Supporting Information

Chimeric fungal – firefly luciferins exhibit red shifted fungal bioluminescence

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Contents

General Experimental

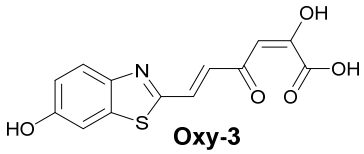
Anhydrous reactions were performed in flame dried glassware under a positive flow of nitrogen gas. Rection temperatures of 0 ºC was achieved by the use of an ice-water bath and of -78 ºC by using a dry ice in acetone bath. All commercially available solvents and chemicals were used without further purification. The anhydrous solvents tetrahydrofuran (THF), dichloromethane (DCM) and toluene were obtained from the in-house drying solvent system. Thin layer chromatography (TLC) was carried out on Merck aluminum backed DC 60 F254 0.2 mm pre-coated plates. Visualization of the TLC was under ultraviolet light (254 nm or 364 nm). Flash column chromatography was prepared on Gedran silca gel 60, 40-63 µm.

The ^{1}H and ^{13}C NMR spectra were recorded on the following machines: Bruker Avance III 400, Bruker Avance III 600, and Bruker Acance Neo 700 spectrometer. All the NMR spectra were manipulated using MestReNova (Version 11.0). The chemical shife (δ) was reported in parts per million (ppm) and coupling constants are quoted in Hertz (Hz). Two-dimensional NMR (COSY, HSQC, and HMBC) were used to assist full assignment where required. A Perkin Elmer Spectrum 100 FT/IR with 100 µATR machine was used to collect IR spectra under thin film conditions or as a fine solid. Mass spectra were obtained on a ThermoMAT900 and an Accela LC-Finnigan LTQ instruments.

Kinetic analysis of chimera 3 and 6.

Bioluminescence activity and kinetic measurements for **3** and **6** chimeras were performed according to standardised luciferase assay protocols.[1] Each reaction mixture contained 2.5 µL substrate solution, 2.5 µL wild-type luciferase (membrane fraction in extraction buffer: 50 mM HEPES, 0.5 M NaCl, 20% glycerol, pH 8.0, 25 mM DDM) and 120 µL assay buffer (0.2 M NaH_2PO_4, 0.5 M Na_2SO_4, 0.05% DDM, pH 8.0). Reactions were conducted at room temperature (20–25 °C). Substrates (**3** and **6**) were dissolved in DMSO and their purity confirmed by LCMS. Substrate concentrations were determined spectrophotometrically using extinction coefficients: **6**, ε = 10,474 $L \cdot mol^{-1} \cdot cm^{-1}$; **3**, ε = 2,666 $L \cdot mol^{-1} \cdot cm^{-1}$. Luminescence was measured using a Promega GloMax luminometer with 1 min light integration. All assays were performed in duplicate or triplicate, and Michaelis–Menten kinetics were analysed using Prism 9.

Computational data files



Oxy-3

Oxychimera **3** (**Oxy-3**) was calculated by density functional calculations (DFT) at the B3LYP/6-311+G(2d,p) level

Geomtry optimisation

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%CHK=output.chk

#N B3LYP/6-311+G(2d,p) SP GFINPUT POP=FULL Geom=Connectivity

$C_{13}H_9O_5NS$

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	5.470639	0.216196	-0.049933
2	6	4.483645	-0.724984	-0.025479
3	6	3.154121	-0.475410	0.010107
4	6	2.458631	0.697117	0.035336
5	6	1.091944	0.700290	0.061973
6	6	0.243649	-0.347809	0.068572
7	6	-1.099563	-0.214579	0.092144
8	16	-2.004968	-1.265887	0.091872
9	6	-3.164096	-0.545355	0.027354
10	6	-2.936481	0.769111	0.035030
11	7	-1.685300	0.914072	0.104634
12	6	-3.922531	1.669252	-0.022912
13	6	-5.179256	1.203032	-0.092531
14	6	-5.462216	-0.119547	-0.106423
15	6	-4.430996	-0.986705	-0.041004
16	1	-4.657424	-2.068667	-0.047718
17	8	-6.749668	-0.611838	-0.173225

18	1	-6.863009	0.082816	0.495086
19	1	-6.012389	1.924414	-0.142129
20	1	-3.706437	2.751217	-0.014286
21	1	0.634485	-1.377473	0.052900
22	1	0.647958	1.711388	0.078766
23	8	2.976163	1.790878	0.036912
24	1	2.587954	-1.415670	0.017654
25	8	4.882539	-2.033428	-0.041465
26	1	4.104247	-2.613967	-0.022406
27	8	6.768793	-0.167254	-0.088025
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Emission calculations for Oxy-4

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%CHK=output.chk

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$C_{13}H_9O_5NS$

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-5.680681	-0.349680	-0.052785
2	6	-4.709308	0.811345	-0.029822
3	6	-3.372808	0.697907	-0.053791
4	6	-2.705093	-0.612233	-0.053393
5	6	-1.228300	-0.689937	-0.051664
6	6	-0.371964	0.342955	-0.015022
7	6	1.070691	0.192616	-0.008988
8	16	2.097376	1.649624	0.040118
9	6	3.500777	0.620214	0.019258
10	6	3.078267	-0.730680	-0.022690
11	7	1.719985	-0.927581	-0.037110
12	6	4.045078	-1.742867	-0.045981
13	6	5.382483	-1.401717	-0.027518
14	6	5.781062	-0.053205	0.014267
15	6	4.845312	0.971418	0.038100
16	1	5.178770	2.000004	0.070031
17	8	7.098538	0.306997	0.033217
18	1	7.654586	-0.480095	0.013148
19	1	6.139549	-2.178784	-0.045402
20	1	3.731451	-2.778274	-0.078272
21	1	-0.734435	1.364002	0.014675
22	1	-0.843862	-1.703048	-0.076888
23	8	-3.348963	-1.656637	-0.054815
24	1	-2.782746	1.606798	-0.085586
25	8	-5.390978	1.975257	-0.035628
26	1	-4.777656	2.721480	-0.075880
27	8	-5.951177	-0.797905	1.182278
28	1	-6.569517	-1.541001	1.089421
29	8	-6.208314	-0.733815	-1.058452

