Electronic Supplementary Information

Click-on Fluorescent Triazolyl Coumarin Peptidomimetics as Inhibitors of Human Breast Cancer Cell line MCF-7

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[1] Experimental Section

Procedure for the synthesis of 4-Bromomethyl umbeliferone BMU: Placed a solution of 4-methylumbeliferone (176 mg, 10 mmol) in 20 ml glacial acetic acid in a 500 ml flask. 1.598 g (0.4982 ml, 10 mmol) of bromine in 10 ml glacial acetic acid was added to the flask very slowly (about 30 minutes) from a dropping funnel. The mixture was stirred vigorously during the addition of bromine and kept the temperature at 0-5°C. When the addition was completed, it was allowed to stand at room temperature for 30 minutes. Then the mixture was poured into 400 ml water and stirred well. The precipitate was filtered with suction, washed with cold water. Recrystallised from dilute ethanol to afford reasonably pure BMU (white powder). 4-(bromomethyl)-7-hydroxy-2H-chromen-2-one. FT-IR (KBr) ν\text{max} \(1698,1620,1600,1548,1511,1441,1375,1352,1263,1232,1204,1141,1075,1000,945,848,836,814,750,739,725,\) 692, 633,618,556,516,499,492,471,458,453,444,434,429,421,413,406,401 cm\(^{-1}\); \(^1\)H-NMR: 2.490 (s, 2H), 6.972-6.994 (d, 2H), 7.682-7.703 (d, 2H), 8.232 (s, 1H) ppm; HRMS (EI) calcd for C\(_{10}\)H\(_7\)BrO\(_3\) [M+H]\(^+\): 254.9579, found: [M+H]\(^+\): 255.0365.

Procedure for the synthesis of 4-Azidomethyl umbeliferone AMU: 4-Bromomethyl umbeliferone (254 mg, 1 mmol), K\(_2\)CO\(_3\) (414 mg, 3 mmol) and sodium azide (65 mg, 1 mmol) were stirred at room temperature in DMF. After completion of the reaction, the reaction mixture was poured into ice cold water. The solid product was filtered and dried under vacuum to afford the 4-AMU in pure form (Brown powder). 4-(azidomethyl)-7-hydroxy-2H-chromen-2-one. FT-IR (KBr) ν\text{max} \(3392,2920,2851,2122,1701,1608, 1550,1507,1445,1377,1340,1315,1261,1201,1161,1146,1105,810,754,725,631,580,552,529,456 cm\(^{-1}\); \(^1\)H-NMR: 2.142 (2, 2H), 6.669 (s, 1H), 6. 732-6. 762 (d, 1H), 6.820-6.841 (d, 1H), 7.361-7.383 (d, 1H), 7.668-7.690 (d, 1H), 8.229 (s, 1H) ppm; \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 167.01,163.01,156.67,154.65,129.31,127.95,125.01,113.65,112.75,102.13,56.30,40.12,39.91,39.70,39.08, 38.07 ppm; HRMS (EI) calcd for C\(_{10}\)H\(_7\)N\(_3\)O\(_3\) [M]\(^+\):217.0487 , found: [M]\(^+\): 217.0118.
General procedure for the synthesis of type 1 alkynes 1-3: Hydroxycoumarin (1 mmol), K$_2$CO$_3$ (414 mg, 3 mmol) in DMF were heated to 50 °C for half an hour. After cooling, propargyl bromide (0.119 ml, 1 mmol) was added and stirred for 4 hours. The resulting solution is poured into ice water. The precipitate formed was filtered and washed with water to afford pure propargylated coumarin.

Procedure for the synthesis of type 1 alkyne 4: A solution of Coumarin-3-carboxylic acid (1.9 g, 0.01 mol), Propargyl alcohol (0.56 ml, 0.01 mol), N,N-dicyclohexyl carbodiimide (2.3 g, 0.11 mol), and 4-dimethylaminopyridine (0.122 g, 0.001 mol) in dichloromethane was stirred at room temperature for 6 h. After 6 h, the precipitated N,N-dicyclohexylurea was filtered off and the filtrate was washed with water, 5% acetic acid, and again with water. It was then dried over magnesiuim sulphate and the solvent was evaporated. The residue obtained was washed with petroleum ether to afford the ester prop-2-yn-1-yl 2-oxo-2'Hchromene-3-carboxylate (2.4 g, 96%).

