# Multicolor GLUT5-Permeable Fluorescent Probes for Fructose Transport Analysis

V. V. Begoyan,<sup>a</sup> Ł. J. Weseliński,<sup>a</sup> S. Xia,<sup>a</sup> J. Fedie,<sup>a</sup> S. Kannan,<sup>b</sup> A. Ferrier,<sup>a</sup> S. Rao,<sup>b</sup> and M. Tanasova<sup>a\*</sup>

<sup>a</sup> Michigan Technological University, Department of Chemistry, 1400 Townsend Dr, Houghton, Michigan 49931, United States

<sup>b</sup> Michigan Technological University, Department of Biomedical Engineering, 1400 Townsend Dr, Houghton, Michigan 49931, United States

\* mtanasov@mtu.edu

## SUPPORTING INFORMATION

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## 1. Supplementary Tables and Figures

ManCou	Absorbance, <sup>a</sup> λ <sub>max</sub> , nm	Fluorescence, <sup>a</sup> λ <sub>max</sub> , nm	Stock Shift, nm	Absolute Quantum Yield, <sup>b</sup> ΦF
1	366	452	86	0.26
2	360	461	101	0.30
3	387	508	121	0.1
4	376	506	130	0.44
5	398	558	160	0.09
6	371	538	167	0.21
7	365	460	95	0.37
8	384	459	75	0.08
9	430	481	51	0.27
10	382	564	182	0.06
11	385	549	164	0.22
12	383	554	171	0.12
13 (2-furyl)	368	531	163	0.29
14 (2-pyridyl)	374	535	161	0.21

Table S1. Spectroscopic properties of ManCous.

<sup>a</sup>All data measured in water/ethanol (70:30 v/v) mixture. <sup>b</sup>Absolute quantum yield was derived with respect to the anthracene as fluorescence standard.

**Table S2.** Comparative analysis of UV  $\lambda_{max}$  and HOMO/LUMO contribution for selected C4-substituted Cous and ManCous.<sup>a</sup>

ManCou	UV,	HOMO/LUMO <sup>b</sup>	$\Delta_{ HOMO/LUMO }$	Cou	UV,	HOMO/LUMO	$\Delta_{ HOMO/LUMO }$		
	λ <sub>max</sub> ,				λ <sub>max</sub> ,				
	nm				nm				
1	366	-445/-186	259	1	352	-459/-190	269		
2	360	-440/-177	263	2	347	-453/-180	273		
3	387	-468/-232	236	3	372	-486/-253	233		
4	376	-441/-193	248	4	358	-453/-196	257		
5	398	-467/-217	250	5	377	-482/-223	259		
13	374	-438/-209	219	13	366	-449/-213	236		
<sup>a</sup> Data provided for probes showing the uptake through GLUT5; <sup>b</sup> HOMO/LUMO orbital energies were calculated with Spartan '14 V1.1.2 (Wavefunction Inc.) using Density Functional, BLYP, 6-									

31G\*



**Figure S1.** ManCous uptake analysis. a) Concentration-dependent uptake of ManCous at 37 °C; b) Comparative uptake of ManCous vs. corresponding coumarins (Cou) at 20  $\mu$ M concentration (37 °C); c) Z-stack imaged of MCF7 cells treated with 7-aminocoumarin (Cou1) at 37 °C; d) Comparative analysis of ManCou1 vs. Cou1 uptake at varied concentrations (37 °C); e) Comparative analysis of ManCou1 uptake at 37 °C vs. 4 °C. All uptake data are measured in triplicates in 96-well plate after 10 min incubation of cells, removal of the probe and repeated cell wash. Data represents the Gained Fluorescence (exc. 385 nm) measured for 20  $\mu$ M solutions of probes. Confocal images obtained with 60X objective using exc/em 405 nm/461 nm.



**Figure S2.** Analysis of ManCou uptake in the presence of fructose (a and b); glucose (c); glucosamine (d); cytochalasin B (e); and culture media (f). All data obtained in triplicates. Data collected for 20 µM ManCou probe. Plots represents average data, error bars represent standart deviation. Data are collected after 10 min incubation of cells with ManCou in 96-well plates, removal of the probe, and cell wash (exc. 360 nm, em. 430 nm for ManCous 1 and 2, and em. 500 nm for ManCou3 and 4).



**Figure S3.** Blight-field and fluorescence overlay images of ManCou-treated cells: A) Images of HepG2 and MCF7 cells treated with 20 µm ManCou1 (40X objective); B) Z-stack images of HepG2 cells treated with ManCou1 and ManCou3 (20X objective). Images taken with EVOS optical microscope, exc/em 405 nm/461 nm.



**Figure S4.** Fluorescence confocal Z-stack images of MCF7 cells treated with ManCous and established stains. a) ManCou1 with DID membrane staining; b) ManCou3 with RedDot nuclear staining; c) ManCou4 with RedDot nuclear staining. ManCous imaged under exc/em 405 nm/425-525 nm; RedDot and DID imaged under exc/em 635/ 655-755 nm. Images taken with 60X objective.



**Figure S5.** GLUT5-ManCou uptake analysis. A-D) Binding affinity. Data derived after fitting the concentration-dependent change in fluorescence into the ligand binding (one site) curve (SigmaPlot 13). The Kd values are calculated from the f = a/(1+exp(-(x-x0)/b)). E) Kinetic analysis of uptake.



Figure S6. ManCous as competitors for NBDM and NBDG uptake in MCF7 cells (Confocal, Z-stack images). a) equimolar NBDM and ManCou3; b) equimolar NBDM and ManCou1; c) Equimolar ManCou1 and NBDG; d) Equimolar ManCou3 and NBDG; e) NBDM; f) NBDG. Z-stack images taken after 10 min treatment and cell wash. Cells imaged with 60X objective using exc/em. 405 nm/425-525 nm for ManCous, and exc/em 450 nm/565 nm for NBDM and NBDG (eGFP, green fluorescence). All images obtained at the same laser intensity and exposure time.

### 2. Materials and Methods

All reagents were used as received unless otherwise stated from Sigma-Aldrich, TCI America, Alfa Aesar, Ark Pharm, AK Scientific, Combi-Blocks or Chem-Impex International. Commercially available coumarins were purchased from: 7-amino-4-methylcoumarin (**Cou2**), Sigma-Aldrich; 7-amino-4-(trifluoromethyl)coumarin (**Cou3**), Alfa Aesar; 2-(7-amino-2-oxo-2*H*-chromen-4-yl)acetic acid (**Cou7**), Ark Pharm; 6-amino-2*H*-chromen-2-one (**Cou8**), Enamine; 7-(diethylamino)-2-oxo-2*H*-chromene-3-carboxylic acid (**Cou9**), Combi-Blocks. *N*,*N*-Dimethylformamide (DMF) was dried and stored over CaH<sub>2</sub> before use. Dry tetrahydrofuran was dispensed from an automated Innovative Technology Pure-Solv 400 Solvent Purification System. Analytical TLC was carried out on commercial SiliCycle SiliaPlate® 0.2 mm F254 plates. Preparative silica chromatography was performed using SiliCycle SiliaFlash® F60 40-63 μm (230-400 mesh). Final purification of compounds was achieved with Agilent-1200 HPLC (high-pressure liquid chromatography) using reversed phase semi-preparative column (Phenomenex® Luna® 10 μm C18(2) 100 Å, LC Column 100 x 10 mm, Ea). <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at room temperature with a Varian

