Supporting information

An improved photo-induced fluorogenic alkene-tetrazole reaction for protein labeling

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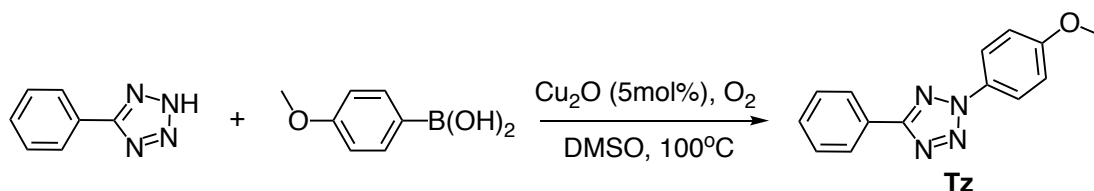
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I. Synthetic procedures

Synthesis of 2-(4-methoxyphenyl)-5-phenyl-2*H*-tetrazole (Tz; Figure 1 of manuscript)

The desired tetrazole (**Tz**) was synthesized through cross-coupling reaction between 5-phenyl tetrazole and 4-methoxylphenylboronic acid by following a literature method.[1] A mixture of 5-phenyl tetrazole (731 mg, 5 mmol), 4-methoxylphenylboronic acid (1.36 g, 8.9 mmol), and Cu_2O (36 mg, 0.25 mmol) in 40 mL DMSO was stirred at 100 $^{\circ}$C under oxygen atmosphere for 5 hours. After being cooled to the room temperature, acetyl acetate (400 mL) was added. The resulting mixture was washed with 50 mL of 1N HCl and 5 × 50 mL of brine. The organic layer was separated and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum. The residue was purified by silica-gel flash chromatography with hexane:ethyl acetate (50:1) to afford the white product (1.135 g) with 90% yield. The ^{1}H-NMR of the product is identical to the reported one.[1] ^{1}H-NMR (400 MHz, CDCl$_3$) δ ppm: 8.25 (d, *J* = 7.9 Hz, 2H), 8.11 (d, *J* = 9.0 Hz, 2H), 7.52 (m, 3H), 7.07 (d, *J* = 9.0 Hz, 2H), 3.90 (s, 3H).

General procedure to synthesize substituted pyrazoline through alkene-tetrazole reaction

In a quartz test tube, tetrazole (**Tz;** 30 mg) was dissolved in 6 mL of ethyl acetate followed by the addition of 10 eq alkene (styrene, acrylamide or hex-5-en-1-ol). The reaction mixture was irradiated with 302 nm UV (UVP, 0.16 Amps, 8 watt) at a distance of 4 cm for 2 hours. The reaction mixture was concentrated under vacuum and the product was purified by silica-gel flash chromatography.
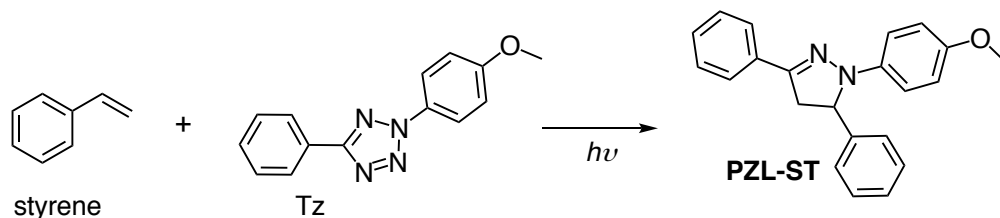
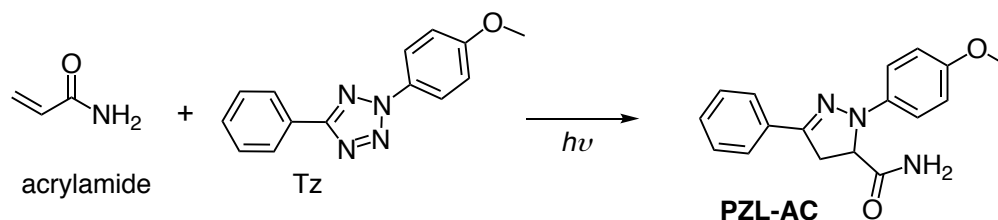
1-(4-methoxyphenyl)-3,5-diphenyl-4,5-dihydro-1*H*-pyrazole (PZL-ST). ^{1}H-NMR (300 MHz, CDCl$_3$) δ ppm: 7.71 (d, *J* = 7.2 Hz, 2H), 7.41-7.26 (m, 8H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 9.3 Hz, 2H), 5.18 (m, 1H), 3.82-3.73 (m, 1H), 3.72 (s, 3H), 3.13 (dd, *J* = 16.2, 8.4 Hz, 1H); ^{13}C-

NMR (400 MHz, CDCl$_3$) δ ppm: 153.42, 148.35, 142.85, 139.79, 132.99, 129.22, 128.64, 128.51, 127.67, 126.21, 125.73, 115.00, 114.52, 65.82, 55.72, 43.84. HRMS (ESI): [M + H]$^+$ calcd for C$_{22}$H$_{20}$N$_2$O 329.1648; found 329.1626.
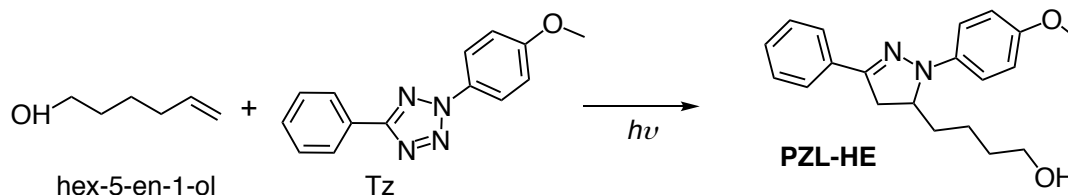
4-(1-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)butan-1-ol (PZL-HE). ^{1}H-NMR (400MHz, CDCl$_3$) δ ppm: 7.70 (d, *J* = 7.6 Hz, 2H), 7.37 (t, *J* = 7.2 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.11 (d, *J* = 9.2 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 4.28 (m, 1H), 3.80 (s, 3H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.40 (dd, *J* = 16.8, 11.6 Hz, 1H), 3.13 (dd, *J* = 16.4, 6.0 Hz, 1H), 1.80-1.90 (m, 1H), 1.60-1.40 (m, 6H); ^{13}C-NMR (400 MHz, CDCl$_3$) δ ppm: 153.57, 147.06, 139.28, 133.31, 128.58, 128.30, 125.60, 115.64, 114.75, 62.78, 61.29, 55.82, 38.24, 32.66, 32.30, 21.43. HRMS (ESI): [M + H]$^+$ calcd for C$_{20}$H$_{24}$N$_2$O$_2$ 325.1911; found 325.1901.

