Electronic Supplementary Information

Chromene-triazole-pyrimidine based chemosensor therapeutics for the in vivo and in vitro detection of Fe$^{3+}$ ions

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Experimental Section

General

IR spectra were recorded on a JASCO-FT/IR-4100 Fourier transform infrared spectrometer and measured as KBr pellets. \(^1\)H and \(^13\)C NMR spectra were determined in DMSO with a Bruker amx 500 MHz spectrometer. The chemical shifts (\(\delta\)) are given relative to tetramethylsilane (TMS) and the coupling constants (J) are reported in hertz (Hz). Electron spray ionization mass spectra were recorded with a Thermo scientific exactive mass spectrometer and on a JEOL JMS600H. Absorption spectra of the compounds were recorded on a JASCO V-550 UV/Vis spectrophotometer and fluorescence measurements were carried out with a Jasco spectrofluorometer.

General procedure for the synthesis of chlorinated 3, 4-dihydropyrimidinone (a1-a3): A mixture of benzaldehyde (1 mmol), ethyl-4-chloroacetoacetate (1mmol), and urea (1.5 mmol) was heated at 80 °C in an oil bath. After 1 hour the reaction mixture was cooled to room temperature and poured into crushed ice. The precipitate of pyrimidinone derivatives obtained was washed with cold water. Filtered, and dried under vacuum to give the crude product in pure form. Recrystallization from hot ethanol provided the analytically pure product.

**a1:** ethyl6-(choloromethyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5 carboxylate: White solid, Mp 234-237°C, Yield: 85%; \(^1\)H-NMR (DMSO-(d_6) 500MHz) \(\delta_{H}\) (ppm): 7.84-7.09 (m,5H), 6.98 (s, 1H), 6.85-6.84 (d, 1H,J=7.5), 4.97 (s, 1H), 4.90 (s,1H), 4.40-4.34 (q, 2H), 1.29 (s, 1H), 1.07-1.02 (t, 3H), FT-IR(KBr)(\(\nu_{max},\text{cm}^{-1}\)): 3386, 3087, 1768, 1701, 1690, 1595, 1513, 1473, 1433, 1351, 1293, 1235, 1201,1137,1105,1024,1000,964,924 ; MS: m/z Calcd for C_{14}H_{14}ClN_{2}O_{5}, [M] \(^{+}\): 328.04, Found: [M] \(^{+}\): 328.0

**a2:** ethyl6-(chloromethyl)-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Brown solid, Mp 236-238°C,Yield: 83%; \(^1\)H-NMR (DMSO-(d_6) 500MHz) \(\delta_{H}\) (ppm): 8.19-8.178(d,1H,J=9),7.90-7.77(d,2H),7.47-7.45(d,1H,J=8.5),7.37-7.35(d,2H,J=8.5),5.92(s,1H),5.18-5.16 (d,1H,J=7.5)3.48 (s,1H), 3.417-3.35 (q,2H),1.66-1.62 (t,3H); FTIR(KBr) (\(\nu_{max},\text{cm}^{-1}\)): 3437, 3291, 1665, 1597, 1520, 1475, 1433, 1351, 1293, 1235, 1201,1137,1105,1024,1000,964,924 ; MS: m/z Calcd for C_{14}H_{14}ClN_{2}O_{5}, [M] \(^{+}\): 339.0, Found: [M] \(^{+}\): 339.0

**a3:** ethyl6-(chloromethyl)-4-(4-hydroxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5 carboxylate:White solid, Mp 235-237°C, Yield: 85%; \(^1\)H-NMR (DMSO-(d_6) 500MHz) \(\delta_{H}\) (ppm): 7.54 -7.15 (m,5H),5.37 (s,1H),5.19-5.17(d,1H,J=9.5),5.07(s,1H),4.97-4.92(d,1H),1.96(s,1H),1.81-1.755(t,3H); FT-IR(KBr) (\(\nu_{max},\text{cm}^{-1}\)): 3568, 3381, 3234, 3105, 2917, 1683, 1645, 1612, 1595, 1513, 1464, 1431, 1389, 1366, 1325,1304,1281,1229,1169; MS: m/z Calcd for C_{14}H_{14}ClN_{2}O_{5}, [M+H\(^{+}\)]: 311.1, Found: [M+H\(^{+}\)]: 311.1

General procedure for the synthesis of azide derivatives of dihydropyrimidinones (P1-P3): Chloroderivative of dihydropyrimidinone (1 mmol), and sodium azide (1 mmol) were stirred at 0°C in acetone. After completion of the reaction (6 h,TLC) the mixture was poured into ice cold water. The solid product obtained was filtered, washed with petroleum ether and ethylacetate(4:1), and dried under vacuum to afford the dihydropyrimidinone azide.

