems were performed by using the CAM-B3LYP functional, followed by single point calculations with the PBE0 functional, in order to partially correct the blue-shift which characterizes CAM-B3LYP results.

References

1) T. Yanai, D. P. Tew and N. C. Handy, *Chem. Phys. Lett.*, 2004, **393**, 51–57.

2) Becke, A. D. Density-functional Thermochemistry. III. The Role of Exact Exchange. *J. Chem. Phys.* **1993**, *98*

3) Lee, C.; Yang, W.; Parr, R. G. Development of the Colle-Salvetti Correlation-Energy Formula into a Functional of the Electron Density. *Phys. Rev. B* **1988**, *37* (2), 785–789.

4) Adamo, C.; Barone, V. Toward Reliable Density Functional Methods without Adjustable Parameters: The PBE0 Model. *J. Chem. Phys.* **1999**, *110* (13), 6158–6170. https://doi.org/10.1063/1.478522.

5) Shimomura, O. Cause of Spectral Variation in the Luminescence of Semisynthetic Aequorins. *Biochem. J.* **1995**, *306* (2), 537–543. https://doi.org/10.1042/bj3060537.

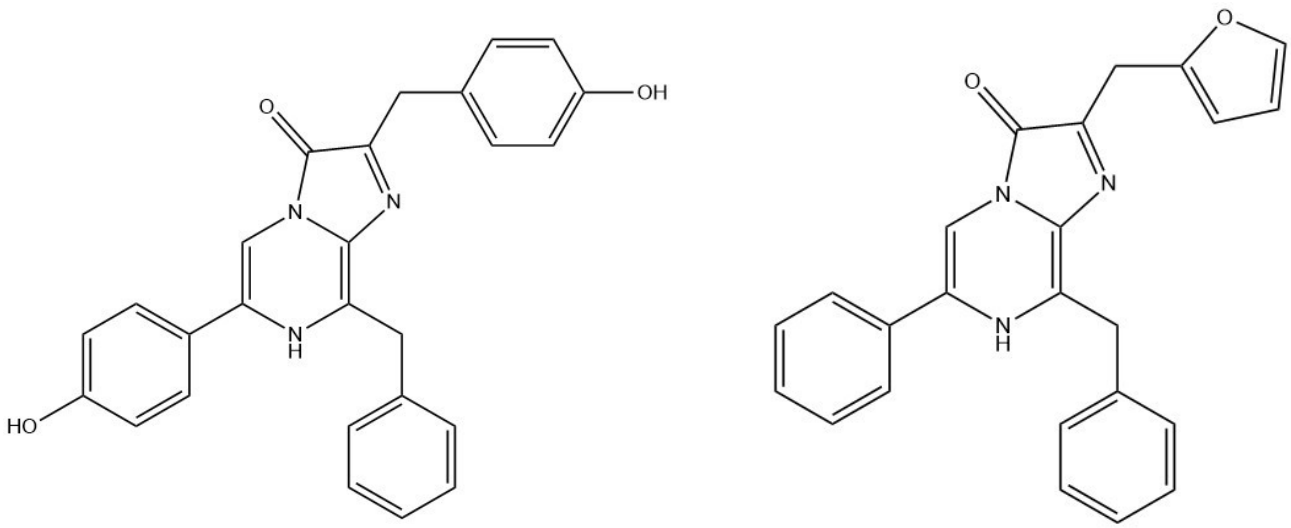

Figure S 1 - Coelenterazine (on the left) and furimazine (on the right)

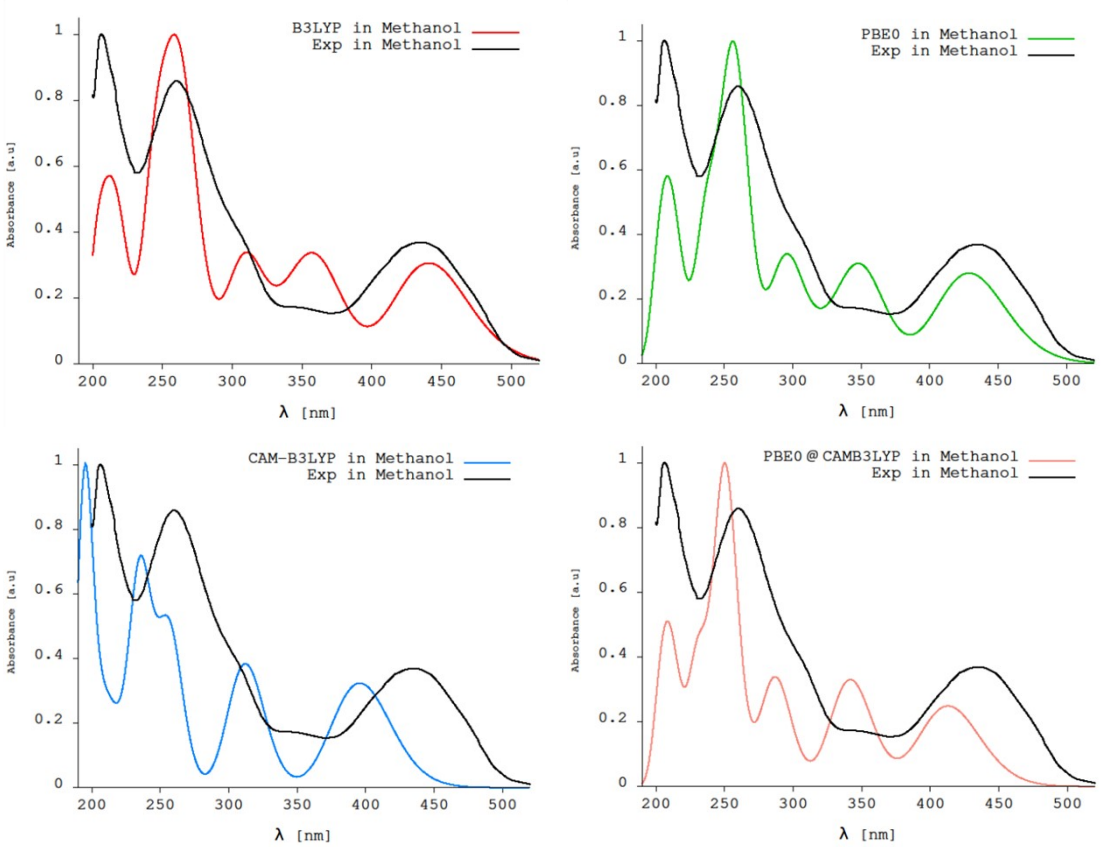

Figure S2. Calculated absorption spectra of coelenterazine in methanol using B3LYP/6-31+G*, CAM-B3LYP/6-31+G*, and PBE0/6-31+G* levels of theory after geometry optimization, and PBE0/6-31+G* on the CAM-B3LYP/6-31+G* (PBE0@CAMB3LYP) optimized geometry.

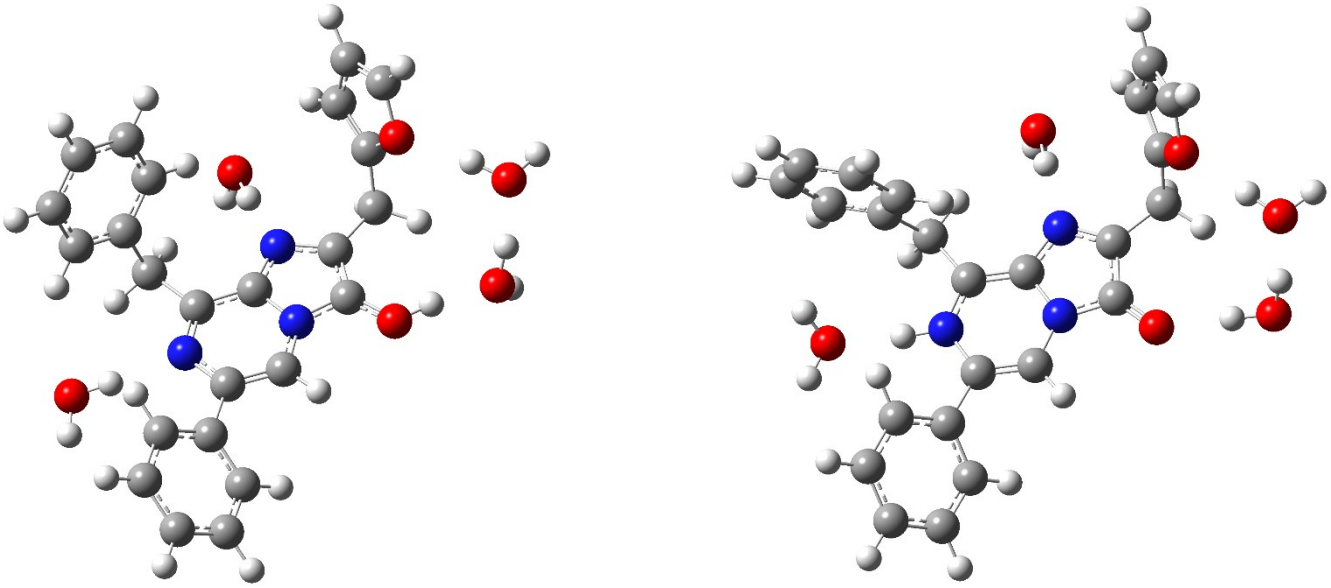

Figure S3. Optimized structures (Level of Theory: CAM-B3LYP/6-31+G(d)) for enolic and ketonic forms of furimazine in presence of 4 explicit H_2O molecules.

