Supporting Information

3D-QSAR Assisted Design, Synthesis and Evaluation of Novobiocin Analogues

Huiping Zhao,[1] Elisabetta Moroni,[2] Bin Yan,[1] Giorgio Colombo,[2] and Brian S. J. Blagg[1,*]

1. Department of Medicinal Chemistry, 1251 Wescoe Hall Drive, Malott 4070, The University of Kansas, Lawrence, Kansas 66045-7563, USA

2. Istituto di chimica del riconoscimento molecolare, CNR. Via Mario Bianco 9, 20131 Milano, Italy

** Author to whom correspondence should be addressed. Phone: (785) 864-2288. Fax: (785) 864-5326. Email: bblagg@ku.edu.*

The University of Kansas

Material and Methods

Conformational search

All compounds were constructed with building fragments from the standard libraries of MAESTRO[1] v 9.3. The minimum energy conformation was obtained by a conformational search using the Low Mode Sampling algorithm implemented[2,3] in MACROMODEL[4] v 9.9 with the Amber*[5,6] molecular force field and the Polak–Ribiere conjugate gradient (PRCG)[7] minimization method, with an energy convergence criterion of 0.05 kJ□mol−1. The generalized Born equation/surface area (GB/SA)[8] continuum model was used for solvation, with a dielectric constant (ε) of 1. The maximum number of Monte Carlo steps was set to 15000. Generated conformations were saved if within an energy window of 50 kJ□mol^{-1} over the global minimum. Similar structures were excluded based on heavy atom superimposition. All other settings were used as default.

3D-QSAR and statistical analysis with PENTACLE

GRIND[7] descriptors were calculated, analyzed and interpreted using the program PENTACLE[7-9] v 1.0.6. The procedure to obtain these descriptors consists in determining a set of molecular interaction fields (MIFs),[10] which are arrays of non-bonded interaction energy values between a molecule of known structure and a probe group, calculated sampling positions of the probe throughout and around the molecule. In this study MIFs of novobiocin-analogues were computed with different chemical probes: the O probe (carbonyl oxygen) to represent hydrogen bond acceptor

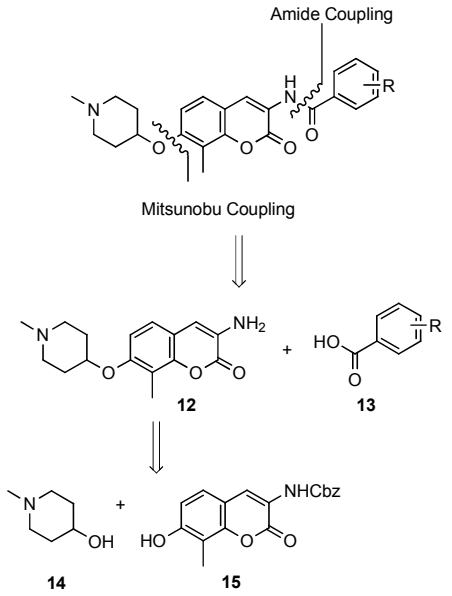
groups, the N1 probe (amide nitrogen) to represent hydrogen bond donor groups, the shape probe TIP[13] to take into account for shape fit between the ligand and the protein and the DRY probe representing hydrophobic interactions. A filtering procedure implemented in PENTACLE (AMANDA[8] algorithm) was applied in order to extract from the computed MIFs highly informative points around each molecule which summarize the most relevant information on binding. The chosen MIFs should represent important types of non-bonded interactions expected to guide the binding of novobiocin-analogues to the active site of Hsp90. All settings were used as default. The encoding procedure to obtain GRIND descriptors from MIFs is an auto- and cross-correlation transform consisting in computing the product of the interaction energy for each pair of points, in such a way that they are no longer dependent on their positions in the 3D space. These products of interaction energies, which encode the geometrical relationship between pairs of non-bonded interactions, are handled according to the distance between the points and only the highest product is stored for a given small range distance (maximum auto- and cross- correlation method[14]). In such a way it is always possible to know which are the chemical groups that produce intense interactions at a certain distance around each molecule.

The statistical analysis, implemented in PENTACLE, consists in calculating the Partial Least-Squares regression (PLS) to derive the 3D-QSAR model. No scaling was applied to the variables. The optimal dimensionality of the model was selected by cross-validation using either LOO or 3RG, recalculating the weights in both cases. The fractional factorial design (FFD),[15] a variable selection methodology

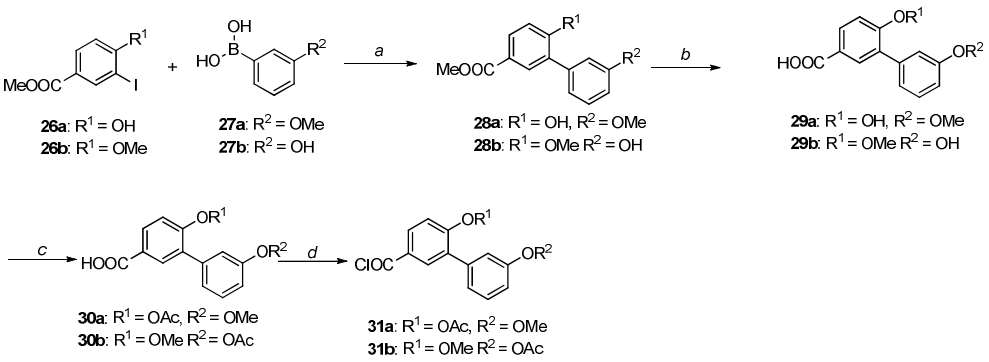
implemented in PENTACLE, allowed the removal of descriptors not correlated with activity.

