A Fluorogenic 1,3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes

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Supporting Information

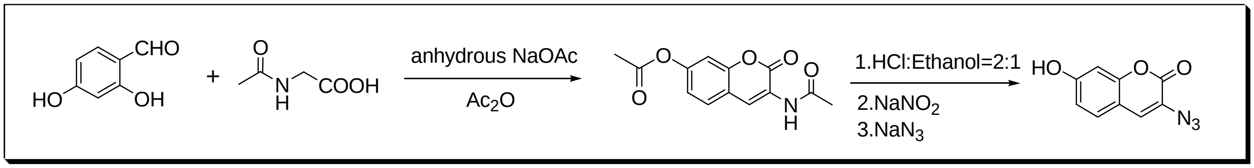
Materials and Reagents. Chemicals used in the syntheses were purchased from Aldrich and Acros without further purification. Melting points were uncorrected. NMR spectra were recorded with a Varian Mercury/VX 300 spectrometer and the δ values are in ppm vs $SiMe_4$ (0 ppm, ^1H, 300MHz). IR spectra were recorded with a Shimadzu FTIR Spectrophotometer. Mass spectra were recorded using Micromass Q-TOF I mass spectrometer. Fluorescence emission and excitation spectra were recorded using a JASCO FP-6500 Spectrofluorometer. Quantum yields were determined in THF using 9,10-diphenylanthracene as the fluorescence standard. The path length was 1 cm with a cell volume of 3.0 mL.

Synthesis of Starting Materials:

3-Azidochromen-2-one (or 3-azidocoumarin) (A).

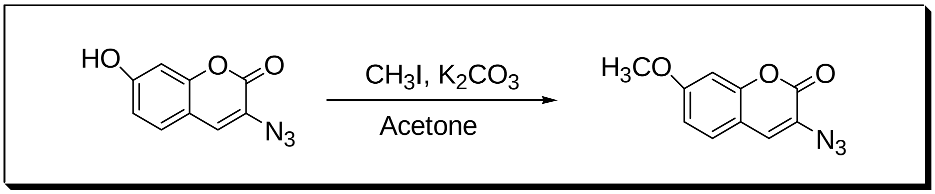
The synthesis followed the literature procedure [1, 2]; mp = 108 - 112°C (lit.[2] 117 - 118°C). ^1H NMR (DMSO-d_6, 300 MHz) δ 7.34 - 7.66 (m, 5 H).

3-Azido-7-hydroxy-chromen-2-one (3-Azido-7-hydroxycoumarin) (B).



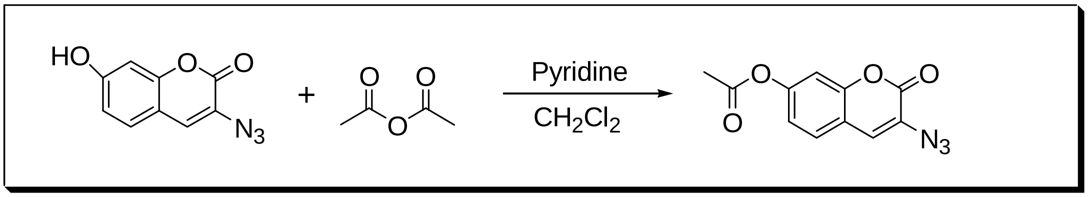
A mixture of 2,4-dihydroxy benzaldehyde (2.76 g, 20 mmol), *N*-acetylglycine (2.34 g, 20 mmol), anhydrous sodium acetate (60 mmol) in acetic anhydride (100 ml) was refluxed under stirring for 4 h. The reaction mixture was poured onto ice to give a yellow precipitate. After filtration, the yellow solid was washed by ice water before it was refluxed in a solution of conc. HCl and ethanol (2:1, 30 mL) for 1 hour, then ice water (40 mL) was added to dilute the solution. The solution was then cooled in an ice bath and $NaNO_2$ (40 mmol) was added. The mixture was stirred for 5-10 minutes and NaN_3 (60 mmol) was added in portions. After stirring for another 15 minutes, the resulting precipitate was filtered off, washed with water, and dried under reduced pressure to afford a brown solid; 2.2 g (54% overall yield). The product was pure enough for further reactions. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.74 (d, *J* = 2.2 Hz, 1 H), 6.79 (dd, *J* = 8.4, 2.2 Hz, 1 H), 7.47 (d, *J* = 8.5 Hz, 1 H), 7.56 (s, 1 H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 161.0, 158.0, 153.4, 128.7, 128.5, 121.8, 114.5, 112.0, 102.7. IR (KBr): 3296 (s), 2125 (vs), 1680 (s), 1620 (m), 1321 (s). EI-HRMS *m/e* calculated for M^+ $C_9H_5N_3O_3$ 203.0331; found 203.0326.

3-Azido-7-methoxychromen-2-one (3-Azido-7-Methoxycoumarin) (C).



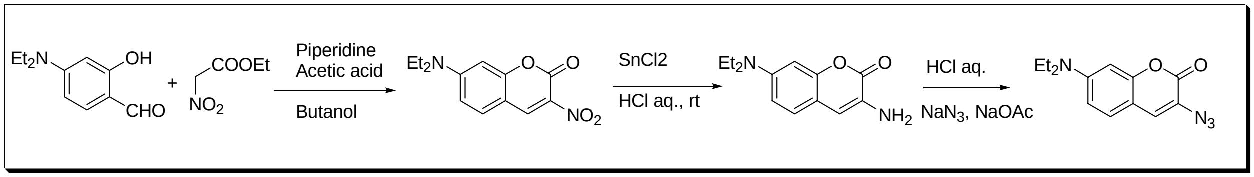
In dry acetone (20 mL), 3-azido-7-hydroxycoumarin (**B**) (203 mg, 1.0 mmol), anhydrous K_2CO_3 (250 mg), CH_3I (0.13 mL, 200 mg, 1.4 mmol) was refluxed overnight. After filtration, acetone was removed using a rotary evaporator, and the residue was dissolved with ethyl acetate 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure providing the product as a yellow solid; 185 mg, 81%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 3.81 (s, 3 H), 6.95 (dd, J = 9.0, 2.7 Hz, 1 H), 7.03 (d, J = 2.7, 1 H), 7.56 (d, J = 9.5, 1 H), 7.62 (s, 1 H). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 56.6, 101.3, 113.2, 113.7, 122.8, 128.0, 129.5, 153.3, 157.9, 162.2. IR (KBr): 3406 (vs), 2125 (vs), 1616 (s), 1155 (m). EI-HRMS *m/e* calculated for M^+ $C_{10}H_7N_3O_3$ 217.0487; found 217.0469.