Unity Inova 400 MHz spectrometer. CD<sub>3</sub>OD, DMSO-d<sub>6</sub>, and D<sub>2</sub>O were used as solvents and referenced to the corresponding residual solvent peaks (3.31 and 49.0 ppm for CD<sub>3</sub>OD, respectively; 2.50 and 39.52 ppm for DMSO-d<sub>6</sub>, respectively; 4.79 ppm for D<sub>2</sub>O).<sup>1</sup> The following abbreviations are used to indicate the multiplicity: s - singlet; d - doublet; t - triplet; q - quartet; m - multiplet; b broad signal; app - approximate. The coupling constants are expressed in Hertz (Hz). The highresolution (HR) MS data (ESI) were obtained using a Thermo Fisher Orbitrap Elite<sup>™</sup> Hybrid Ion Trap-Orbitrap Mass Spectrometer at Chemical Advanced Resolution Methods (ChARM) Laboratory at Michigan Technological University. UV-vis spectra were recorded on a Cary 100 Bio-spectrophotometer from Agilent Technologies. Fluorescence spectra were obtained with FluoroMax-4 spectrophotometer. 96-well plate analysis of cell fluorescence was carried out with Victor3 fluorescence plate reader (excitation at 385 nm). Confocal images were taken with Olympus FluoViewTM FV1000 using the FluoView software. Fluorescence imaging was done with EVOS FLAuto inverted microscope.

RPMI-1640, Penicillin/Streptomycin, FBS (Fetal Bovine Serum), 25% Trypsin-EDTA (1X), and PBS (phosphate buffered saline solution) were purchased from Life Technologies, USA. MEM Non-Essential Amino Acids 100X were purchased from Quality Biological, USA. Sterile DMSO (25-950-CQC, 250mL) was purchased from Sigma. MCF7 and Hep G2 cells were purchased from ATCC, USA and cultured according to the suggested growth methods.

### 3. Synthesis of ManCous 1-14



Scheme S1: Synthesis of 2,5-anhydro-2-carbaldehyde-D-mannitol 2.

(2S,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-carbaldehyde (2):<sup>2</sup> D-Glucosamine hydrochloride 1 (4.00 g, 18.5 mmol) was dissolved in water (100 ml) and stirred at room temperature for 5 h. Sodium nitrite (3.19 g, 45.3 mmol) was then added, followed by cautious addition of Amberlite 120 H<sup>+</sup> resin (90 g) by portion, maintaining the temperature of ice bath for 4 h. After the reaction, the resin was removed by filtration and the solution was then neutralized by adding sodium carbonate. The remaining solution was vacuum dried and then methanol was added to the residue to precipitate the inorganic salts. After removing the salts by filtration, the solution was vacuum dried to get the compound **2** as a yellow sticky solid (2.49 g, 70%) that was used directly without further purification.



Scheme S2: Synthesis of ManCous1-14.

**General procedure for the synthesis of ManCous1-8, 10-14**:<sup>3</sup> Typically, (2S,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-carbaldehyde<sup>2</sup> (up to 1 mmol) and the corresponding coumarin (0.8 equiv.) were dissolved in methanol (10 ml). AcOH (1 ml) was used to adjust the pH to <6, followed by portionwise addition of NaBH<sub>3</sub>CN to the reaction mixture (3 X 0.8 equiv, every 20-30 minutes). Water could also be added to improve solubility of some coumarin substrates (up to 20% v/v). The solutions were stirred at room temperature for up to 24 h. The mixtures were then concentrated to dryness under reduced pressure and purified by column chromatography on silica gel using CH<sub>2</sub>Cl<sub>2</sub>: MeOH (up to 90 : 10), EtOAc : MeOH (up to 80 : 20) or water : isopropanol : EtOAc (1 : 2 : 7 up to 2 : 4 : 4) mixtures. The final purification was achieved by semi-preparative HPLC using water-acetonitrile gradient starting with 2-20% acetonitrile to obtain the final products as yellow solids or semi-solids, 10-30 mg samples. No attempts were made to optimize the yields of the products, which could be estimated as 30-40% average. The composition and purity of the final products was confirmed by HRMS, <sup>1</sup>H NMR, and <sup>13</sup>C NMR.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2Hchromen-2-one (ManCou1**): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ, 7.76-7.36 (d, J = 9.2, 1H), 7.32-7.30 (d, J = 8.4, 1H), 6.68-6.65 (dd,  $J_1 = 2.4$ ,  $J_2 = 8.4$ , 1H), 6.53 (d, J = 2.4, 1H), 6.01-5.99 (d, J = 9.2, 1H), 4.02-3.98 (m, 2H), 3.95-3.92 (m, 1H), 3.88-3.85 (m, 1H), 3.73-3.69 (app dd,  $J_1 = 3.2$ ,  $J_2 = 12.0$ , 1H), 3.66-3.61 (app dd,  $J_1 = 5.6$ ,  $J_2 = 12.0$ , 1H), 3.48-3.44 (app dd,  $J_1 = 3.6$ ,  $J_2 = 13.6$ , 1H), 3.38-3.32 (app dd,  $J_1 = 6.8$ ,  $J_2 = 13.6$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ, 164.7, 158.1, 154.5, 146.5, 130.2, 112.3, 110.6, 109.1, 98.0, 85.3, 83.2, 80.3, 78.9, 63.3, 46.2 ppm. HRMS (ESI): m/z [M + Na]<sup>+</sup> calcd for C<sub>15</sub>H<sub>17</sub>NNaO<sub>6</sub>: 330.09539; found 330.09434. **7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-4-methyl-2H-chromen-2-one (ManCou2**): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ, 7.48-7.45 (dd,  $J_1$  = 3.2,  $J_2$  = 8.8, 1H), 6.71-6.67 (dt,  $J_1$  = 2.4,  $J_2$  = 8.8, 1H), 6.52-6.51 (t, J = 2.4, 1H), 5.93 (m, 1H), 4.02-3.98 (m, 2H), 3.96-3.93 (m, 1H), 3.88-3.85 (m, 1H), 3.73-3.69 (app dd,  $J_1$  = 3.6,  $J_2$  = 11.6, 1H), 3.66-3.61 (app dd,  $J_1$  = 5.6,  $J_2$  = 12.0, 1H), 3.48-3.43 (app dd,  $J_1$  = 3.6,  $J_2$  = 13.6, 1H), 3.37-3.32 (app dd,  $J_1$  = 6.8,  $J_2$  = 13.6, 1H), 2.38 (d, J = 0.8, 3H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ, 164.7, 157.3, 156.5, 154.4, 126.9, 112.1, 111.1, 108.6, 98.2, 85.3, 83.2, 80.3, 78.9, 63.3, 46.2, 18.5 ppm. HRMS (ESI): m/z [M + Na]<sup>+</sup> calcd for C<sub>16</sub>H<sub>19</sub>NNaO<sub>6</sub>: 344.1104; found 344.11043.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-4-(tri-fluoromethyl)-2H-chromen-2-one (ManCou3**): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ, 7.44-7.41 (dd,  $J_1 = 2.0$ ,  $J_2 = 9.2$ , 1H), 6.74-6.71 (dt,  $J_1 = 2.4$ ,  $J_2 = 9.2$ , 1H), 6.60-6.59 (d, J = 2.4, 1H), 6.37 (s, 1H), 4.02-3.98 (m, 2H), 3.95-3.92 (m, 1H), 3.89-3.85 (m, 1H), 3.73-3.69 (app dd,  $J_1 = 3.6$ ,  $J_2 = 11.6$ , 1H), 3.66-3.61 (app dd,  $J_1 = 5.6$ ,  $J_2 = 12.0$ , 1H), 3.50-3.46 (app dd,  $J_1 = 3.6$ ,  $J_2 = 14.0$ , 1H), 3.40-3.35 (app dd,  $J_1 = 6.4$ ,  $J_2 = 13.6$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ, 162.2, 158.6, 155.0, 143.3, 143.0, 124.8, 122.1, 112.8, 108.3, 104.1, 98.5, 85.4, 83.2, 80.2, 78.8, 63.3, 46.1 ppm. HRMS (ESI): m/z [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>17</sub>F<sub>3</sub>NO<sub>6</sub>: 376.10082; found 376.09955.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-4-phenyl-2H-chromen-2-one (ManCou4**): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): *δ*, 7.50-7.48 (m, 3H), 7.42-7.39 (m, 2H), 7.15-7.13 (d, J = 8.8, 1H), 6.59-6.56 (m, 2H), 5.90 (s, 1H), 4.03-3.99 (m, 2H), 3.97-3.94 (m, 1H), 3.90-3.86 (m, 1H), 3.74-3.70 (app dd,  $J_1 = 3.6$ ,  $J_2 = 11.6$ , 1H), 3.66-3.61 (app dd,  $J_1 = 5.6$ ,  $J_2 = 12.0$ , 1H), 3.47-3.43 (app dd,  $J_1 = 3.6$ ,  $J_2 = 14.0$ , 1H), 3.37-3.32 (app dd,  $J_1 = 6.8$ ,  $J_2 = 13.6$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): *δ*, 164.2, 158.7, 157.9, 154.2, 137.2, 130.4, 129.7, 129.3, 128.8, 112.0, 109.6, 108.2, 98.4, 85.2, 83.1, 80.2, 78.8, 63.2, 46.2 ppm. HRMS (ESI): m/z [M + Na]<sup>+</sup> calcd for C<sub>21</sub>H<sub>21</sub>NNaO<sub>6</sub>: 406.12669; found 406.12520.