Table S1. Relevant variables with high impact on the GRIND PLS model

Probe pairs	Variable number	Impact	Interpretation
O-O	OO-120	Direct	Interaction of the NH group of the piperidine ring and the hydroxyl group in 4'-position with the probe O
O-O	OO-102	Direct	Interaction of the NH group of the piperidine ring and the amide/carbamide nitrogen with the probe O
O-N1	ON-529	Direct	Interaction of the NH group of the piperidine ring with the probe O and the oxygen atom in position 3 in the first aromatic ring of the amide side chain with the probe N1
DRY-DRY	DD-43	Direct	Interaction of the first aromatic ring of the amide/carbamide side chain and the carbon chain of the piperidine ring with the probe DRY
O-O	OO-80	Inverse	Interaction of the hydroxyl groups in the sugar moiety with the probe O
N1-N1	NN-141	Inverse	Interaction of the oxygen atoms in the sugar moiety with the probe N



Scheme S1. Retrosynthesis of novobiocin analogues.



Reagents and conditions: *a* $Pd(dppf)_2$, K_2CO_3, dioxane/H_2O *b* LiOH, THF/H_2O/MeOH. *c* Ac_2O, pyridine *d* $SOCl_2$, THF

Scheme S2. Synthesis of intermediate **31a** and **31b**.

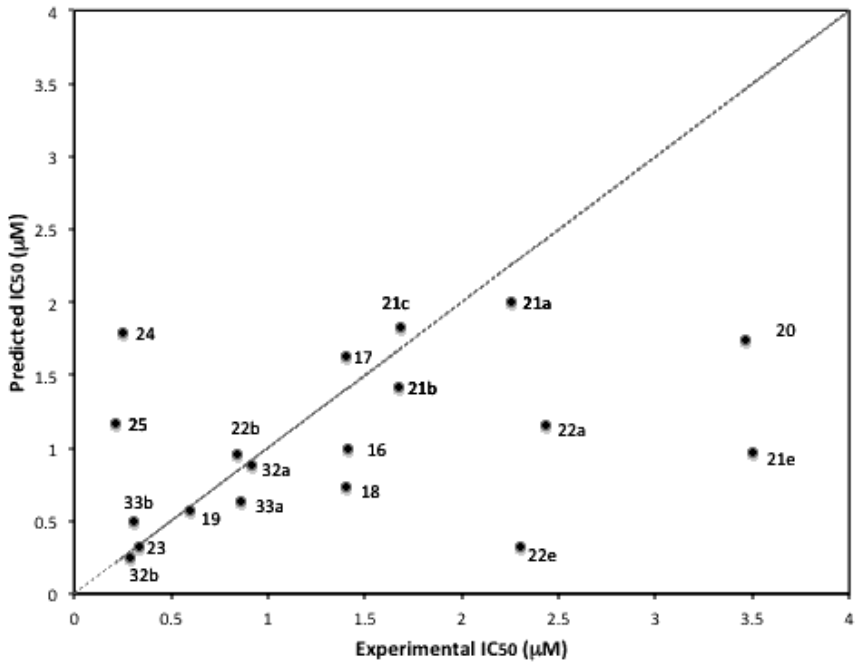
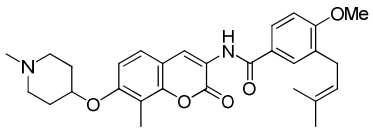


Figure S1. Plot of the predicted versus experimental IC_{50} of the newly synthesized compounds. Prediction of compound **21d** has not been shown. Activities are expressed as μM units. The dashed line represents the trend of the theoretical optimal predictions. The correlation coefficient is r^2=0.68.

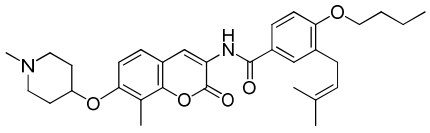
Synthesis and characterization

4-methoxy-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)-3-(3-methylbut-2-enyl)benzamide (16): General procedure for phenol alkylatioon.