General procedure for the synthesis of type 2 alkynes 5-10: A round bottom flask is charged with propargyl derivative of hydroxy benzaldehyde (2 mmol), ketone (2 mmol), acetyl chloride (2 mL), acetonitrile (1 mL) and catalytic amount of BF$_3$.Et$_2$O and kept under stirring for 3h at room temperature. The progress of the reaction was monitored by TLC analysis at regular intervals. After 3h, the mixture was quenched with cold water. The solid residue separated was collected and purified by passing it through a silica gel column. Elution of the column with petroleum ether (60-80 °C) / ethyl acetate (9:1 v/v) afforded 5-10.

General procedure for the Cu (I) 1, 3-dipolar cycloaddition reactions to synthesis TC1-4 & ATC1-6: An equimolar amount of 4-Azidomethyl umbeliferone 4-AMU (1 mmol) and the alkynes 1-10 (1 mmol) are dissolved in minimum amount of DMSO. To this, 2 ml of t-BuOH, 1 ml of water, CuSO$_4$.5H$_2$O (200 mg) and sodium ascorbate (150 mg) are added and stirred at room temperature for 12 h, and then poured in to cold water. The precipitated click product was filtered, washed with water and dried under vacuum to afford TC1-4 & ATC1-6 in pure form.
1. 7-hydroxy-4-(((2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-2H-chromen-2-one (TC1- white powder).
   FT-IR, KBr, $\nu_{\text{max}}$: 3439,2922,2852,1715,1619,1572,1509,1467,1389,1349,1326,1295,1225,1206,1151,1063,1019,984,941, 844, 822,765,686 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 2.297-2.321 (d, 3H), 4.022-4.070 (q, 2H), 5.023 (s, 1H), 5.464-5.468 (d, 2H), 5.998 (s, 2H), 6.165 (s, 1H), 7.314-7.438 (m, 3H), 7.554 (s, 1H), 7.570-80121 (m, 4H) ppm; HRMS (EI) calcd for C$_{23}$H$_{17}$N$_3$O$_6$ [M+H]$^+$: 432.1117 , found: [M+H]$^+$: 432.1188.

2. 7-hydroxy-4-(((4-methyl-2-oxo-2H-chromen-7-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-2H-chromen-2-one (TC2- white powder).
   FT-IR, KBr, $\nu_{\text{max}}$: 3422,3165,2923,2853,1726,1685,1615,1573,1557,1508,1466,1402,1354,1322,1283,1228,1159, 1135, 1089,1045,1016,949,906,836,802,765,703,656,620,566,534,507,458 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 4.693-4.622 (q, 1H), 5.238 (s, 2H),5.559 (s,1H), 6.502-6.876 (d, 1H), 7.090-7.354 (m, 8H), 7.963 (s, 1H) ppm; HRMS (EI) calcd for C$_{23}$H$_{17}$N$_3$O$_6$ [M+H]$^+$: 418.0961 , found: [M+H]$^+$: 418.1016.

3. 7-hydroxy-4-(((2-oxo-2H-chromen-4-yl)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-2H-chromen-2-one (TC3- white powder).
   FT-IR, KBr, $\nu_{\text{max}}$: 3421,2919,2850,1728,1710,1610,1563,1509,1493,1449,1425,1387,1354,1332,1273,1244, 1197,1156, 1128,1107, 1085,1047,947,882,764,747,655 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$4.2660-4.3096 (q, 2H), 5.3050 (s,1H), 5.7121-5.7860 (d, 3H), 6.3132 (s, 2H), 6.7602-6.7765 (d, 2H), 7.3075-7.4262 (m, 5H) ppm; $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 168.20,160.80,160.27,156.05,141.43,135.53,135.07,131.75,130.04,127.80,127.47,127.27,114.52,114.33,87.47,66.48,60.66,48.33,45.3 3,40.12,39.31,33.24,33.07,30.87,30.66ppm; HRMS (EI) calcd for C$_{22}$H$_{15}$N$_3$O$_6$[M+H]$^+$: 418.0961 , found: [M+H]$^+$: 418.1029.
4. (1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl 2-oxo-2H-chromene-3-carboxylate (TC4- white powder). FT-IR, KBr, $\nu_{\text{max}}$: 3431,2925,2852,1717,1693,1648,1617,1576,1555,1456,1401,1369,1289,1258,1233,1181,1033,923,882, 779,759,644,595,464,418cm$^{-1}$; HRMS (EI) calcd for C$_{23}$H$_{15}$N$_{3}$O$_{7}$[M+H]$^+$: 446.0910 , found: [M+H]$^+$: 446.1774.