Molecular Orbital calculation for the optimised Oxy-3

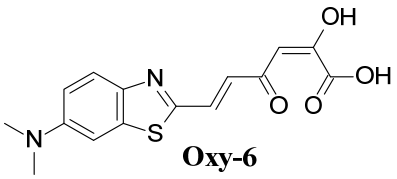
%NProcShared=10

%CHK=output.chk

#N B3LYP/6-311+G(2d,p) SP GFINPUT POP=FULL Geom=Connectivity

$C_{13}H_9O_5NS$

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	-5.680681	-0.349680	-0.052785
2	6	-4.709308	0.811345	-0.029822
3	6	-3.372808	0.697907	-0.053791
4	6	-2.705093	-0.612233	-0.053393
5	6	-1.228300	-0.689937	-0.051664
6	6	-0.371964	0.342955	-0.015022
7	6	1.070691	0.192616	-0.008988
8	16	2.097376	1.649624	0.040118
9	6	3.500777	0.620214	0.019258
10	6	3.078267	-0.730680	-0.022690
11	7	1.719985	-0.927581	-0.037110
12	6	4.045078	-1.742867	-0.045981
13	6	5.382483	-1.401717	-0.027518
14	6	5.781062	-0.053205	0.014267
15	6	4.845312	0.971418	0.038100
16	1	5.178770	2.000004	0.070031
17	8	7.098538	0.306997	0.033217
18	1	7.654586	-0.480095	0.013148
19	1	6.139549	-2.178784	-0.045402
20	1	3.731451	-2.778274	-0.078272
21	1	-0.734435	1.364002	0.014675
22	1	-0.843862	-1.703048	-0.076888
23	8	-3.348963	-1.656637	-0.054815
24	1	-2.782746	1.606798	-0.085586
25	8	-5.390978	1.975257	-0.035628
26	1	-4.777656	2.721480	-0.075880
27	8	-5.951177	-0.797905	1.182278
28	1	-6.569517	-1.541001	1.089421
29	8	-6.208314	-0.733815	-1.058452



Oxy-6

Oxychimera **6** (**Oxy-6**) was calculated by density functional calculations (DFT) at the B3LYP/6-311+G(2d,p) level

Geomtry optimisation

%NProcShared=10

%CHK=output.chk

#N B3LYP/6-311+G(2d,p) OPT Geom=Connectivity

$C_{15}H_{14}O_4N_2S$

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	7.083915	0.541170	-0.550027

2	7	6.055716	-0.442628	-0.265488
3	6	4.739441	0.029191	-0.154666
4	6	4.423777	1.342433	-0.072277
5	6	3.159882	1.784830	0.036146
6	6	2.183879	0.874120	0.052132
7	7	0.931454	0.995847	0.141598
8	6	0.362902	-0.137846	0.053600
9	16	1.284544	-1.172486	-0.023485
10	6	2.433078	-0.431991	-0.038304
11	6	3.706735	-0.846631	-0.141659
12	1	3.910224	-1.927988	-0.239544
13	6	-0.978046	-0.292157	0.022232
14	6	-1.848656	0.734494	0.096900
15	6	-3.215438	0.701188	0.072276
16	6	-3.882385	-0.482127	-0.049311
17	6	-5.205285	-0.764707	-0.090630
18	6	-6.217392	0.144768	0.001495
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24	1	-3.293056	-1.403484	-0.135561
25	8	-3.759741	1.778416	0.159537
26	1	-1.425732	1.750086	0.194021
27	1	-1.347069	-1.325798	-0.071037
28	1	2.927105	2.860925	0.109353
29	1	5.201786	2.121619	-0.078034
30	6	6.432400	-1.346063	0.809504
31	1	5.749493	-2.219462	0.904912
32	1	7.445971	-1.770652	0.624370
33	1	6.451863	-0.804595	1.783999
34	1	7.279463	1.200309	0.327718
35	1	8.040517	0.028580	-0.802765
36	1	6.824581	1.153288	-1.445389

Energy calculation for Oxy-6

%NProcShared=10

%CHK=output.chk

#N TD(NStates=5,Root=1) B3LYP/6-311+G(2d,p) OPT Geom=Connectivity

$C_{15}H_{14}O_4N_2S$

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	7.371049	-0.922091	-0.128463
2	7	6.393036	0.127899	0.105825
3	6	5.042734	-0.161064	0.054215
4	6	4.591938	-1.511582	0.005141
5	6	3.253731	-1.832826	-0.015557
6	6	2.289332	-0.817131	0.005178
7	7	0.931852	-0.998135	-0.015053
8	6	0.293283	0.130126	0.003551
9	16	1.342221	1.574079	0.049149
10	6	2.733819	0.524485	0.041465
11	6	4.079804	0.863771	0.060080