Sodium hydride (4.2 mg, 60% in mineral oil, 0.10 mmol) was added to a solution of **2** (50 mg, 0.10 mmol) in DMF (2 mL) at 0 ^{o}C, followed by iodomethane (14 mg, 0.10 mmol). The resulting solution was stirred at 0 ^{o}C for 4 hours, quenched with saturated aqueous ammonium chloride solution and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried (MgSO4), filtered, and concentrated. The residue was purified by column chromatography (SiO2; 10:1, CH_2Cl_2:MeOH) to afford methylether **16** as light brown amorphorous solid (13 mg, 27%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.47 (s, 1H), 7.85 (d, J = 8.5 Hz, 1H), 7.73 (s, 1H), 7.12 (d, J = 8.6 Hz, 1H), 7.00 (d, J = 8.6 Hz, 1H), 5.29~5.17 (m, 1H), 4.34~5.30 (m, 1H), 4.61 (m, 1H), 3.89 (s, 3H), 3.30 (d, J = 7.1 Hz, 2H), 2.66 (m, 2H), 2.42 (s, 3H), 2.29 (s, 3H), 2.25 (s, 3H), 1.99~1.96 (m, 2H), 1.77~1.74 (m, 2H), 1.66 (s, 3H), 1.65 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.1, 159.9, 158.2, 156.6, 149.5, 132.1, 129.2, 128.6, 128.5, 127.1, 126.0, 125.2, 121.9, 121.2, 113.4, 112.6, 110.6, 110.2, 71.6, 55.7, 51.6, 45.2, 29.8, 28.0, 25.4, 17.6, 8.1. HRMS (ESI^{+}) m/z [M+H^{+}] calcd for C29H35N2O5 491.2546, found 491.2541.

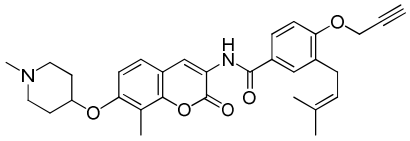
4-butoxy-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)-3-(3-methylbut-2-enyl)benzamide (17).



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.67 (s, 1H), 7.74 (d, J = 8.5 Hz, 1H),

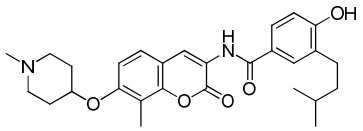
7.71 (s, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 4.34~5.30 (m, 1H), 4.54 (m, 1H), 4.05 (t, *J* = 6.3 Hz, 2H), 3.38 (d, *J* = 6.3 Hz,2H), 2.80~2.77 (m, 2H), 2.62 (m, 2H), 2.45 (s, 3H), 2.35 (s, 3H), 2.18~2.14 (m, 2H), 2.03~1.98 (m, 2H), 1.85~1.80 (m, 2H), 1.77 (s, 3H), 1.74 (s, 3H), 1.56~1.52 (m, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 160.5, 159.7, 156.7, 147.6, 133.5, 131.0, 128.7, 126.7, 125.8, 125.5, 124.1, 122.1, 121.9, 115.3, 113.8, 110.7, 110.6, 71.6, 68.1, 52.0, 45.9, 31.5, 30.1, 28.8, 26.0, 19.5, 18.1, 14.1, 8.6. HRMS (ESI^+) m/z [$M+H^+$] calcd for C32H41N2O5 Exact Mass: 533.3015, found 533.3021.

N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)-3-(3-methyl but-2-enyl)-4-(prop-2-ynyloxy)benzamide (18).



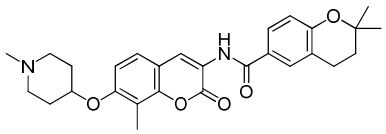
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.66 (s, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 7.27 (s, 1H), 6.99 (d, *J* = 8.7 Hz, 1H), 6.83 (d, *J* = 8.4 Hz, 1H), 5.26~5.23 (m, 1H), 4.55 (m, 1H), 3.32 (d, *J* = 7.3 Hz, 1H), 2.80 ~2.75 (m, 4H), 2.54 (s, 1H), 2.46 (s, 3H), 2.28 (s, 3H), 2.12 (m, 2H), 1.99~1.97 (m, 2H), 1.70 (s, 3H), 1.67 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 159.6, 158.8, 156.4, 149.5, 133.8, 131.5, 128.8, 126.44, 126.39, 125.9, 124.6, 121.7, 121.3, 115.1, 113.7, 111.5, 110.4, 78.1, 76.1, 70.1, 56.1, 51.3, 45.1, 19.1, 28.4, 25.8, 17.9, 8.4. HRMS (ESI^+) m/z [$M+H^+$] calcd for C31H35N2O5 515.2546, found 515.2539.

4-hydroxy-3-isopentyl-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)benzamide (19).