5. N-(1-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-oxo-3-phenylpropyl)acetamide (ATC1- white powder). FT-IR, KBr, $\nu_{\text{max}}$: 3414,2923,2851,1725,1648,1589,1489,1455,1400,1376,1292,1240,1121,1091,1024,818, 754,620,529,470,321,243,198,141,132,1240,1160,1122,1088,1016,949,817,754,691,504cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.517 (s, 3H), 3.271-3.278 (d, 1H), 3.296-3.313 (d, 1H), 4.617-4.653 (q, 2H), 5.229 (s, 1H), 5.485-5.564 (d, 3H), 6.576-6.617 (q, 1H), 6.870 (s, 1H), 7.087-7.368 (m, 11H), 7.998 (s, 1H),8.248 (s, 1H) ppm;HRMS (EI) calcd for C$_{30}$H$_{26}$N$_{4}$O$_{6}$[M+H]$^+$: 539.1852 , found: [M+H]$^+$: 539.2055.

6. N-(3-(4-bromophenyl)-1-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-oxopropyl)acetamide (ATC2- white powder). FT-IR, KBr, $\nu_{\text{max}}$: 3420,2922,1731,1648,1600,1556,1490,1450,1375,1358,1320, 1240,1160,1122,1088,1016,949,817,754,691,504cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.502 (s, 3H), 2.297 -2.321 (d, 2H), 4.014-4.074 (m, 2H), 5.023 (s,2H), 5.468-5.613 (t, 1H), 6.147 (s, 1H), 7.299-8.121 (m, 13 H),10.074 (s, 1H), ppm;HRMS (EI) calcd forC$_{30}$H$_{25}$BrN$_{4}$O$_{6}$[M+H]$^+$: 617.0957 , found: [M+H]$^+$: 617.1911.

7. N-(1-(2-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy) phenyl)-3-oxo-3-phenylpropyl)acetamide (ATC3- white powder). FT-IR, KBr, $\nu_{\text{max}}$: 3423,2922,2852,1728,1655,1599,1508,1466,1419,1383,1323,1248,1171,1125, 1088, 1019,832,763,725,550,502,466cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.772 (s, 3H), 2.791-2.864 (d, 2H), 4.795-4.794 (d, 2H), 4.836-4.864 (d,1H), 5.875 (s, 1H), 5.891 (s, 1H), 7.225-7.313 (m, 6H), 7.706-7.930 (m, 9H), 10.059 (s, 1H) ppm;HRMS (EI) calcd forC$_{30}$H$_{26}$N$_{4}$O$_{6}$[M+H]$^+$: 539.1852 , found: [M+H]$^+$: 539.1747.
8. N-(3-(4-chlorophenyl)-1-(2-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-oxopropyl)acetamide (ATC4 - white powder). FT-IR, KBr, $\nu_{\text{max}}$ : 3385, 2983, 1700, 1647, 1579, 1591, 1445, 1351, 1301, 1223, 1097, 1040, 923, 808, 759, 735, 689, 458, 421 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.6795-1.7685 (m, 3H), 2.4337-2.5221 (t, 2H), 4.4094-4.4133 (d, 2H), 5.2202 (s, 1H), 5.3973 (s, 1H), 5.4245 (s, 1H), 6.6648-6.9757 (m, 10 H), 7.3284-7.5221 (m, 2H), 7.9360 (s, 1H), 8.3214 (s, 1H) ppm; $^{13}$C NMR (100 MHz, DMSO-d$_6$): $\delta$ 194.01, 194.00, 168.43, 168.73, 161.04, 142.20, 132.62, 130.31, 129.92, 129.83, 127.62, 127.74, 122.22, 114.74, 76.68, 55.68, 48.73, 48.01, 39.50, 39.07, 34.06, 22.52 ppm; HRMS (EI) calcd for C$_{30}$H$_{25}$ClN$_4$O$_6$ [M]+: 572.1463, found: [M]+: 572.1482.

9. N-(3-(4-chlorophenyl)-1-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-oxopropyl)acetamide (ATC5 - white powder). FT-IR, KBr, $\nu_{\text{max}}$ : 3433, 2923, 1787, 1765, 1650, 1596, 1557, 1529, 1500, 1449, 1421, 1349, 1325, 1272, 1230, 1158, 1000, 886, 860, 809, 776, 700, 623, 542 cm$^{-1}$; $^1$H NMR (400 MHz, DMSO-d$_6$): $\delta$ 1.6057 (s, 3H), 3.3075-3.2340 (d, 2H), 4.2811-4.2951 (d, 2H), 5.1848 (s, 1H), 5.3050-5.3096 (d, 2H), 5.5231 (s, 1H), 6.7602-6.7765 (d, 1H), 7.2837-7.3146 (m, 2H), 7.4336-7.6278 (m, 10 H), 8.5555 (s, 1H) ppm; HRMS (EI) calcd for C$_{30}$H$_{25}$ClN$_4$O$_6$ [M+H]+: 573.1463, found: [M+H]+: 573.1219.