1-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazole-5-carboxamide (PZL-AC). PZL-AC[2] is a known molecule. It was confirmed by ^{1}H-NMR. ^{1}H-NMR (400MHz, DMSO-d$_6$) δ ppm: 7.71 (m, 3H), 7.42 (m, 2H), 7.36 (m, 1H), 7.28 (br, 1H), 6.99 (d, *J* = 9.1 Hz, 2H), 6.88 (d, *J* = 9.1 Hz, 2H), 4.54 (dd, *J* = 12.6, 8.4 Hz, 1H), 3.80 (s, 3H), 3.71 (dd, *J* = 17.3, 12.6 Hz, 1H), 3.21 (dd, *J* = 17.3, 8.4 Hz, 1H).

II. Computational Results

Vertical excitation

PZL-AC. Based on the FixSol/B3LYP/6-31++G(d,p) optimized molecular geometry, the FixSol/TD-B3LYP/6-31++G(d,p) calculated lowest absorption energy is 3.070 eV (403.8 nm) for the $S_0 \rightarrow S_1$ transition, with an oscillator strength 0.6678. The calculated wavelength 403.8 nm is about 44 nm larger than the experimental value ~360 nm (Figure S4: PZL-AC in acetonitrile). The second lowest absorption energy is 3.984 eV (311.2 nm) for the $S_0 \rightarrow S_2$ transition, with an oscillator strength 0.1429. This 311.2 nm absorption should be the shoulder peak at 320 nm in experiment (Figure S4: PZL-AC in acetonitrile). For $S_0 \rightarrow S_6$, $S_0 \rightarrow S_9$, $S_0 \rightarrow S_{11}$ and $S_0 \rightarrow S_{13}$, the calculated energies are 4.064 eV (269.3 nm), 4.851 eV (255.6 nm), 4.970 eV (249.5 nm) and 5.024 eV (246.8 nm), respectively, with a combined oscillator strength 0.6599. These transitions should be the peak at ~250 nm in experiment (Figure S4: PZL-AC in acetonitrile).

PZL-AC-H$^+$. Based on the FixSol/B3LYP/6-31++G(d,p) optimized molecular geometry, the FixSol/TD-B3LYP/6-31++G(d,p) calculated lowest absorption energy for PZL-AC-H$^+$, the protonated form of PZL-AC, is 3.083 eV (402.2 nm) for the $S_0 \rightarrow S_1$ transition, with an oscillator strength 0.3917. The second lowest absorption energy is 3.984 eV (325.1 nm) for the $S_0 \rightarrow S_2$ transition, with an oscillator strength 0.4075. This second transition should correspond to the ~320 nm peak in experiment (PZL-AC in the PBS buffer, Figure S4: PZL-AC in PBS), which has an intensity comparable to the peak at ~360 nm. In acetonitrile (Figure S4: PZL-AC in acetonitrile), this 320 nm peak appears as a shoulder. These results suggest that PZL-AC is protonated in PBS solution but not protonated in acetonitrile. For $S_0 \rightarrow S_8$, $S_0 \rightarrow S_9$ and $S_0 \rightarrow S_{11}$, the calculated energies are 4.968 eV (249.6 nm), 5.004 eV (247.8 nm) and 5.255 eV (235.9 nm), respectively. These transitions should be the peak at ~240 nm in experiment (Figure S4: PZL-AC).

PZL-HE. Based on the FixSol/B3LYP/6-31++G(d,p) optimized molecular geometry, the FixSol/TD-B3LYP/6-31++G(d,p) calculated lowest absorption energy is 3.405 eV (364.1 nm) for the $S_0 \rightarrow S_1$ transition, with an oscillator strength 0.7784. The calculated wavelength is in good agreement with the experimental value (~370 nm, Figure S4: PZL-HE in acetonitrile). For $S_0 \rightarrow S_2$ and $S_0 \rightarrow S_3$, the calculated excitation energies are 4.067 eV (304.9 nm, with an oscillator strength 0.0320) and 4.223 eV (293.6 nm, with an oscillator strength as 0.0410), corresponding to the shoulder peak at ~320 nm in experiment (Figure S4: PZL-HE in acetonitrile). For $S_0 \rightarrow S_4$,

$S_0 \to S_6$, and $S_0 \to S_{10}$, the calculated energies are 4.490 eV (276.1 nm), 4.661 eV (266.0 nm) and 4.985 eV (248.7 nm), respectively, with a combined oscillator strength 0.3344. These transitions should be the peak at ~260 nm in experiment (Figure S4: PZL-HE in acetonitrile).

PZL-HE-H$^+$. Based on the FixSol/B3LYP/6-31++G(d,p) optimized molecular geometry, the FixSol/TD-B3LYP/6-31++G(d,p) calculated lowest absorption energy for PZL-HE-H$^+$, the protonated form of PZL-HE, is 3.150 eV (393.6 nm) for the $S_0 \to S_1$ transition, with an oscillator strength 0.3628. The second lowest absorption energy is 3.742 eV (331.3 nm) for the $S_0 \to S_2$ transition, with an oscillator strength 0.4288. So, for protonated PZL-HE, the peak at ~330 nm should be comparable to the one at ~390 nm. For PZL-HE in PBS solution, the peak at ~320 nm has an intensity larger than the one at ~380 nm. While in acetonitrile, the peak at ~330 nm has a much lower intensity as compared to the one at ~390 nm. Thus, it is very likely that PZL-HE is protonated in the PBS buffer. For $S_0 \to S_7$, $S_0 \to S_8$ and $S_0 \to S_9$, the calculated energies are 4.867 eV (254.7 nm), 5.028 eV (246.6 nm) and 5.153 eV (240.6 nm), respectively, with a combined oscillator strength 0.4378. These transitions should be the peak at ~240 nm in experiment (Figure S4: PZL-HE).