Ethyl 7-((((2*R*,3*S*,4*S*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2oxo-2*H*-chromene-4-carboxylate (ManCou5): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$ , 7.84-7.82 (d, *J* = 8.8, 1H), 6.64-6.61 (dd, *J*<sub>1</sub> = 2.4, *J*<sub>2</sub> = 8.8, 1H), 6.49-6.48 (d, *J* = 2.4, 1H), 6.36 (s, 1H), 4.43-4.38 (q, *J* = 7.2, 2H), 4.03-3.99 (m, 2H), 3.96-3.94 (m, 1H), 3.90-3.86 (m, 1H), 3.74-3.70 (app dd, *J*<sub>1</sub> = 3.6, *J*<sub>2</sub> = 11.6, 1H), 3.66-3.62 (app dd, *J*<sub>1</sub> = 5.6, *J*<sub>2</sub> = 12.0, 1H), 3.47-3.43 (app dd, *J*<sub>1</sub> = 3.6, *J*<sub>2</sub> = 14.0, 1H), 3.38-3.32 (app dd, *J*<sub>1</sub> = 6.8, *J*<sub>2</sub> = 14.0, 1H), 1.42-1.38 (t, *J* = 7.2, 2H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD):  $\delta$ , 165.8, 163.6, 158.3, 154.5, 145.3, 128.6, 112.5, 111.0, 106.9, 98.2, 85.3, 83.1, 80.3, 78.8, 63.30, 63.25, 46.1, 14.4 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>18</sub>H<sub>22</sub>NO<sub>8</sub>: 380.13458; found 380.13345.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2-oxo-2H-chromene-4-carboxylic acid (ManCou6)**: <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ, 7.47-7.45 (d, *J* = 8.8, 1H), 6.73-6.70 (d, *J* = 8.8, 1H), 6.53 (s, 1H), 6.07 (m, 1H), 4.07 (bs, 3H), 3.96-3.93 (m, 1H), 3.80-3.76 (app dd,  $J_1$  = 3.2,  $J_2$  = 12.4, 1H), 3.73-3.68 (app dd,  $J_1$  = 6.0,  $J_2$  = 12.0, 1H), 3.48-3.36 (m, 2H) ppm. <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): *δ*, 172.6, 165.9, 156.4, 155.4, 152.8, 127.7, 112.1, 106.7, 103.4, 97.7, 82.7, 81.0, 78.4, 76.8, 61.4, 44.6 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>18</sub>NO<sub>8</sub>: 352.10328; found 352.10300.

2-(7-((((2*R*,3*S*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2oxo-2*H*-chromen-4-yl)acetic acid (ManCou7): <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$ , 7.48-7.45 (d, *J* = 8.8, 1H), 6.77-6.74 (dd, *J*<sub>1</sub> = 2.4, *J*<sub>2</sub> = 8.8, 1H), 6.59 (d, *J* = 2.4, 1H), 6.06 (s, 1H), 4.10 (bs, 2H), 3.98-3.94 (m, 1H), 3.81-3.65 (m, 5H), 3.54-3.46 (m, 2H) ppm. We were not able to obtain satisfactory <sup>13</sup>C NMR data in D<sub>2</sub>O. HRMS (ESI): *m*/*z* [M + H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>20</sub>NO<sub>8</sub>: 366.11893; found 366.11771.

6-((((2*R*,3*S*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2*H*chromen-2-one (ManCou8): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ, 7.84-7.82 (d, J = 9.2, 1H), 7.13-7.11 (d, J = 8.8, 1H), 6.99-6.96 (dd,  $J_1 = 2.4$ ,  $J_2 = 9.2$ , 1H), 6.80-6.79 (d, J = 2.4, 1H), 6.36-6.33 (d, J = 9.2, 1H), 4.03-3.96 (m, 3H), 3.89-3.85 (m, 1H), 3.74-3.70 (app dd,  $J_1 = 3.6$ ,  $J_2 = 12.0$ , 1H), 3.66-3.62 (app dd,  $J_1 = 5.6$ ,  $J_2 = 11.6$ , 1H), 3.42-3.38 (app dd,  $J_1 = 3.6$ ,  $J_2 = 13.6$ , 1H), 3.30-3.32 (app dd,  $J_1 = 6.4$ ,  $J_2 = 13.6$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ, 163.7, 147.59, 147.56, 146.1, 120.8, 119.9, 118.0, 116.7, 109.4, 85.2, 83.2, 80.4, 78.9, 63.3, 47.3 ppm. HRMS (ESI): m/z [M + H]<sup>+</sup> calcd for C<sub>15</sub>H<sub>18</sub>NO<sub>6</sub>: 308.11344; found 308.11234.