12	1	4.372413	1.902322	0.078822
13	6	-1.144166	0.298143	-0.010018
14	6	-2.019243	-0.721365	-0.044207
15	6	-3.491226	-0.616894	-0.053423
16	6	-4.135482	0.706533	-0.062744
17	6	-5.469031	0.844757	-0.044146
18	6	-6.461919	-0.297578	-0.063121
19	8	-6.742897	-0.735460	1.173781
20	1	-7.373796	-1.467922	1.081846
21	8	-6.998121	-0.675774	-1.066814
22	8	-6.129578	2.022354	-0.058974
23	1	-5.500789	2.755341	-0.100760
24	1	-3.528908	1.604472	-0.097378
25	8	-4.159144	-1.647185	-0.053737
26	1	-1.651734	-1.740845	-0.061487
27	1	-1.492336	1.324499	0.011682
28	1	2.934250	-2.866747	-0.051774
29	1	5.312485	-2.315304	-0.016232
30	6	6.832831	1.506747	-0.010221
31	1	6.406133	2.122382	0.785860
32	1	7.915294	1.545218	0.090185
33	1	6.559923	1.956948	-0.974357
34	1	7.282032	-1.366601	-1.128254
35	1	8.369650	-0.501735	-0.031096
36	1	7.278827	-1.721300	0.610719

Molecular Orbital calculation from the optimised geometry of Chimera 6

%NProcShared=10

%CHK=output.chk

#N B3LYP/6-311+G(2d,p) SP GFINPUT POP=FULL Geom=Connectivity

$C_{15}H_{14}O_4N_2S$

Center Number	Atomic Number	Coordinates (Angstroms) X	Y	Z
1	6	7.371049	-0.922091	-0.128463
2	7	6.393036	0.127899	0.105825
3	6	5.042734	-0.161064	0.054215
4	6	4.591938	-1.511582	0.005141
5	6	3.253731	-1.832826	-0.015557
6	6	2.289332	-0.817131	0.005178
7	7	0.931852	-0.998135	-0.015053
8	6	0.293283	0.130126	0.003551
9	16	1.342221	1.574079	0.049149
10	6	2.733819	0.524485	0.041465
11	6	4.079804	0.863771	0.060080
12	1	4.372413	1.902322	0.078822
13	6	-1.144166	0.298143	-0.010018
14	6	-2.019243	-0.721365	-0.044207
15	6	-3.491226	-0.616894	-0.053423
16	6	-4.135482	0.706533	-0.062744
17	6	-5.469031	0.844757	-0.044146
18	6	-6.461919	-0.297578	-0.063121
19	8	-6.742897	-0.735460	1.173781
20	1	-7.373796	-1.467922	1.081846
21	8	-6.998121	-0.675774	-1.066814
22	8	-6.129578	2.022354	-0.058974

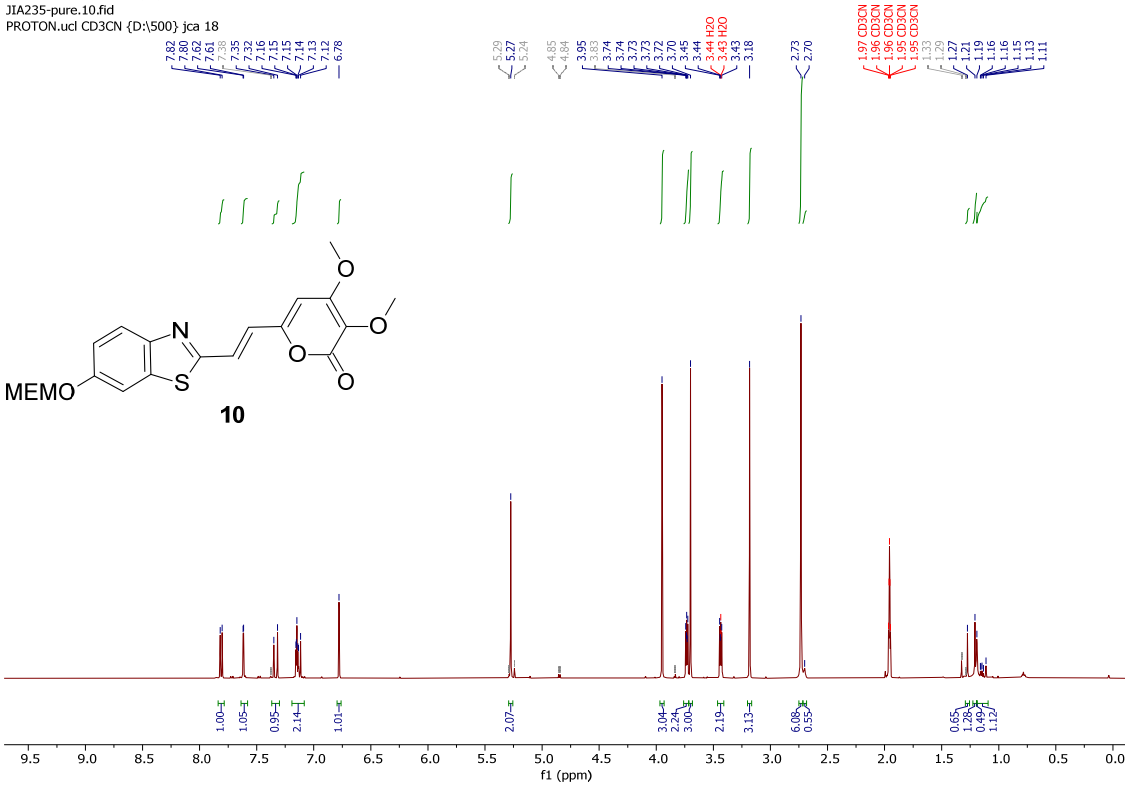
23	1	-5.500789	2.755341	-0.100760
24	1	-3.528908	1.604472	-0.097378
25	8	-4.159144	-1.647185	-0.053737
26	1	-1.651734	-1.740845	-0.061487
27	1	-1.492336	1.324499	0.011682
28	1	2.934250	-2.866747	-0.051774
29	1	5.312485	-2.315304	-0.016232
30	6	6.832831	1.506747	-0.010221
31	1	6.406133	2.122382	0.785860
32	1	7.915294	1.545218	0.090185
33	1	6.559923	1.956948	-0.974357
34	1	7.282032	-1.366601	-1.128254
35	1	8.369650	-0.501735	-0.031096
36	1	7.278827	-1.721300	0.610719