PZL-ST. Based on the FixSol/B3LYP/6-31++G(d,p) optimized molecular geometry, the FixSol/TD-B3LYP/6-31++G(d,p) calculated lowest absorption energy is 3.387 eV (366.0 nm) for the $S_0 \to S_1$ transition, with an oscillator strength 0.7695. It is in accordance with the one observed in experiment (~360 nm, Figure S4: PZL-ST in acetonitrile). For $S_0 \to S_2$, $S_0 \to S_3$, and $S_0 \to S_4$, the calculated energies are 3.951 eV (313.8 nm, oscillator strength 0.0430), 4.220 eV (293.8 nm, oscillator strength 0.0401) and 4.271 eV (290.3 nm, oscillator strength 0.0395), respectively, with a combined oscillator strength 0.1226. These transitions contribute to a peak at ~300 nm, corresponding to the shoulder peak at ~300 nm in experiment (Figure S4: PZL-ST in acetonitrile). For $S_0 \to S_6$, $S_0 \to S_8$ and $S_0 \to S_{12}$, the calculated energies are 4.521 eV (274.2 nm), 4.713 eV (263.1 nm) and 4.958 eV (250.1 nm), respectively, with a combined oscillator strength 0.3751. These transitions should be the peak at ~260 nm in experiment (Figure S4: PZL-ST in acetonitrile).

PZL-ST-H$^+$. Based on the FixSol/B3LYP/6-31++G(d,p) optimized molecular geometry, the FixSol/TD-B3LYP/6-31++G(d,p) calculated lowest absorption energy is 3.074 eV (403.3 nm) for the $S_0 \to S_1$ transition, with an oscillator strength 0.3941. The second lowest absorption energy is 3.712 eV (334.0 nm) for the $S_0 \to S_2$ transition, with an oscillator strength 0.3105. These two

peaks have similar oscillator strength, indicating that for protonated PZL-ST, the peak at ~330 nm should be comparable to the one at ~390 nm. For PZL-ST in PBS solution (Figure S4: PZL-ST in PBS), the case was not found in experiment: the absorption spectra of PZL-ST in PBS buffer and in acetonitrile are similar. Thus, it is likely that PZL-ST is not protonated in PBS solution, or the protonation portion is insignificant.

De-excitation from $S_1 \rightarrow S_0$

The S1→S0 de-excitation energy was calculated for each species using the FixSol/TDB3LYP/6-31++G(d,p) geometry optimization method. The calculated $S_1 \rightarrow S_0$ de-excitation energies for PZL-AC, PZL-HE and PZL-ST are 2.355 eV (526.5 nm, oscillator strength 0.3105), 2.312 eV (536.2 nm, oscillator strength 0.7332) and 2.348 eV (528.0 nm, oscillator strength 0.7242), respectively. Therefore, fluorescence can be observed for all of them at ~530 nm. This is in good agreement with the experiment data (Figure 2B, in acetonitrile). For the protonated forms, PZL-AC-H$^+$, PZL-HE-H$^+$ and PZL-ST-H$^+$, the calculated $S_1 \rightarrow S_0$ de-excitation energies are 0.230 eV (oscillator strength 0.0005), 0.434 eV (oscillator strength 0.0046) and 0.063 eV (oscillator strength 0.0001), respectively. These indicate that if the three molecules are protonated, there would be no observable fluorescence. Given the measured fluorescence spectrum, it is likely that PZL-AC and PZL-HE are protonated in the PBS buffer solution (with 5% DMSO), while PZL-ST is not protonated, or the protonation portion is insignificant.

Computational Data

PZL-AC

Vertical Excitation

STATE	ENERGY	EXCITATION	TRANSITION DIPOLE, A.U.			OSCILLATOR
0 ->	HARTREE	EV	X	Y	Z	STRENGTH
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1 A	-972.1385147432	3.070	1.5514	2.5439	-0.0346	0.6678
2 A	-972.1049366957	3.984	-1.1197	-0.3678	0.2744	0.1429
3 A	-972.1000365242	4.117	0.1421	-0.5717	-0.0724	0.0355
4 A	-972.0976405564	4.182	0.1633	0.4919	0.1118	0.0288
5 A	-972.0896428905	4.400	-0.1198	0.0435	0.0066	0.0018
6 A	-972.0821520770	4.604	0.9763	1.2277	0.0453	0.2777
7 A	-972.0816003639	4.619	-0.3799	-0.3576	0.1611	0.0337
8 A	-972.0773706537	4.734	-0.3608	0.1639	0.0685	0.0188
9 A	-972.0730467367	4.851	-0.2419	1.0721	0.0610	0.1440
10 A	-972.0715738845	4.891	-0.2491	-0.1413	-0.0120	0.0099
11 A	-972.0686703961	4.970	-0.0843	-0.9800	-0.1295	0.1199
12 A	-972.0670111621	5.016	-0.8170	0.2094	-0.0473	0.0877
13 A	-972.0666946161	5.024	-0.1259	-0.9363	0.2614	0.1183
14 A	-972.0636356122	5.107	-0.0047	0.1009	0.1725	0.0050

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15  A       -972.0611201894   5.176    -0.2009 -0.0466 -0.0858    0.0063
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De-Excitation

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PZL-HE

Vertical Excitation

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 2  A      -1035.8037162956   4.067    0.4466  0.0292  0.3482    0.0320
 3  A      -1035.7979857215   4.223   -0.0612 -0.6139 -0.1250    0.0410
 4  A      -1035.7881531758   4.490    0.0066  1.1121 -0.3350    0.1484
 5  A      -1035.7866887833   4.530    0.2834  0.3879 -0.0913    0.0265
 6  A      -1035.7818704086   4.661   -0.7707  0.1833 -0.1737    0.0751
 7  A      -1035.7784677172   4.754    0.2148 -0.1076  0.0128    0.0067
 8  A      -1035.7738785468   4.879    0.1977 -0.0642  0.1650    0.0084
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De-Excitation

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PZL-ST

Vertical Excitation

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 5  A      -1034.3663148028   4.319    0.0859 -0.3068  0.1342    0.0126
 6  A      -1034.3588758382   4.521    0.7507  0.9291 -0.1190    0.1596
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10  A    -1034.3482201226   4.811    0.2562 -0.0854  0.1229    0.0104
11  A    -1034.3438936803   4.929   -0.2475  0.0171 -0.1284    0.0094
12  A    -1034.3428401070   4.958   -0.0273  0.8238  0.4080    0.1027
13  A    -1034.3396194446   5.045   -0.2434 -0.4414 -0.2997    0.0425
14  A    -1034.3389061726   5.065   -0.2108 -0.3124 -0.0504    0.0179
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De-Excitation

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PZL-AC-H$^+$

Vertical Excitation

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 8  A     -972.5105488215   4.968   -0.6673  0.5642  0.3010    0.1040
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De-Excitation

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PZL-HE-H$^+$

Vertical Excitation

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11  A        -1036.2040563080     5.411     0.1042 -0.0533 -0.0378     0.0020
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De-Excitation

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 1  A        -1036.3233775574     0.434    -0.3110 -0.5046  0.2893     0.0046
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PZL-ST-H$^+$