7-(Diethylamino)-N-(((2R,3S,4S,5R)-3,4-dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)-2-oxo-2H-chromene-3-carboxamide (ManCou9): This compound was synthesized according to the reported general procedure.<sup>4</sup> To a solution of 1-amino-2,5-anhydro-D-mannitol<sup>2</sup> (100 mg, 0.61 mmol), 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (120 mg, 0.46 mmol), 1-hydroxybenzotriazole monohydrate (HOBt; 93 mg, 0.69 mmol) and diisopropylethylamine (DIEA; 0.24 ml, 1.4 mmol) in dry DMF (4 ml), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI; 132 mg, 0.69 mmol) was added and the mixture was stirred at room temperature for 12 h. It was diluted with EtOAc (100 ml), washed with brine (3 X 20 ml), and dried with MgSO<sub>4</sub>. After filtration and concentration, the residue was purified by column chromatography on silica gel, eluting with 0-100% EtOAc in hexanes, followed by 20% MeOH in EtOAc. The final purification was achieved by semi-preparative HPLC using water-acetonitrile gradient starting with 50% acetonitrile. A sample of compound was obtained as a yellow semi-solid (~20 mg). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$ , 8.60 (s, 1H), 7.53-7.51 (d, J = 9.2, 1H), 6.81-6.78 (dd,  $J_1 = 2.4$ ,  $J_2 = 9.2$ , 1H), 6.54-6.53 (d, J = 3.2, 1H), 7.55-6.53 (d, J = 3.2, 2H), 7.55-6.53 (d, J = 3.2, 2H), 7.55-6.53 (d, J = 3.2, 2 2.4, 1H), 4.04-3.86 (m, 4H), 3.75-3.62 (m, 4H), 3.54-3.49 (q, J = 7.2, 4H), 1.25-1.21 (t, J = 7.2, 6H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ, 165.4, 163.8, 159.0, 154.4, 149.2, 132.5, 111.6, 109.9, 109.4, 97.2, 85.0, 82.7, 79.8, 78.4, 63.2, 46.0, 42.3, 12.8 ppm. HRMS (ESI): m/z [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>27</sub>N<sub>2</sub>O<sub>7</sub>: 407.18187; found 407.18109.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2-oxo-2H-chromene-4-carboxamide (ManCou10**): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ, 7.52-7.50 (d, J = 8.8, 1H), 6.70-6.68 (dd,  $J_1 = 2.4$ ,  $J_2 = 8.8$ , 1H), 6.57-6.56 (d, J = 2.4, 1H), 6.08 (s, 1H), 4.02-3.98 (m, 2H), 3.95-3.92 (m, 1H), 3.88-3.84 (m, 1H), 3.73-3.69 (app dd,  $J_1 = 3.6$ ,  $J_2 = 12.0$ , 1H), 3.65-3.61 (app dd,  $J_1 = 5.6$ ,  $J_2 = 12.0$ , 1H), 3.49-3.45 (app dd,  $J_1 = 4.0$ ,  $J_2 = 13.6$ , 1H), 3.39-3.34 (app dd,  $J_1 = 6.4$ ,  $J_2 = 13.6$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): *δ*, 170.0, 163.8, 158.3, 154.7, 151.7, 128.2, 112.4, 106.9, 98.2, 85.3, 83.1, 80.2, 78.8, 63.2, 46.2 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>19</sub>N<sub>2</sub>O<sub>7</sub>: 351.11927; found 351.11826.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-***N*-methyl-2-oxo-2*H*-chromene-4-carboxamide (ManCou11): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$ , 7.46-7.44 (d, *J* = 8.8, 1H), 6.69-6.66 (dd, *J*<sub>1</sub> = 2.4, *J*<sub>2</sub> = 8.8, 1H), 6.56 (d, *J* = 2.4, 1H), 6.04 (s, 1H), 4.02-3.92 (m, 3H), 3.88-3.84 (m, 1H), 3.73-3.69 (app dd, *J*<sub>1</sub> = 3.6, *J*<sub>2</sub> = 11.6, 1H), 3.65-3.61 (app dd, *J*<sub>1</sub> = 5.6, *J*<sub>2</sub> = 11.6, 1H), 3.49-3.45 (app dd, *J*<sub>1</sub> = 3.6, *J*<sub>2</sub> = 13.6, 1H), 3.39-3.33 (app dd, *J*<sub>1</sub> = 6.4, *J*<sub>2</sub> = 13.6, 1H), 2.92 (s, 3H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD):  $\delta$ , 168.4, 163.9, 158.4, 154.8, 151.9, 128.3, 112.4, 107.1, 98.3, 85.3, 83.2, 80.3, 78.8, 63.3, 46.2, 26.5 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>17</sub>H<sub>21</sub>N<sub>2</sub>O<sub>7</sub>: 365.13492; found 365.13368.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-2-oxo-***N*-phenyl-2*H*-chromene-4-carboxamide (ManCou12): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$ , 7.70-7.67 (m, 2H), 7.50-7.47 (d, *J* = 9.2, 1H), 7.40-7.35 (m, 2H), 7.21-7.16 (m, 1H), 6.71-6.68 (dd, *J*<sub>1</sub> = 2.4, *J*<sub>2</sub> = 8.8, 1H), 6.59-6.58 (d, *J* = 2.4, 1H), 6.18 (s, 1H), 4.02-3.98 (m, 2H), 3.95-3.93 (app dd, *J*<sub>1</sub> = 5.2, *J*<sub>2</sub> = 6.0, 1H), 3.88-3.85 (m, 1H), 3.73-3.69 (app dd, *J*<sub>1</sub> = 3.6, *J*<sub>2</sub> = 12.0, 1H), 3.65-3.61 (app dd, *J*<sub>1</sub> = 5.6, *J*<sub>2</sub> = 12.0, 1H), 3.50-3.45 (app dd, *J*<sub>1</sub> = 3.6, *J*<sub>2</sub> = 14.0, 1H), 3.40-3.34 (app dd, *J*<sub>1</sub> = 6.8, *J*<sub>2</sub> = 14.0, 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD):  $\delta$ , 165.7, 163.7, 158.3, 154.7, 151.7, 139.0, 129.9, 128.0, 126.0, 121.6, 112.4, 107.01, 106.95, 98.3, 85.3, 83.1, 80.2, 78.8, 63.2, 46.2 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>23</sub>H<sub>23</sub>N<sub>2</sub>O<sub>7</sub>: 427.15057; found 427.15000.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-4-(furan-2-yl)-2H-chromen-2-one (ManCou13)**: <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ, 7.92-7.90 (d, J = 8.8, 1H), 7.81-7.80 (dd,  $J_1 = 0.4$ ,  $J_2 = 1.6$ , 1H), 7.17-7.16 (dd,  $J_1 = 0.4$ ,  $J_2 = 3.6$ , 1H), 6.70-6.67 (m, 2H), 6.54-6.53 (d, J = 2.4, 1H), 6.24 (s, 1H), 4.04-4.00 (m, 2H), 3.97-3.44 (app dd,  $J_1 = 5.2$ ,  $J_2 = 6.0$ , 1H), 3.90-3.87 (m, 1H), 3.74-3.70 (app dd,  $J_1 = 3.6$ ,  $J_2 = 12.0$ , 1H), 3.67-3.62 (app dd,  $J_1 = 5.6$ ,  $J_2 = 12.0$ , 1H), 3.49-3.44 (app dd,  $J_1 = 3.6$ ,  $J_2 = 14.0$ , 1H), 3.39-3.34 (app dd,  $J_1 = 6.8$ ,  $J_2 = 14.0$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD): δ, 164.5, 158.1, 154.1, 150.2, 146.5, 144.6, 128.4, 115.5, 113.3, 112.3, 107.0, 104.3, 98.5, 85.3, 83.1, 80.2, 78.8, 63.3, 46.2 ppm. HRMS (ESI): m/z [M + H]<sup>+</sup> calcd for C<sub>19</sub>H<sub>20</sub>NO<sub>7</sub>: 374.12401; found 374.12338.