References

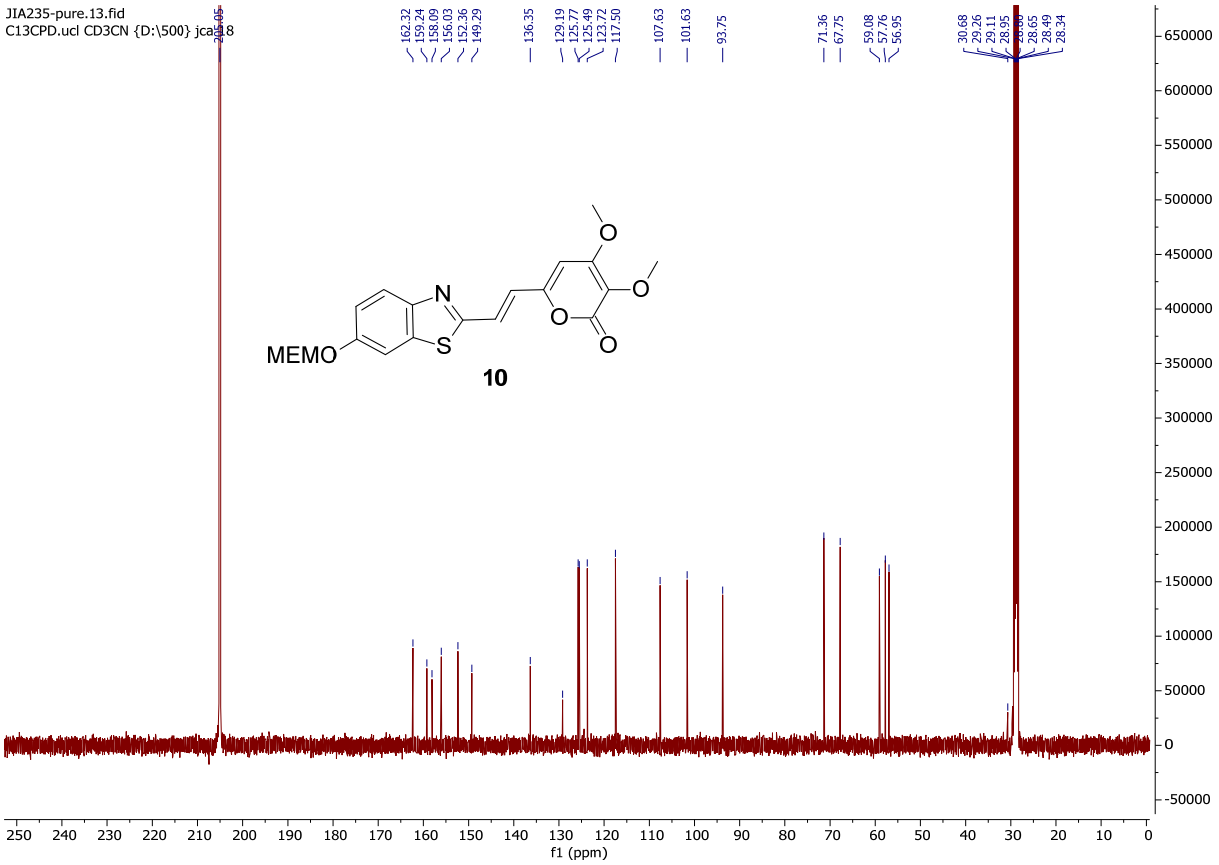
1 E. Hawkins, M. Valley, M. Scurria, J. Unch, T. Good, D. Good, L. Bernad, D. H. Klaubert and K. V. Wood, K. V. *Promega* 2007, **97**, 30.

NMR spectra.