Acetic acid 3-azido-2-oxo-2H-chromen-7-yl ester (7-Acetic-3-azidocoumarin) (D).



To a stirred suspension of 3-azido-7-hydroxycoumarin (**B**) (203 mg, 1.0 mmol) in dry CH_2Cl_2 (15 mL), acetic anhydride (1.5 mmol) and pyridine (one drop) were added. Upon stirring overnight at room temperature, the solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and washed with H_2O. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. Purification with column chromatography over silica gel (hexane-EtOAc 4:1) afforded the final product as a yellow solid (210 mg, 86%). ^{1}H-NMR (DMSO-d$_6$, 300 MHz) δ 2.29 (s, 3 H), 7.17 (dd, *J* = 8.0, 2.0 Hz, 1 H), 7.32 (d, *J* = 2.1 Hz, 1 H), 7.67 (s, 1 H), 7.70 (d, *J* = 7.9 Hz, 1 H). ^{13}C NMR (DMSO-d$_6$, 75 MHz) δ 21.5, 110.6, 117.8, 119.9, 125.8, 126.8, 129.2, 151.9, 152.3, 157.5, 169.6. IR (KBr): 2133(vs), 1749, 1730(vs), 1622(s), 1234(s). EI-HRMS *m/e* calculated for M$^+$ $C_{11}H_7N_3O_4$ 245.0437; found 245.0432.

3-Azido-7-diethylaminochromen-2-one (3-azido-7-diethylaminocoumarin) (E).

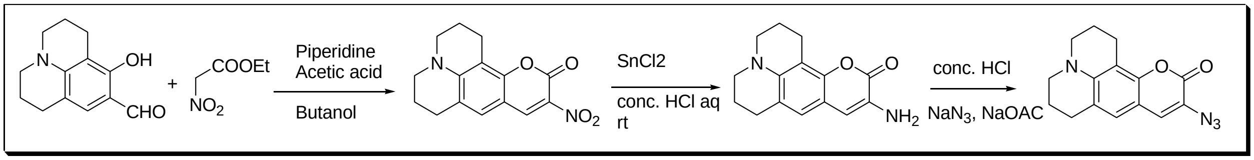


A mixture containing n-butanol (20 mL), 4-diethylamino salicylaldehyde (1.4 g, 7.2 mmol), ethyl nitroacetate (0.8 mL, 7.2 mmol), molecular sieves 4 Å (100 mg), piperidine (0.1 mL) and acetic acid (0.2 mL) was refluxed for a period of 24 h. Upon cooling to room temperature, a bright yellow solid formed, which was collected and dissolved in DMF (15 mL) at 80 °C. It was filtered again to remove the molecular sieves. The filtrate, upon addition to 100 ml of ice-cold water, yielded 3-nitro-7-diethylamino coumarin as a bright yellow solid: 1.40 g, 73 %. A small amount of this compound was recrystallized from DMF to give an analytical sample; mp 193-195 °C. ^{1}H NMR (CDCl$_3$, 300 MHz) δ1.27 (t, *J* = 7.14 Hz, 6 H), 3.48 (q, *J* = 7.86 Hz, 4 H), 6.50 (s, 1 H), 6.70 (d, *J* = 6.71 Hz, 1 H), 7.42 (d, *J* = 7.42 Hz, 2 H), 8.72 (s, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{13}H_{14}N_2O_4$ 263.1041; found 263.1032.

In a 25 mL round bottomed flask equipped with a magnetic stirrer, were placed in order, 37.4% HCl (5 mL), stannous chloride dihydrate (1.6 g, 7.12 mmol). To this suspension 3-nitro-7-diethylamino coumarin (0.25 g, 0.95 mmol) was added at room temperature in small portions, over a period of thirty minutes. Stirring was continued for 4 h before the solution was poured onto 20 g of ice and made alkaline using sodium hydroxide solution (5 M) at 15 °C using an ice-water bath. The resulting suspension was then extracted with diethyl ether (2 X 25 mL). The organic layer was washed with water (50 mL), dried over anhydrous sodium sulfate and concentrated to a pasty residue which upon triturating using hexane yielded 3-amino-7-diethylaminocoumarin as a pale yellow solid: 0.15 g, 68%. A small amount of this compound was recrystallized in ethyl acetate / hexane to give an analytical sample; mp 85-87 °C. IR (KBr): 3323, 3398 (m), 1697 (m), 1589 (m), 1517(m). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.27 (t, *J* = 7.17 Hz, 6 H), 3.48 (q, *J* = 7.22 Hz, 4 H), 3.85 (s, 2 H), 6.6 (m, 2 H), 6.7 (s, 1 H), 7.10 (d, *J* = 8.65 Hz, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{13}H_{16}N_2O_2$ 233.1282; found 233.1290.