10. N-(1-(2-bromophenyl)-3-(4-((1-((7-hydroxy-2-oxo-2H-chromen-4-yl)methyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-3-oxopropyl)acetamide (ATC6 - white powder). FT-IR, KBr, $\nu_{\text{max}}$ : 3410, 2923, 1757, 1687, 1490, 1446, 1408, 1359, 1325, 1272, 1230, 1180, 1157, 1091, 1014, 952, 833, 754, 727, 616, 585, 508, 486 cm$^{-1}$; HRMS (EI) calcd for C$_{30}$H$_{25}$BrN$_4$O$_6$ [M+H]+: 617.0957, found: [M+H]+: 617.0110.
Mass Spectra of compounds

[Image: Mass spectrum of a compound with molecular formula BMU. Peaks at m/z 233.0808, 215.0703, 199.1188, 177.1007, 255.0365, and 299.9865.]

TC2
[3] Infrared Spectra of compounds
[4] $^1$H NMR spectra of compounds
[5] $^{13}$C NMR Spectra of compounds

4-AMU
**Table S1**: Drug like properties of click products TC1-4 & ATC1-6

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Cytotoxicity evaluation results of TC1 against Human Breast Cancer Cell line MCF-7

The MCF-7 cells were maintained in RPMI medium 1640 supplemented with 10% fetal bovine serum as well as 100 µg/mL streptomycin, 100 U/mL penicillin, 2 mM L-glutamine and Earle’s BSS adjusted to contain 1.5 g/L Na bicarbonate, 0.1 mM nonessential amino acids, and 1.0 mM of Na pyruvate in a humidified atmosphere containing 5% CO₂ at 37 °C. The MCF-7 cells in the log phase were seeded in 96-well plates at a concentration of 1.0 x 10^4 cells/well and incubated overnight at 37 °C in 5%CO₂ humidified environment. The cells were then treated with different concentrations of the TC1 such as 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µM/mL (dissolved with RPMI medium 1640), respectively. Controls were also cultivated under same conditions without the addition of TC1. The treated cells were incubated for 48 h and then subjected for MTT assay.

![Figure S1](image)

Figure S1: MTT assay results confirming the in vitro cytotoxicity effect of TC1 against the MCF-7 cells. The detected IC50 concentration was 30 µM/mL.

The stock concentration (5 mg/mL) of MTT-(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was prepared and 100 µL of MTT was added in each TC1 treated wells and incubated for 4h. Purple colourformazan crystals were observed and these crystals were dissolved in 100 µL of dimethyl sulphoxide (DMSO), and read at 620 nm in a multiwell ELISA plate reader (Thermo, Multiskan). The dose dependent cytotoxicity was observed in TC1 treated MCF-7 cells. Fifty percentage of cell death, which determines the inhibitory concentration (IC50) value of TC1 against MCF-7 cells holds at 30 µM in 48 h (figure S1).

In order to study the morphological changes, MCF-7 cells were grown (1 x 10^5 cells/cover slip) and incubated with TC1 at its IC50 concentration (30 µM/mL) and then they were fixed in a mixture of methanol and acetic acid (3:1, v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Morphological changes of MCF-7 cells were analyzed under a Nikon (Japan) bright field inverted light microscope at 40x magnification. The most recognizable morphological changes of TC1 treated cells observed in this study were the cytoplasmic condensation, cell shrinkage, production of numerous cell surface protuberances at the plasma membrane blebbing and hyper condensed chromatin as shown in treated MCF-7 cells.
Followed by this, DAPI (4,6-diamidino-2-phenylindole, dihydrochloride) staining was carried out. For this, the MCF-7 cells incubated with TC1 at its IC50 concentration (30 µM/mL) for 48 h and then they were fixed in a mixture of methanol and acetic acid (3:1, v/v) prior to washing with PBS. The washed cells were then stained with 1 mg/mL DAPI (4,6-diamidino-2-phenylindole, dihydrochloride) for 20 min. in a dark atmosphere. The stained images were recorded using a fluorescent microscope with appropriate excitation filter. The bright field and fluorescence microscopic images are shown in Figure S2. As shown in Figure S2, the strong bluish fluorescence and cellular uptake observed in the imaging studies with TC1 reveals that these molecules have high potency against breast cancer cell lines (MCF-7).

Figure S2. Bright field inverted light microscopy images (a) (Control), (b) (IC25) and (c) (IC50) and fluorescence microscopy images (d) (Control), (e) (IC25) and (f) (IC50) of TC1 treated MCF-7 cells.