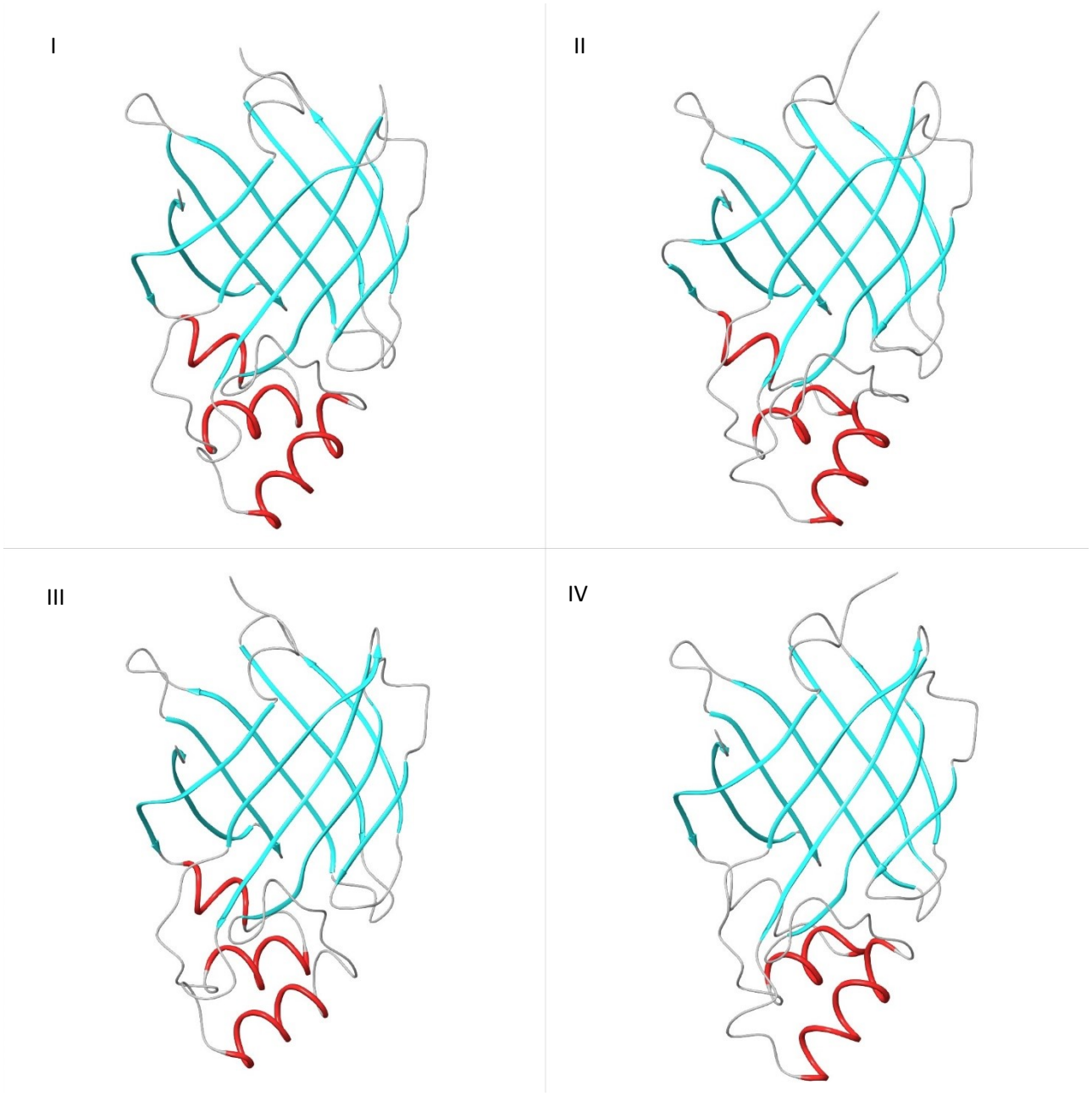

Figure S4. Centres of the four main clusters from the NanoLuc MD trajectory. Protein structure coloured according to secondary structure: alpha-helices in red and beta-strands in sky blue.

NLuc				
	% conf / cluster			
N° Clusters	**I**	**I-II**	**I-III**	**I-IV**
8	84	94	97	98

Table S1. Results of the cluster analysis on the NLuc MD trajectory

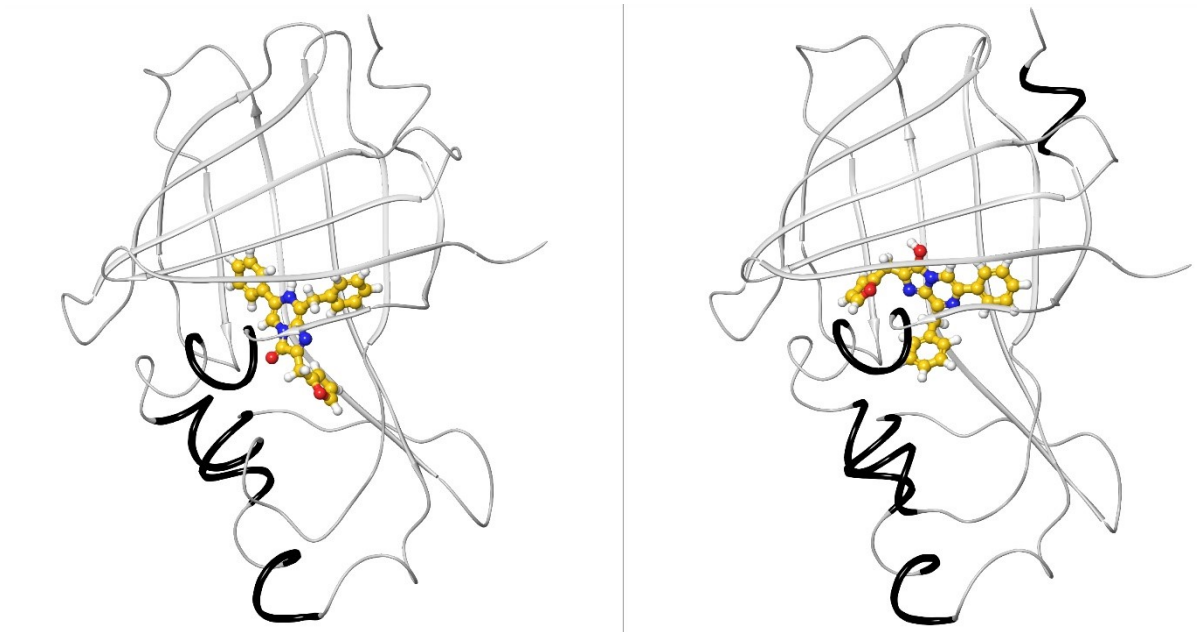

Figure S5. Comparison between the best-scoring docking pose of ketonic (left) and enolic (right) forms of furimazine.

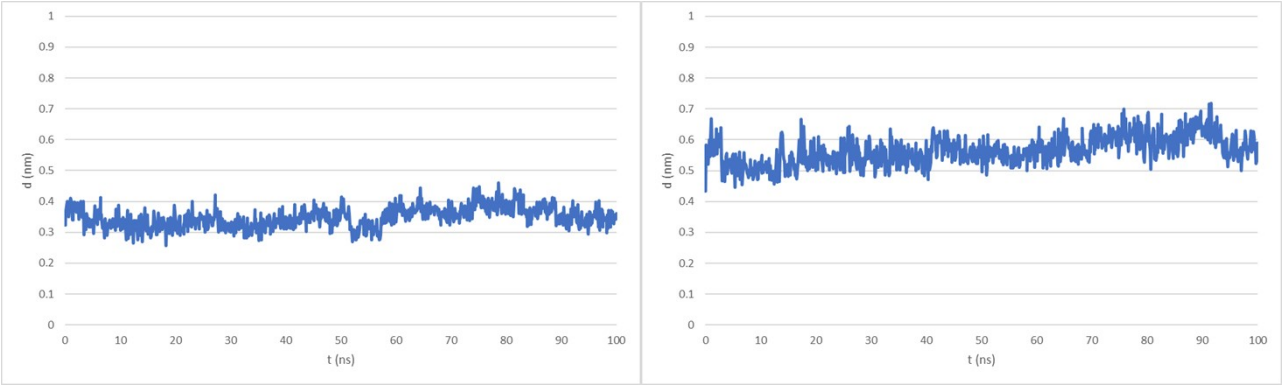

Figure S6. Distance between center of mass of NanoLuc and center of mass of furimazine enolic form (left) and ketonic form (right) in the corresponding MD simulations.

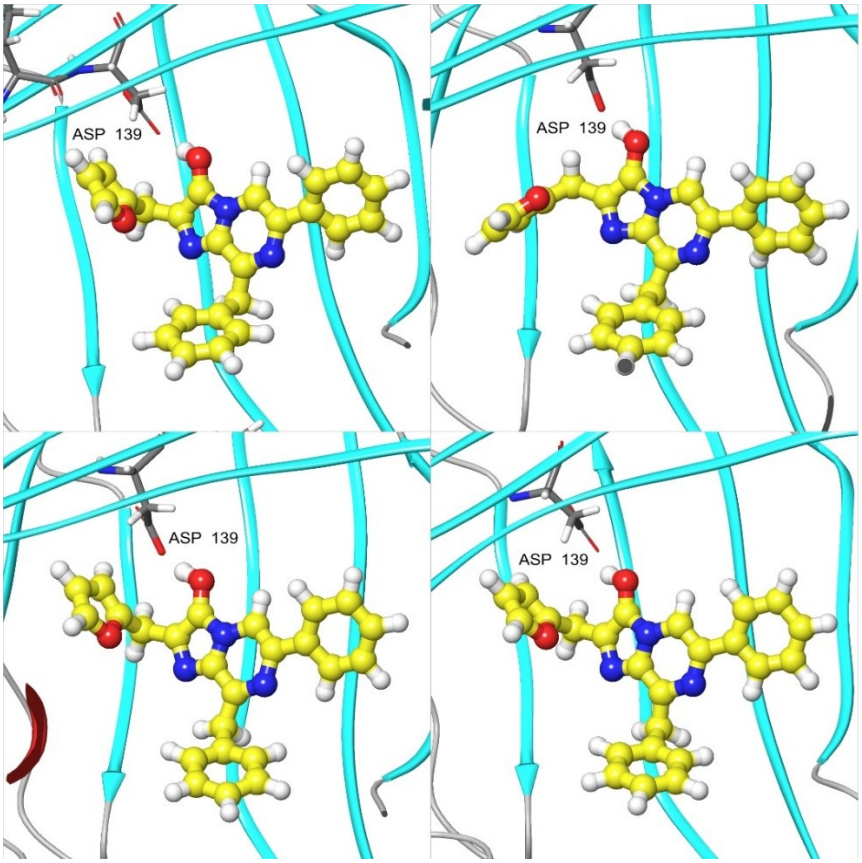

Figure S7. Centres of the four main clusters from the NanoLuc-furimazine (eno) MD trajectory, zoom on furimazine. All residues hidden, only ASP139 shown in tubes.