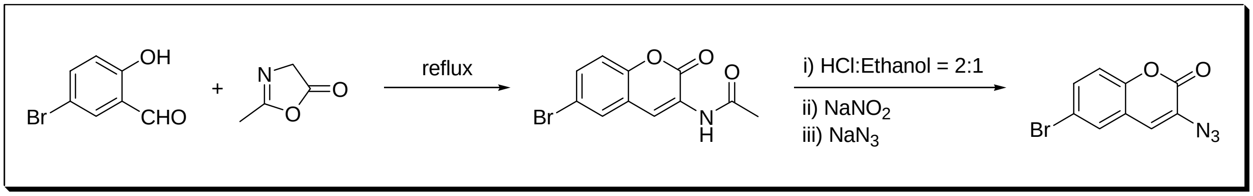
7-Diethylamino-3-amino coumarin (100 mg, 0.43 mmol) was dissolved slowly in HCl aq. (17.2%, 4 mL) at room temperature. Upon cooling to 0-5 °C and addition of a solution of $NaNO_2$ (30 mg, 0.43 mmol), the reaction mixture was stirred for 1 hour at 0-5 °C. This was followed by the addition of potassium acetate (2 g) in water (5 mL) to adjust the pH of the resulting solution to 4. Sodium azide (57 mg, 0.88 mmol) was added in portions at 0-5 °C, the mixture stirred at 0-5 °C for another five hours. The precipitated product was rapidly filtered, washed with ice-cold water (10 mL) and dried under vacuum to yield the final product as a yellow solid: 84 mg, 80%. The product was stored at -20 °C. IR (KBr): 2113 (vs), 1710 (s), 1625 (m), 1512 (m); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.26 (t, *J* = 7.25 Hz, 6 H), 3.48 (q, *J* = 7.24 Hz, 4 H), 6.50 (s, 1 H), 6.70 (d, *J* = 8.04 Hz, 1 H), 7.10 (s, 1 H), 7.20 (d, *J* = 9.07 Hz, 1 H); EI-MS for $C_{13}H_{14}N_4O_2$ *m/z* (MH^+): 259.

9-Azido-2,3,5,6-tetrahydro-1H,4H-11-oxa-3a-aza-benzo[de]anthracen-10-one (F). [3]



10-Amino-2,3,6,7-tetrahydro-11-oxo-1H-benzo[b]pyrano-[6,7,8-*i,j*] isoquinolizine (200 mg, 0.78 mmol) was dissolved slowly in 2 ml of 17.2 % HCl at room temperature. After cooling to 0-5 °C, a solution of $NaNO_2$ (64 mg, 0.93 mmol) was added drop-wise to the mixture, which was stirred for one hour at 0-5 °C. This was followed by dilution of the reaction using 25 ml of ice cold water and stirring continued for thirty minutes at 0-5 °C. Maintaining the same temperature, sodium azide (130 mg, 0.88 mmol) was added in portions and the mixture stirred for fifteen minutes. The pH of the resulting solution was adjusted to 4 by the addition of saturated potassium acetate solution in water and the stirring continued another five hours. The precipitated product was rapidly filtered, washed with 20 ml of ice-cold water and dried under vacuum to yield 180 mg of the product as a greenish yellow solid (82% yield). The product was stored at -20 °C. IR (KBr): 2113 (vs), 1701 (s), 1625 (m), 1512 (m); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.84 - 1.89 (m, 4 H), 2.64 - 2.70 (m, 4 H), 3.23 (m, 4 H), 6.96 (s, 1 H), 7.39 (s, 1 H); EI MS for $C_{15}H_{14}N_4O_2$ *m/z* (MH^+): 283.

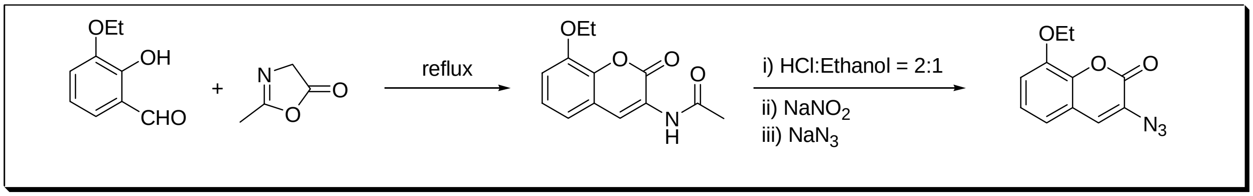
3-Azido-6-bromo-chromen-2-one (3-Azido-6-bromocoumarin) (G).



Benzenesulfonyl chloride (4.4 g, 25 mmol) was added into a solution of *N*-acetylglycine (2.93 g, 25 mmol) and triethylamine (6.3 g, 62.5 mmol) in dry benzene (125 ml). The mixture was stirred at room temperature for 4 h before the triethylamine hydrochloride salt was removed by filtration. To the filtrate, 5-bromosalicylaldehyde (5.0 g, 25 mmol) was added. After refluxing for 2 h, the reaction mixture was cooled to room temperature and the solvents were removed under reduced pressure. The residue was then washed with hot ethanol (100 mL) and the light yellow precipitation was collected as 3-acetamido-6-bromocoumarin. More product could be recovered from the ethanol solution. The overall yield was 3.5 g (52%).

3-Acetamido-6-bromocoumarin (1 g, 3.7 mmol) was dissolved in a mixture of concentrated HCl aq. and ethanol (v/v = 2:1, 18 mL) and refluxed for 2 h. After cooling the mixture to room temperature, ice water (10 mL) was poured into the reaction system, which was stirred in an ice bath until the interior temperature was lower than 5 °C. Sodium nitrite (621 mg, 9 mmol) was added in portions during 10 min and stirred vigorously for 5 min before NaN_3 (720 mg, 11 mmol) was added. The reaction mixture was then stirred in ice bath for one hour at 0 – 5 °C and 24 h at room temperature. The crude product thus obtained was collected by filtration and purified on silica gel (hexane-EtOAc= 4:1) to yield the product as white solid; 320 mg, 33%. IR (KBr): 2133 (vs), 1693 (vs), 1413 (m), 1355 (m). ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 7.10 (s, 1 H), 7.21 (d, *J* = 9.5 Hz, 1 H), 7.54 (m, 3 H). EI-HRMS *m/e* calculated for (M^+) $C_9H_4BrN_3O_2$ 264.9487; found 264.9489.

3-Azido-8-ethoxy-chromen-2-one (H).



The synthesis is analogous to **G** with 6-ethoxysalicylaldehyde as the starting materials. The product was obtained as a light yellow solid; 120 mg, 20%. IR (KBr): 3413 (vs), 2125 (vs), 1616 (s), 1467 (m), 1336 (m). ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 1.40 (t, *J* = 6.9 Hz, 3 H), 4.17 (q, *J* = 7.0 Hz, 2 H), 7.23 (m, 3 H), 7.64 (s, 1 H). ^{13}C NMR (DMSO-d$_6$, 75 MHz) δ 15.3, 65.0, 114.5, 119.7, 120.7, 125.8, 126.4, 127.4, 140.9, 146.3, 157.4. EI-HRMS *m/e* calculated for M+ $C_{11}H_9N_3O_3$ 231.0644; found 231.0640.