Palladium on carbon (10%, 5 mg) was added to a solution of **2** (48 mg, 0.10 mmol) in anhydrous THF (3 mL) and the solution was placed under an atmosphere of hydrogen. After 12 h, the solution was filtered through SiO2 (10:1, CH_2Cl_2:Methanol) and the eluent was concentrated to afford **19** as a colorless amorphous solid (42 mg, 88%): ^{1}H NMR (400 MHz, $CDCl_3$/MeOD) δ 8.44 (s, 1H), 7.41 (s, 1H), 7.34 (d, *J* = 8.4 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.67 (d, *J* = 8.1 Hz, 1H), 6.61 (d, *J* = 8.0 Hz, 1H), 4.46 (m, 1H), 2.55 ~2.45 (m, 2H), 2.40~2.38 (m, 2H), 2.36~2.31 (m, 2H), 2.14 (s, 3H), 2.07 (s, 3H), 1.84~1.78 (m, 2H), 1.72~1.70 (m, 2H), 1.40~1.37 (m, 1H), 1.29~1.23 (m, 2H), 0.71 (s, 3H), 0.69 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$/MeOD) δ 165.5, 159.5, 156.7, 152.2, 149.6, 136.1, 131.7, 129.6, 125.89, 125.85, 124.67, 123.1, 121.7, 115.3, 113.6, 110.5, 71.1, 51.7, 45.6, 39.3, 29.7, 28.3, 28.1, 22.6, 21.1, 8.6. HRMS (ESI^+) m/z [$M+Na^+$] calcd for C28H34N2NaO5 501.5697, found 501.5694.

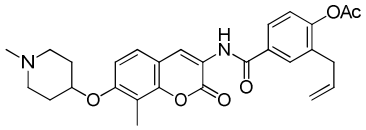
2,2-dimethyl-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl) chroman-6-carboxamide (20).



1 mL of 6 M hydrochloride in dioxane was added to a solution of **2** (25 mg, 0.05 mmol) in dioxane (2 mL) and the resulting solution was stirred at room temperature for 48 hours. The solvent was evaporated and residues was purified (SiO2; 10:1, CH_2Cl_2:Methanol) to afford **20** as a colorless amorphous solid (17 mg, 68%): ^{1}H

NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.59 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.25 (d, *J* = 8.7 Hz, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H), 4.49 (m, 1H), 2.78~2.74 (m, 4H), 2.64 (m, 2H), 2.38 (s, 3H), 2.23 (s, 3H), 2.05~2.01 (m, 2H), 1.98~1.91 (, 4H), 1.55 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 159.7, 159.5, 156.5, 149.4, 129.4, 129.0, 126.7, 125.8, 124.7, 124.4, 121.6, 115.0, 114.9, 113.6, 110.4, 77.2, 70.9, 51.4, 45.4, 45.1, 32.3, 29.2, 26.2, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C28H33N2O5 477.2389, found 477.2394.

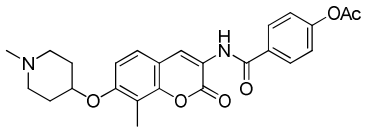
2-allyl-4-((8-methyl-7-((1-methylpiperidin-4-yl)oxy)-2-oxo-2H-chromen-3-yl)carbamoyl)phenyl acetate (21a): General procedure for amide coupling.



Freshly prepared acid chloride from carboxylic acid **13a** (44 mg, 0.2 mmol) was added to a solution of amine **12** (29 mg, 0.1 mmol) in dichloromethane, followed by pyridine (100 μL). The resulting solution was stirred at room temperature for 4 hours, quenched with water and extracted with ethyl acetate; combined organic fractions were dried (MgSO4), filtered, and concentrated. The residue was purified by column chromatography (SiO2, 10:1 CH_2Cl_2:MeOH) to afford **21a** as colorless amorphous solid (32 mg, 65%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.65 (s, 1H, NH), 7.75 (s, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.1 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 5.95~6.04 (m, 1H), 5.09 (d, *J* = 12.0 Hz, 2H), 4.61 (m, 1H), 3.48 (d, *J* = 7.6 Hz, 2H), 2.91~2.80 (m, 4H), 2.56 (s, 3H), 2.32 (s, 3H), 2.29~2.11 (m, 2H), 2.12~2.02 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 165.5,

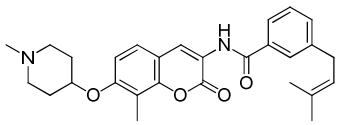
159.6, 157.2, 152.3, 149.7, 135.1, 133.2, 131.9, 129.9, 126.6, 125.9, 124.9, 123.3, 121.6, 117.3, 115.5, 113.5, 110.7, 72.1, 52.3, 46.3, 34.9, 30.6, 21.2, 8.6. HRMS (ESI^+) m/z [$M+H^+$] calcd for C28H31N2O6 491.5555, found 491.5557.

4-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-ylcarbamoyl)phenyl acetate (21b).



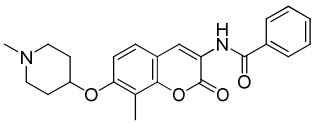
^1H NMR (500 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.52 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.31 (d, *J* = 8.7 Hz, 2H), 7.17 (d, *J* = 7.8 Hz, 1H), 4.92 (m, 1H), 3.46 ~3.12 (m, 4H), 2.76 (s, 3H), 2.31 (s, 3H), 2.25~2.18 (m, 2H), 2.05 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 169.0, 165.1, 158.1, 153.3, 149.8, 131.1, 129.3, 126.3, 122.1, 121.3, 113.6, 113.0, 110.5, 66.9, 48.7, 42.3, 28.4, 20.9, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C25H27N2O6 451.1869, found 451.1875.