**P1:** ethyl6-(azidomethyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: White Solid, Mp 164-166°C, Yield: 80%; \(^1\)H-NMR (DMSO-(d_6) 500MHz) \(\delta_{H}\) (ppm):1.00-0.97 (t,3H),1.62 (s,1H),2.17 (s,2H),3.98-3.94 (q,2H),4.97-4.95 (d, 1H,J=11.5), 5.19 (s, 1H), 6.12 (s, 1H), 7.26 (s, 1H),7.36-
7.31 (m, 5H); FT-IR (KBr) ($\nu_{\text{max}}$, cm$^{-1}$): 3421,3298,3212, 2923, 2749, 2122, 1723, 1677, 1602, 1577, 1508,1427,1302,1250,1137,1021,861,828,761,653,611,511; MS: m/z Calculated for C$_{14}$H$_{12}$ClN$_5$O$_3$, [M]+: 335.08, Found: [M]+ : 335.0

**P2:** ethyl6-(azidomethyl)-4-(3-nitrophenoxy)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Yellow solid, Mp 163-165°C, Yield: 82%; $^1$H-NMR (DMSO-($d_6$) 500MHz) $\delta$H (ppm): 0.97-1.00 (t, 3H), 2.17 (s, 2H); 3.93-4.03 (m, 2H); 4.97 (s, 1H); 5.19 (s, 1H); 6.12 (s, 1H); 7.26-7.36 (m, 4H); FT-IR(KBr)$\nu_{\text{max}}$ 3383, 3090, 2982, 2931, 2113, 1698, 1652, 1530, 1476, 1446, 1350, 1303, 1096, 1018, 904, 861, 809, 761,736,691,553,479; HRMS (EI)m/z calcld for C$_{14}$H$_{15}$N$_{10}$O$_3$ [M+Na]+: 369.09234, found [M+Na]+: 369.09718.

**P3:** ethyl6-(azidomethyl)-4-(4-hydroxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Yellow solid, Mp 166-167°C, Yield: 85%; $^1$H-NMR (DMSO-($d_6$) 500MHz) $\delta$H (ppm): 1.13-1.15 (t,3H); 2.24-2.25 (d, H); 4.01 (s,1H); 4.05 (s,1H); 5.22 (s,1H); 5.54 (s,1H); 6.82 (s,1H); 6.84 (s,1H); 7.00-7.35 (m,4H); FT-IR(KBr)$\nu_{\text{max}}$, cm$^{-1}$): 3442,2922,2852,2057, 1638, 1529, 1418, 1350, 1212, 1157,1113,1020,873,664,553; HRMS (EI) m/z calcld for C$_{14}$H$_{13}$N$_5$O$_4$ [M+Na]+:340.10217 , found: [M+Na]+: 340.10574.

*General experimental procedure for the synthesis alkynes (C1-C3):* A mixture of propargylated aromatic aldehyde (160 mg, 1 mmol), 2-naphthol (144mg, 1 mmol), malononitrile (66mg, 1mmol), and sodium carbonate (0.106 mg, 0.01mmol) were mixed by using a mortar and pestle. The resulting solid was heated in an oven at 80°C for 10 min. After cooling, the mixture was washed with hot water and the solid separated was filtered and dried. Recrystallized from hot ethanol to obtain pure product