Vertical Excitation

```
 STATE            ENERGY   EXCITATION  TRANSITION DIPOLE, A.U.  OSCILLATOR
 0 ->            HARTREE        EV       X       Y       Z      STRENGTH
 0  A        -1034.9722793555     0.000
 1  A        -1034.8593251480     3.074    -1.5036 -1.7241  0.0161     0.3941
 2  A        -1034.8358800898     3.712     1.5141  1.0492 -0.1446     0.3105
 3  A        -1034.8272103981     3.948    -0.9280 -0.7570  0.0070     0.1387
 4  A        -1034.8246962390     4.016    -0.1869 -0.3767  0.0278     0.0175
 5  A        -1034.8199188713     4.146    -0.0456  0.7948  0.2732     0.0720
 6  A        -1034.8189377192     4.173    -0.0801 -0.1365 -0.1604     0.0052
 7  A        -1034.7991447871     4.711    -0.5700  0.0356 -0.4740     0.0636
 8  A        -1034.7931659241     4.874    -0.6887  0.5353  0.0505     0.0911
 9  A        -1034.7925744289     4.890    -0.7057 -0.6640  0.2838     0.1221
10  A        -1034.7892830967     4.980    -0.2197 -0.8341 -0.2598     0.0990
11  A        -1034.7832713421     5.143    -0.0054  0.9085 -0.0410     0.1042
12  A        -1034.7824744815     5.165     0.1495 -0.7435 -0.0001     0.0728
13  A        -1034.7792189571     5.253    -0.2014 -0.0446  0.1513     0.0084
14  A        -1034.7751008733     5.365     0.0570 -0.0305 -0.0668     0.0011
15  A        -1034.7740298348     5.395    -0.1226  0.3276 -0.0463     0.0165
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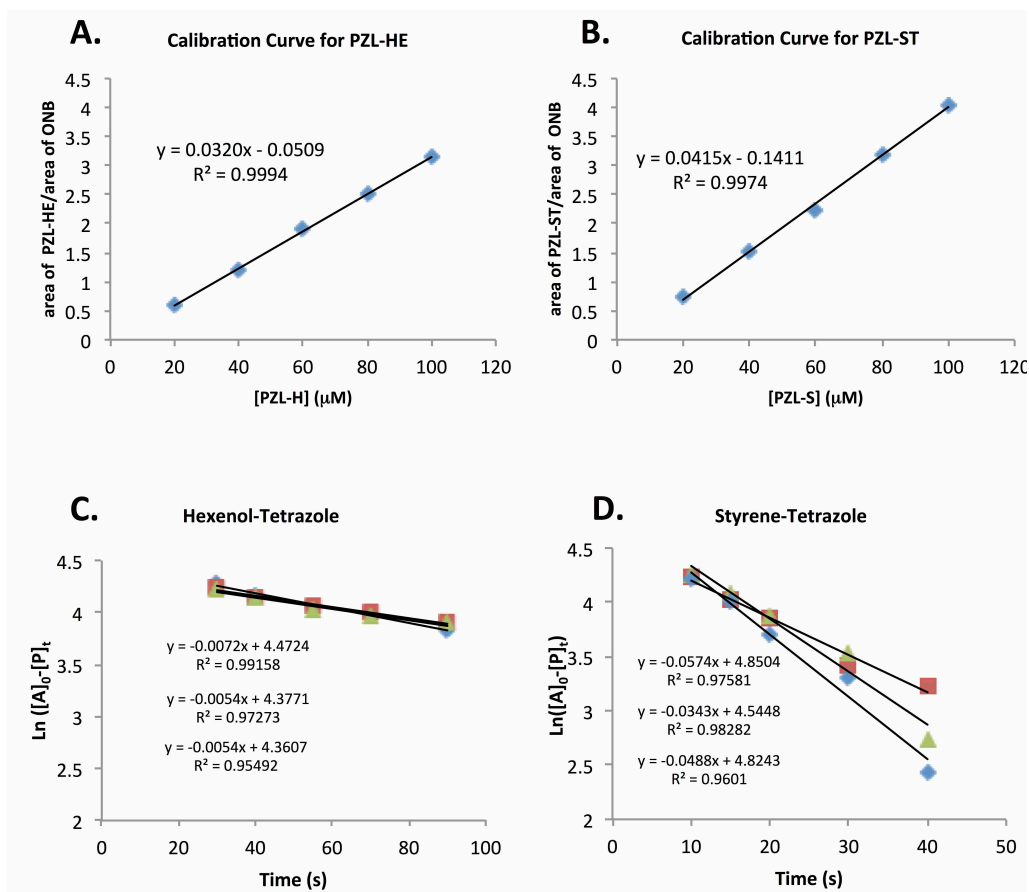
De-Excitation