NLuc-furimazine (Enolic form)				
	% conf / cluster			
N° Clusters	**I**	**I-II**	**I-III**	**I-IV**
15	60	71	81	89

Table S2. Results of the cluster analysis on the NLuc-furimazine (enolic form) MD trajectory

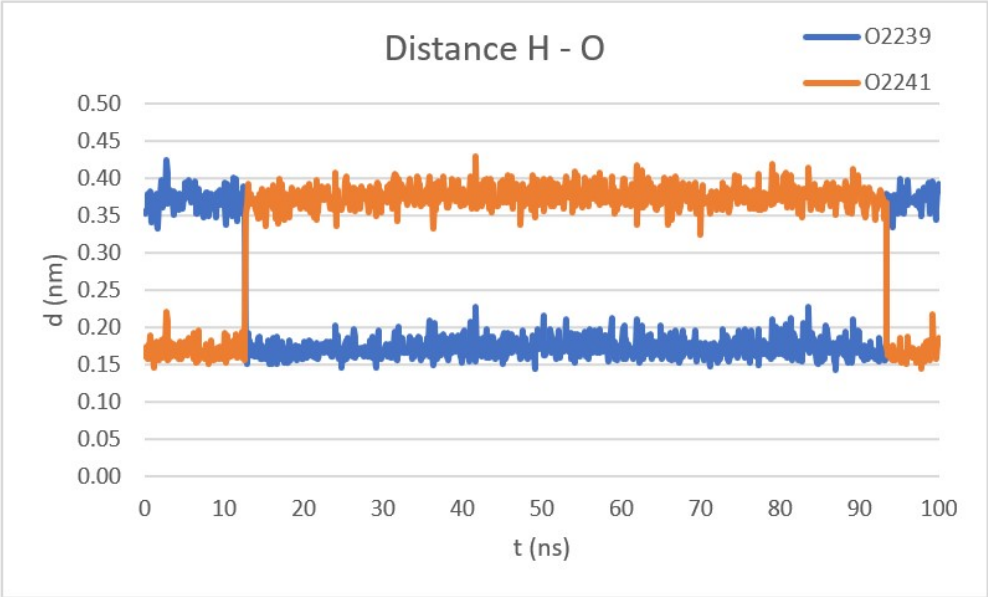

Figure S8. Distances between the enolic group hydrogen and the Asp139 carboxylate oxygens

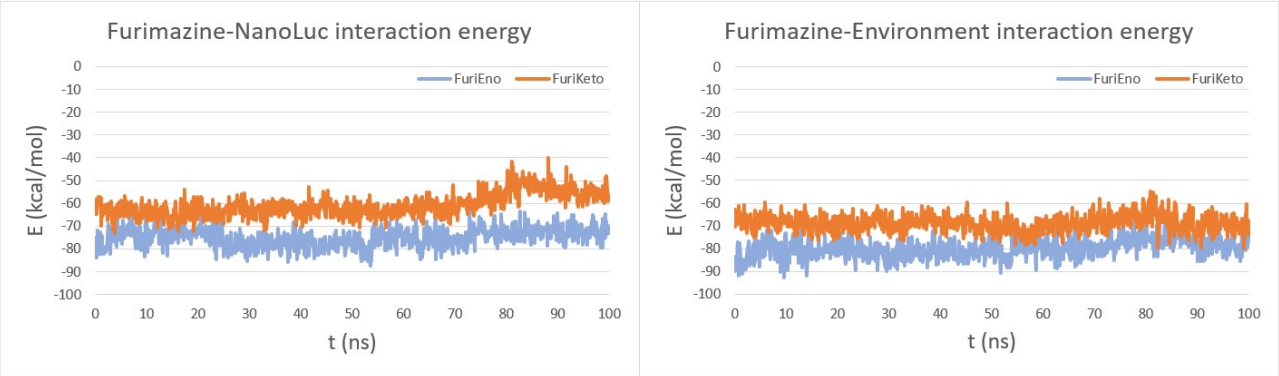

Figure S9. Interaction energies trends from the MD simulations for the NanoLuc-reactants pairs without (left) and including (right) H_2O contributions. Energy reported in kcal/mol.

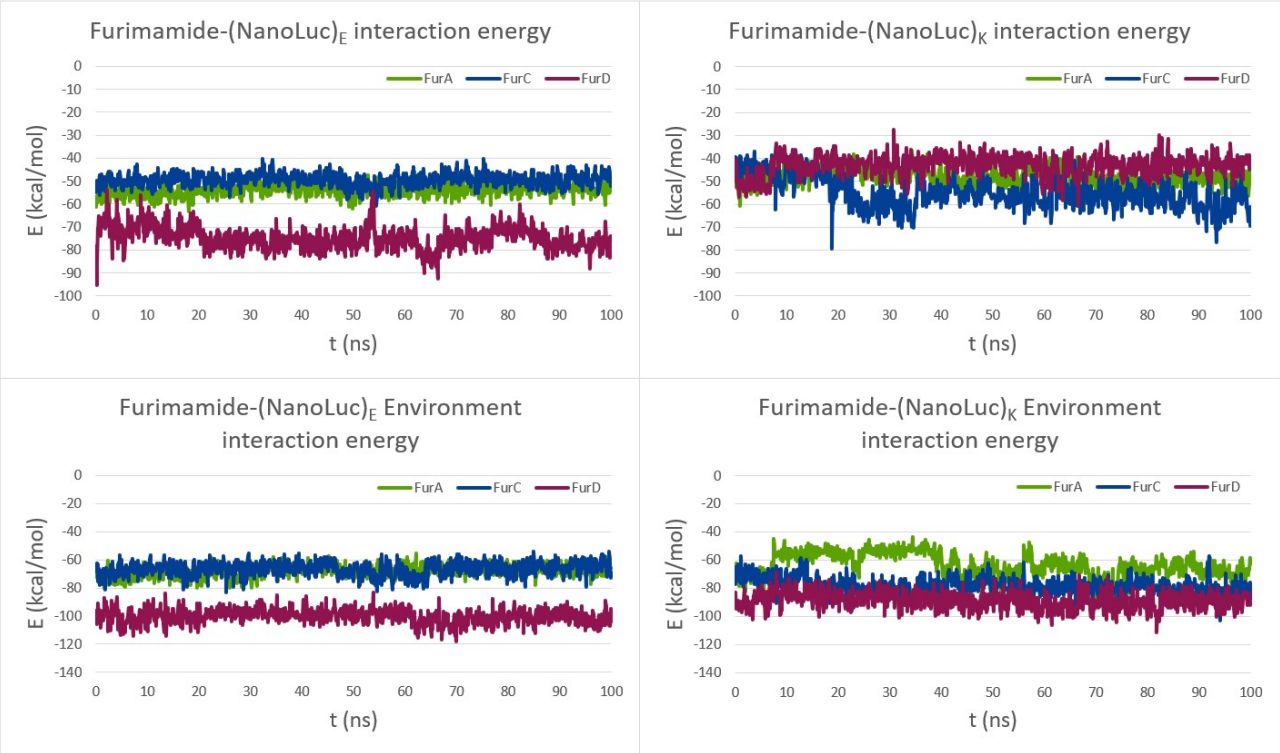

Figure S10. Interaction energies trends from the MD simulations for the products pairs, without (above) and including (below) H_2O contributions. Energy reported in kcal/mol.

Avg. Interaction Energy for (NanoLuc) - furimazine (kcal/mol)		
	(NanoLuc) - *FuriEno*	**(NanoLuc) - *FuriKeto***
Energy (kcal/mol)	-74.94	-60.85

Table S3. Average interaction energies from the MD simulations for the NanoLuc – furimazine pairs.

Avg. Interaction Energy for (NanoLuc) - furimazine (kcal/mol)		
	(NanoLuc) - *FuriEno*	**(NanoLuc) - *FuriKeto***
Energy (kcal/mol)	-79.96	-68.74

Table S4. Average interaction energies (including interactions with surrounding water molecules) from the MD simulations for the NanoLuc – furimazine pairs.

Avg. MMGBSA Binding Energy for (NanoLuc) - furimazine (kcal/mol)		
	(NanoLuc) - *FuriEno*	**(NanoLuc) - *FuriKeto***
Energy (kcal/mol)	-53.28	-45.48

Table S17. Binding free energies from the MD simulations for the NanoLuc – furimazine pairs. As computed according to the MMGBSA model.