**C1:** 3-amino-1-(2-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[f]chromene-2-carbonitrile: Yellow solid, M.p. 110-112°C,Yield:93%; $^1$H NMR (500 MHz, DMSO): $\delta$H (ppm): 3.31 (s,1H), 3.68 (s,2H),4.97 (s,1H),7.09 (d,2H, J=9Hz),7.11(S,2H),7.67-7.77(m,3H),8.00-7.90(d,1H, J=8.5Hz), 8.42(d, 1H), $^{13}$C NMR (DMSO-($d_6$), 125 MHz) $\delta$c (ppm):22.4, 56.4, 59.2, 78.2, 79.2, 112.9, 117.1, 117.9, 121.7, 122.8, 126.9, 128.5, 129.8, 130.5, 130.5, 132.7, 133.8, 133.9, 156.5, 171.9, 200.8. FT-IR (KBr) ($\nu_{\text{max}}$, cm$^{-1}$): 3420, 3274, 3030, 2224, 2130, 1586, 1559, 1509; EI-MS: m/z calculated for C$_{25}$H$_{18}$N$_{2}$O$_{2}$:353.3847 and found EI-MS: 353.37 (M+).

**C2:** 3-amino-1-(4-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[f]chromene-2-carbonitrile: Yellow solid, M.p. 110-113°C, Yield:96%; $^1$H-NMR (DMSO-($d_6$) 500MHz) $\delta$H (ppm): 3.31 (s,1H), 3.68 (s,2H), 4.978 (s,1H), 7.09(d,2H, J=9Hz),7.11(S,2H),7.67-7.77(m,3H),8.00-7.90(d,1H, J=8.5Hz), 8.42( d,1H). FT-IR (KBr) ($\nu_{\text{max}}$, cm$^{-1}$): 3420, 3274, 3030, 2224, 2130, 1586, 1559, 1509; EI-MS: m/z calculated for C$_{25}$H$_{18}$N$_{2}$O$_{2}$:353.3847 and found EI-MS: 353.37 (M+).

**C3:** 3-amino-1-(3-methoxy-4-(prop-2-yn-1-yloxy)phenyl)-1H-benzo[f]chromene-2-carbonitrile: Yellow solid, M.p.111-114°C, Yield: 98%; $^1$H-NMR (DMSO-($d_6$) 500MHz) $\delta$H (ppm): 3.33 (s, 1H), 3.67(s, 1H), 3.82(s, 3H) 4.98 (s, 1H), 7.08(d, 2H, J=8.5Hz),7.13 (s, 2H), 7.20 (d, 2H J=7.5Hz),7.37(d, 1H, J=8Hz),7.67-7.77(m, 3H), 8.00 (d, 1H, J=8Hz), 8.022(d, 1H, J=8Hz). $^{13}$C NMR (DMSO-($d_6$), 125 MHz) $\delta$c (ppm):27.9, 54.3, 56.2, 59.3, 78.2, 112.9, 117.1, 117.9, 121.8, 122.8, 128.7, 128.8, 128.9, 129.8, 129.8, 130.9, 130.9, 132.7, 134.8, 139.8, 157.4, 172.9. FT-IR (KBr) ($\nu_{\text{max}}$, cm$^{-1}$): 3363, 3279, 3223, 2129, 1629, 1598, 1417; EI-MS: m/z calculated for C$_{23}$H$_{18}$N$_{2}$O$_{2}$:382.4106 and found EI-MS: 382.32 (M+).

*General procedure for the synthesis of 1, 4-disubstituted chromene pyrimidinone diad via Cu(I) catalyzed Huisgen cycloaddition(CP1-CP6):* An equimolar mixture of azide and alkyne was mixed with 0.2 equiv of CuSO$_4$ and 0.4 equiv of sodium ascorbate in a mixed solvent system containing tert-butanol, water, and
DMSO (4:2:1) at room temperature. After 12 h, the reaction mixture was diluted with cold water, filtered, and washed with water to afford the click product in solid form.