```
 STATE            ENERGY   EXCITATION  TRANSITION DIPOLE, A.U.  OSCILLATOR
```

```
0 ->              HARTREE         EV       X        Y       Z     STRENGTH
0  A       -1034.9015335012     0.000
1  A       -1034.8992584506     0.062    0.1477 -0.1137  0.1980    0.0001
```

A. Calibration Curve for PZL-HE

y = 0.0320x - 0.0509
R^2 = 0.9994

B. Calibration Curve for PZL-ST

y = 0.0415x - 0.1411
R^2 = 0.9974

C. Hexenol-Tetrazole

y = -0.0072x + 4.4724
R^2 = 0.99158

y = -0.0054x + 4.3771
R^2 = 0.97273

y = -0.0054x + 4.3607
R^2 = 0.95492

D. Styrene-Tetrazole

y = -0.0574x + 4.8504
R^2 = 0.97581

y = -0.0343x + 4.5448
R^2 = 0.98282

y = -0.0488x + 4.8243
R^2 = 0.9601

Figure S1. Kinetic studies of alkene-tetrazole reaction. Reactions were carried out in ACN/PBS (1:1) and irradiated at 302 nm using a handheld UV lamp. Internal reference was 2-nitrobenzyl alcohol (ONB). (A) Calibration curve of purified pyrazoline PZL-HE formed from a reaction between 5-hexen-1-ol and tetrazole; (B) Calibration curve of purified pyrazoline PZL-ST formed from a reaction between styrene and tetrazole; (C) Kinetic measurement of the reaction between tetrazole and 5-hexen-1-ol; (D) Kinetic measurement of the reaction between tetrazole and styrene.
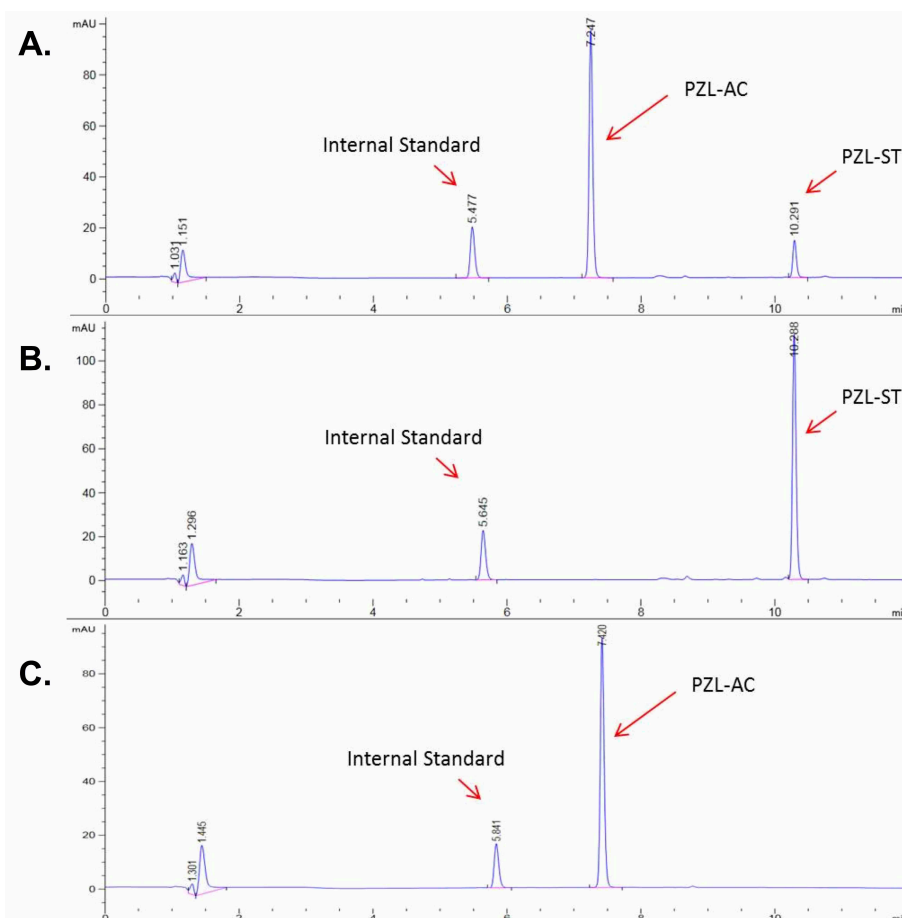