4-Azidocoumarin (I).

The synthesis followed the literature procedure [4,5]; mp = 159 – 161 °C (lit.[6] 164 - 165°C). ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 7.26 - 7.74 (m, 4 H), 6.18 (s, 1 H).

4-(3-Ethynyl-phenylcarbamoyl)-butyric acid (23). Glutaric anhydride (215 mg, 1.9 mmol) was dissolved in anhydrous dichloromethane (7 mL) and followed by the addition of 3-ethynylaniline (200 mg, 1.7 mmol). The mixture was allowed to stir at room temperature for 6 hours before the solvent was evaporated under reduced pressure to provide the product as a white solid (363 mg, 99%); mp 150-154 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.77 (p, J = 7.77 Hz, 2 H), 2.25 (t, J = 8.10 Hz, 2 H), 2.33 (t, J = 7.13 Hz, 2 H), 4.15 (s, 1H), 7.12 (d, J=7.60 Hz, 1 H), 7.25 (t, J = 7.60 Hz, 1 H), 7.51 (d, J = 8.29 Hz, 1 H), 7.76 (s, 1 H), 9.96 (s, 1 H); HRMS *m/e* calculated for $(M+H)^+$ $C_{13}H_{13}NO_3$ 231.0895; found 231.0895.

4-(4-Ethynyl-phenylcarbamoyl)-butyric acid (24). Glutaric anhydride (540 mg, 4.70 mmol) was dissolved in anhydrous dichloromethane (7 mL) and followed with the addition of *p*-aminophenyl-acetylene (500 mg, 4.27 mmol). The mixture was allowed to stir at room temperature for 6 hours before the solvent was evaporated under reduced pressure to provide 900 mg of a yellow white product **24** (96%); mp 135-140 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.77 (p, J = 8.21 Hz, 2 H), 2.25 (t, J = 9.30 Hz, 2 H), 2.34 (t, J = 6.56 Hz, 2 H), 4.05 (s, 1 H), 7.38 (d, J = 8.52 Hz, 2 H), 7.59 (d, J = 7.91 Hz, 2 H) 9.90 (s, 1 H); HRMS *m/e* calculated for $(M+H)^+$: $C_{13}H_{13}NO_3$ 231.0895; found 231.0895.

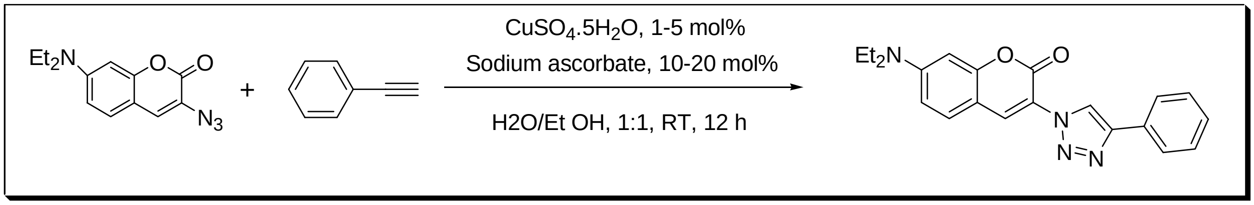
Typical Experimental Procedure of Well-plate Screening Reaction:

A 96-well microtier plate was used in the experiments. The overall volume in each well was 200 µL containing a solution of coumarin azide (1 mM), alkyne (1 mM), $CuSO_4$ (16 mM) and sodium ascorbate (16 mM) in DMSO/H_2O (1:1). The reactions could be monitored with long wavelength UV irradiation conveniently (Figure 1). The plate was incubated at room temperature for 12 h before diluted to 0.01 mM in DMSO for fluorescent emission measurement. For 7-diethylaminocoumarin and Julolidine derived click products, the reactions were performed at 4 °C using 80:20 ethanol and water as the reaction medium. The excitation and emission spectra were recorded for 1 µM concentration after dilution using DMF. The results are listed in Table S1.

Table S1. Fluorescent data of coumarin click products.