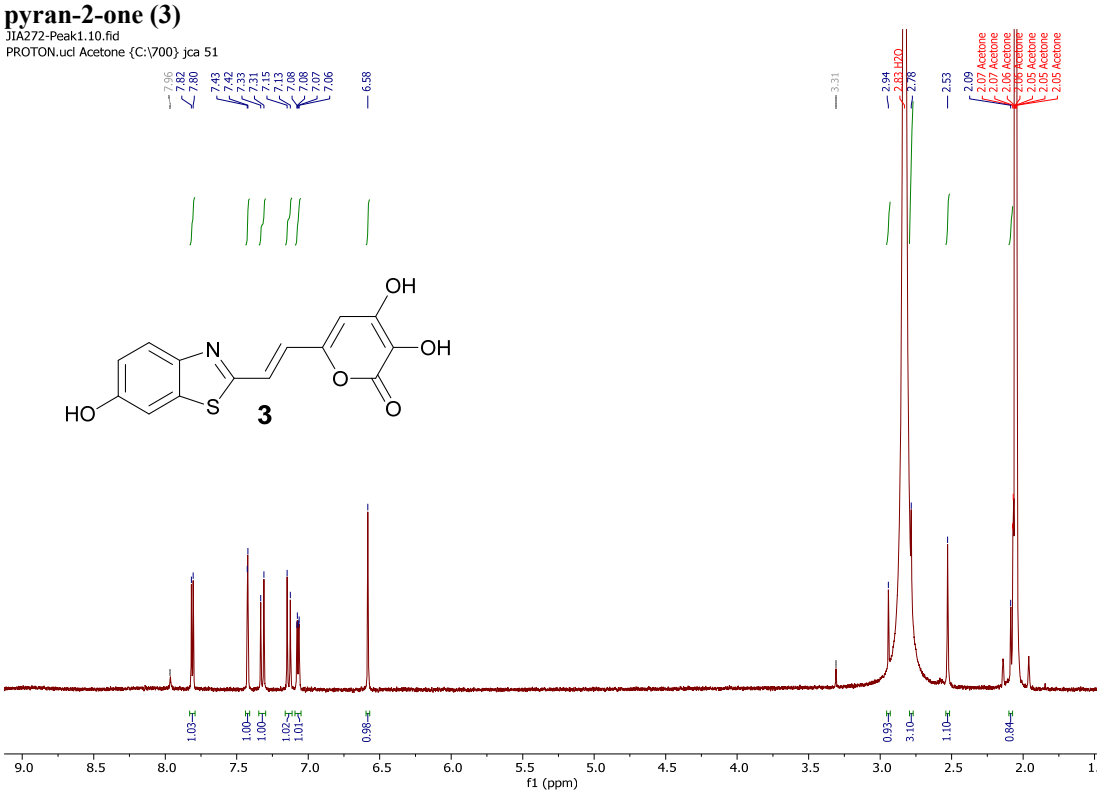
^{1}H NMR (500 MHz, CD_3CN): **(*E*)-3,4-Dimethoxy-6-(2-((2-methoxy)methoxy)benz0[*d*]thiazol-2-yl)vinyl)-2*H*-pyran-2-one (10)**



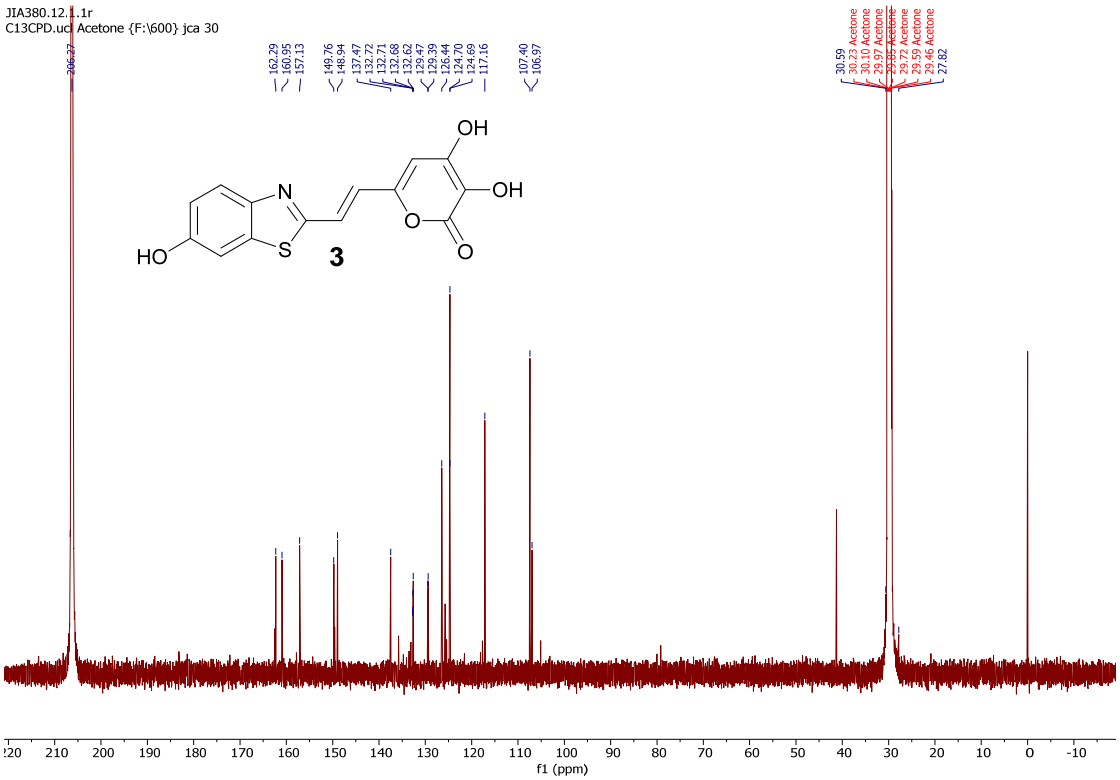
^{13}C NMR (125 MHz, CD_3CN): **(*E*)-3,4-Dimethoxy-6-(2-((2-methoxy)methoxy)benz0[*d*]thiazol-2-yl)vinyl)-2*H*-pyran-2-one (10)**