Figure S2. Competitive study of acrylamide and styrene in the 1,3-cycloaddition reaction with tetrazole. Reactions were carried out with 0.1 mM tetrazole and 10 mM alkenes in acetonitrile/PBS (1/1) under 302 nm irradiation for 2 minutes. 2-Nitrobenzyl alcohol (ONB, 0.5 mM) was used as internal standard. (A) Styrene (10 mM) and acrylamide (10 mM); (B) Styrene (10 mM) only; (C) Acrylamide (10 mM) only. Based on the area ratio relative to the internal standard, around 84% PZL-AC and 16% PZL-ST were formed in the competitive reaction (A).
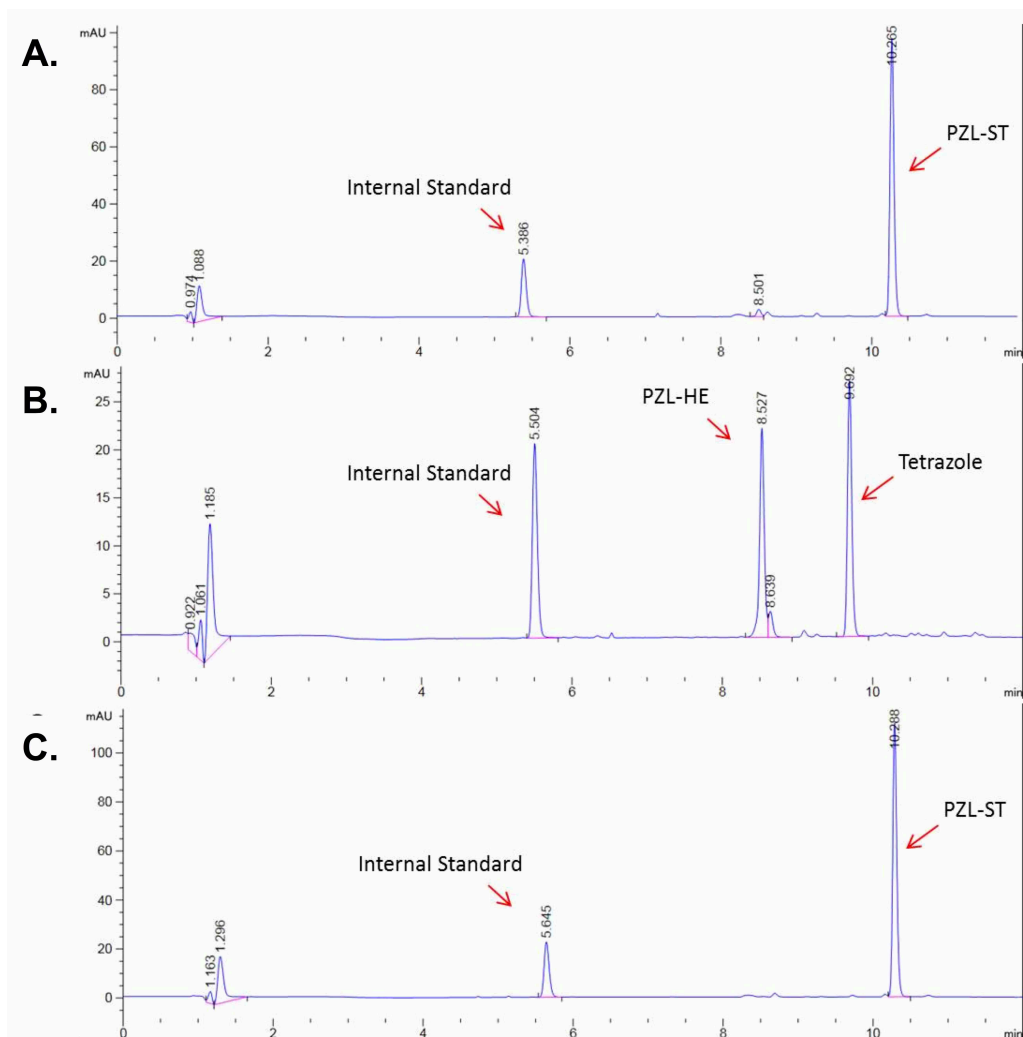
Figure S3. Competitive study of 5-hexen-1-ol and styrene in the 1,3-cycloaddition with the tetrazole. Reactions were carried out with 0.1 mM tetrazole and 10 mM alkenes in acetonitrile/PBS (1/1) under 302 nm irradiation for 2 minutes. 2-Nitrobenzyl alcohol (ONB, 0.5 mM) was used as internal standard. (A) Styrene (10 mM) and 5-hexen-1-ol (10 mM); (B) 5-Hexen-1-ol (10 mM) only; (C) Styrene (10 mM) only. Nearly 100% PZL-ST was formed in the competitive reaction (A).
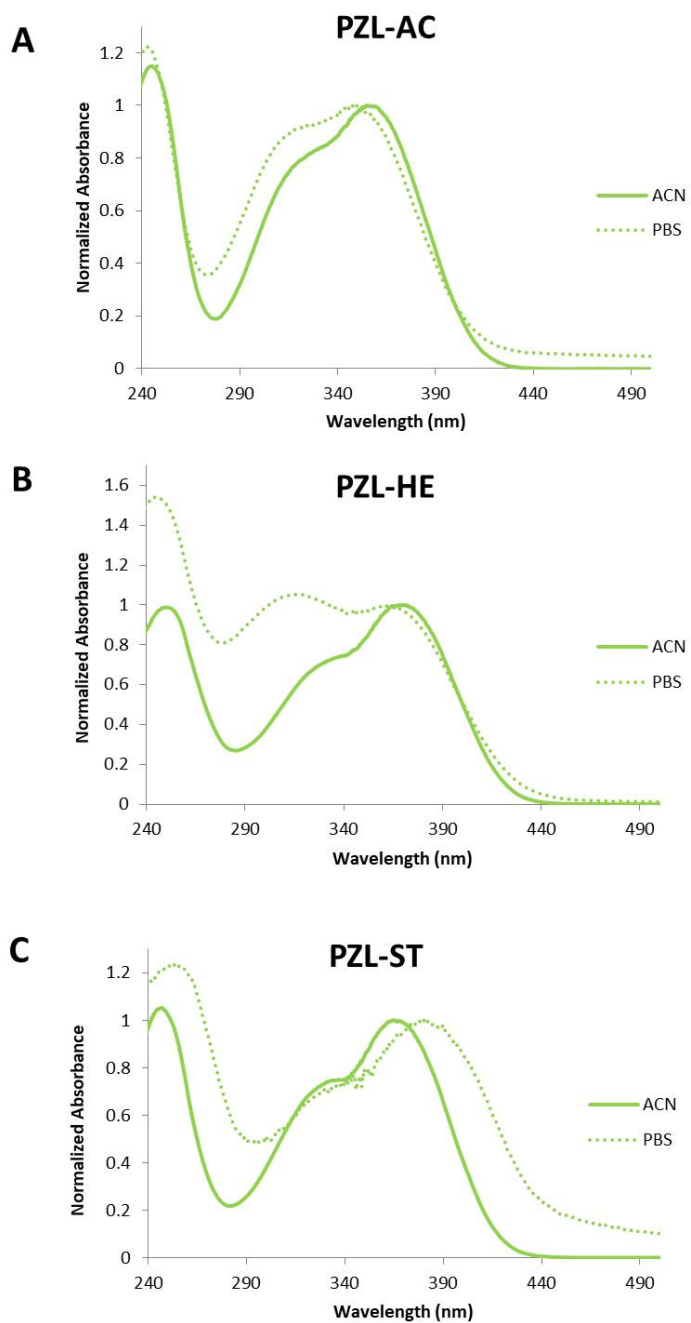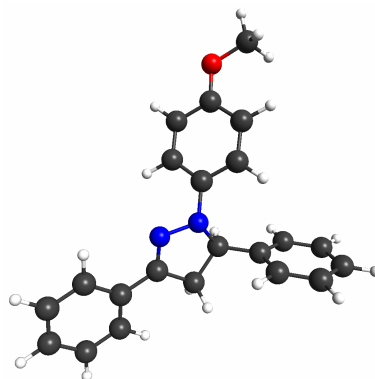
Figure S4. Absorption spectra of pyrazolines. Dashed lines: in PBS buffer (pH 7.4, 5% DMSO); Solid lines: in acetonitrile.
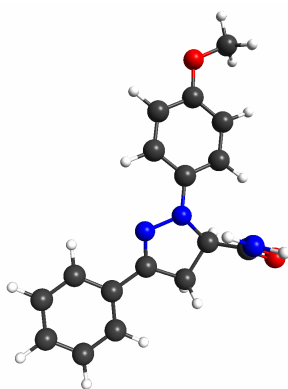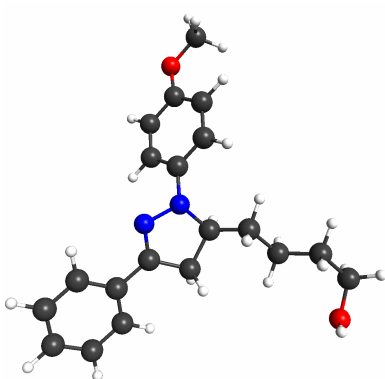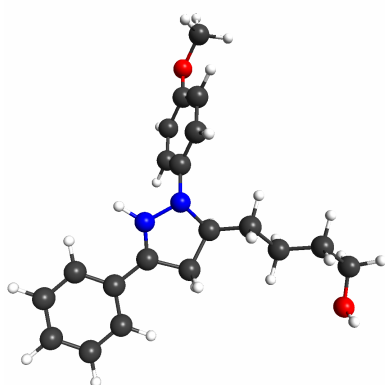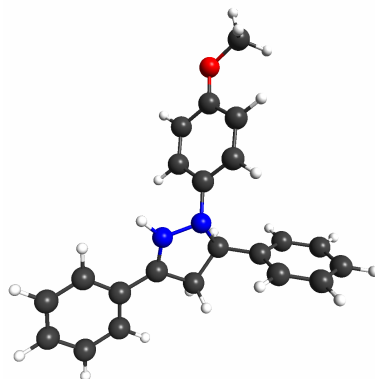
PZL-AC

PZL-HE

PZL-ST
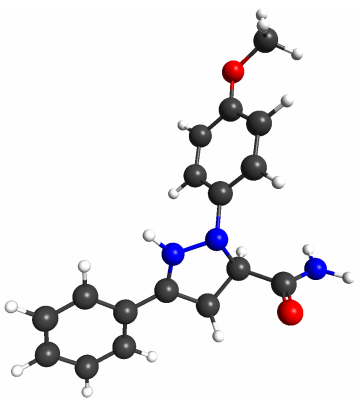
PZL-AC-H$^+$

PZL-HE-H$^+$

PZL-ST-H$^+$
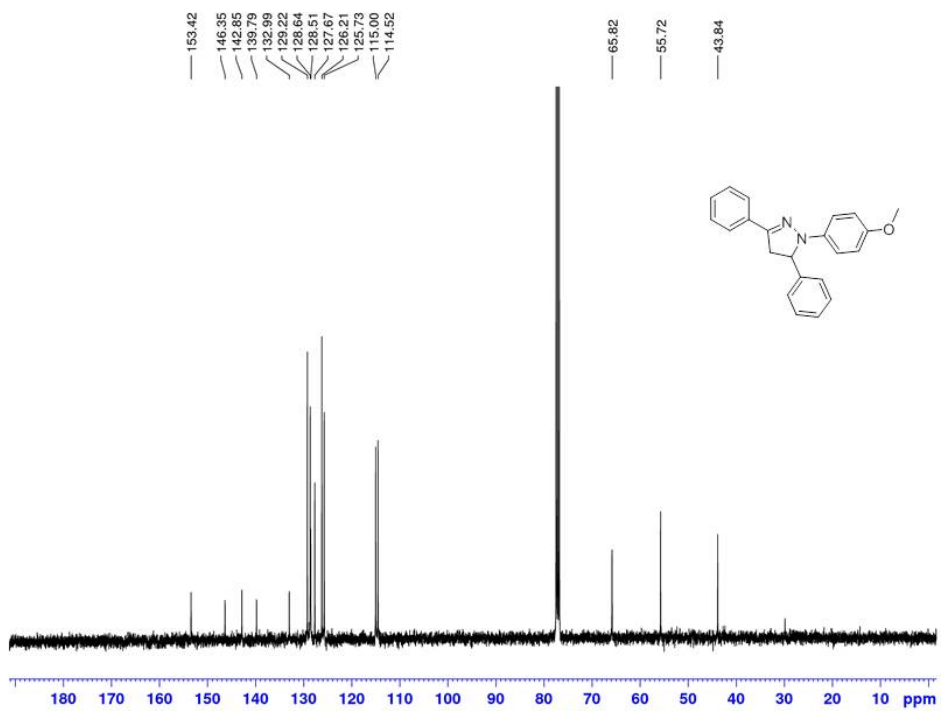
