Selective Affinity-Based Probe for Oncogenic Kinases Suitable for Live cell Imaging

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Supplemental infomation

General Techniques. Chemicals were purchased and used as received. Reactions were carried out under ambient conditions unless stated otherwise. Anhydrous solvents were obtained by passing them through commercially available alumina columns (Innovative Technology Inc. RVA). Standard syringe techniques were applied for the transfer of dry solvents and air- or moisture-sensitive reagents. Reactions were followed using TLC on silica gel-coated alumina plates (Merck 60 F_{254}) with the indicated solvent mixture. Detection was performed with UV-light (254 nm), and/or by heating after dipping into a solution of either $KMnO_4$ (10 g/L), K_2CO_3 (67 g/L) and NaOH (0.83 g/L) in H_2O or ninhydrin (33 g/L) in ethanol. $^{1}H/^{13}C$ NMR spectra were recorded at ambient temperature on a Bruker DMX 400 machine in the specified deuterated solvents. Chemical shifts are given in ppm with respect to the residual undeuterated solvent signal as internal standard. Coupling constants are reported as *J*-values in Hz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = doublet of doublet, m = multiplet, br = broad. Column or flash chromatography was carried out using Merck silica gel (particle size 40-63 μm). LC-MS were recorded using an HP1100 series or Thermo Electron Corporation HPLC with a Thermo Finnezau Surveyor MSQ mass spectrometer system; a Thermo Scientific column (50 x 2.1 mm) was used. Fluorescence microscopic images were recorded using a Nikon Eclipse Ti inverted fluorescence microscope and a Photometrices Cool Snap HQ^2 CCD camera (Nikon). The filter sets used were Semrock Bright Line Exciter 325-375, Dichroic 405, and Emitter 435-485 for DAPI; Exciter 460-495,

Dichroic 505, and Emitter 510-560 for **FA**; Exciter 520-555, Dichroic 558, and Emitter 560-590 for **Cy3-RA1 (8).** Nikon NIS-Elements AR 3.10 imaging software was used for imaging.

Abreviations. DIPEA = *N*,*N*-Diisopropylethylamine**;** EOMCl = (chloromethoxy)ethane; PE = petroleum ether; DMF = *N,N*-Dimethylformamide.

Procedure A: General procedure for the esterification with DIC activation: To a stirred solution of the corresponding acid (1.0 equiv) in DMF, was added DIC (1.0 equiv) and the resulting solution was stirred at 23°C for 10 min. Then the corresponding alcohol (1.0 equiv) and Et_3N (1.2 equiv) were added and the mixture was stirred overnight. Evaporation of the solvents followed by column chromatography (PE/ EtOAc) gave the desired ester.

Procedure B: General procedure for the Mitsunobu esterificaton: To a stirred solution of the acid (1.0 equiv), alcohol (1.0 equiv) and triphenylphosphine (1.0 equiv) in THF, was added dropwise DIAD (1.0 equiv), the resulting solution was stirred at 23°C for 14 hours. The reaction mixture was evaporated in vacuo and purified by column chromatography (PE/EtOAc) to give the corresponding ester.

Procedure C: General procedure for the esterification with NHS: To a stirred solution of acid (1.0 equiv) in THF, NHS (1.0 equiv) and DCC (1.0 equiv) were added and the resulting mixture was stirred at 23 °C for 4 hours. Evaporation of solvents gave the crude NHS intermediate. The crude NHS intermediate was reacted with the corresponding alcohol which was pre-treated with NaH (1.0 equiv) in THF at 23 °C. The reaction mixture was quenched with water and extracted with ethyl acetate, dried over Na_2SO_4 evaporated and purified by column chromatography to give the corresponding ester.

Procedure D: General procedure for the amidation with DIC or EDC activation: 2-hydroxy-5-nitro benzoic acid (**1a**) (1.0 equiv) and DIC or EDC.HCl (1.0 equiv) in DMF (0.2 M) were stirred at 23°C for 15 min, then the corresponding amine (1.0 equiv) was added and stirring was continued for 18 hours. Evaporation of the solvents followed by column chromatography (PE/EtOAc 10:1) gave the desired amide.