**CP1:** ethyl 6-((4-((3-amino-2-cyano-1H-benzo[f]chromen-1-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-(4-hydroxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Brown solid, Mp 145-147°C, Yield 85%; 1H NMR (500 MHz, DMSO): δH (ppm): 8.57 (s, 2H), 7.85-7.22 (m,14H),7.58 (s,1H),6.21(s,2H),5.96(s,1H),5.52(s,1H),5.23-5.11(d,1H),5.02(s,2H),4.30(s,2H),4.15-4.09 (q,2H),1.35-1.31(t,3H); 13CNMR(DMSO-d6),500MHz): 35.80, 39.50, 39.67, 39.83, 40.00, 40.17, 40.26, 40.33, 40.42,40.50,40.59,55.79,71.25,79.45,80.32,81.00,104.50,114.73,115.79,116.21,116.42,123.78,124.43,128.15,131.83,132.15,133.64,152.76,158.16,165.13,171.23; FT-IR(KBr)(μmax(cm⁻¹): 3383, 2928, 2227, 1697, 1552, 1504, 1512, 1487, 1457, 1369,1307, 1227, 1175, 1097, 1030, 756; HRMS m/z calcd for C37H31N7O6 [M⁺]:669.23358,found: [M⁺]: 669.23352.

**CP2:** ethyl 6-((4-((3-amino-2-cyano-1H-benzo[f]chromen-1-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-(4-hydroxyphenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Brown solid, Yield 87%, M.p 148-151°C; 1H NMR (500 MHz, DMSO): δH(ppm): 8.23(s,2H), 7.91-6.94 (m,13H),7.59(s,1H),5.69(s,1H),5.50(s,1H),5.35(s,1H),4.98 (s,1H),4.77-4.71 (q,2H), 3.76 (s,3H),1.34-1.32 (t,3H); 13CNMR(DMSO-d6),500MHz): 34.05, 39.51, 39.67, 39.84, 40.01, 40.17, 40.27, 40.34, 40.43, 40.51,56.53,57.13,68.05,78.25,79.31,103.70,113.64,116.93,121.73,122.94,125.01,128.87,130.27,131.62,133.13,152.60,154.53,156.08,165.01,173.03; FT-IR(KBr)(μmax(cm⁻¹):3403, 2920, 2223, 1696, 1585, 1510, 1451, 1425, 1375, 1341,1274, 1145,1097,1015,804,624; HRMS m/z calcd for C38H33N7O7 [M⁺]: 669.24415 , found: [M⁺]: 669.24405.

**CP3:** ethyl 6-((4-((3-amino-2-cyano-1H-benzo[f]chromen-1-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Yellow solid, M.p 144-147°C, Yield 86%. 1H NMR (500 MHz, DMSO) δH (ppm): 8.53(s, 2H),7.83-7.19 (m,13H),7.53 (s,1H),6.19 (s,1H),5.90(s,1H),5.16-5.11 (d,1H),4.98 (s,2H),4.25 (s,2H),4.13-4.07 (q,2H),1.33-1.29 (s,3H); 13CNMR(DMSO-d6),500MHz): 27.78, 36.29, 39.50, 39.66, 39.83, 40.00, 40.17, 40.26, 40.33, 40.42, 40.50,59.13,63.16,69.19,71.13,76.94,78.94,101.64,104.13,108.53,110.04,113.35,116.75,122.09,122.80,123.51,126.30,136.64,137.04,149.63,151.51,155.20,157.07,158.62,159.63,174.59; FT-IR(KBr)(μmax cm⁻¹):3083, 3031, 2923, 2224, 1745, 1683, 1601, 1587, 1559,1509,1454,1429,1375, 1318, 1262, 1238, 1186, 1158, 1126, 1071,1009; HRMS m/z calcd for C37H30N6O7[M+Na⁺]:725.22375,found: [M+Na⁺]: 725.23396

**CP4:** ethyl 6-((4-((2-(3-amino-2-cyano-1H-benzo[f]chromen-1-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Brown solid, M.p 155-157°C, Yield 83%. 1H NMR (500 MHz, DMSO) δH (ppm): 8.08 (s,2H), 7.69 (s, 1H), 7.59-7.05 (m,14H),6.28 (s,1H),6.01 (s,1H),5.18-5.14 (d,1H), 5.08-4.95 (d, 2H), 4.83-4.75 (d,1H), 1.39-1.35 (t,3H); 13CNMR (DMSO-d6), 500 MHz): 23.07, 39.51, 39.68, 39.84, 40.01, 40.18, 40.34, 40.51, 44.76, 48.85, 64.81,66.12,67.62,69.18,84.88,103.36,106.94,111.73,113.14,128.45,128.62,129.04,129.19,131.80,133.74,136.98,142.55,156.80,158.93,160.18,168.89,182.39; FT-IR(KBr)(μmax cm⁻¹): 3376, 2923, 2853, 2227, 1747,1675,1583,1562,1486,1456,1393,1365, 1224, 1108, 1016, 849, 810, 754, 727, 614,570; HRMS m/z calcd for C37H30N6O7[M⁺]: 698.22375 , found: [M⁺]: 698.02643