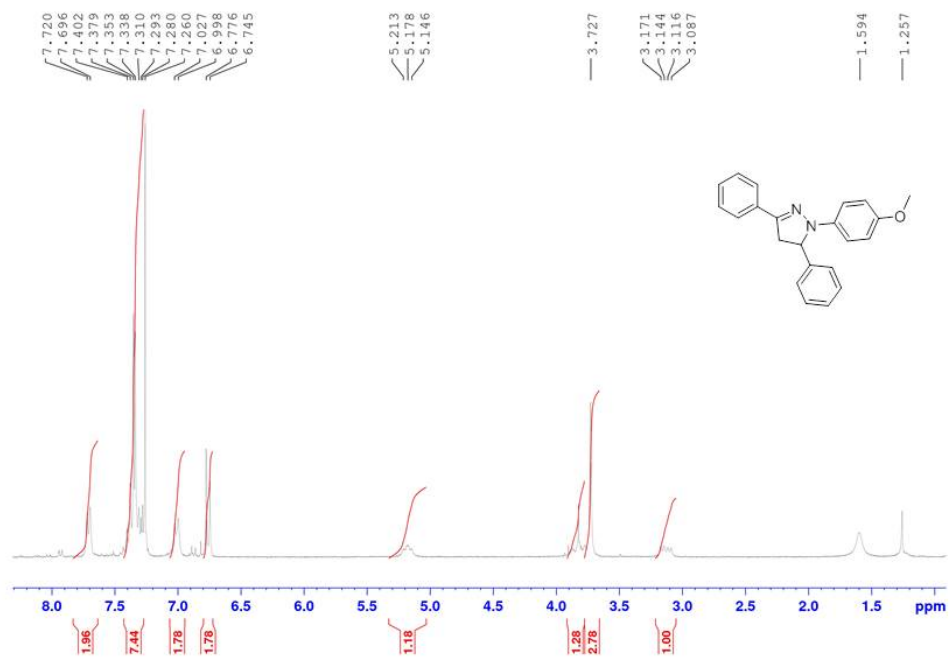**Figure S5. B3LYP/FixSol optimized structures.**

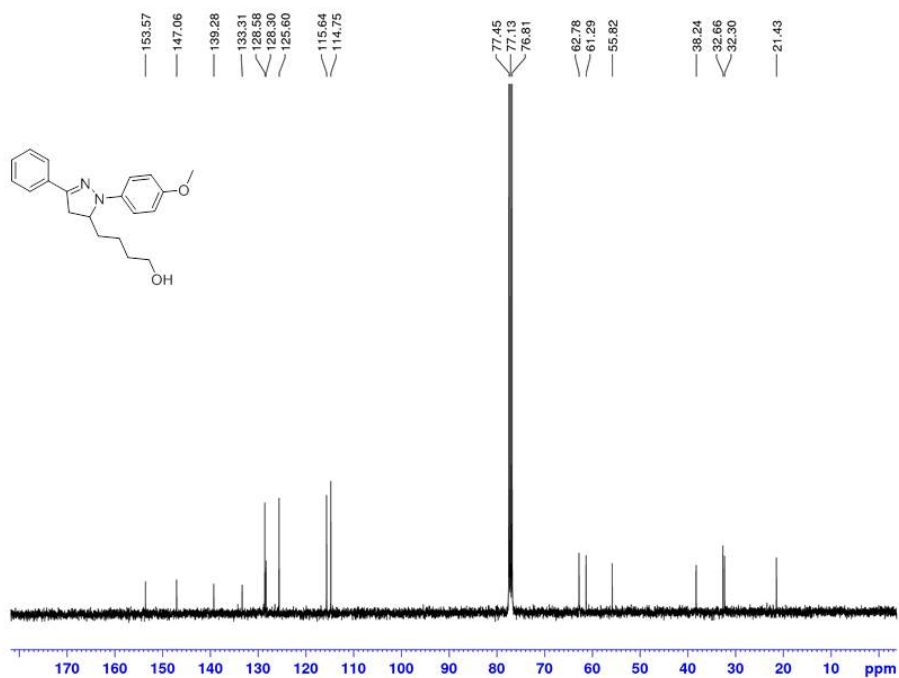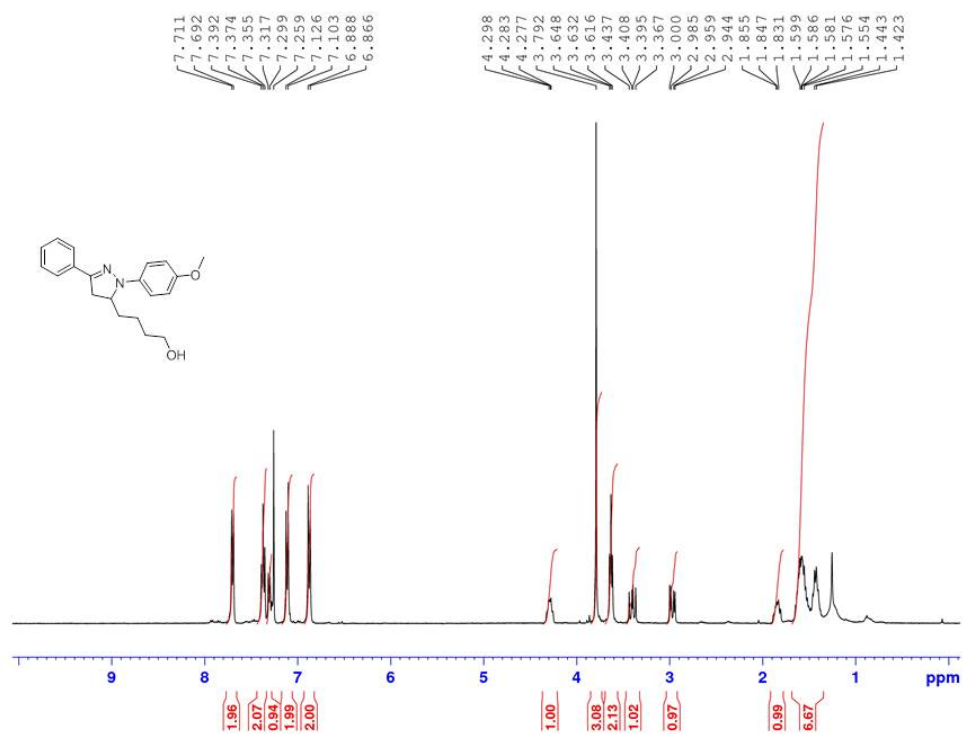**Figure S6** ^{1}H-NMR and ^{13}C-NMR of PZL-ST.

Figure S7 ^{1}H-NMR and ^{13}C-NMR of PZL-HE.

Table S1. Vertical excitation energies of pyrazolines and protonated pyrazolines. The absorption spectra range from 240 nm to 400 nm.

Molecule	Absorption (eV)	Oscillator strength	Wavelength (nm)
PZL-AC	$S_0 \rightarrow S_1$: 3.070	0.6678	403.8
	$S_0 \rightarrow S_2$: 3.984	0.1429	311.2
	$S_0 \rightarrow S_6$: 4.604	0.2777	269.3
	$S_0 \rightarrow S_9$: 4.851	0.1440	255.6
	$S_0 \rightarrow S_{11}$: 4.970	0.1199	249.5
	$S_0 \rightarrow S_{13}$: 5.024	0.1183	246.8
PZL-AC-H$^+$	$S_0 \rightarrow S_1$: 3.083	0.3917	402.2
	$S_0 \rightarrow S_2$: 3.814	0.4075	325.1
	$S_0 \rightarrow S_8$: 4.968	0.1040	249.6
	$S_0 \rightarrow S_9$: 5.004	0.1324	247.8
	$S_0 \rightarrow S_{11}$: 5.004	0.1081	235.9
PZL-HE	$S_0 \rightarrow S_1$: 3.405	0.7784	364.1
	$S_0 \rightarrow S_2$: 4.067	0.0320	304.9
	$S_0 \rightarrow S_3$: 4.223	0.0410	293.6
	$S_0 \rightarrow S_4$: 4.490	0.1484	276.1
	$S_0 \rightarrow S_6$: 4.661	0.0751	266.0
	$S_0 \rightarrow S_{10}$: 4.985	0.1109	248.7
PZL-HE-H$^+$	$S_0 \rightarrow S_1$: 3.150	0.3628	393.6
	$S_0 \rightarrow S_2$: 3.742	0.4288	331.3
	$S_0 \rightarrow S_7$: 4.867	0.1391	254.7
	$S_0 \rightarrow S_8$: 5.028	0.1582	246.6