FurA							
Excitation				Emission			
Excited state	**ΔE (eV)**	**λ (nm)**	**f**	**ΔE (eV)**	**λ (nm)**	**f**	**Contribution of LUMO -> HOMO**
S1	4.35	285	0.20	3.54	350	0.49	82%
S2	4.53	273	0.41				
S3	4.96	250	0.15				
S4	5.05	245	0.03				
S5	5.20	239	0.04				
FurC							
Excitation				Emission			
Excited state	**ΔE (eV)**	**λ (nm)**	**f**	**ΔE (eV)**	**λ (nm)**	**f**	**Contribution of LUMO -> HOMO**
S1	3.83	324	0.28	3.05	406	0.23	92%
S2	3.85	322	0.10				
S3	4.49	276	0.31				
S4	4.52	274	0.21				
S5	4.85	255	0.00				
FurD							
Excitation				Emission			
Excited state	**ΔE (eV)**	**λ (nm)**	**f**	**ΔE (eV)**	**λ (nm)**	**f**	**Contribution of LUMO -> HOMO**
S1	3.65	340	0.51	3.14	395	0.29	75%
S2	3.94	314	0.26				
S3	4.15	299	0.04				
S4	4.48	277	0.02				
S5	4.52	274	0.02				

Table S6. In vacuo vertical excitations corresponding to the first five singlet ESs at the GS optimized geometries, emissions of the optimized ES at the ES optimized geometries, and corresponding oscillator strengths (f), calculated at the CAM-B3LYP/6-31+G(d) level

FurA							
Excitation				Emission			
Excited state	**ΔE (eV)**	**λ (nm)**	**f**	**ΔE (eV)**	**λ (nm)**	**f**	**Contribution of LUMO -> HOMO**
S1	4.30	289	0.58	3.21	386	0.94	95%
S2	4.47	278	0.33				
S3	4.97	249	0.25				
S4	5.09	244	0.08				
S5	5.20	238	0.07				
FurC							
Excitation				Emission			
Excited state	**ΔE (eV)**	**λ (nm)**	**f**	**ΔE (eV)**	**λ (nm)**	**f**	**Contribution of LUMO -> HOMO**
S1	3.77	329	0.54	2.98	417	0.56	95%
S2	3.99	311	0.00				
S3	4.53	274	0.52				
S4	4.62	269	0.03				
S5	4.99	249	0.00				
FurD							
Excitation				Emission			
Excited state	**ΔE (eV)**	**λ (nm)**	**f**	**ΔE (eV)**	**λ (nm)**	**f**	**Contribution of LUMO -> HOMO**
S1	3.97	312	0.32	3.03	409	0.88	94%
S2	4.31	288	0.18				
S3	4.56	272	0.37				
S4	4.81	258	0.10				
S5	4.99	249	0.00				

Table S7. Aqueous solution vertical excitations corresponding to the first five singlet ESs at the GS optimized geometries and emissions of the optimized ES at the ES optimized geometries, and corresponding oscillator strengths (f), calculated at the CAM-B3LYP/6-31+G(d) level. Solvent model: CPCM.

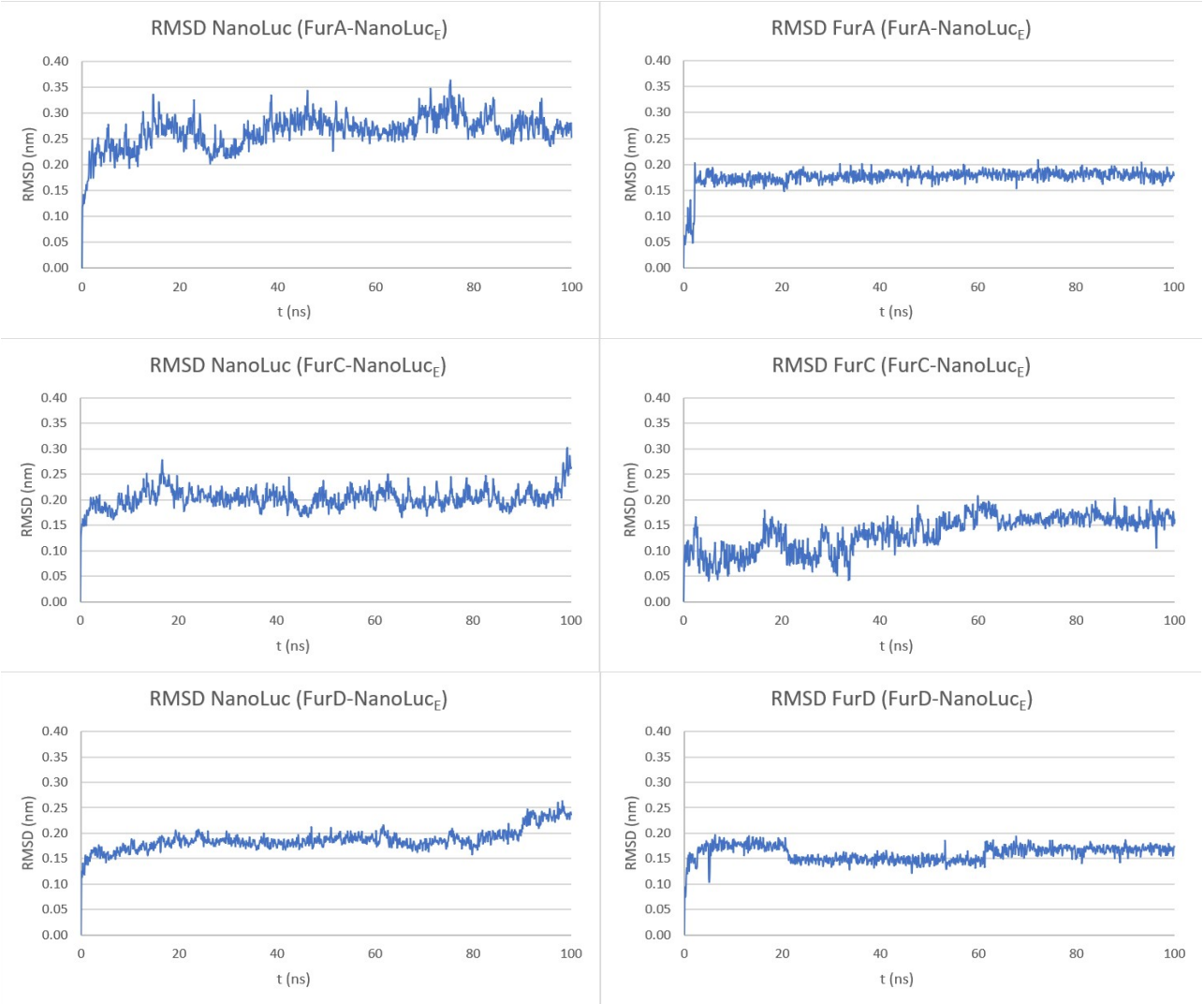

Figure S11. NanoLuc and Fur* RMSD for Eno pose MD simulations. The RMSD trends for the protein structure are reported in the plots on the left, while the ones for the ligand are on the right.

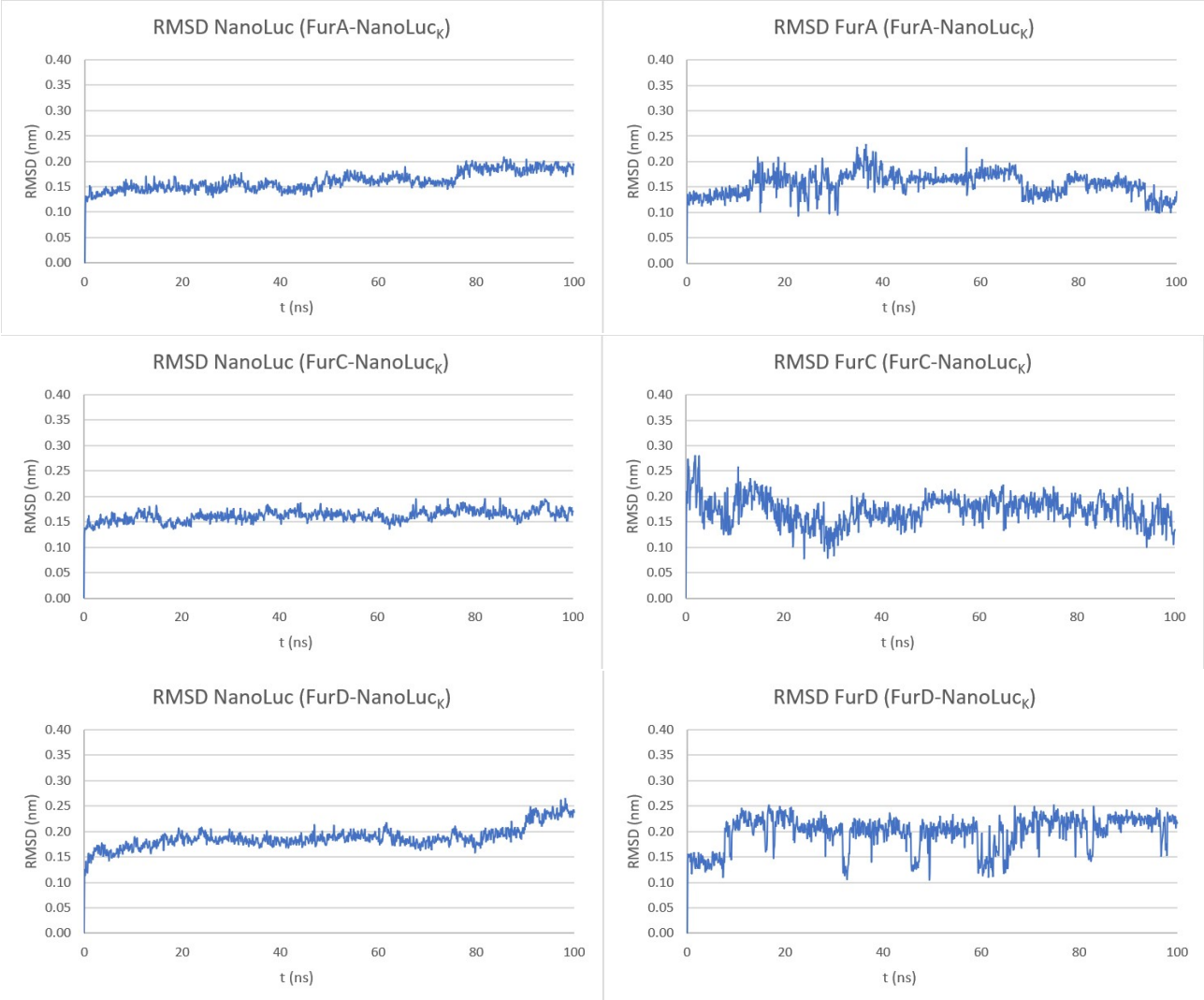

Figure S12. NanoLuc and Fur* RMSD for Keto pose MD simulations. The RMSD trends for the protein structure are reported in the plots on the left, while the ones for the ligand are on the right.