**CP5:** ethyl 6-((4-((2-(3-amino-2-cyano-1H-benzo[f]chromen-1-yl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: pale yellow solid, M.p
143-145°C, Yield 84%. 1H NMR (500 MHz, DMSO) δH (ppm): 7.99 (s, 2H), 7.97-7.26 (m, 13H), 7.41 (s, 1H), 5.83-5.82 (d, 1H), 5.68-5.63 (d, 1H), 4.13-4.07 (q, 2H), 1.41-1.37 (t, 3H); 13C NMR (DMSO-d6, 500 MHz): 23.10, 39.52, 39.69, 39.86, 40.02, 40.19, 40.28, 40.36, 40.52, 48.78, 48.87, 56.60, 78.61, 79.92, 111.44, 114.62, 116.59, 118.77, 124.76, 125.48, 131.24, 135.02, 137.04, 145.86, 147.78, 149.56, 155.02, 158.96, 168.69, 195.29; FT-IR(KBr) (v max, cm⁻¹): 3353, 2924, 2205, 1654, 1530, 1457, 1350, 1225, 1158, 1100, 1028, 755; HRMS m/z calcd for C₃₇H₃₀ClN₇O₅[M+Na]⁺: 710.19969, found: [M+Na]⁺: 710.19903

CP6: ethyl 6-((4-(3-amino-2-cyano-1H-benzo[γ]chromen-1-yl)-2-methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)-4-(4-chlorophenyl)-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate: Brown solid, M.p 141-144°C, Yield 83%; 1H NMR (500 MHz, DMSO) δH (ppm): 8.19 (s, 2H), 7.79-7.36 (m, 13H), 7.49 (s, 1H), 5.93 (s, 1H), 5.68 (s, 1H), 4.81-4.79 (d, 2H), 4.27-3.25 (d, 2H), 3.11-3.07 (t, 3H), 1.41-1.38 (t, 3H); 13C NMR (DMSO-d6, 500 MHz): 36.50, 39.50, 39.67, 39.84, 40.00, 40.17, 40.26, 40.34, 40.43, 40.50, 40.59, 71.29, 71.47, 82.19, 83.07, 111.69, 118.04, 121.60, 123.27, 124.21, 125.17, 137.06, 140.59, 145.42, 147.02, 155.84, 157.76, 159.58, 165.02, 168.92, 178.89; FT-IR(KBr) (v max, cm⁻¹): 3403, 2920.18, 2223, 1696, 1585, 1510, 1451, 1425, 1375, 1341, 1274, 1145, 1097, 1015, 804, 624; HRMS m/z calcd for C₃₈H₃₂ClN₇O₆ [M⁺]: 717.21026, found: [M⁺]: 717.21045

**Preparation of stock solutions for the measurement**

The stock solution of probe CP1 (100 mL of 1.0×10⁻⁴ M) was prepared in DMSO. Stock solutions of different ions in 0.1mM concentration were prepared in distilled water from their respective salts. From the above stock solutions, 1 equivalent (0.8 ml) of each of the ions were added to 2.5ml of probe solution taken in the cuvette. After mixing, absorbance and fluorescence were measured using a UV-Visible spectrophotometer and fluorescent spectrometer at room temperature respectively.

**Analysis of Fe³⁺ in normal drinking water and distilled water**

Stock solutions of Fe³⁺ were prepared in distilled water and normal drinking water at .01mM, .02mM and .03mM concentrations. Calculated amounts of stock solutions of Fe³⁺ and sample solutions (0.01mM) were added to a vial containing different concentrations of normal water and distilled water.