Procedure E: General procedure amidation with oxalyl chloride activation. To a stirred solution of the corresponding acid (1.0 equiv) in dry CH_2Cl_2 (0.2 M) under N_2 were added $(COCl)_2$ (1.3 equiv) and dropwise DMF (cat.) and stirred until the gas evolution had ceased (5 min). This solution was added directly to the appropriate amine (1.1 equiv) and NEt_3 (1.5 equiv) in dry CH_2Cl_2 (0.3 M) under N_2 at 0 °C and then stirred for 10 min at 23 °C. After quenching with sat. $NaHCO_3$ (aq) the mixture was extracted with EtOAc twice and the combined organic layers were dried over Na_2SO_4. Evaporation of the solvents followed by column chromatography (PE/EtOAc 10:1) gave the desired amide.

Procedure F: General procedure for the reduction with zinc.[1] Following a literature procedure, to a solution of the appropriate nitro compound (1.0 equiv) in dry CH_2Cl_2 (0.036 M) were added at 0 °C zinc dust (14.0 equiv) and acetic acid (150 equiv). After stirring 10 min at room temperature, the mixture was filtered over Celite©, quenched with sat. $NaHCO_3$ (aq.) and extracted with EtOAc (3x). The organic layers were dried over Na_2SO_4 and concentrated to give the crude amine, which was used in the subsequent reaction without further purification.

Procedure G: General procedure for the addition of the Michael acceptor: To a stirred solution of the corresponding crude amine (1.0 equiv) in dry CH_2Cl_2 (0.2 M) was added at −40 °C (or at 0 °C when protected substrates were used) DIPEA (1.2 equiv) and dropwise acryloyl chloride (0.9 equiv) and stirring was continued for 15 min. After quenching with sat. $NaHCO_3$ (aq), the mixture was extracted with CH_2Cl_2 (3x) dried over Na_2SO_4 and the solvent was evaporated. Column chromatography (PE/EtOAc) gave the desired acrylate. In the case of propiolic acid the following procedure GI was used. **GI:** To a solution of propiolic acid (2.0 equiv) in DCM was added DCC (2.0 equiv) and stirred at 23 °C for 10 min and the amine (1.0 equiv) and DMAP (20 mol%) were added and further stirred for 15 min. The reaction mixture was treated with aq $NaHCO_3$ and extracted with ethyl acetate. The crude material was purified by flash chromatography to give the corresponding amide.

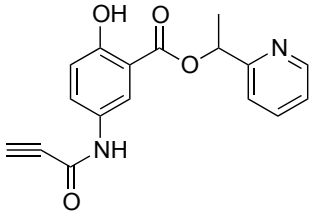
Procedure H: General procedure for the EOM-protection To a stirred solution of the alcohol (1.0 equiv) in DMF (0.16 M) was added at 0 °C DIPEA (1.5 equiv) and dropwise EOMCl (1.5 equiv) after which stirring was continued for 24 hours. $NaHCO_3$ (sat. aq) was added to the reaction mixture after which an extraction with Et_2O (3x) was carried out. The combined organic layers were dried over Na_2SO_4, after which the solvent was evaporated and column chromatography (PE/EtOAc 3:1) was performed to give the protected derivative.

Procedure I: General procedure for the EOM deprotection. To a stirred solution of the corresponding EOM-ether protected compound (1.0 equiv) in MeOH (0.05 M) was added sulfonic acid resin (10.0 equiv, 3.0 mmol/g) at 23°C. After 6 hours the mixture was filtered and the solvent was evaporated. The crude product was purified by column chromatography (PE/EtOAc 3:1) to afford the desired alcohol.

Procedure J: General procedure for reduction with Pd/C: To a solution of the corresponding nitro compound (1.0 equiv.) in MeOH (3 mL) was added 5% Pd/C (40 mol%). The mixture was stirred overnight at 23°C under a H_2 atmosphere (balloon). Then, the catalyst was removed upon filtration through a short pad of celite eluting with MeOH. The filtrate was concentrated under reduced pressure and the residue was purified by flash chromatography to afford the corresponding amine .