**7-((((2R,3S,4S,5R)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)methyl)amino)-4-(pyridin-2-yl)-2H-chromen-2-one (ManCou14**): <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$ , 8.71-8.69 (ddd,  $J_1 = 1.2$ ,  $J_2 = 1.6$ ,  $J_2 = 5.2$ , 1H), 8.02-7.98 (dt,  $J_1 = 1.6$ ,  $J_2 = 8.0$ , 1H), 7.66-7.64 (d, J = 8.0, 1H), 7.56-7.53 (ddd,  $J_1 = 0.8$ ,  $J_2 = 5.2$ ,  $J_2 = 8.0$ , 1H), 7.30-7.28 (d, J = 8.8, 1H), 6.63-6.60 (app dd,  $J_1 = 2.4$ ,  $J_2 = 8.8$ , 1H), 6.59 (d, J = 2.4, 1H), 6.09 (s, 1H), 4.02-3.99 (m, 2H), 3.96-3.93 (m, 1H), 3.89-3.85 (m, 1H), 3.73-3.69 (app dd,  $J_1 = 3.6$ ,  $J_2 = 12.0$ , 1H), 3.66-3.61 (app dd,  $J_1 = 5.6$ ,  $J_2 = 12.0$ , 1H), 3.48-3.44 (app dd,  $J_1 = 3.6$ ,  $J_2 = 14.0$ , 1H), 3.38-3.33 (app dd,  $J_1 = 6.8$ ,  $J_2 = 14.0$ , 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD):  $\delta$ , 164.0, 158.2, 155.7, 155.6, 154.3, 150.3, 139.0, 128.8, 125.6, 125.5, 112.2, 109.0, 108.8, 98.3, 85.2, 83.1, 80.2, 78.8, 63.2, 46.2 ppm. HRMS (ESI): m/z [M + H]<sup>+</sup> calcd for C<sub>20</sub>H<sub>21</sub>N<sub>2</sub>O<sub>6</sub>: 385.14000; found 385.13907.

#### 4. Synthesis of Coumarins



7-Aminocoumarin (**Cou1**) was synthesized according to the reported procedure.<sup>5</sup>

Scheme S3: Synthesis of C4-aryl coumarins Cou4 and Cous13-14.

(3-Hydroxyphenyl)carbamic acid ethyl ester (6):<sup>6</sup> This compound was synthesized according to the reported procedure.<sup>6</sup> 3-Aminophenol **4** (10.0 g, 91.6 mmol) and ethyl acetate (350 ml) were refluxed for 1 hour with vigorous stirring. Ethyl chloroformate **5** (11.92 g, 109.9 mmol) was then added via addition funnel over a 30 minute period. The reaction mixture was refluxed for an additional hour and then allowed to cool to room temperature. Upon cooling a grey/white precipitate formed within the flask. The precipitate was removed via filtration, and washed with ethyl acetate (3 x 150 ml). The combined filtrates were concentrated to afford **6** as a grey solid (9.46 g, 57%) that was used without further purification. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 9.45 (s, 1H), 9.29 (bs, 1H), 7.04-7.00 (m, 2H), 6.86-6.84 (m, 1H), 6.39-6.36 (ddd,  $J_1 = 1.2, J_2 = 2.4, J_3 = 8.0, 1H$ ), 4.12-4.07 (q, J = 7.2, 2H), 1.24-1.21 (t, J = 7.2, 3H) ppm.

**7-Amino-4-phenyl-2***H***-chromen-2-one (Cou4):<sup>7</sup>** This compound was synthesized according to the reported procedure.<sup>7</sup> **6** (1.00 g, 5.52 mmol) and ethyl benzoylacetate (1.27g, 6.62 mmol) were added to a 100 ml round-bottom flask equipped with a stir bar. 70% H<sub>2</sub>SO<sub>4</sub> (30 ml) was added and the mixture was stirred at room temperature. More 70% H<sub>2</sub>SO<sub>4</sub> was added until the reaction mixture turned from yellow/cloudy to amber/clear and then stirring was maintained overnight at room temperature. The reaction mixture was then poured over 100 ml of crushed ice to give a bright yellow precipitate. The solid was filtered and recrystallized from hot methanol to afford clear large crystals of **Cou4-precursor** (0.717g, 42%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 10.14 (s, 1H), 7.62-7.61 (d, *J* = 2.4, 1H), 7.54-7.48 (m, 5H), 7.36-7.30 (m, 2H), 6.20 (s, 1H), 4.18-4.13 (q, *J* = 7.2, 2H), 1.27-1.24 (t, *J* = 7.2, 3H) ppm.

**Cou4-precursor** (0.408 g, 1.32 mmol) was added to a 50 ml round-bottom flask, followed by H<sub>2</sub>SO<sub>4</sub> conc. (5 ml) and glacial AcOH (5 ml). The reaction mixture was heated to 125 °C for 2 hours under reflux condenser, after which it was cooled to room temperature and poured over 50 ml of crushed ice. The resulting suspension was neutralized till weakly basic using 4M NaOH, affording a yellow precipitate. The precipitate was filtered and recrystallized from hot methanol to give **Cou4** as a fine yellow powder (0.175 g, 56%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 7.54-7.46 (m, 5H), 7.09-7.07 (d, *J* = 8.8, 1H), 6.54-6.51 (app dd,  $J_1 = 2.0, J_2 = 8.8, 1H$ ), 6.50 (d, *J* = 2.0, 1H), 6.24 (bs, 2H), 5.90 (s, 1H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-

d<sub>6</sub>): *δ*, 160.3, 156.0, 155.6, 153.1, 135.5, 129.2, 128.6, 128.1, 127.6, 111.3, 107.2, 107.0, 98.8 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>15</sub>H<sub>12</sub>NO<sub>2</sub>: 238.08681; found 238.08597.

**7-Amino-4-(furan-2-yl)-2H-chromen-2-one (Cou13):** This compound was synthesized according to the reported general procedure.<sup>8</sup> **6** (1.00g, 5.52 mmol) and 3-furan-2-yl-3-oxo-propionic acid ethyl ester (1.27 g, 6.62 mmol) were added to a 50 ml round-bottom flask equipped with a stir bar. Methanesulfonic acid (10 ml) was added and the mixture was stirred at room temperature for overnight while the colour turned from clear/amber to black. The reaction mixture was then poured over 50 ml of ice water. It was then extracted with ethyl acetate (3 x 75 ml), and the combined organics were washed with brine (2 x 50 ml) and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration, a brown solid was obtained which was then recrystallized from hot methanol to afford **Cou13-precursor** as grey-black crystals (0.446 g, 27 %). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 10.18 (s, 1H), 8.16-8.14 (d, *J* = 8.8, 1H), 8.05-8.04 (d, *J* = 1.6, 1H), 7.59 (d, *J* = 2.0, 1H), 7.47-7.46 (d, *J* = 3.6, 1H), 7.44-7.42 (dd, *J*<sub>1</sub> = 2.0, *J*<sub>2</sub> = 8.8, 1H), 6.80-6.78 (dd, *J*<sub>1</sub> = 1.6, *J*<sub>2</sub> = 3.6, 1H), 6.53 (s, 1H), 4.20-4.14 (q, *J* = 7.2, 2H), 1.28-1.25 (t, *J* = 7.2, 3H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 159.8, 154.6, 153.1, 147.6, 146.2, 142.8, 140.9, 127.0, 115.3, 114.3, 112.7, 110.0, 107.6, 104.7, 60.7, 14.4 ppm.