Avg. Interaction Energy for $(NanoLuc)_*$ - *Fur** (kcal/mol)			
	FurA	***FurC***	***FurD***
$(NanoLuc)_E$	-53.82	-49.42	-74.80
$(NanoLuc)_K$	-47.99	-54.91	-43.04

Table S8. Average interaction energies from the MD simulations for the (NanoLuc) – furimamide pairs.

Avg. Interaction Energy for $(NanoLuc)_*$ - *Fur** (kcal/mol)			
	FurA	***FurC***	***FurD***
$(NanoLuc)_E$	-68.10	-66.48	-100.14
$(NanoLuc)_K$	-63.58	-78.14	-100.19

Table S9. Average interaction energies (including interactions with surrounding water molecules) for the (NanoLuc) – furimamide pairs.

Avg. MMGBSA Binding Energy for (NanoLuc)$_*$ - *Fur** (kcal/mol)			
	FurA	***FurC***	***FurD***
(NanoLuc)$_E$	-40.23	-41.35	-48.57
(NanoLuc)$_K$	-34.73	-40.57	-27.13

Table S18. Binding free energies from the MD simulations for the (NanoLuc) – furimamide pairs. As computed according to the MMGBSA model.

Maximum emission wavelength (nm)			
	In vacuo @ (NanoLuc$_E$)-Fur*	***In vacuo @ (NanoLuc$_K$)-Fur****	***In vacuo***
FurA	339	375	367
FurC	430	-	451
FurD	416	426	426

Table S11. Maximum emission wavelength (nm) calculated *in vacuo* (PBE0/6-31G*+) for the different forms of Fur* at the geometries adopted in the enzymatic pockets. For comparison, values calculated *in vacuo* at the *in vacuo* ES optimized geometries (PBE0/6-31G*+//CAM-B3LYP/6-31G*+) are also reported.

Torsional angles of FurA optimized geometries				
	1-2-3-4 (°)	**7-6-9-10 (°)**	**8-9-11-12 (°)**	**9-11-12-13 (°)**
Vacuum	6.0	-140.7	178.5	-72.1
Protein	12.9	-128.8	76.2	-69.7

Table S12. Comparison of torsional angles of furimamide neutral form excited state optimized geometries in vacuum and in $NanoLuc_E$ protein environment.

Torsional angles of FurC optimized geometries				
	1-2-3-4 (°)	**7-6-9-10 (°)**	**8-9-11-12 (°)**	**9-11-12-13 (°)**
Vacuum	-8.7	-14.9	-37.6	-66.6
Protein	18.0	29.2	79.1	175.8

Table S13. Comparison of torsional angles of furimamide zwitterionic form excited state optimized geometries in vacuum and in $NanoLuc_E$ protein environment.

Torsional angles of FurD optimized geometries				
	1-2-3-4 (°)	**7-6-9-10 (°)**	**8-9-11-12 (°)**	**9-11-12-13 (°)**
Vacuum	-2.1	58.6	163.0	-92.7
Protein	10.8	107.9	-144.9	-178.2

Table S14. Comparison of torsional angles of furimamide anionic form excited state optimized geometries in vacuum and in $NanoLuc_E$ protein environment.

Scan of the FurA C9-C11-C12-O13 dihedral angle	
α (°)	**λ (nm)**
-69.7	339
-59.7	339
-49.7	339
-39.7	339
-29.7	339
-19.7	339
-9.7	339
0.3	340
10.3	340
20.3	340
30.3	340
40.3	341

Table S15. Emission wavelength of FurA at different values of the C9-C11-C12-O13 dihedral angle. Scan performed on the optimized geometry obtained in $NanoLuc_E$ protein environment. (Level of Theory: PBE0/6-31+G(d))

FurA predicted maximum emission depending on single group modification					
	In vacuo @ $NanoLuc_E$	***Core***	***Phenyl***	***Core+Phenyl***	***Vacuum Opt***
λ (nm)	339	345	356	367	367
ΔE (eV)	3.53	3.58	3.48	3.38	3.38

Table S16. Predicted emission wavelength and corresponding ΔE of FurA $NanoLuc_E$ optimized geometry in vacuum with moiety geometrical parameters modifications. Each column represents a specific modification to the geometry of the system. These modifications are based on the gas-phase optimized structure. "Core" is the pyrazinic ring, "Phenyl" is the ring directly linked to the central core. Values calculated in vacuo at the in vacuo ES optimized geometries reported for comparison. (Level of Theory: PBE0/6-31+G(d))

FurC predicted maximum emission depending on single group modification			
	In vacuo @ NanoLuc$_E$	***Furan***	***Vacuum Opt***
λ (nm)	430	456	451
ΔE (eV)	2.88	2.72	2.75

Table S17. Comparison of predicted emission wavelength and corresponding ΔE of FurC (NanoLuc$_E$) optimized geometry in vacuum and after modification of furan moiety geometrical parameters based on the gas-phase optimized structure. Values calculated in vacuo at the in vacuo ES optimized geometries reported for comparison. (Level of Theory: PBE0/6-31+G(d))

Scan of the FurC N7-C6-N8-C9 dihedral angle	
α (°)	***λ (nm)***
-17.7	433
-12.7	427
-7.7	425
-2.7	425
2.3	427
7.3	428
12.3	429
17.3	431
22.3	433
27.3	434
32.3	436

Table S18. Emission wavelength of FurC at different values of the N7-C6-N8-C9 dihedral angle. Scan performed on the optimized geometry obtained in NanoLuc$_E$ protein environment. (Level of Theory: PBE0/6-31+G(d))

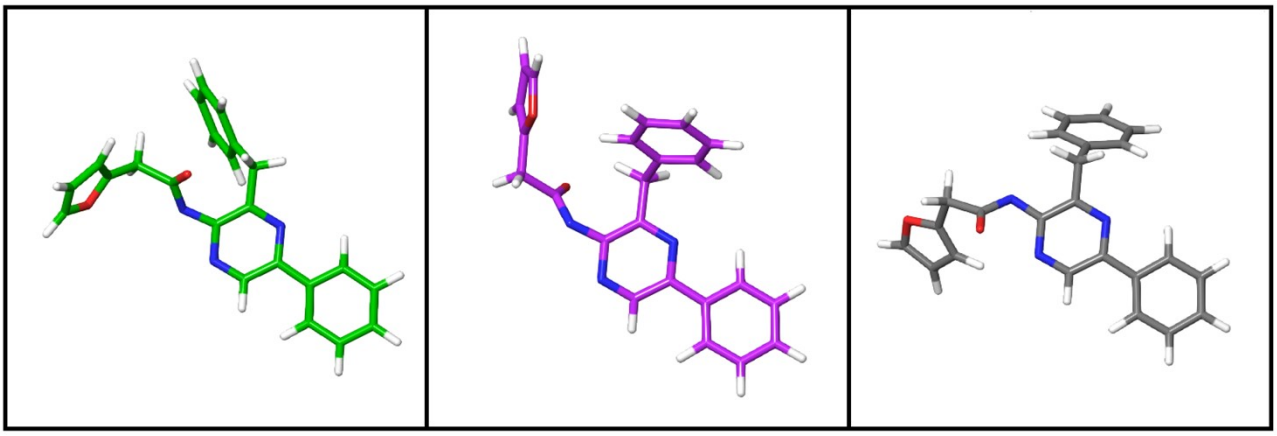

Figure S13. Geometries of FurD optimized in $NanoLuc_E$ (left), $NanoLuc_K$ (middle) and vacuum (right)

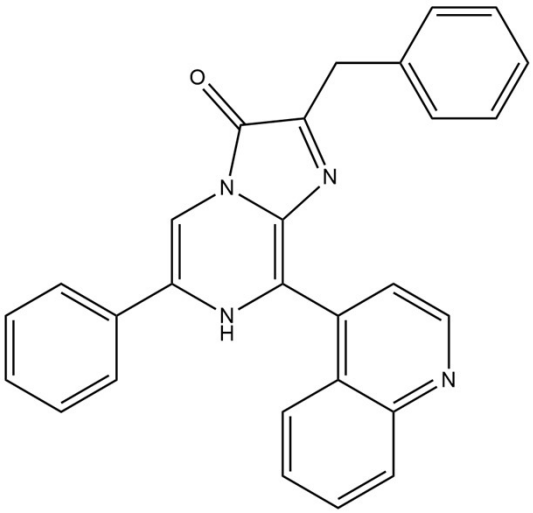

Figure S14. QTZ, the coelenterazine analogue characterized by a 4-quinolinyl substituent, designed by Xiong et al.