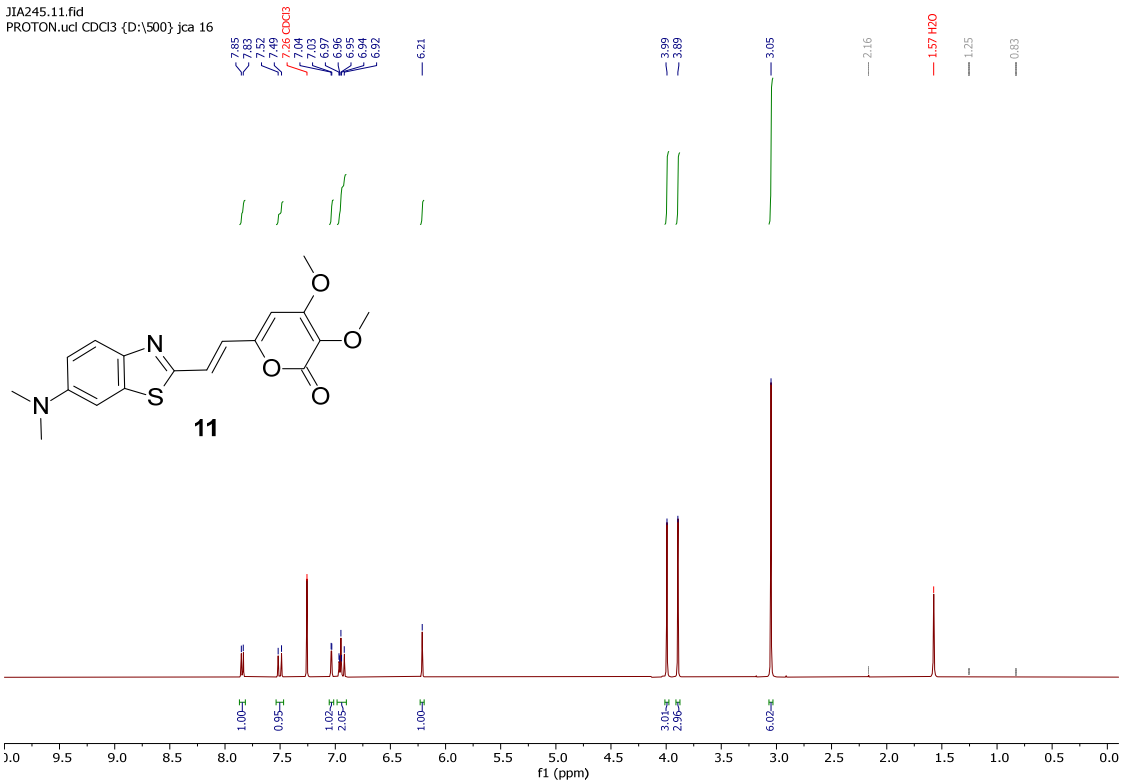
^{1}H NMR (700 MHz, acetone-d6): **(*E*)-3,4-Dimhydroxy-6-(2-hydroxy)benzo[*d*]thiazol-2-yl)vinyl)-2*H*-pyran-2-one (3)**



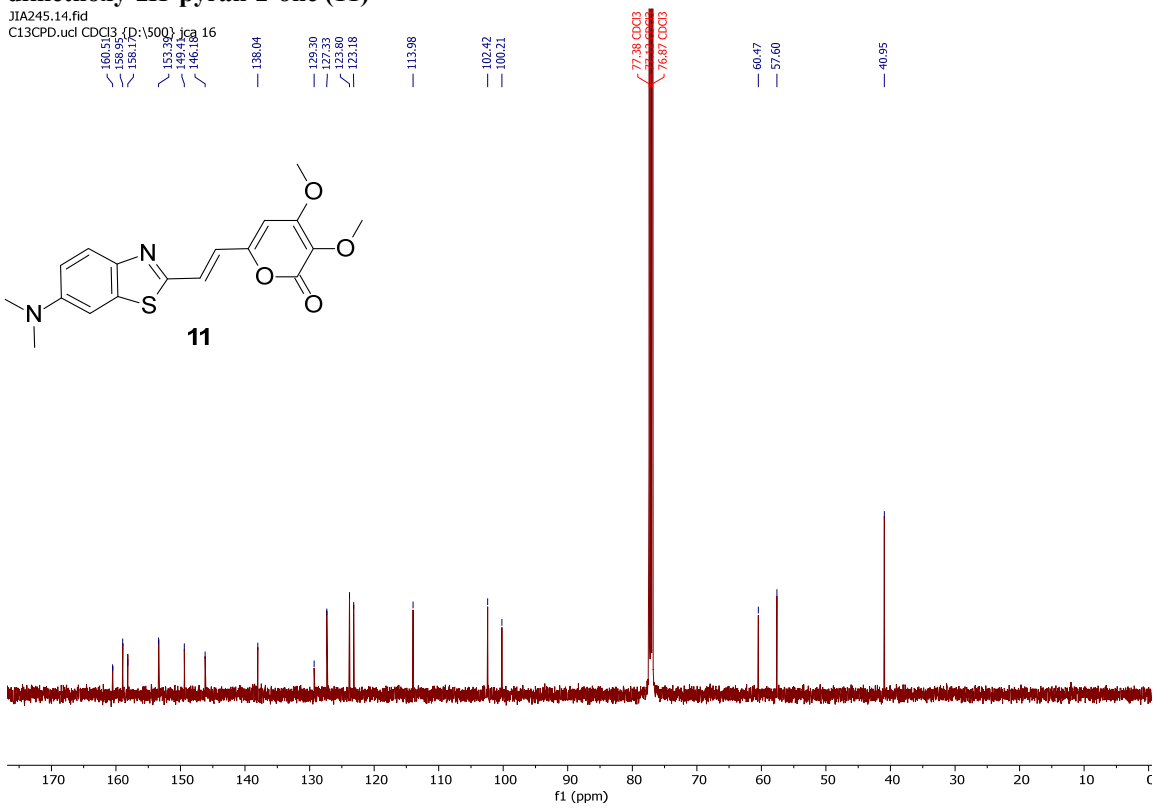
^{13}C NMR (150 MHz, acetone-d6): **(*E*)-3,4-Dimhydroxy-6-(2-hydroxy)benzo[*d*]thiazol-2-yl)vinyl)-2*H*-pyran-2-one (3)**