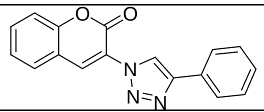
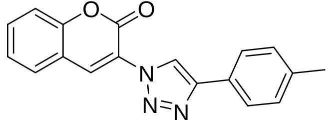
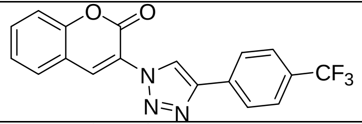
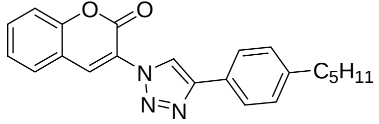
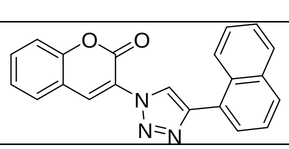
Code	**λex (nm)**	**λem (nm)**	**Code**	**λex (nm)**	**λem (nm)**	**Code**	**λex (nm)**	**λem (nm)**	**Code**	**λex (nm)**	**λem (nm)**
A1	340	478	**B1**	441	494	**C1**	350	430	**D1**	443	495
A2	341	507	**B2**	441	495	**C2**	353	443	**D2**	440	494
A3	346	405	**B3**	444	495	**C3**	350	426	**D3**	442	495
A4	0	0	**B4**	445	495	**C4**	0	0	**D4**	440	495
A5	0	0	**B5**	442	497	**C5**	0	0	**D5**	444	497
A6	342	406	**B6**	442	494	**C6**	350	425	**D6**	444	495
A7	341	504	**B7**	443	495	**C7**	353	444	**D7**	441	495
A8	342	515	**B8**	444	496	**C8**	353	473	**D8**	441	495
A9	353	473	**B9**	441	495	**C9**	352	429	**D9**	443	495
A10	338	485	**B10**	443	494	**C10**	351	430	**D10**	440	494
A11	0	0	**B11**	444	495	**C11**	351	426	**D11**	440	494
A12	348	403	**B12**	444	494	**C12**	351	425	**D12**	441	494
A13	342	425	**B13**	442	494	**C13**	350	426	**D13**	444	494
A14	0	0	**B14**	444	495	**C14**	350	426	**D14**	441	494
A15	0	0	**B15**	444	495	**C15**	351	427	**D15**	441	494
A16	343	427	**B16**	445	495	**C16**	351	426	**D16**	442	495
A17	0	0	**B17**	444	495	**C17**	0	0	**D17**	441	495
A18	0	0	**B18**	440	494	**C18**	351	507	**D18**	439	494
A19	0	0	**B19**	444	495	**C19**	351	432	**D19**	441	494
A20	303	388	**B20**	440	494	**C20**	349	420	**D20**	442	494
A21	339	400	**B21**	440	492	**C21**	348	421	**D21**	442	492
A22	348	390	**B22**	440	493	**C22**	350	421	**D22**	442	494
A23	0	0	**B23**	441	495	**C23**	0	0	**D23**	443	496
A24	0	0	**B24**	442	495	**C24**	0	0	**D24**	443	495
E1	417	489	**F1**	435	508	**G1**	348	501	**H1**	307	429
E2	417	489	**F2**	434	508	**G2**	348	521	**H2**	333	497
E3	417	489	**F3**	435	509	**G3**	348	410	**H3**	306	415
E4	418	489	**F4**	435	508	**G4**	347	408	**H4**	310	408
E5	415	488	**F5**	433	507	**G5**	0	0	**H5**	0	0
E6	417	489	**F6**	435	508	**G6**	346	406	**H6**	312	451
E7	417	489	**F7**	435	508	**G7**	349	520	**H7**	310	408
E8	416	489	**F8**	435	508	**G8**	353	518	**H8**	298	512
E9	417	488	**F9**	435	507	**G9**	348	499	**H9**	309	448
E10	416	488	**F10**	433	507	**G10**	346	506	**H10**	310	420
E11	416	489	**F11**	435	507	**G11**	343	405	**H11**	307	414
E12	417	489	**F12**	435	508	**G12**	349	412	**H12**	308	421
E13	416	489	**F13**	434	508	**G13**	346	410	**H13**	307	413
E14	417	489	**F14**	435	509	**G14**	354	395	**H14**	309	414
E15	417	489	**F15**	435	508	**G15**	0	0	**H15**	308	418
E16	418	489	**F16**	436	508	**G16**	0	0	**H16**	307	416
E17	416	488	**F17**	435	507	**G17**	325	380	**H17**	0	0
E18	416	489	**F18**	434	508	**G18**	0	0	**H18**	0	0
E19	417	489	**F19**	435	507	**G19**	353	413	**H19**	307	415
E20	415	488	**F20**	434	507	**G20**	355	397	**H20**	310	409
E21	413	484	**F21**	431	503	**G21**	358	416	**H21**	310	446
E22	415	486	**F22**	433	506	**G22**	359	418	**H22**	309	411
E23	416	488	**F23**	433	507	**G23**	355	397	**H23**	308	412
E24	417	488	**F24**	435	507	**G24**	353	395	**H24**	307	413

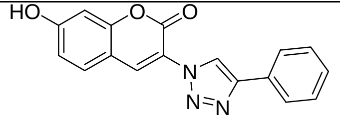
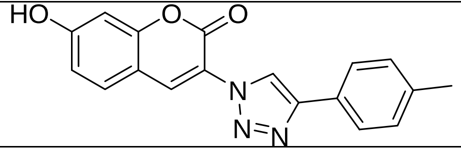
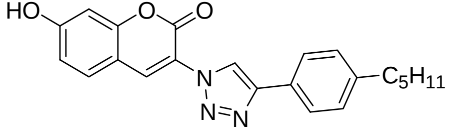
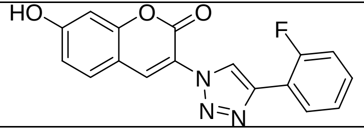
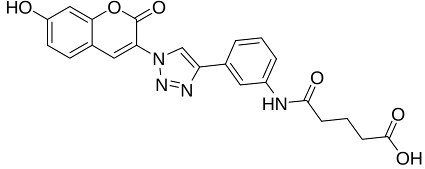
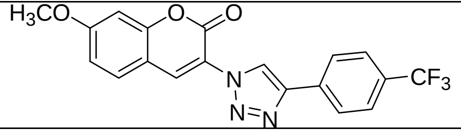
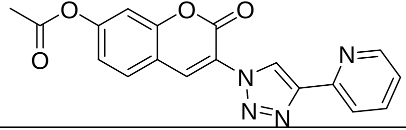
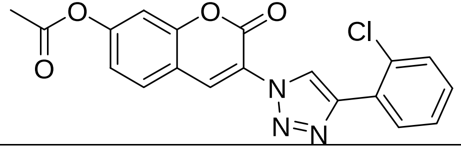
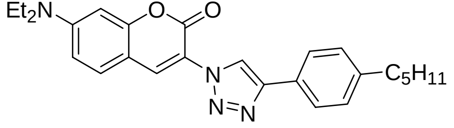
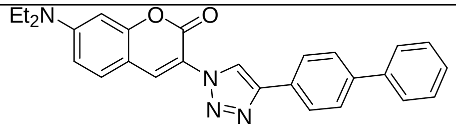
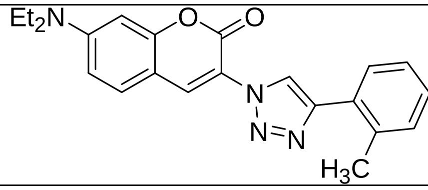
Typical Experimental Procedure of Click Reaction:

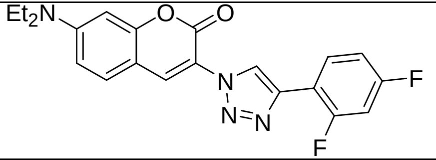
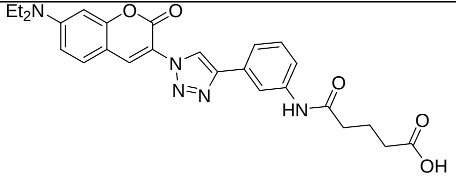
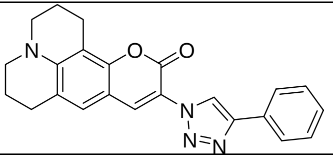
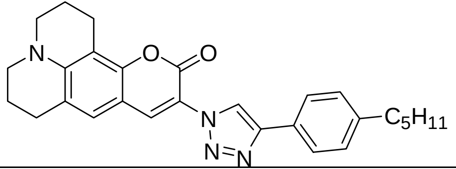
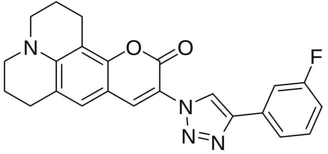
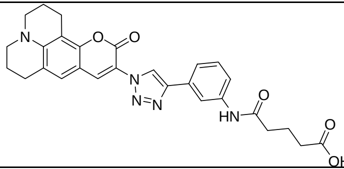
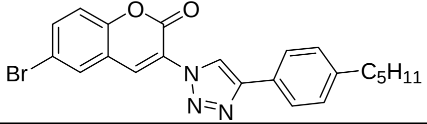
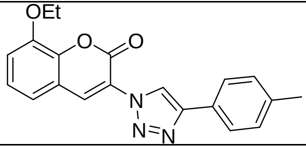
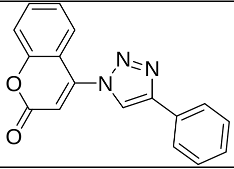
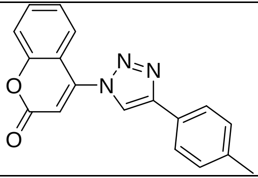


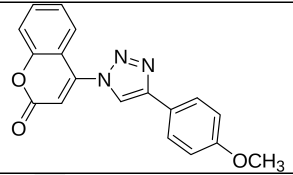
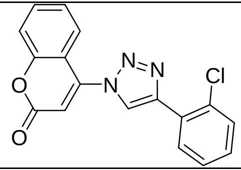
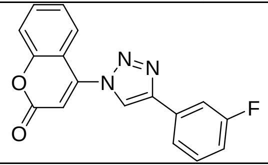
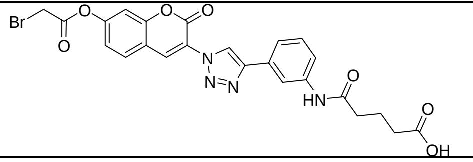
Synthesis of 7-Diethylamino-3-(4-phenyl-[1,2,3]triazol-1-yl)-chromen-2-one (E1). In a mixture of phenylacetylene (18 mg, 0.17 mmol) and 7-diethylamino-3-azidocoumarin (45 mg, 0.17 mmol) in water and ethyl alcohol (v/v = 1:1, 5 mL), sodium ascorbate (34 μL, 0.034 mmol) of freshly prepared 1 M solution in water was added, followed by the addition of copper (II) sulfate pentahydrate 7.5 % in water (28 μL, 0.0085 mmol). The heterogeneous mixture was stirred vigorously overnight in the dark at room temperature. TLC analysis indicated complete consumption of the reactants in 12 h. The ethanol was removed and the residue was diluted with water (5 mL), cooled in ice, and then the precipitate was collected by filtration. After washing the precipitate with cold water (10 ml), it was dried under vacuum to afford 53 mg of pure product as a yellow powder (84%); mp 242 – 244 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, J = 6.94 Hz, 6 H), 3.48 (q, J = 7.13 Hz, 4 H), 6.50 (s, 1 H), 6.70 (d, J = 8.93 Hz, 1 H), 7.4 - 7.6 (m, 4 H), 7.9 (d, J = 6.29 Hz, 1 H), 8.5 (s, 1 H), 8.8 (s, 1 H). HRMS *m/e* calculated for $(M+H)^+$ $C_{21}H_{20}N_4O_2$ 361.1663; found 361.1664.

Analytical Data of Triazolylcoumarin Products:

Code	Structure	Analysis
A1		Mp = 199 – 201 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.3 - 7.8 (m, 9 H), 8.8 (s, 1 H), 9.2 (s, 1 H); HRMS *m/e* calculated for MH^+ $C_{17}H_{11}N_3O_2$ 290.0922, found 290.0930.
A2		Mp 220 - 222 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.39 (s, 3 H), 7.31 (d, J = 8.88 Hz, 2 H), 7.54 (m, 2 H), 7.78 (t, 1 H), 7.91 (d, J = 10.15 Hz, 2 H), 7.98 (d, J = 6.34 Hz, 1 H), 8.73 (s, 1 H), 8.93 (s, 1 H); HRMS *m/e* calculated for M^+ $C_{18}H_{13}N_3O_2$ 303.1001, found 304.1008.
A3		Mp > 250 °C; ^{1}H NMR ($CDCl_3$, 300 MHz,) δ 7.44 - 7.74 (m, 6 H), 8.07 (d, J = 8.05 Hz, 2 H), 8.70 (s, 1 H), 9.03 (s, 1 H); HRMS *m/e* calculated for MH^+ $C_{10}H_{10}F_3N_3O_2$ 358.0809, found 358.0803.
A7		Mp = 186 - 188°C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, J = 6.89, 3 H), 1.33 (m, 4 H), 1.56 (m, 2 H), 2.64 (t, J = 7.80 Hz, 2 H), 7.30 - 7.47 (m, 3 H), 7.69 (m, 3 H), 7.82 (d, J = 8.21, 2 H), 8.67 (s, 1 H), 8.88 (s, 1 H). HRMS *m/e* calculated for MH^+ $C_{22}H_{21}N_3O_2$ 360.1711, found 360.1712.
A18		Mp = 180 - 185 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.61 - 7.98 (m, 11 H), 8.81 (s, 1 H), 9.10 (s, 1 H); EI-HRMS *m/e* calculated for M^+ $C_{21}H_{13}N_3O_2$ 340.1086, found 340.1098.