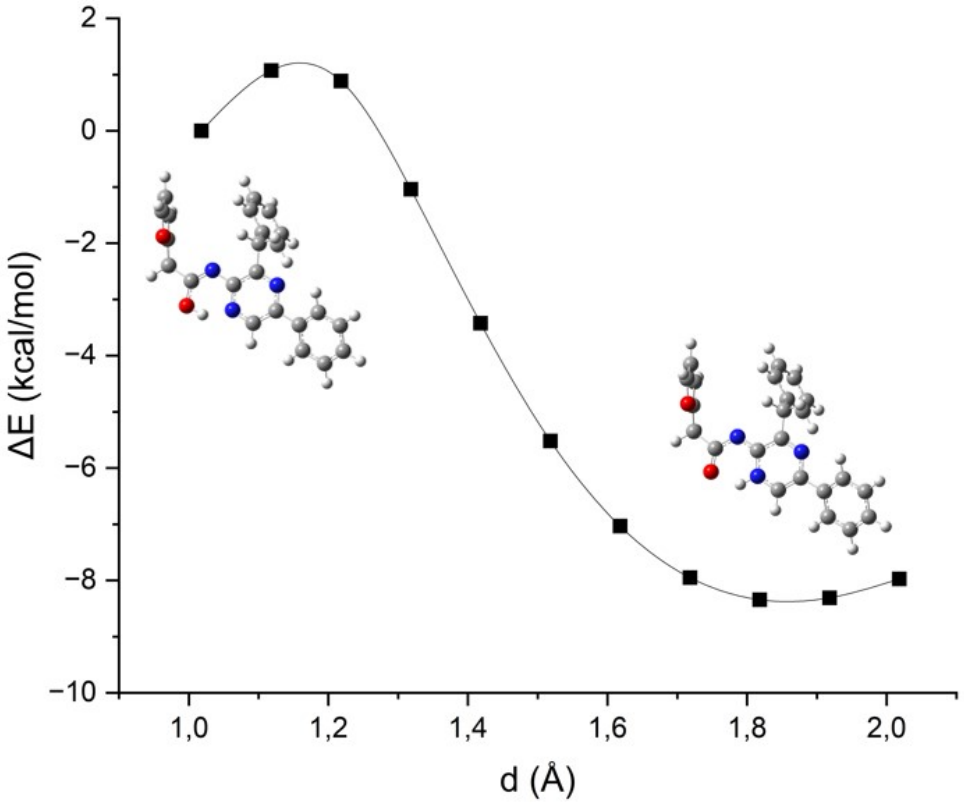

Figure S15. Relaxed scan performed to evaluate the energy barrier for the O10-N7 proton transfer in furimamide (d is the O10-H distance). Level of theory: CAM-B3LYP/6-31+G(d), implicit solvation with CPCM, solvent=water.

Table S19 Cartesian coordinates of the molecular species**.**

Coordinates of the optimized geometries of furimazine keto and enol form in H_2O (implicit solvation)

Enol form:
Energy at CAM-B3LYP/6-31+G(d) level: -1240.089387 Hartree

C	0.13078700	4.58111500	0.99708000
C	0.13671800	3.65320800	-0.04137800
C	-1.06151800	3.20096600	-0.60067900
C	-2.26592000	3.70088000	-0.10107800
C	-2.27561500	4.62960600	0.93762800
C	-1.07638300	5.07198500	1.49107500
C	-1.05605100	2.17392300	-1.71737900
C	-0.95406700	0.76312300	-1.19072600
C	0.32048700	0.14525200	-1.08506600
N	0.35834400	-1.13830600	-0.55354300
C	-0.78387800	-1.78597700	-0.14674700
C	-1.97093700	-1.13653900	-0.30529000
N	-2.04283200	0.13876100	-0.82271300
C	-3.25329200	-1.76851000	0.09479900
C	-3.42361200	-3.15722800	0.05889800
C	-4.62419200	-3.73560600	0.45947700
C	-5.67771900	-2.93488000	0.89483000
C	-5.52149600	-1.55058100	0.92243100
C	-4.32075700	-0.97221300	0.52334900
C	1.66474900	-1.51632800	-0.54737800
C	2.38200400	-0.45846900	-1.07812600
N	1.53876200	0.56442400	-1.39924200
O	1.99972500	-2.74122300	-0.09787700
C	3.86416400	-0.38429500	-1.32102500
C	4.69606300	-0.60358100	-0.10118900
C	5.59220200	0.14675300	0.58420300
C	6.05485400	-0.65579600	1.68359900
C	5.40630000	-1.83869400	1.59399900
O	4.57388800	-1.82957800	0.50647900
H	1.07143800	4.92369200	1.41878100
H	1.07970700	3.27163600	-0.42311800
H	-3.20504900	3.36295100	-0.53101400
H	-3.22190700	5.01011400	1.31135300
H	-1.08186700	5.79771800	2.29901000
H	-1.98165800	2.24927900	-2.29330600
H	-0.21730600	2.36427400	-2.39193100
H	-0.65799600	-2.76689100	0.28960700
H	-2.62305000	-3.79451900	-0.30430700
H	-4.73927600	-4.81470600	0.41982800
H	-6.61558700	-3.38619100	1.20446800
H	-6.33723000	-0.91712700	1.25847600
H	-4.19975100	0.10528100	0.54605500
H	2.93662500	-2.72634000	0.18264100
H	4.15285400	-1.11227500	-2.09140600
H	4.09280300	0.60617000	-1.71975800
H	5.89286700	1.15569600	0.33861700
H	6.77686900	-0.37777000	2.43836900
H	5.41134600	-2.74741600	2.17545100

Keto form:
Energy at CAM-B3LYP/6-31+G(d) level: -1240.093178 Hartree

C	-4.93451200	-3.35961600	0.33119200

C	-3.65714300	-2.97307100	-0.05996200
C	-3.20267900	-1.67377000	0.19038900
C	-4.05661300	-0.76550600	0.82668400
C	-5.33755600	-1.15242600	1.20797700
C	-5.77960500	-2.45026800	0.96361600
C	-1.83741000	-1.27276300	-0.20660300
N	-1.67521700	0.00930500	-0.74134700
C	-0.47366000	0.51494000	-1.12404600
C	0.62656000	-0.27736100	-0.97629200
N	0.48628200	-1.58500100	-0.46040700
C	-0.75412900	-2.06230400	-0.06325400
C	-0.41913100	1.90232600	-1.69388600
C	-0.97079800	2.95339600	-0.74928000
C	-2.15575200	3.62758200	-1.04376100
C	-2.65966100	4.59230300	-0.17149400
C	-1.98096500	4.89069700	1.00577400
C	-0.79423000	4.22217800	1.30653600
C	-0.29375700	3.26126500	0.43458700
C	1.72114600	-2.17390100	-0.41014600
C	2.59078500	-1.12886100	-0.94096400
N	1.92722300	-0.03434800	-1.26182900
C	4.07550900	-1.27114500	-1.09306100
C	4.85109100	-0.55543100	-0.03606500
C	5.72405600	0.48448700	-0.05896200
C	6.12798700	0.71152000	1.30029600
C	5.46764200	-0.20601200	2.04699100
O	4.68554000	-0.98707800	1.24854200
O	1.96977900	-3.32635700	-0.00174600
H	-5.27455600	-4.37056300	0.12851000
H	-3.01426000	-3.67741400	-0.57858000
H	-3.71534800	0.24078900	1.05304000
H	-5.98714700	-0.43900800	1.70560300
H	-6.77913000	-2.75109300	1.26214700
H	-2.50485500	0.56863800	-0.89829600
H	-0.78167400	-3.05069200	0.37314900
H	0.62235000	2.11530200	-1.94402400
H	-0.98538100	1.91410400	-2.63224500
H	-2.68800000	3.40359400	-1.96476000
H	-3.58237900	5.10996100	-0.41633200
H	-2.37088600	5.64210800	1.68582300
H	-0.25627000	4.45246100	2.22141700
H	0.63327800	2.74595900	0.67347500
H	4.32640700	-2.33745900	-1.08007300
H	4.38277400	-0.87164700	-2.06360400
H	6.04346700	1.02670000	-0.93822100
H	6.81791400	1.45992000	1.66445200
H	5.44008800	-0.43343800	3.10164200

Coordinates of the optimized geometries of furimazine keto and enol form in H_2O (explicit microsolvation and implicit solvation)

Enol form:

Energy at CAM-B3LYP/6-31+G(d) level: -1545.745457 Hartree

C	-5.26233500	-3.59451700	0.35001100
C	-4.02686400	-3.08195900	-0.03496900
C	-3.69550300	-1.74967800	0.23246700
C	-4.62975100	-0.93907700	0.88604200
C	-5.86543400	-1.45117700	1.26807700
C	-6.18587100	-2.78108700	1.00219400
C	-2.37283000	-1.19965000	-0.15261400
N	-2.34249100	0.10039400	-0.61485500

C	-1.20728500	0.65809000	-0.95929800
C	0.00969100	-0.06776900	-0.88345800
N	-0.05202200	-1.36973500	-0.41151300
C	-1.24092300	-1.94491100	-0.03272900
C	-1.19929200	2.09643300	-1.41481700
C	-1.16070200	3.06053400	-0.24260700
C	-2.34302300	3.59786500	0.27129700
C	-2.31219300	4.47120700	1.35652600
C	-1.09669000	4.81794200	1.94207000
C	0.08744100	4.28731900	1.43415200
C	0.05441500	3.41477200	0.34911700
C	1.22121200	-1.86232900	-0.41301800
C	2.02404400	-0.83977800	-0.89354100
N	1.25915600	0.25640200	-1.18236300
C	3.51098900	-0.82263000	-1.11424600
C	4.23643300	0.11620800	-0.20493800
C	4.65171100	1.40016400	-0.33321000
C	5.24240900	1.77540700	0.92125400
C	5.14643100	0.69312900	1.72642800
O	4.53497100	-0.33387500	1.05789500
O	1.40175800	-3.11336900	0.01359600
H	-5.50672600	-4.62928500	0.12946300
H	-3.32435200	-3.71762300	-0.56585300
H	-4.37861600	0.09332000	1.10737800
H	-6.57767500	-0.81067000	1.77969500
H	-7.15110900	-3.18011700	1.29906000
H	-1.19594000	-2.95292400	0.35496200
H	-0.33470500	2.26464700	-2.06093600
H	-2.10042500	2.27108600	-2.00761500
H	-3.29311600	3.33107400	-0.18393600
H	-3.24011700	4.88375100	1.74224200
H	-1.07159400	5.50056000	2.78639700
H	1.04053800	4.55540000	1.88099900
H	0.98090200	3.00593100	-0.04452700
H	3.91780600	-1.83193500	-1.00938800
H	3.71002000	-0.51003000	-2.14405100
H	4.52607800	2.01373000	-1.21453500
H	5.67978500	2.72920400	1.18110500
H	5.44752000	0.48047200	2.74067000
O	3.73183600	-4.31400100	-0.10806000
H	4.42476900	-3.91678700	0.47798000
H	2.33150200	-3.46551600	-0.10048600
O	-4.66795600	1.49508400	-1.59400700
H	-5.43660800	0.90727300	-1.55731800
H	-3.92469300	0.97763700	-1.20393400
O	2.38740400	2.36339900	-2.76454300
H	1.97964900	1.65406300	-2.21397900
H	2.04381300	2.22703000	-3.65928500
O	5.38980800	-3.02730300	1.59749900
H	6.34750600	-3.09693400	1.46556100
H	5.16504500	-2.08602300	1.46509900
H	4.14919900	-4.49328600	-0.96383700