**Calculation of the Fluorescence Quantum Yield, Limit of detection (LOD) and Association constant**

The fluorescence quantum yield was determined using quinine sulfate (0.54) as standard and was calculated using equation

\[ \Phi_f = \Phi_f^R \frac{F_S A_R \eta_S^2}{F_R A_S \eta_R^2} \]

where \( F_S \) and \( F_R \) are the integrated fluorescence intensities of the sample and reference, \( A_S \) and \( A_R \) are the absorbance of the sample and reference at the excitation wavelength, and \( \eta_S \) and \( \eta_R \) are the refractive indexes of the solvents used for the sample and reference.

The limit of detection (LOD) was calculated from the fluorescence spectra. It was calculated using the plots of concentration versus fluorescence intensity using the equation

\[ \text{LOD} = 3\sigma / K \]

where \( \sigma \) stands for the standard deviation and \( K \) stands for the slope of the graph.

Association constant, \( K_a \) was calculated using the equation

\[ \frac{1}{(F-F_0)} = \frac{1}{[K_a (F_{max}-F_0)]} \times \frac{1}{[\text{ion}]} + \frac{1}{(F_{max}-F_0)} \]

where \( F \) = fluorescence intensity after the addition of metal ions, \( F_0 \) = fluorescence intensity before the addition of metal ions.
intensity before the addition of metal ions, and Fmax=maximum fluorescence intensity observed after the addition of metal ions.

**Molecular docking to find the binding affinities with CDK2**

Docking studies were carried out using Auto Dock Vina 1.1.2. The pdb structure of the protein was recovered from the Brookhaven protein database (http://www.rcsb.org). Water molecules were removed in the first step and then have removed the original inhibitors from the protein structure. The synthesized molecules were optimized through the B3LYP/6-31G basis set using the Gaussian 09 program and were changed to pdbqt coordinates by Autodock Tools, version 1.5.4. After deleting the water molecules, polar hydrogens were added. The docking sites were assigned by making 1Å spacing between grid points by the Auto grid. The center of the grid box was placed at the center of donepezil with coordinates x = 23.903, y = 29.002, z = 25.954, and with exhaustiveness 8. The dimensions of the active site box were set at 24 × 22 × 18 Å. Then these parameters were noted into a conf file split out file using vinasplit and the docking interaction was scanned using Autodock tools.

**In vitro cytotoxicity against human cervical cancer cell line HeLa**

Hela cells were procured from National Centre for Cell Science (Pune, India). The cells were maintained in Dulbecco’s Modified Eagles medium supplemented with 10% FBS, 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 media were changed every two days, and the cells were passaged by trypsinization before the confluence. Cell viability was determined by MTT assay. Cytotoxicity of different sized compounds in the log phase was seeded in 96-well plates at a concentration of 1.0x10⁴ cells/well and incubated overnight at 37°C in a 5% CO₂ humidified environment. The cells were then treated with different concentrations of the samples such as 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL (dissolved with RPMI medium 1640), respectively. Controls were also cultivated under the same conditions without the addition of samples. The treated cells were incubated for 24 h for cytotoxicity analysis. The cells were then subjected to MTT assay. The stock concentration (5 mg/mL) of MTT-(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) was prepared and 100 µL of MTT was added in each sample treated wells and incubated for 4 h. Purple color formazone crystals were observed and these crystals were dissolved with 100 µL of dimethyl sulphoxide (DMSO) and read at 620 nm in a multi well ELISA plate reader (Thermo, Multiskan). The dose-dependent cytotoxicity was observed when the samples treated Hela cells. Fifty percentage of cell death, which determines the inhibitory concentration (IC50) value of samples against Hela cells in 48 h. To study the morphological changes, Hela cells were grown (1 × 10⁵ cells/coverslip) and incubated with samples at their IC50 concentrations, and then they were fixed in a mixture of methanol and acetic acid (3:1, v/v). The coverslips were gently mounted on glass slides for the morphometric analysis. Morphological changes of these cells were analyzed under a Nikon (Japan) bright-field inverted light microscope at 40 magnifications.
Scheme S1: Three component MCR synthesis of Chromene alkynes

Scheme S2: Biginelli multicomponent synthesis of dihydropyrimidinone azides
Table S1: Cheminformatics descriptors of CP1-CP6