**Cou13-precursor** (1.00 g, 3.34 mmol) was added to a 50 ml round-bottom flask, followed by H<sub>2</sub>SO<sub>4</sub> conc. (5 ml) and glacial AcOH (5 ml). The reaction mixture was heated to 125 °C for 2 hours under reflux condenser, after which it was cooled to room temperature and poured over 50 ml of crushed ice. The resulting suspension was neutralized till weakly basic using 4M NaOH, extracted with ethyl acetate (3 x 75 ml) and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration, the residue was purified by column chromatography on silica gel (dry loading), eluting with 40-100% EtOAc in hexanes, followed by 5% MeOH in EtOAc. The residue was triturated with hexanes containing little CH<sub>2</sub>Cl<sub>2</sub>. The product was filtered, washed with hexanes and dried in air to obtain **Cou13** as a yellow-brown powder (0.11 g, 14%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 8.02 (s, 1H), 7.92-7.90 (d, *J* = 8.4, 1H), 7.36 (s, 1H), 6.76 (s, 1H), 6.63-6.61 (d, *J* = 8.4, 1H), 6.48 (s, 1H), 6.26 (bs, 2H), 6.24 (s, 1H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 160.7, 156.4, 153.2, 148.6, 145.9, 142.0, 127.5, 114.7, 112.6, 111.6, 104.6, 103.3, 99.1 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>13</sub>H<sub>10</sub>NO<sub>3</sub>: 228.06608; found 228.06500.

**7-Amino-4-(pyridin-2-yl)-2H-chromen-2-one (Cou14):** This compound was synthesized according to the reported general procedure.<sup>8</sup> **6** (2.00 g, 11.03 mmol) and ethyl 3-oxo-3-(pyridin-2-yl)propanoate (2.56 g, 13.24 mmol) were added to a 50 ml round-bottom flask with a stir bar. Methanesulfonic acid (10 ml) was added and the mixture was stirred at room temperature for overnight while the colour turned from clear/amber to black. The reaction mixture was then poured over 50 ml of ice water. It was then extracted with ethyl acetate (3 x 75 ml), and the combined organics were washed with brine (2 x 50 ml) and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtration and concentration, a grey solid was obtained which was then recrystallized from hot methanol to afford **Cou14-precursor** as fine white crystals of 1 : 1 MeOH solvate (1.403 g, 41 %). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 10.17 (s, 1H), 8.78-8.76 (ddd,  $J_1 = 1.2$ ,  $J_2 = 2.0$ ,  $J_2 = 8.8$ , 1H), 8.03-8.00 (dt,  $J_1 = 2.0$ ,  $J_2 =$ 

8.0, 1H), 7.77-7.75 (m, 1H), 7.70-7.68 (d, J = 8.8, 1H), 7.64-7.63 (d, J = 2.0, 1H), 7.58-7.54 (m, 1H), 7.36-7.34 (dd,  $J_1 = 2.0$ ,  $J_2 = 8.8$ , 1H), 4.19-4.14 (q, J = 7.2, 2H), 4.11-4.07 (q, J = 5.2, 1H), 3.18-3.16 (d, J = 5.2, 3H), 1.28-1.24 (t, J = 7.2, 3H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 159.8, 154.6, 153.3, 153.1, 152.2, 149.3, 142.8, 137.5, 127.8, 124.4, 124.2, 114.1, 112.7, 112.0, 104.6, 60.7, 48.6, 14.4 ppm.

**Cou14-precursor** (0.50 g, 1.61 mmol) was added to a 50 ml round-bottom flask, followed by H<sub>2</sub>SO<sub>4</sub> conc. (5 ml) and glacial AcOH (5 ml). The reaction mixture was heated to 125 °C for 2 hours under reflux condenser, after which it was cooled to room temperature and poured over 50 ml of crushed ice. The resulting suspension was neutralized till weakly basic using 4M NaOH, affording a beige precipitate. The precipitate was filtered and recrystallized from hot methanol to give **Cou14** as fine yellow crystals (0.162 g, 68%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD):  $\delta$ , 8.72-8.70 (ddd,  $J_1 = 1.2$ ,  $J_2 = 1.6$ ,  $J_2 = 4.8$ , 1H), 8.04-8.00 (dt,  $J_1 = 2.0$ ,  $J_2 = 8.0$ , 1H), 7.69-7.66 (dt,  $J_1 = 1.2$ ,  $J_2 = 8.0$ , 1H), 7.58-7.54 (ddd,  $J_1 = 0.8$ ,  $J_2 = 4.8$ ,  $J_2 = 8.0$ , 1H), 7.29-7.26 (app dd,  $J_1 = 3.2$ ,  $J_2 = 6.4$ , 1H), 6.60-6.57 (m, 2H), 6.11 (s, 1H) ppm. <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD):  $\delta$ , 164.1, 158.2, 156.0, 155.8, 155.0, 150.5, 139.2, 129.2, 125.7, 125.6, 113.2, 109.2, 109.1, 100.7 ppm. HRMS (ESI): m/z [M + H]<sup>+</sup> calcd for C<sub>14</sub>H<sub>11</sub>N<sub>2</sub>O<sub>2</sub>: 239.08207; found 239.08078.



Scheme 4: Synthesis of Cou5.

**Ethyl 7-amino-2-oxo-2H-chromene-4-carboxylate** (**Cou5**):<sup>9</sup> 3-Aminophenol (**4**) (5.00 g, 45.8 mmol), diethyl oxaloacetate sodium salt (**8**) (14.4 g, 68.7 mmol) and EtOH (20 ml) were heated at reflux overnight. The mixture was cooled and concentrated to dryness and the residue was purified by silica gel chromatog-raphy (50% EtOAc in hexanes). **Cou5** was obtained as an yellow-orange solid (4.17 g, 39%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 7.69-7.66 (d, *J* = 8.8, 1H), 6.60-6.57 (dd, *J*<sub>1</sub> = 2.4, *J*<sub>2</sub> = 8.8, 1H), 6.46-6.45 (d, *J* = 2.4, 1H), 6.37 (bs, 2H), 6.31 (s, 1H), 4.39-4.33 (q, *J* = 7.2, 1H), 1.35-1.31 (t, *J* = 7.2, 1H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 164.3, 160.3, 156.5, 153.6, 143.8, 127.4, 111.8, 109.2, 104.4, 98.8, 62.0, 13.9 ppm. HRMS (ESI): *m*/*z* [M + H]<sup>+</sup> calcd for C<sub>12</sub>H<sub>12</sub>NO<sub>4</sub>: 234.07665; found 234.07600.



Scheme 5: Synthesis of Cou6 and Cous10-12.