N-(8-methyl-7-((1-methylpiperidin-4-yl)oxy)-2-oxo-2H-chromen-3-yl)-3-(3-methylbut-2-en-1-yl)benzamide (21c)



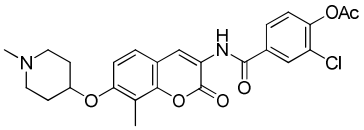
^1H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.71 (s, 1H, NH), 7.70~7.68 (m, 2H), 7.40~7.38 (m, 2H), 7.31 (d, *J* = 8.0 Hz, 1H), 6.85 (d, *J* = 8.1 Hz, 1H), 5.32 (m, 1H), 4.61 (m, 1H), 3.41 (d, *J* = 7.6 Hz, 2H), 2.77 (m, 2H), 2.62 (m, 2H), 2.44 (s, 3H), 2.32 (s, 3H), 2.15 (m, 2H), 1.98 (m, 2H). 1.76 (s, 3H), 1.73 (s, 3H). HRMS (ESI^+) m/z [$M+H^+$] calcd for C28H33N2O4 461.5726, found 461.5729.

N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)benzamide (21d).



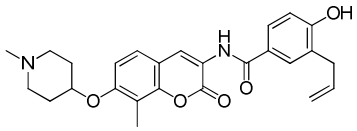
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.58 (s, 1H), 8.45 (s, 1H), 7.94 (d, J = 6.9 Hz, 2H), 7.61 (m, 1H), 7.54 (m, 3H), 7.09 (d, J = 8.2 Hz, 2H), 4.54 (m, 1H), 2.67 (m, 2H), 2.46 (m, 2H), 2.29 (s, 3H), 2.21 (s, 3H), 1.96 (m, 2H), 1.76 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 166.1, 158.4, 156.6, 150.0, 133.7, 132.4, 129.7, 128.9, 127.8, 126.5, 121.4, 113.9, 113.1, 110.8, 70.0, 50.8, 43.5, 28.3, 8.3. HRMS (ESI$^+$) m/z [M+H$^+$] calcd for C23H25N2O4 393.1814, found 393.1819.

2-chloro-4-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-ylcarbamoyl)phenyl acetate (21e).



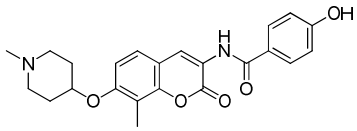
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (s, 1H), 7.99 (s, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 8.7 Hz, 1H), 7.26 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 7.8 Hz, 1H), 4.56 (m, 1H), 2.78 (m, 2H), 2.67 (m, 2H), 2.43 (s, 3H), 2.35 (s, 3H), 2.28 (s, 3H), 2.13~2.08 (m, 2H), 1.95~1.93 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 168.4, 164.3, 159.4, 156.9, 150.1, 149.6, 132.8, 129.7, 129.6, 127.9, 126.7, 126.0, 125.9, 124.2, 121.1, 115.1, 113.3, 110.4, 70.6, 51.5, 45.3, 29.4, 20.5, 8.2. HRMS (ESI$^+$) m/z [M+H$^+$] calcd for C25H26ClN2O6 485.1479, found 485.1486.

3-allyl-4-hydroxy-N-(8-methyl-7-((1-methylpiperidin-4-yl)oxy)-2-oxo-2H-chromen-3-yl)benzamide (22a): General procedure for ester hydrolysis.



Triethylamine (0.1 mL) was added to a solution of **21a** (18 mg, 0.037 mmol) in methanol (1 mL). The solution was stirred at room temperature overnight and concentrated. The residue was purified by column chromatography on silica by using methylene chloride and methanol (10:1) to give **22a** as a white, amorphous solid (13 mg, 79 %). ^{1}H NMR (500 MHz, $CDCl_3$/MeOD) δ 8.68 (s, 1H), 7.64 (s, 1H), 7.60 (d, *J* = 8.7 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.03~5.95 (m, 1H), 5.07~5.05 (m, 2H), 4.50 (m, 1H), 3.39 (d, *J* = 7.6 Hz, 2H), 2.69 (m, 2H), 2.52 (m, 2H), 2.35 (s, 3H), 2.29 (s, 3H), 2.03~2.01 (m, 2H), 1.93~1.92 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4, 159.7, 159.2, 156.6, 149.3, 136.0, 129.4, 127.3, 126.9, 125.7, 124.7, 124.4, 121.5, 115.9, 115.0, 114.9, 113.4, 110.5, 71.7, 51.7, 45.6, 34.0, 29.8, 29.6, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C26H29N2O5 449.2076, found 449.2074.