<table>
<thead>
<tr>
<th>Entry</th>
<th>MW</th>
<th>TPSA</th>
<th>miLog P</th>
<th>Number of rotatable bonds</th>
<th>Number of Violations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1</td>
<td>669.70</td>
<td>186.66</td>
<td>4.91</td>
<td>10</td>
<td>2</td>
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<tr>
<td>CP2</td>
<td>699.72</td>
<td>195.89</td>
<td>4.50</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>CP3</td>
<td>698.70</td>
<td>212.25</td>
<td>5.35</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>CP4</td>
<td>698.70</td>
<td>212.25</td>
<td>5.30</td>
<td>11</td>
<td>3</td>
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<tr>
<td>CP5</td>
<td>688.14</td>
<td>166.43</td>
<td>6.02</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>CP6</td>
<td>718.17</td>
<td>175.66</td>
<td>5.66</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

Table S2: Docking scores were obtained for CP1-CP6 with CDK2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Docking score/Binding affinity(kcal mol(^{-1}))</th>
<th>Interaction with receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP1</td>
<td>-9.8</td>
<td>ASN132(HB), ASP145, GLN131, GLY13, GLY11, GLU12, ILE10, LEU83, LEU134, LYS88, LYS89, LYS129, LYS33, THR14, PHE80, VAL18</td>
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<tr>
<td>CP2</td>
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<td>ASP145, ALA144, GLN131, ILE10, LEU134, LYS89, LYS33, PHE80, VAL8</td>
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<tr>
<td>CP3</td>
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<td>ALA31, GLN131, LEU134, LYS129, HIS84, PHE80, VAL18</td>
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<tr>
<td>CP4</td>
<td>-9.6</td>
<td>ALA31, ALA144, ASP145, GLU12, GLY11, GLY13, ILE10, LEU134, LYS88, LYS89, PHE80, PHE82, VAL18, VAL64</td>
</tr>
<tr>
<td>CP5</td>
<td>-9.6</td>
<td>ALA31, ASP145(HB), GLN131, HIS84, ILE10, LEU134, LYS89, PHE80, VAL18</td>
</tr>
<tr>
<td>CP6</td>
<td>-9.5</td>
<td>ALA144, ASP145, GLU12, GLY13, ILE10, LYS89, PHE80, THR14(HB), VAL18, VAL64</td>
</tr>
</tbody>
</table>
Figure S1. Docking interaction of CP1-CP6 with CDK2 protein
Figure S2. a, In vitro cytotoxicity assay results with the CP1 against Human cervical cancer cell line HeLa cells (IC50, 15 μg/mL). b, Bright field inverted light microscopy images of CP1 against HeLa cells.
Table S3: Determination of Fe$^{3+}$ in tap water

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added amount (μM)</th>
<th>Found (μM)</th>
<th>Recovery (%)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>0.243</td>
<td>0.232</td>
<td>95.4</td>
<td>4.7</td>
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<tr>
<td></td>
<td>0.476</td>
<td>0.453</td>
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<td>1.082</td>
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<tr>
<td></td>
<td>1.304</td>
<td>1.275</td>
<td>97.7</td>
<td>2.9</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added amount (μM)</th>
<th>Found (μM)</th>
<th>Recovery (%)</th>
<th>Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap Water</td>
<td>0.243</td>
<td>0.229</td>
<td>94.2</td>
<td>6.1</td>
</tr>
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<td>0.458</td>
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<td>0.992</td>
<td>109.1</td>
<td>8.3</td>
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<tr>
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<td></td>
<td>1.304</td>
<td>1.276</td>
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</tr>
<tr>
<td>Sample</td>
<td>Added amount (μM)</td>
<td>Found (μM)</td>
<td>Recovery (%)</td>
<td>Error (%)</td>
</tr>
<tr>
<td>------------</td>
<td>-------------------</td>
<td>------------</td>
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<td>-----------</td>
</tr>
<tr>
<td>Tap water</td>
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<td>0.229</td>
<td>94.2</td>
<td>6.1</td>
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<td>1.281</td>
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</table>

Error(%) is in the range 1.1-8.4

**Figure S3.** Repeat experiments for Fluorescence signaling of Fe³⁺ ions by CP1 in distilled water and tap water for finding out the error range.
Figure S4: Repeat experiments for Job’s plot for receptor Fe$^{3+}$