B1		Mp > 250 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 6.87 (m, 6.89, 2 H), 7.38 (m, 1 H), 7.44 (t, *J* = 6.77 Hz, 2 H), 7.52 (d, *J* = 8.27 Hz, 1 H), 8.63 (s, 1 H), 8.98 (s, 1 H). HRMS *m/e* calculated for M$^+$ $C_{17}H_{11}N_3O_3$ 306.0878, found 376.0892.
B2		Mp > 250 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 2.33 (s, 3 H), 6.89 (m, 2 H), 7.28 (d, *J* = 9.75 Hz, 2 H), 7.75 (d, *J* = 7.80 Hz, 1 H), 7.82 (d, *J* = 5.85 Hz, 2 H), 8.63 (s, 1 H), 8.92 (s, 1 H), 10.93 (s, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{18}H_{13}N_3O_3$ 320.1035, found 320.1044.
B7		Mp 209 - 211 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 0.85 (t, *J* = 5.11 Hz, 3 H), 1.26 (m, 4 H), 1.56 (p, *J* = 7.15 Hz, 2 H), 2.59 (t, *J* = 8.17 Hz, 2 H), 6.92 (m, 2 H), 7.29 (d, *J* = 6.54 Hz, 2 H), 7.76 (d, *J* = 10.46 Hz, 1 H), 7.83 (d, *J* = 7.85 Hz, 3 H), 8.63 (s, 1 H), 8.92 (s, 1 H). EI-HRMS *m/e* calculated for M$^+$ $C_{22}H_{21}N_3O_3$ 376.1661, found 376.1664.
B11		Mp > 250 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 7.39 (m, 5 H), 8.18 (t, *J* = 7.94 Hz, 2 H), 8.65 (s, 1 H), 8.81 (d, *J* = 3.49 Hz, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{17}H_{10}FN_3O_3$ 324.0784, found 324.0783.
B23		Mp 212 - 218 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 1.82 (p, *J* = 8.37 Hz, 2 H), 2.26 (t, *J* = 7.53 Hz, 2 H), 2.34 (t, *J* = 6.90 Hz, 2 H), 6.92 (m, 2 H), 7.38 (t, *J* = 7.44 Hz, 1 H), 7.57 (t, *J* = 8.68 Hz, 2 H), 7.64 (d, *J* = 8.68 Hz, 1 H), 8.21 (s, 1 H), 8.65 (s, 1 H), 8.92 (s, 1 H), 10.0 (s, 1 H), 10.91 (s, 1 H); EI-HRMS *m/e* calculated for MS$^+$ $C_{22}H_{18}N_4O_6$ 435.1304, found 435.1299.
C3		^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 3.90 (s, 3 H), 7.09(dd, *J* = 8.9, 2.8 Hz, 1 H), 7.19 (d, *J* = 2.2 Hz, 1 H), 7.85 (m, 3 H), 8.19 (d, *J* = 8.4 Hz, 2H), 8.74 (s, 1 H), 9.22 (s, 1 H).
D6		Mp > 250 °C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 2.32 (s, 3 H), 7.28 (d, *J* = 6.87 Hz, 2 H), 7.46 (s, 2 H), 8.00 (d, *J* = 8.80 Hz, 3 H), 8.87 (s, 2 H). HRMS *m/e* calculated for MH$^+$ $C_{18}H_{12}N_4O_4$ 349.0937, found 349.0937.
D13		Mp decomp. at 205 °C; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.31 (s, 1 H), 7.28 (d, *J* = 7.8 Hz, 2 H), 7.47 (m, 3 H), 7.61 (d, *J* = 7.97 Hz, 2 H), 8.12 (d, *J* = 8.44, 1 H), 8.78 (s, 1 H), 9.09 (s, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{18}H_{12}ClN_3O_4$ 382.0594, found 382.0591.
E7		Mp = 173 -175 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 0.90 (t, *J* = 6.27 Hz, 3 H), 1.22 (t, *J* = 7.33 Hz, 6 H), 1.32 (m, 4 H), 1.55 (m, 2 H), 2.64 (t, *J* = 8.02 Hz, 2 H), 3.47 (q, *J* = 7.46 Hz, 4 H), 6.70 (d, *J* = 6.7 Hz, 1 H), 6.56 (d, *J* = 6.56, 1 H), 7.41 (d, *J* = 9.25 Hz, 2 H), 7.81 (d, *J* = 8.38 Hz, 3 H), 8.45 (s, 1 H), 8.78 (s, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{26}H_{30}N_4O_2$ 431.2451, found 431.2447
E8		Mp = 203 – 205 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.25 (t, *J* = 7.25 Hz, 6 H), 3.48 (q, *J* = 7.21 Hz, 4 H), 6.57 (s, 1 H), 6.70 (d, *J* = 6.66 Hz, 1 H), 7.36 - 7.48 (m, 4 H), 7.64 - 7.71 (m, 4 H), 8.01 (d, *J* = 8.27 Hz, 1 H), 8.48 (s, 1 H), 8.86 (s, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{27}H_{24}N_4O_2$ 437.1981, found 437.1977.
E10		Mp = 165 – 167 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.25 (t, *J* = 6.93 Hz, 6 H), 2.54 (s, 3 H), 3.47 (q, *J* = 7.26 Hz, 4 H), 6.68 (s, 1 H), 6.71 (m, 1 H), 7.29 - 7.40 (m, 5 H), 7.80 (m, 1 H), 8.46 (s, 1 H), 8.50 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{22}H_{22}N_4O_2$ 375.1824, found 375.1821.