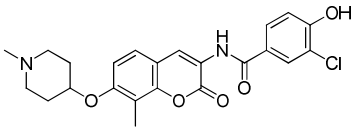
4-hydroxy-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)benzamide (22b).



^{1}H NMR (500 MHz, DMSO-d_6) δ 9.28 (s, 1H), 8,.48 (s, 1H), 7.83 (d, *J* = 8.7 Hz, 2H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.1 1 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 7.8 Hz, 1H), 4.59 (m, 1H), 2.62 (m, 2H), 2.37 (m, 2H), 2.25 (s, 3H), 2.23 (s, 3H), 1.97~1.94 (m, 2H), 1.75~1.73 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.2, 161.1, 158.3, 156.6, 149.5, 129.6, 127.9, 126.0, 124.1, 121.3, 115.2, 113.4, 112.7, 110.7, 71.9, 51.7, 45.5,

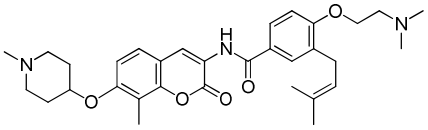
30.0, 8.1. HRMS (ESI^+) m/z [$M+H^+$] calcd for C23H25N2O5 409.1763, found 409.1776.

3-chloro-4-hydroxy-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)benzamide (22e).



^1H NMR (400 MHz, DMSO-d_6) δ 8,.45 (s, 1H), 7.96 (s, 1H), 7.77 (d, J = 8.7 Hz, 2H), 7.51 (d, J = 8.7 Hz, 1H), 7.09~7.06 (m, 2H), 4.56 (m, 1H), 2.59 (m, 2H), 2.32 (m, 2H), 2.24 (s, 3H), 2.23 (s, 3H), 1.97~1.94 (m, 2H), 1.79~1.68 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.3, 158.2, 157.0, 156.9, 149.8, 129.7, 129.4, 128.2, 126.1, 124.8, 121.1, 119.8, 116.4, 113.4, 112.6, 110.8, 72.3, 52.0, 25.9, 30.4, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C23H24ClN2O5 443.1374, found 443.1371.

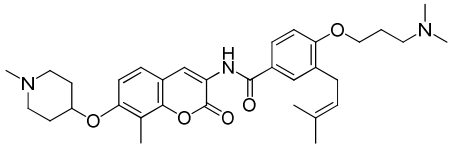
4-(2-(dimethylamino)ethoxy)-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)-3-(3-methylbut-2-enyl)benzamide (23): General procedure for Mitsunobu esterification.



Diisopropylazodicarboxylate (17 mg, 0.08 mmol) was added to a solution of 2-(dimethylamino)ethanol (3.7 mg, 0.04 mmol), phenol **2** (20 mg, 0.04 mmol) and triphenylphosphine (22 mg, 0.08 mmol) in anhydrous THF (5 mL). After 2 h, the solvent was concentrated and the residue purified via column chromatography (SiO_2, 10:1 CH_2Cl_2:Methanol) to afford compound **23** as a colorless amorphous solid (18 mg, 78%). ^1H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.68 (s, 1H), 7.75 (d, J = 8.5 Hz,

1H), 7.72 (s, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 1H), 6.89 (d, *J* = 8.7 Hz, 1H), 5.34~5.30 (m, 1H), 4.47 (m, 1H), 4.17 (t, *J* = 4.6 Hz, 2H), 3.39 (d, *J* = 7.3 Hz, 2H), 2.81 (t, *J* = 4.6 Hz, 2H), 2.65 (m, 2H), 2.38 (s, 6H),2.38~2.35 (m, 2H), 2.35 (s, 3H), 2.32 (s, 3H), 2.05~2.01 (m, 2H), 1.93~1.89 (m, 2H), 1.77 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 160.1, 159.8, 157.1, 149.6, 133.7, 131.1, 128.8, 126.7, 125.9, 125.7, 124.3, 121.9, 121.8, 115.5, 113.6, 110.9, 110.7, 72.7, 67.2, 58.4, 52.6, 46.5, 46.4, 31.0, 28.6, 26.0, 18.1, 8.6. HRMS (ESI^+) m/z [$M+H^+$] calcd for C32H42N3O5 548.6930, found 548.6931.

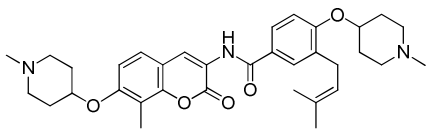
4-(3-(dimethylamino)propoxy)-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)-3-(3-methylbut-2-enyl)benzamide (24).