**7-Amino-2-oxo-2***H***-chromene-4-carboxylic acid (Cou6):**<sup>9</sup> Ethyl 7-amino-2-oxo-2*H*-chromene-4-carboxylate (**Cou5**) (1.00 g, 4.30 mmol) and 2M KOH solution (25 ml) were added to the flask and refluxed for 4 h. After cooling, the reaction mixture was washed with AcOEt (25 ml, discarded). AcOH was used to neutralize the solution that was concentrated to dryness. The residue was purified by column chromatog-raphy on silica gel, eluting with water : isopropanol : EtOAc mixtures (1 : 2 : 7 to 2 : 4 : 4). The residue was triturated with CH<sub>2</sub>Cl<sub>2</sub> containing little EtOH, and then refluxed in CHCl<sub>3</sub> overnight. The product was filtered, washed with CHCl<sub>3</sub> and dried in air to obtain orange solid (0.38 g, 43%) in satisfying purity. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 7.73-7.70 (d, *J* = 8.8, 1H), 6.53-6.50 (dd, *J*<sub>1</sub> = 2.0, *J*<sub>2</sub> = 8.8, 1H), 6.40 (d, *J* = 2.0, 1H), 5.93 (s, 1H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 167.1, 161.6, 156.3, 152.6, 128.6, 111.0, 106.2, 104.8, 98.4 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>10</sub>H<sub>8</sub>NO<sub>4</sub>: 206.04535; found 206.04487.

**7-Amino-2-oxo-2H-chromene-4-carboxamide (Cou10):** This compound was synthesized according to the modified general procedure.<sup>10</sup> **Cou5** (1.00 g, 4.30 mmol) was placed in the flame-dried flask under the flow of Ar. Then 7N NH<sub>3</sub> in absolute MeOH (20 ml) was added and the mixture was allowed to react at room temperature under Ar for 24 h. Then the mixture was concentrated to dryness. The residue was purified by column chromatography on silica gel, eluting with 100% CH<sub>2</sub>Cl<sub>2</sub> to 100% EtOAc, followed by 10-40% MeOH in EtOAc. The residue was triturated with CH<sub>2</sub>Cl<sub>2</sub>, and filtered washing sequentially with CH<sub>2</sub>Cl<sub>2</sub>, water, ethanol, again CH<sub>2</sub>Cl<sub>2</sub> and dried in air. The product was obtained as a yellow solid (0.12 g, 14%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 8.16 (bs, 1H), 7.83 (bs, 1H), 7.43-7.41 (d, *J* = 8.8, 1H), 6.57-6.55 (dd, *J*<sub>1</sub> = 2.0, *J*<sub>2</sub> = 8.8, 1H), 6.44 (d, *J* = 2.0, 1H), 6.27 (bs, 2H), 5.99 (s, 1H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 166.8, 160.8, 156.4, 153.5, 150.2, 127.6, 111.6, 105.5, 105.0, 98.7 ppm. HRMS (ESI): *m*/*z* [M + H]<sup>+</sup> calcd for C<sub>10</sub>H<sub>9</sub>N<sub>2</sub>O<sub>3</sub>: 205.06134; found 205.06064.

**7-Amino-N-methyl-2-oxo-2H-chromene-4-carboxamide (Cou11):** This compound was synthesized according to the modified general procedure.<sup>10</sup> **Cou5** (1.00 g, 4.30 mmol) was placed in the flame-dried flask under the flow of Ar. 33 wt. % CH<sub>3</sub>NH<sub>2</sub> in MeOH (20 ml) was added and the mixture was allowed to react at room temperature under Ar for 24 h. Then the mixture was concentrated to dryness. The residue was purified by column chromatography on silica gel, eluting with 50-100% EtOAc in hexanes, followed by

10% MeOH in EtOAc. The residue was stirred overnight in CH<sub>2</sub>Cl<sub>2</sub>/EtOAc, filtered, washed with CH<sub>2</sub>Cl<sub>2</sub> and dried in air. The product was obtained as a yellow solid (0.08 g, 9%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 8.66 (d, *J* = 4.0, 1H), 7.38-7.36 (d, *J* = 8.8, 1H), 6.56-6.54 (d, *J* = 8.8, 1H), 6.44 (s, 1H), 6.28 (bs, 2H), 5.98 (s, 1H), 2.77 (d, *J* = 4.0, 3H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 165.0, 160.5, 156.2, 153.4, 150.0, 127.5, 111.5, 105.6, 105.0, 98.6, 25.8 ppm. HRMS (ESI): *m/z* [M + H]<sup>+</sup> calcd for C<sub>11</sub>H<sub>11</sub>N<sub>2</sub>O<sub>3</sub>: 219.07699; found 219.07602.

**7-Amino-2-oxo-N-phenyl-2H-chromene-4-carboxamide (Cou12)**: This compound was synthesized according to the modified general procedure.<sup>11</sup> A solution of aniline (0.729 ml, 8 mmol) in dry THF (20 ml) was cooled to 0 °C under Ar and a commercial 2.0 M solution of trimethylaluminum in hexanes (10 ml, 20 mmol) was added dropwise over 10 minutes. Then, to the resulting mixture a solution of **Cou5** (0.932 g, 4 mmol) in dry THF (40 ml) was added dropwise over 20 minutes at 0 °C. The mixture was allowed to warm to rt and stirred for overall 22 h. It was diluted with EtOAc (100 ml) and carefully neutralized with aq. NH<sub>4</sub>Cl (100 ml) with vigorous stirring. The phases were separated, and the aqueous phase was additionally extracted with EtOAc (100 ml). The combined organic extracts were washed with brine (50 ml) and dried over MgSO<sub>4</sub>. After filtration and concentration, the residue was purified by column chromatography on silica gel, eluting with 0-80% EtOAc in hexanes. The product was further purified by recrystallization from EtOAc and filtered washing copiously with CH<sub>2</sub>Cl<sub>2</sub> to obtain an orange solid (0.165 g, 14%). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 10.7 (bs, 1H), 7.74-7.72 (d, *J* = 8.0, 2H), 7.39-7.36 (m, 3H), 7.16-7.13 (t, *J* = 7.2, 1H), 6.60-6.58 (d, *J* = 8.0, 1H), 6.49 (s, 1H), 6.34 (bs, 2H), 6.20 (s, 1H) ppm. <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>):  $\delta$ , 163.2, 160.5, 156.2, 153.5, 149.6, 138.2, 128.7, 127.2, 124.2, 119.9, 111.7, 105.8, 104.8, 98.6 ppm. HRMS (ESI): *m/z* [M + H]\* calcd for C<sub>16</sub>H<sub>13</sub>N<sub>2</sub>O<sub>3</sub>: 281.09264; found 281.09189.

#### 5. Cell Imaging

For confocal microscopy cells were plated (100,000/plate) in 35 mm glass-bottom confocal dishes (MatTek) and allowed to grow in their respective growth media for 24 hours. For optical microscope analysis cells were plated in a 6-well plates (3 x 105) and allowed to adhere and grow for 24 h. For treatment, cell media was removed and ManCou solution in PBS (1 ml) was added. Cells were incubated with ManCous at 37 C° for 10 min. After incubation, probe solution was removed, and cells were washed with warmed PBS (3 x 1 ml) and leaving 1 ml of PBS for imaging. Cell images were taken using Olympus FluoView<sup>TM</sup> FV1000 using the FluoView software. 60X oil suspended lens was used to observe fluorescent activity with the following conditions: DAPI (ManCous) and eGFP (NBDM and NBDG) filters; lasers 405 nm (45% intensity), 450/490 nm (30% intensity); 10 µs/pixel. Z-stacking was done using FluoView software and depth command.