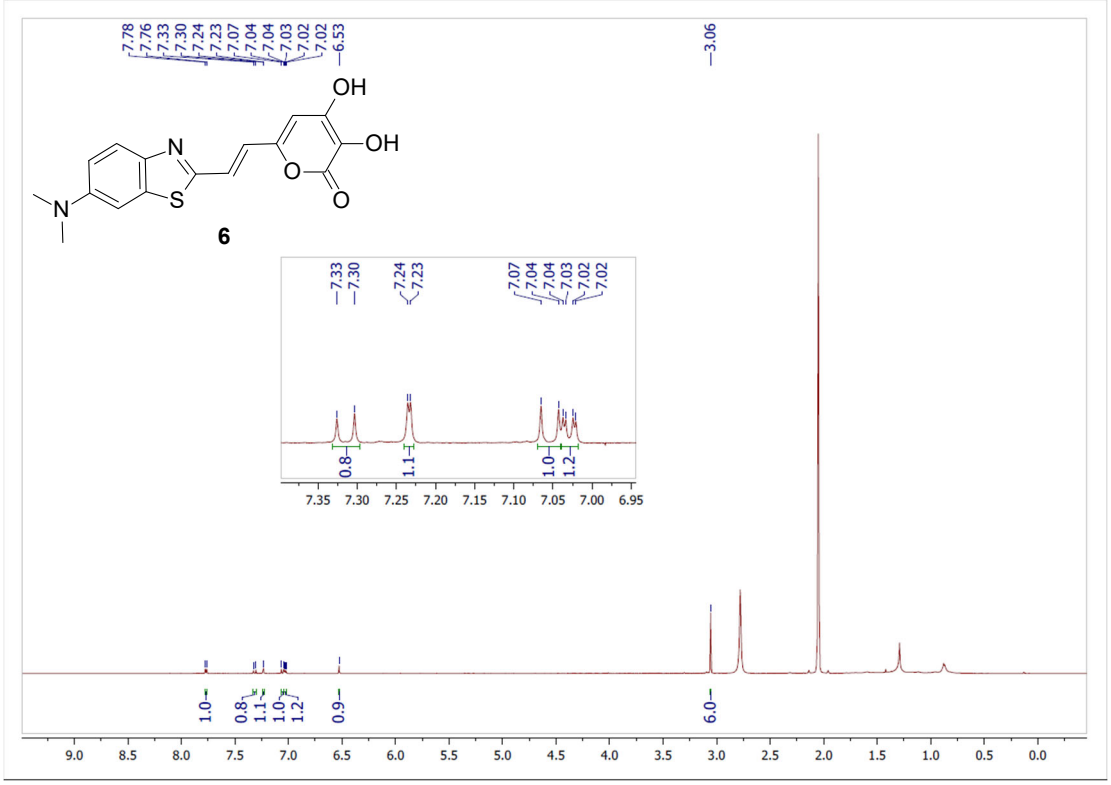
^{1}H NMR (500 MHz, $CDCl_3$): **(*E*)-6-(2-((6-dimethylamino)benzo[*d*]thiazol-2-yl)vinyl)-3,4-dimethoxy-2*H*-pyran-2-one (11)**