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 8.68 (s, 1H), 7.74 (d, *J* = 8.5 z, H), 7.72 (s, 1H), 7.32 (d, *J* = 8.6 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 1H), 5.34~5.31 (m, 1H), 4.47 (m, 1H), 4.11 (t, *J* = 4.6 Hz, 2H), 3.38 (d, *J* = 7.2 Hz, 2H), 2.66 (m, 2H), 2.50 (t, *J* = 4.6 Hz, 2H), 2.48~2.42 (m, 2H), 2.41 (s, 3H), 2.33 (s, 3H), 2.28 (s, 6H), 2.05~1.99 (m, 4H), 1.94~1.87 (m, 2H), 1.77 (s, 3H), 1.75 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 160.3, 159.8, 157.1, 149.6, 133.5, 131.0, 128.7, 126.7, 125.6, 124.3, 124.2, 121.9, 121.8, 115.5, 113.6, 110.8, 110.7, 72.8, 66.6, 56.6, 52.6, 46.5, 45.8, 31.0, 28.8, 27.7, 26.0, 18.1, 8.7. HRMS (ESI^+) m/z [$M+H^+$] calcd for C33H44N3O5 562.7196, found 562.7194.

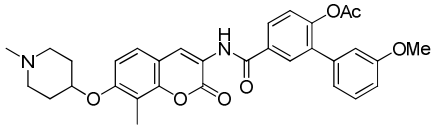
N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)-3-(3-methyl

but-2-enyl)-4-(1-methylpiperidin-4-yloxy)benzamide (25).



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.66 (s, 1H), 7.74~7.72 (m, 2H), 7.31 (d, *J* = 8.6 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 6.87 (d, *J* = 8.7 Hz, 1H), 5.35~5.31 (m, 1H), 4.47 (m, 2H), 3.38 (d, *J* = 7.2 Hz, 2H), 2.70~2.65 (m, 4H), 2.38~2.36 (m, 4H), 2.34 (s, 3H), 2.32 (s, 6H), 2.05~2.00 (m, 4H), 1.93~1.88 (m, 4H), 1.76 (s, 3H), 1.74 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.1, 159.8, 159.7, 157.1, 149.6, 133.4, 131.8, 129.1, 126.6, 125.7, 124.3, 122.0 (2C), 121.9, 115.5, 113.6, 112.0, 110.7, 72.7, 72.0, 53.5, 52.6, 46.5, 46.3, 31.0, 30.9, 28.9, 26.0, 18.2, 8.6. HRMS (ESI^+) m/z [$M+H^+$] calcd for C34H44N3O5 574.7303, found 574.7306.

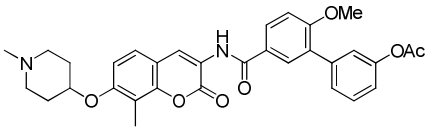
3'-methoxy-5-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-ylca rbamoyl)biphenyl-2-yl acetate (31a).



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.63 (s, 1H), 7.87 (d, *J* = 2.3 Hz, 1H), 7.80 (dd, *J* = 8.5 2.3 Hz, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 7.24 (t, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.93 (dt, *J* = 9.0 1.0 Hz, 1H), 6.89 (t, *J* = 2.4 Hz, 1H), 6.84 (d, *J* = 9.0 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 1H), 4.49 (m, 1H), 3.74 (s, 3H), 2.77~2.73 (m, 2H), 2.70~2.61 (m, 2H), 2.41 (s, 3H), 2.23 (s, 3H), 2.15~2.12 (m, 2H), 2.04 (s, 3H), 1.94~1.91 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.0, 165.1, 159.7, 159.4, 156.7, 151.0, 149.6, 137.8, 135.7, 132.0, 130.1, 129.6, 127.4, 125.9, 124.8, 123.8, 121.6,

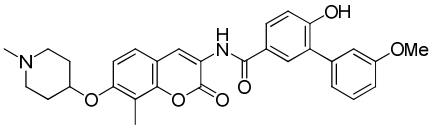
121.3, 115.2, 114.4, 113.9, 113.5, 110.4, 70.6, 55.4, 51.6, 45.5, 29.5, 21.0, 8.5. HRMS (ESI^+) m/z [$M+H^+$] calcd for C32H33N2O7 557.2288, found 557.2288.

2'-methoxy-5'-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-ylcarbamoyl)biphenyl-3-yl acetate (31b).



^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.49 (s, 1H), 8.00 (d, *J* = 2.3 Hz, 1H), 7.94 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.51~7.45 (m, 2H), 7.33 (s, 1H), 7.28 (d, *J* = 8.7 Hz, 1H), 7.17~7.15 (m, 2H), 4.76 (m, 1H), 3.87 (s, 3H), 3.16~3.03 (m, 4H), 2.64 (s, 3H), 2.30 (s, 3H), 2.27 (s, 3H), 2.12~2.11 (m, 2H), 1.94~1.91 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.3, 165.2, 159.0, 158.1, 156.4, 150.3, 149.8, 138.7, 130.0, 129.5, 129.4, 129.1, 128.3, 126.8, 126.2, 125.9, 122.6, 121.4, 120.8, 113.6, 113.0, 111.6, 110.6, 71.6, 56.0, 54.9, 50.6, 28.1, 21.1, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C32H33N2O7 557.2288, found 557.2273.