Keto form:
Energy at CAM-B3LYP/6-31+G(d) level: -1545.745466 Hartree

C	-4.54617100	-4.31552800	0.04696600
C	-3.40176800	-3.61032700	-0.31196800
C	-3.20261900	-2.30508000	0.14811100
C	-4.17219100	-1.71450200	0.96636200
C	-5.31882700	-2.41947800	1.31731800

C	-5.50861200	-3.72166200	0.85979300
C	-1.97093900	-1.56944200	-0.21014400
N	-2.10373400	-0.23647900	-0.59888600
C	-1.05351700	0.54797700	-0.92349500
C	0.20233600	-0.00206600	-0.87067300
N	0.35517400	-1.35151800	-0.50231900
C	-0.74104400	-2.12290600	-0.15865800
C	-1.30543300	1.96938300	-1.33233100
C	-1.93721300	2.82583400	-0.24854500
C	-2.94175700	3.73750000	-0.58077200
C	-3.50885100	4.55796900	0.39273200
C	-3.07708500	4.47463900	1.71405900
C	-2.07276900	3.57047900	2.05263300
C	-1.50674200	2.75318400	1.07785000
C	1.68561400	-1.67021700	-0.52227600
C	2.30737200	-0.42901900	-0.92891700
N	1.41265100	0.53237100	-1.12922600
C	3.78222000	-0.21495800	-1.11215300
C	4.37832100	0.70732900	-0.09788700
C	4.61089900	2.04286000	-0.07777300
C	5.15098800	2.35246100	1.21656200
C	5.20981400	1.18340600	1.89418900
O	4.74235100	0.16393100	1.10888800
O	2.15571000	-2.80083000	-0.24054900
H	-4.68994000	-5.32734700	-0.31938900
H	-2.66644900	-4.06810100	-0.96649700
H	-4.02125900	-0.70952300	1.34927600
H	-6.06121700	-1.95254300	1.95727400
H	-6.40391700	-4.27063600	1.13508900
H	-3.05090600	0.15219600	-0.70958900
H	-0.54599800	-3.13633200	0.16227300
H	-0.35317800	2.40666100	-1.64308500
H	-1.95193400	1.96456400	-2.21676400
H	-3.28077600	3.81144200	-1.61112600
H	-4.28967600	5.26019100	0.11609100
H	-3.51973000	5.11034100	2.47487600
H	-1.72744900	3.49904000	3.07979000
H	-0.72342000	2.05290100	1.35616100
H	4.28951700	-1.18371100	-1.08135700
H	3.96087900	0.21183500	-2.10414200
H	4.39856000	2.72840500	-0.88654400
H	5.45445700	3.32208700	1.58573100
H	5.54248000	0.90199900	2.88149200
O	4.65995600	-3.96622100	-0.41188100
H	5.19716400	-3.48842500	0.25268300
H	3.77841300	-3.53215600	-0.38311600
O	-4.65539700	1.00912300	-0.99924600
H	-4.71753300	1.85386700	-0.52377700
H	-5.45196500	0.51029000	-0.75911700
O	2.15593200	3.03247800	-2.37073700
H	1.90280100	2.18104300	-1.94792900
H	1.94822400	2.92700700	-3.31049900
O	5.99461000	-2.41214500	1.54169800
H	6.94689400	-2.27995800	1.41968400
H	5.58987200	-1.53346500	1.41424600

Coordinates of the optimized geometries of furimamide GS and ES in vacuum

FurA ES:

Energy at CAM-B3LYP/6-31+G(d) level: -1201.861074 Hartree

C	2.06507300	2.65188800	-2.01457400

C	0.97789900	2.25023100	-1.24579700
C	1.06090500	2.21960800	0.14856300
C	2.25739200	2.60235700	0.75521200
C	3.34632800	3.01274100	-0.01127600
C	3.25436000	3.03660900	-1.39918800
C	-0.13993500	1.83675900	0.99033300
C	-0.87552700	0.60095900	0.53280300
N	-2.12825100	0.74343200	0.16756500
C	-2.83374500	-0.31705500	-0.33788400
C	-2.06059000	-1.41142600	-0.84828800
N	-0.80145500	-1.58213200	-0.55323800
C	-0.23798900	-0.65691000	0.30939100
C	-4.26262600	-0.31881100	-0.25927300
C	-4.92958500	0.83819900	0.22521300
C	-6.30949900	0.87165400	0.32358800
C	-7.07606900	-0.22810300	-0.06471000
C	-6.43689000	-1.37545600	-0.55051100
C	-5.05979900	-1.43040600	-0.64091000
N	1.08535800	-0.93518700	0.58160500
C	1.79364600	-0.67759800	1.73514600
O	1.31805200	-0.10399500	2.70002900
C	3.23408400	-1.18639800	1.76520500
C	3.86018000	-1.56825500	0.47226200
O	3.41367400	-2.72534700	-0.10719600
C	4.09971500	-2.88799800	-1.27300000
C	4.97132300	-1.86684300	-1.44910600
C	4.81272300	-1.00574500	-0.31399900
H	1.98206500	2.66932300	-3.09755600
H	0.04981700	1.96153600	-1.73316200
H	2.33444800	2.58004500	1.83920300
H	4.26699100	3.31641200	0.47967000
H	4.10163900	3.35597100	-1.99912600
H	0.17047300	1.69144800	2.02803900
H	-0.87449700	2.64762400	0.97191300
H	-2.53886500	-2.19686700	-1.42907100
H	-4.33026000	1.70381800	0.48404700
H	-6.79807000	1.76942000	0.69156600
H	-8.15907300	-0.19439600	0.00441100
H	-7.02646400	-2.23857800	-0.84568000
H	-4.59253700	-2.34810200	-0.98130900
H	1.49817200	-1.59681300	-0.07256100
H	3.82394700	-0.39278300	2.22989900
H	3.24992600	-2.03272700	2.46362900
H	3.85102000	-3.76851200	-1.84469000
H	5.64607500	-1.73517900	-2.28289800
H	5.33355000	-0.07970600	-0.11572300

FurA GS:
Energy at CAM-B3LYP/6-31+G(d) level: -1202.008383 Hartree

C	1.70982300	2.83657600	-1.97055500
C	0.72284500	2.32384900	-1.13531400
C	0.94406300	2.19204600	0.23794500
C	2.17712400	2.59053500	0.75490000
C	3.16626100	3.11015400	-0.07707800
C	2.93709500	3.23243600	-1.44380300
C	-0.14645800	1.68037800	1.15970000
C	-0.83517200	0.42317300	0.69121800
N	-2.15023500	0.50580000	0.50433000
C	-2.82949200	-0.53728400	0.01486600
C	-2.14156700	-1.70552100	-0.31371400

N	-0.83422000	-1.82460100	-0.11772400
C	-0.18155500	-0.79330300	0.40296100
C	-4.29537200	-0.39100400	-0.15847500
C	-4.99079900	0.55631200	0.59949900
C	-6.36573500	0.70141200	0.46168600
C	-7.06863400	-0.09276500	-0.44092900
C	-6.38421200	-1.03133000	-1.20746700
C	-5.00849600	-1.17904600	-1.06788100
N	1.20296500	-1.00511400	0.55345500
C	1.93769200	-0.77478000	1.68344000
O	1.48868300	-0.25426900	2.69088400
C	3.39419000	-1.24079400	1.64287600
C	4.01292100	-1.47356100	0.31103800
O	3.56468500	-2.55803900	-0.39389000
C	4.24684100	-2.58772300	-1.57359000
C	5.11807000	-1.55362900	-1.63529700
C	4.96340900	-0.82668000	-0.40883900
H	1.51824400	2.93239900	-3.03560000
H	-0.23578300	2.02813900	-1.55454100
H	2.36159700	2.49294700	1.82162000
H	4.11739200	3.42185300	0.34607900
H	3.70679300	3.63684100	-2.09477800
H	0.27122900	1.51249700	2.15512300
H	-0.93273500	2.43623700	1.24901300
H	-2.64701700	-2.57415300	-0.72286800
H	-4.43773500	1.17525800	1.29714100
H	-6.89112600	1.43780500	1.06272700
H	-8.14307000	0.02201200	-0.54944100
H	-6.92060100	-1.64693400	-1.92351200
H	-4.49015800	-1.90018400	-1.69199900
H	1.58487200	-1.67059000	-0.11050100
H	3.96756500	-0.48282600	2.18059200
H	3.44154700	-2.15398300	2.24965600
H	3.99727400	-3.39896000	-2.23960200
H	5.79069100	-1.32858800	-2.45063800
H	5.48742800	0.06941000	-0.10797000