E14		Mp = 204 – 206 °C; ^{1}H NMR (CDCl$_3$, 300 MHz): δ 1.25 (t, *J* = 7.38 Hz, 6 H), 3.47 (q, *J* = 7.15 Hz, 4 H), 6.57 (s, 1 H), 6.70 (m, *J* = 6.58 Hz, 1 H), 6.71 - 7.01 (m, 2 H), 7.33 - 7.44 (m, 3 H), 8.30 (m, 1 H), 8.28 (s, 1 H) 8.85 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{21}H_{18}N_4O_2F_2$ 397.1482, found 397.1476.
E23		Mp 223 - 225 ^{o}C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 1.56 (t, *J* = 7.66 Hz, 6 H), 2.81 (p, *J* = 8.36 Hz, 2 H), 2.30 (t, *J* = 7.16 Hz, 2 H), 2.39 (t, *J* = 8.36 Hz, 2 H), 3.47 (q, *J* = 7.91 Hz, 4 H), 6.68 (s, 1 H), 6.84 (d, *J* = 7.79 Hz, 1 H), 7.38 (m, 1 H), 7.61 (m, 2 H), 8.12 (s, 1 H), 8.54 (s, 1 H), 8.87 (s, 1 H), 10.0 (s, 1 H), 12.1 (s, 1 H); EI-HRMS *m/e* calculated for MS$^+$ $C_{26}H_{27}N_5O_5$ 490.2090, found 490.2095.
F1		mp > 250 °C; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.97 - 2.03 (m, 4 H), 2.80 - 2.96 (m, 4 H), 3.35 (m, 4 H), 7.02 (s, 1 H), 7.34 - 7.51 (m, 3 H), 7.91 (d, *J* = 8.16 Hz, 2 H), 8.37 (s, 1 H), 8.82 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{23}H_{20}N_4O_2$ 385.1660, found 385.1664.
F7		Mp = 158 – 160 °C; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.85 (t, *J* = 7.25 Hz, 3 H), 1.26 - 1.90 (m, 8 H), 1.94 (m, 2 H), 2.57 - 2.89 (m, 6 H), 3.28 (m, 4 H), 7.07 (s, 1 H), 7.20 (d, 2 H), 7.78 (d, *J* = 8.16 Hz, 2 H), 8.30 (s, 1 H), 8.72 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{28}H_{30}N_4O_2$ 455.2447, found 455.2447.
F15		Mp = 203 -205 °C; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.97 - 2.03 (m, 4 H), 2.80 - 2.96 (m, 4 H), 2.39 (s, 3 H),3.35 (m, 4 H), 7.01 (s, 1 H), 7.25 - 7.33 (m, 2 H), 7.82 (d, *J* = 8.17 Hz, 2 H), 8.36 (s, 1 H), 8.77 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{23}H_{19}FN_4O_2$ 403.1574, found 403.1570.
F23		Mp 177 - 178 ^{o}C; ^{1}H NMR (DMSO-d$_6$, 300 MHz) δ 1.87 (m, 7 H), 2.23 - 2.41 (m, 5 H), 2.76 (m, 5 H), 7.28 (s, 1 H), 7.38 (t, *J* = 5.7 Hz, 1 H), 7.59 (m, 3 H), 8.18 (s, 1 H), 8.20 (s, 1 H), 8.84 (s, 1 H), 10.0 (s, 1 H); EI-HRMS *m/e* calculated for M$^+$ $C_{28}H_{27}N_5O_5$ 514.2090, found 514.2076.
G7		^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.88 (t, *J* = 6.7 Hz, 3 H),1.32 (m, 4 H), 1.64 (m, 2 H), 2.63 (t, *J* = 7.6 Hz, 2 H), 7.26 (d, *J* = 8.0 Hz, 2 H), 7.33 (d, *J* = 9.0 Hz, 1 H), 7.71 (dd, *J* = 8.7, 2.3 Hz, 1 H), 7.82 (m, 3 H), 8.59 (s, 1 H), 8.89 (s, 1 H).
H2		^{1}H NMR (CDCl$_3$, 300 MHz) δ 2.38 (s, 1 H), 4.18 (q, *J* = 7.3 Hz, 2 H), 4.23 (t, *J* = 7.0 Hz, 3 H), 7.24 (m, 5 H), 7.80 (d, *J* = 8.2 Hz, 2 H), 8.62 (s, 1 H), 8.89 (s, 1 H).
I1		Mp = 166 – 168 °C; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.6 (s, 1 H), 7.4 - 8.0 (m, 9 H), 8.2 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{17}H_{11}N_3O_2$ 290.0934, found 290.0930.
I2		Mp = 181 - 183 °C; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 2.40 (s, 3 H), 7.29 - 7.83 (m, 8 H), 8.66 (s, 1 H), 8.88 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{18}H_{13}N_3O_2$ 304.1090, found 304.1086.

I4		Mp = 183 – 185 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.87 (s, 3 H), 6.61 (s, 1 H), 7.00 (d, *J* = 8.58 Hz, 2 H), 7.30 - 7.50 (m, 2 H), 7.84 (d, *J* = 8.83 Hz, 1 H), 7.86 (m, 1 H), 7.90 (d, *J* = 8.8 Hz, 2 H), 8.11 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{18}H_{13}N_3O_3$ 320.1041, found 320.1035.
I13		Mp = 156 - 158 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.67 (s, 1 H), 7.30 - 7.70 (m, 6 H), 8.00 (d, *J* = 6.71 Hz, 1 H), 8.35 (d, *J* = 6.03 Hz, 1 H), 8.66 (s, 1 H). HRMS *m/e* calculated for MH$^+$ $C_{17}H_{10}ClN_3O_2$ 324.0547, found 324.0540.
I15		Mp = 177 – 179 °C; ^{1}H NMR (DMSO-d_6, 300 MHz,) δ 6.99 (S, 1 H), 7.24 (m, 1 H), 7.40 - 7.60 (m, 3 H), 7.75 - 7.89 (m, 3 H), 7.94 (d, *J* = 7.21 Hz, 1 H), 9.35 (s, 1 H); HRMS *m/e* calculated for MH$^+$ $C_{17}H_{10}FN_3O_2$ 308.0843, found 308.0835
J23		Mp decomp. at 180 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.18 (p, *J* = 7.87 Hz, 2 H), 2.06 (s, 2 H), 2.30 (t, *J* = 8.99 Hz, 2 H), 2.37 (t, *J* = 5.62 Hz, 2 H), 7.17 - 7.72 (m, 5 H), 8.02 (d, *J* = 7.62 Hz, 1 H), 8.24 (s, 1 H), 8.80 (s, 1 H), 8.98 (s, 1 H), 10.0 (s, 1 H), 12.1 (s, 1 H). EI-HRMS *m/e* calculated for M$^+$ $C_{24}H_{19}BrN_4O_6$ 555.0515, found 555.0499.

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[3] Chaurasia, C. S.; Kauffman, J. M.; *J. Heterocyclic Chem.* **1990**, *27,* 727 - 733.

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