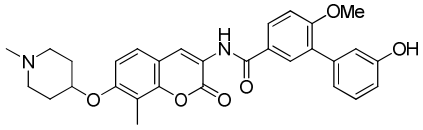
6-hydroxy-3'-methoxy-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)biphenyl-3-carboxamide (32a)



^{1}H NMR (500 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.47 (s, 1H), 7.91 (d, *J* = 2.3 Hz, 1H), 7.81 (dd, *J* = 8.5 2.3 Hz, 1H), 7.56 (d, *J* = 8.7 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.19~7.16 (m, 2H0, 7.13 (d, *J* = 8.9 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.92 (dd, *J* = 8.5 2.3 Hz, 1H), 4.62 (m, 1H), 3.80 (s, 3H), 2.71 (m, 2H), 2.49 (m, 2H), 2.32 (s, 3H), 2.24 (s, 3H), 1.99 (m, 2H), 1.78 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.3,

159.0, 158.3, 158.0, 156.6, 149.7, 139.1, 130.3, 129.1, 128.9, 128.7, 127.4, 126.1, 124.5, 121.6, 121.3, 116.0, 115.1, 113.5, 112.8, 112.3, 110.7, 71.6, 55.1, 51.5, 45.1, 29.8, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C30H31N2O6 515.2182, found 515.2188.

3'-hydroxy-6-methoxy-N-(8-methyl-7-(1-methylpiperidin-4-yloxy)-2-oxo-2H-chromen-3-yl)biphenyl-3-carboxamide (32b).



^{1}H NMR (500 MHz, DMSO-d_6) δ 9.67 (s, 1H), 9.47 (s, 1H), 7.91 (d, J = 2.3 Hz, 1H), 8.48 (s, 1H), 7.99 (dd, J = 8.5 2.1 Hz, 1H), 7.89 (d, J = 2.3 Hz, 1H), 7.57 (d, J = 8.7 Hz, 1H), 7.26~7.22 (m, 2H), 7.15 (d, J = 8.9 Hz, 1H), 6.96~6.94 (m, 2H), 6.77 (dd, J = 8.6 2.1 Hz, 1H), 4.66 (m, 1H), 3.86 (s, 3H), 2.80 (m, 2H), 2.41~2.37 (m, 2H), 2.32 (s, 3H), 2.25 (s, 3H), 2.01 (m, 2H), 1.82 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 165.2, 159.1, 158.2, 157.0, 149.8, 138.6, 129.9, 129.7, 129.5, 129.1, 128.9, 126.2, 125.7, 121.3, 120.1, 116.3, 114.2, 113.5, 111.5, 110.7, 71.8, 55.9, 54.9, 48.6, 29.9, 8.2. HRMS (ESI^+) m/z [$M+H^+$] calcd for C30H31N2O6 515.2182, found 515.2179.

Reference:

(1) Schrödinger LLC, *http://www.schrodinger.com*

(2) Kolossváry, I.; Guida, W. C. *J. Am. Chem. Soc.* **1996**, 118, 5011.

(3) Kolossváry, I.; Guida, W. C. *J. Comp. Chem.* **1999**, 20, 1671.

(4) Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem*. **1990**, 11, 440–467.

(5) Weiner, S. J.; Kollman, P.; Case, D.; Singh, U.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner,P., *J. Am. Chem. Soc.***1984**, 106, 765.

(6) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, 7, 230

(7) Polak, E.; Ribiere, G. *Note sur la Convergence de Méthodes de Directions Conjuguées.* Revenue Francaise Informat. Recherche Operationelle, Serie Rouge, **1969**, 16, 35.

(8) W. C. Still, A. Tempczyk, R. C. Hawley, T. Hendrickson, *J. Am. Chem. Soc.* **1990**, 112, 6127–6129.

(9) M. Pastor, G. Cruciani, I. McLay, S. Pickett, S. Clementi, *J. Med. Chem.* **2000**, 43, 3233–3243

(10) Duran, A.; Comesaña G, Pastor, M. *J. Chem. Inf. Model.* **2008**, 48 (9), 1813-1823.

(11) Duran, A.; Zamora, I; Pastor, M.. *J. Chem. Inf. Model.* **2009**, 49 (9), 2129-2138

(12) P.J. Goodford, *J.Med. Chem.* **1985**, 28, 849-857

(13) Fontaine, F.; Pastor, M.; Sanz, F. *J. Med. Chem*. **2004**, 47 (11), 2805–2815

(14) Clementi, M.; Clementi, S.; Clementi, S.; Cruciani, G.; Pastor, M.. In *Molecular Modelling and Prediction of Bioreactivity*; Gundertofte, K., Jorgensen, F. S., Eds.; Kluwer Academic/Plenum Publishers: New York, **2000**; 207−212.

(15) M. Baroni, G. Costantino, G. Cruciani, D. Riganelli, R. Valigi, S. Clementi, *Quant. Struct.-Act. Relat.* **1993**, 12, 9–20.