	$S_0 \rightarrow S_9$: 5.153	0.1405	240.6
PZL-ST	$S_0 \rightarrow S_1$: 3.387	0.7695	366.0
	$S_0 \rightarrow S_2$: 3.951	0.0430	313.8
	$S_0 \rightarrow S_3$: 4.220	0.0401	293.8
	$S_0 \rightarrow S_4$: 4.271	0.0395	290.3
	$S_0 \rightarrow S_6$: 4.521	0.1596	274.2

	$S_0 \rightarrow S_8$: 4.713	0.1128	263.1
	$S_0 \rightarrow S_{12}$: 4.958	0.1027	250.1
PZL-ST-H$^+$	$S_0 \rightarrow S_1$: 3.074	0.3941	403.3
	$S_0 \rightarrow S_2$: 3.712	0.3105	334.0
	$S_0 \rightarrow S_3$: 3.948	0.1387	314.0
	$S_0 \rightarrow S_9$: 4.890	0.1221	253.5
	$S_0 \rightarrow S_{11}$: 5.143	0.1042	241.1

Table S2. De-excitation energy from $S_1 \rightarrow S_0$ of pyrazolines and protonated pyrazolines.

Molecule	Emission ($S_1 \rightarrow S_0$, eV)	Oscillator strength	Wavelength (nm)
PZL-AC	2.355	0.6876	526.5
PZL-AC-H$^+$	0.230	0.0005	N/A
PZL-HE	2.312	0.7332	536.2
PZL-HE-H$^+$	0.434	0.0046	N/A
PZL-ST	2.348	0.7242	528.0
PZL-ST-H$^+$	0.063	0.0001	N/A

IV. References

1. Li, Y., Gao, L.-X., and Han, F.-S. (2012) Efficient synthesis of 2,5-disubstituted tetrazoles via the Cu_2O-catalyzed aerobic oxidative direct cross-coupling of N-H free tetrazoles with boronic acids. *Chem. Commun. 48*, 2719-2721.
2. Yu, Z., Pan, Y., Wang, Z., Wang, J., and Lin, Q. (2012) Genetically encoded cyclopropene directs rapid, photoclick-chemistry-mediated protein labeling in mammalian cells. *Angew. Chem., Int. Ed. 51*, 10600-10604.