FurC ES:
Energy at CAM-B3LYP/6-31+G(d) level: -1201.869730 Hartree

C	0.38664600	-1.73589600	-4.88547300
C	0.65913200	-1.56363500	-3.53245600
C	-0.06079500	-2.26922100	-2.56456200
C	-1.06290000	-3.14588300	-2.98081200
C	-1.33919100	-3.31986400	-4.33456200
C	-0.61503100	-2.61510600	-5.29150300
C	0.23656800	-2.06238400	-1.09188600
C	-0.44794800	-0.83910400	-0.55069700
N	-1.77958300	-0.94739300	-0.30597700
C	-2.43139200	0.15115500	0.14001400
C	-1.75363700	1.34409100	0.42216300
N	-0.40933900	1.38836300	0.32761900
C	0.27664900	0.33166500	-0.29965900
C	-3.89016200	0.06931600	0.25937300
C	-4.65863000	1.06792300	0.88495700
C	-6.03691800	0.95239100	0.96833300
C	-6.68093700	-0.16402300	0.43309100
C	-5.93201300	-1.16611400	-0.17915400
C	-4.55112300	-1.05637800	-0.26101600
N	1.56839700	0.47865500	-0.60289600
C	2.30428800	1.57876500	-0.27656100

O	1.84633700	2.70779900	-0.02889400
C	3.80819200	1.36808900	-0.34266200
C	4.24743400	0.02557500	0.12152600
O	4.06573900	-0.24698900	1.44539700
C	4.52033900	-1.50778200	1.66801400
C	4.99332300	-2.04790200	0.51777000
C	4.81514400	-1.04664500	-0.49087600
H	0.95859600	-1.18341600	-5.62576400
H	1.43759800	-0.87513700	-3.21431800
H	-1.63098600	-3.69639100	-2.23577200
H	-2.12079700	-4.00923500	-4.64158400
H	-0.82786500	-2.75101500	-6.34792000
H	-0.12853700	-2.92445000	-0.52256500
H	1.31389000	-1.97625900	-0.93523200
H	-2.25317200	2.27346900	0.66065700
H	0.12965100	2.26273200	0.37596400
H	-4.18194700	1.93673600	1.32575200
H	-6.61374000	1.73171500	1.45684900
H	-7.76125000	-0.25219700	0.49966500
H	-6.42747200	-2.03961200	-0.59221000
H	-3.95383100	-1.83006300	-0.72760500
H	4.12158300	1.49806500	-1.38490300
H	4.27776000	2.16650700	0.23910300
H	4.44104300	-1.86435700	2.68331200
H	5.41682100	-3.03499000	0.39749700
H	5.06916500	-1.11907100	-1.53913600

FurC GS:
Energy at CAM-B3LYP/6-31+G(d) level: -1201.99846978 Hartree

C	1.44027200	-2.22937100	-4.23826900
C	1.31448100	-2.07449200	-2.86005500
C	0.09954800	-2.33883400	-2.22697600
C	-0.98693900	-2.75644400	-2.99867000
C	-0.86369900	-2.90905700	-4.37612500
C	0.35280000	-2.64554500	-5.00072800
C	-0.04992400	-2.17428000	-0.72446300
C	-0.73730200	-0.88440400	-0.36151000
N	-2.02609200	-0.88838900	-0.17288400
C	-2.70424100	0.26156900	0.12895500
C	-2.02097400	1.44123700	0.20805600
N	-0.67571600	1.44532400	0.02905100
C	0.04921900	0.33018200	-0.24533900
C	-4.16738800	0.16284800	0.33482600
C	-4.86549000	1.11364800	1.08713300
C	-6.24221900	1.01601000	1.25252100
C	-6.94342800	-0.03896400	0.67530600
C	-6.25488800	-0.99760200	-0.06297600
C	-4.87849600	-0.90014100	-0.22977100
N	1.36582600	0.30928600	-0.39469800
C	2.07428200	1.45985500	-0.27108200
O	1.60516400	2.59316200	-0.05231700
C	3.58201300	1.34336800	-0.45659900
C	4.16582800	-0.01223300	-0.29375300
O	4.14372100	-0.54142200	0.96030800
C	4.72048600	-1.77050100	0.89580400
C	5.12070200	-2.03650000	-0.37131900
C	4.76046900	-0.88579700	-1.14735600
H	2.39348400	-2.02442000	-4.71777600
H	2.15928700	-1.73976800	-2.26587700
H	-1.93657500	-2.96608600	-2.51288600

H	-1.71729900	-3.23880400	-4.96159900
H	0.45270800	-2.76735800	-6.07543000
H	-0.65777600	-2.98827400	-0.32146200
H	0.93468700	-2.19929000	-0.25308000
H	-2.48079900	2.40333200	0.39254800
H	-0.09066700	2.29684000	0.08518600
H	-4.33255800	1.92572300	1.57302300
H	-6.76608300	1.76026500	1.84491800
H	-8.01844900	-0.11713200	0.80682200
H	-6.79227000	-1.82714000	-0.51304400
H	-4.33904600	-1.64755600	-0.80036600
H	3.80689700	1.70449800	-1.46677900
H	4.04338000	2.05684800	0.23403100
H	4.77353800	-2.31498500	1.82584100
H	5.61171000	-2.93573300	-0.71562800
H	4.92291600	-0.73097300	-2.20510900

FurD ES:
Energy at CAM-B3LYP/6-31+G(d) level: -1201.337984 Hartree

C	1.15454300	-4.18434200	-3.39775600
C	1.17894100	-3.56728000	-2.15047100
C	0.05082600	-3.57025700	-1.32556000
C	-1.10959500	-4.19767800	-1.78730700
C	-1.13965400	-4.81175200	-3.03701200
C	-0.00682600	-4.81142200	-3.84682300
C	0.07949400	-2.87592600	0.02078800
C	-0.43804900	-1.46790500	-0.07111100
N	-1.79708900	-1.34172600	-0.14966400
C	-2.25920900	-0.06526500	-0.19017000
C	-1.37427800	1.03439600	-0.16479800
N	-0.05457500	0.93485300	-0.11379700
C	0.42438400	-0.35978300	-0.04304300
C	-3.70815600	0.11606700	-0.31516700
C	-4.34332300	1.37320500	-0.32086700
C	-5.71980100	1.48376500	-0.44480900
C	-6.51590600	0.33813700	-0.56761100
C	-5.90537700	-0.91255900	-0.55770700
C	-4.52546900	-1.02922200	-0.42951900
N	1.76766300	-0.53813200	-0.01936400
C	2.57255400	0.40158900	0.54943100
O	2.32640200	1.03260100	1.57758400
C	3.92004200	0.57482200	-0.15092300
C	3.81283600	1.53453500	-1.28844700
O	4.96832800	1.80198400	-1.97101500
C	4.65911500	2.69237300	-2.95384300
C	3.33885300	2.99617600	-2.91166300
C	2.78525600	2.24141400	-1.82438800
H	2.04353400	-4.17059400	-4.02351300
H	2.07605400	-3.05784500	-1.80881000
H	-1.99681600	-4.18705100	-1.16082100
H	-2.05378300	-5.29020700	-3.38037800
H	-0.02930300	-5.29122700	-4.82206000
H	-0.56032900	-3.42709700	0.72401900
H	1.09943400	-2.87071200	0.40858100
H	-1.76301000	2.04565400	-0.24862900
H	-3.75775300	2.28075000	-0.22345100
H	-6.17927800	2.46905100	-0.44547100
H	-7.59491800	0.42627900	-0.66475700
H	-6.51117300	-1.81150400	-0.64747600
H	-4.04096100	-1.99776600	-0.41293600

H	4.64429700	0.94812800	0.58114700
H	4.26646600	-0.39633900	-0.52218900
H	5.47919900	3.00045300	-3.58438700
H	2.81438500	3.67279400	-3.57257900
H	1.76002200	2.20856400	-1.47626000

FurD GS:
Energy at CAM-B3LYP/6-31+G(d) level: -1201.463275 Hartree

C	1.27714500	-3.50642400	-3.46098500
C	1.09370300	-2.90360000	-2.22097400
C	0.10616100	-3.36726800	-1.34348100
C	-0.68860400	-4.44219100	-1.74468400
C	-0.50544400	-5.04857400	-2.98636300
C	0.48070700	-4.58285900	-3.85015900
C	-0.09849400	-2.71164700	0.00722300
C	-0.66482500	-1.31150900	-0.08055500
N	-1.97300500	-1.18819600	-0.11068200
C	-2.51368100	0.04662900	-0.19354300
C	-1.66394200	1.14393500	-0.28272400
N	-0.33962400	1.05564800	-0.24411500
C	0.21203000	-0.16871700	-0.11488500
C	-3.98821400	0.15406000	-0.21395800
C	-4.65303300	1.34876000	0.09734100
C	-6.04112100	1.43133500	0.05738000
C	-6.80512500	0.31844000	-0.28418700
C	-6.15767900	-0.87948200	-0.58202500
C	-4.77104400	-0.96055900	-0.54719900
N	1.54584700	-0.36485500	-0.12942400
C	2.39705400	0.50712800	0.44129400
O	2.17910300	1.30811400	1.35836200
C	3.82401900	0.41250900	-0.12094400
C	3.95762900	1.21668900	-1.36905400
O	5.19888900	1.25786800	-1.94638200
C	5.10620800	2.03822600	-3.05805500
C	3.83816300	2.49531500	-3.20340600
C	3.08984100	1.96012500	-2.10308200
H	2.04753000	-3.13053900	-4.12965300
H	1.70192600	-2.05736000	-1.91174700
H	-1.46565300	-4.80517500	-1.07620600
H	-1.13759500	-5.88394800	-3.27784600
H	0.62670000	-5.05162300	-4.82011800
H	-0.79477900	-3.31612400	0.59627100
H	0.85844400	-2.66199800	0.53418700
H	-2.06422700	2.14737900	-0.41862100
H	-4.08093600	2.22182900	0.39487300
H	-6.52814500	2.37080200	0.30702600
H	-7.88969700	0.38192900	-0.31213700
H	-6.73873900	-1.75935000	-0.84778800
H	-4.26430800	-1.89105500	-0.77813200
H	4.52268000	0.78781200	0.63434500
H	4.07005100	-0.63379700	-0.33589400
H	6.01737900	2.16408300	-3.62293500
H	3.47319400	3.13481700	-3.99577100
H	2.04151000	2.09741500	-1.87559000