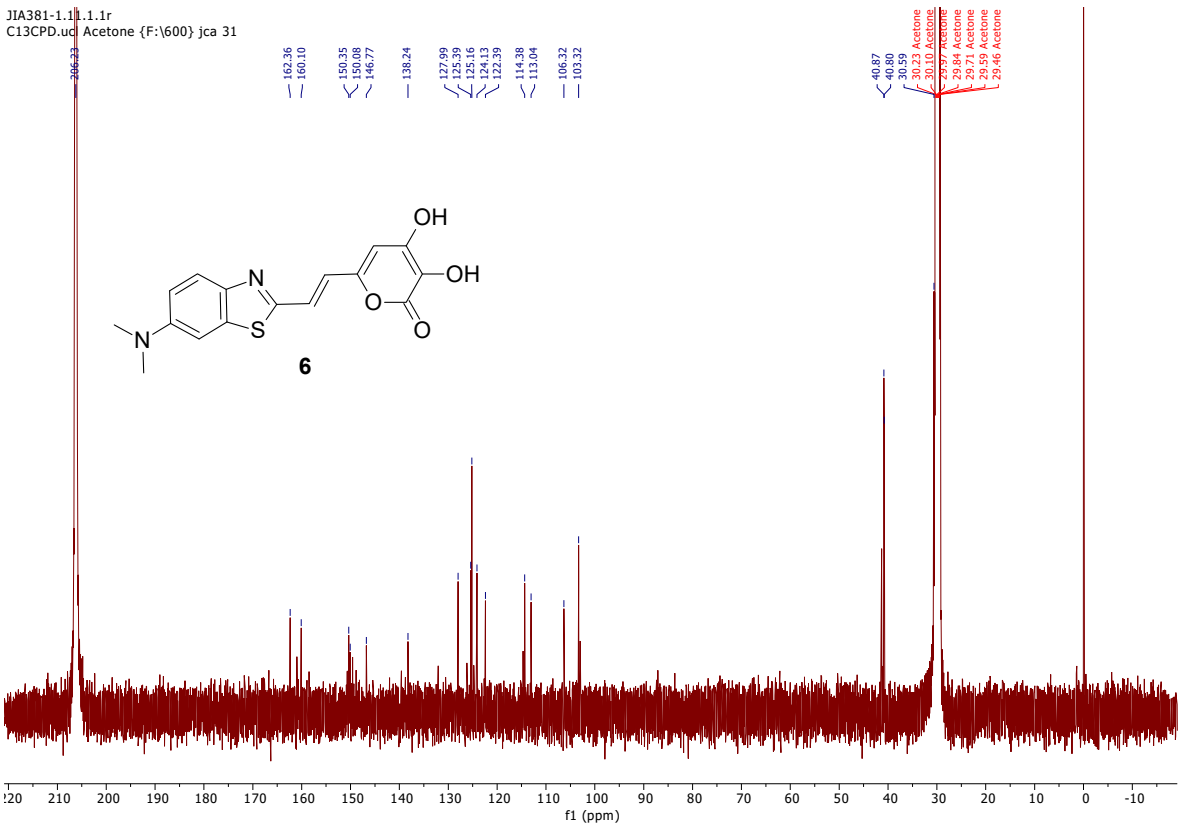
^{13}C NMR (125 MHz, $CDCl_3$): **(*E*)-6-(2-((6-dimethylamino)benzo[*d*]thiazol-2-yl)vinyl)-3,4-dimethoxy-2*H*-pyran-2-one (11)**



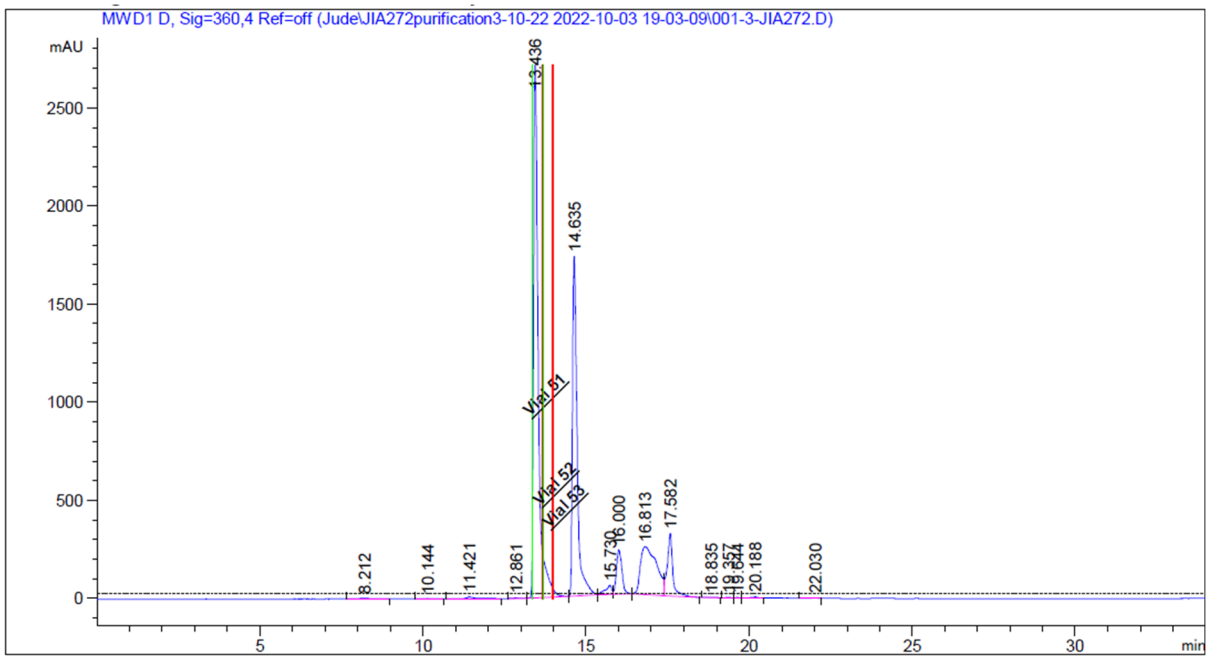
^{1}H NMR (700 MHz, acetone-d6): **(*E*)-6-(2-(6-(dimethylamino)benzo[d]thiazol-2-yl)vinyl)-3,4-dihydroxy-2*H*-pyran-2-one (6)**



^{13}C NMR (150 MHz, acetone-d6): **(*E*)-6-(2-(6-(dimethylamino)benzo[d]thiazol-2-yl)vinyl)-3,4-dihydroxy-2*H*-pyran-2-one (6)**



HPLC trace of compound **3** with fraction collection



HPLC trace of compound **6** with fraction collection