#### 6. Microplate uptake and inhibition assays

For microplate assays, at ~80% confluence cells were collected and plated in 96-well flat bottom plates (20,000 cells/well) and allowed to grow for 24 hours. Cells were then washed with warmed (37 °C) PBS

solution, treated with ManCou probes (concentration varies) in PBS and incubated at 37 °C and 5% CO<sub>2</sub> for 10 min. After incubation, cells were carefully washed with warmed PBS (3 x 100 µl). Fluorescent data were immediately collected using Victor3 plate reader and using WallacTM umbelliferone (excitation 355 nm, emission 460 nm, 1.0 s) protocol. All trials were done in triplicate on each plate.

Uptake Inhibition studies were carried out in microplate format. Using 96-well plate method fluorescence of ManCou probes in cells was measured in the presence of varying concentrations of fructose, glucose, glucosamine, and cytochalasin B. For this part, PBS solution containing 20  $\mu$ M ManCou and the specific concentration of a sugar were prepare and introduced to cells. Separately, complete culture media were used to establish the impact of nutrients on ManCou uptake. Cell incubation, and data collection were conducted as stated above.

## 7. References

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Figure S7. <sup>1</sup>H NMR spectrum of ManCou1 (CD<sub>3</sub>OD, 400 MHz).



Figure S8. <sup>13</sup>C NMR spectrum of ManCou1 (CD<sub>3</sub>OD, 100 MHz).



Figure S9. <sup>1</sup>H NMR spectrum of ManCou2 (CD<sub>3</sub>OD, 400 MHz).



Figure S10. <sup>13</sup>C NMR spectrum of ManCou2 (CD<sub>3</sub>OD, 100 MHz).



Figure S11. <sup>1</sup>H NMR spectrum of ManCou3 (CD<sub>3</sub>OD, 400 MHz).



Figure S12. <sup>13</sup>C NMR spectrum of ManCou3 (CD<sub>3</sub>OD, 100 MHz).



Figure S13. <sup>1</sup>H NMR spectrum of ManCou4 (CD<sub>3</sub>OD, 400 MHz).



Figure S14. <sup>13</sup>C NMR spectrum of ManCou4 (CD<sub>3</sub>OD, 100 MHz).



Figure S15. <sup>1</sup>H NMR spectrum of ManCou5 (CD<sub>3</sub>OD, 400 MHz).



Figure S16. <sup>13</sup>C NMR spectrum of ManCou5 (CD<sub>3</sub>OD, 100 MHz).



Figure S17. <sup>1</sup>H NMR spectrum of ManCou6 (D<sub>2</sub>O, 400 MHz).



Figure S18. <sup>13</sup>C NMR spectrum of ManCou6 (D<sub>2</sub>O, 100 MHz).



Figure S19. <sup>1</sup>H NMR spectrum of ManCou7 (D<sub>2</sub>O, 400 MHz).



Figure S20. <sup>1</sup>H NMR spectrum of ManCou8 (CD<sub>3</sub>OD, 400 MHz).



Figure S21. <sup>13</sup>C NMR spectrum of ManCou8 (CD<sub>3</sub>OD, 100 MHz).



Figure S22. <sup>1</sup>H NMR spectrum of ManCou9 (CD<sub>3</sub>OD, 400 MHz).



Figure S23. <sup>13</sup>C NMR spectrum of ManCou9 (CD<sub>3</sub>OD, 100 MHz).


Figure S24. <sup>1</sup>H NMR spectrum of ManCou10 (CD<sub>3</sub>OD, 400 MHz).



Figure S25. <sup>13</sup>C NMR spectrum of ManCou10 (CD<sub>3</sub>OD, 100 MHz).



Figure S26. <sup>1</sup>H NMR spectrum of ManCou11 (CD<sub>3</sub>OD, 400 MHz).



Figure S27. <sup>13</sup>C NMR spectrum of ManCou11 (CD<sub>3</sub>OD, 100 MHz).



Figure S28. <sup>1</sup>H NMR spectrum of ManCou12 (CD<sub>3</sub>OD, 400 MHz).



Figure S29. <sup>13</sup>C NMR spectrum of ManCou12 (CD<sub>3</sub>OD, 100 MHz).



Figure S30. <sup>1</sup>H NMR spectrum of ManCou13 (CD<sub>3</sub>OD, 400 MHz).



Figure S31. <sup>13</sup>C NMR spectrum of ManCou13 (CD<sub>3</sub>OD, 100 MHz).



Figure S32. <sup>1</sup>H NMR spectrum of ManCou14 (CD<sub>3</sub>OD, 400 MHz).



Figure S33. <sup>13</sup>C NMR spectrum of ManCou14 (CD<sub>3</sub>OD, 400 MHz).



Figure S34. <sup>1</sup>H NMR spectrum of 6 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S35. <sup>1</sup>H NMR spectrum of Cou4-precursor (DMSO-d<sub>6</sub>, 400 MHz).



Figure S36. <sup>1</sup>H NMR spectrum of Cou4 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S37. <sup>13</sup>C NMR spectrum of Cou4 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S38. <sup>1</sup>H NMR spectrum of Cou5 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S39. <sup>13</sup>C NMR spectrum of Cou5 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S40. <sup>1</sup>H NMR spectrum of Cou6 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S41. <sup>13</sup>C NMR spectrum of Cou6 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S42. <sup>1</sup>H NMR spectrum of Cou10 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S43. <sup>13</sup>C NMR spectrum of Cou10 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S44. <sup>1</sup>H NMR spectrum of Cou11 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S45. <sup>13</sup>C NMR spectrum of Cou11 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S46. <sup>1</sup>H NMR spectrum of Cou12 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S47. <sup>13</sup>C NMR spectrum of Cou12 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S48. <sup>1</sup>H NMR spectrum of Cou13-precursor (DMSO-d<sub>6</sub>, 400 MHz).



Figure S49. <sup>13</sup>C NMR spectrum of Cou13-precursor (DMSO-d<sub>6</sub>, 100 MHz).



Figure S50. <sup>1</sup>H NMR spectrum of Cou13 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S51. <sup>13</sup>C NMR spectrum of Cou13 (DMSO-d<sub>6</sub>, 100 MHz).



Figure S52. <sup>1</sup>H NMR spectrum of Cou14-precursor (DMSO-d<sub>6</sub>, 400 MHz).



Figure S53. <sup>13</sup>C NMR spectrum of Cou14-precursor (DMSO-d<sub>6</sub>, 100 MHz).



Figure S54. <sup>1</sup>H NMR spectrum of Cou14 (DMSO-d<sub>6</sub>, 400 MHz).



Figure S55. <sup>13</sup>C NMR spectrum of Cou14 (DMSO-d<sub>6</sub>, 100 MHz).

## 9. HRMS Data for ManCous 1-14.



Figure S56. HRMS data for ManCou1.



Figure S57. HRMS data for ManCou2.



Figure S58. HRMS data for ManCou3.



Figure S59. HRMS data for ManCou4.



Figure S60. HRMS data for ManCou5.



Figure S61. HRMS data for ManCou6.



Figure S62. HRMS data for ManCou7.



Figure S63. HRMS data for ManCou8.


Figure S64. HRMS data for ManCou9.



Figure S65. HRMS data for ManCou10.



## Figure S66. HRMS data for ManCou11.



Figure S67. HRMS data for ManCou12.



Figure S68. HRMS data for ManCou13.



Figure S69. HRMS data for ManCou14.