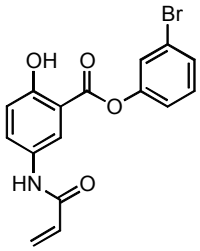
Procedure K: General procedure for deprotection of Trimethyl silyl ethyl group: To a solution of the corresponding TMSE ester in THF was added TBAF (4 equiv) and stirred at 23°C for 6 hours. The reaction mixture was quenched with aq. NH_4Cl and extracted with ethylacetae. The organic layer was extracted with sat. $NH_4Cl_{(aq)}$ (X 2). The crude acid was used as such in next step.

[1] Belén Cid, M.; Duce, S.; Morales, S.; Rodrigo, E.; Luis García Ruano J. Nitrophenylacetonitriles as Versatile Nucleophiles in Enantioselective Organocatalytic Conjugate Additions. *Org. Lett.* **2010**, *12*, 3586-3589.



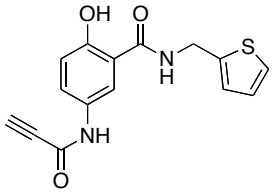
RA1

1-(pyridin-2-yl) ethyl 2-hydroxy-5-propiolamidobenzoate (RA1): Starting from compound **1a** and using procedure B followed by F and finally GI yielded **RA1** in a 43% over all yield. R_f = 0.37 (PE/EtOAc: 1/1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.06 (d, J = 2.8 Hz, 1H), 7.66 (dd, J = 8.8, 2.8 Hz, 1H), 7.47-7.35 (m, 5H), 6.98 (d, J = 8.8, Hz, 1H), 6.16 (q, J = 6.8 Hz, 1H), 2.95 (s, 1H), 1.72 (d, J = 6.8 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{17}H_{15}N_2O^+$: 311.10; found: 311.24.



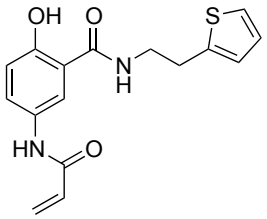
RA2

3-bromophenyl 5-acrylamido-2-hydroxybenzoate (RA2): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA2** in a 15% over all yield. R_f = 0.43 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.2 (s, 1H), 8.38 (d, J = 2.0 Hz, 1H), 7.68 (dd, J = 8.8, 2.0 Hz, 1H), 7.49-7.37 (m, 4H), 6.48 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 16.8, 10.0 Hz, 1H), 5.81 (dd, J = 10.4, 0.8 Hz, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{16}H_{13}BrNO_4^+$: 360.90; found: 362.09.



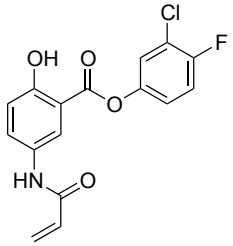
RA3

2-Hydroxy-5-propiolamido-*N*-(thiophen-2-ylmethyl)benzamide (RA3) Starting from compound **1a** and using procedure D followed by F and finally E yielded **RA3** in a 20% over all yield. R_f = 0.35 (PE/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.25 (s, 1H), 8.02 (d, J = 2.4 Hz, 1H), 7.77 (br s, 1H), 7.24 (dd, J = 5.2, 0.8 Hz, 1H), 7.19 (dd, J = 8.4, 2.4 Hz, 1H), 7.04 (d, J = 3.2 Hz, 1H), 6.97-6.93 (m, 3H), 4.78 (d, J = 5.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{15}H_{13}N_2O_3S^+$: 301.07; found: 301.17.



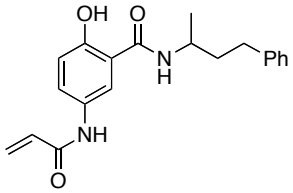
RA4

5-acrylamido-2-hydroxy-N-(2-(thiophen-2-yl)ethyl)benzamide(RA4) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA4** in a 20% over all yield. R_f = 0.35 (PE/EtOAc 1:1);^{1}H (MeOD, 400 MHz, 25 °C) δ = 8.02 (d, *J* = 2.4 Hz, 1H), 7.58 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.24 (dd, *J* = 4.0, 0.8 Hz, 1H), 6.97-6.89 (m, 3H), 6.39 (t, *J* = 2.8 Hz, 2H), 5.78 (dd, *J* = 9.2, 2.4 Hz, 1H), 3.67 (d, *J* = 7.2 Hz, 2H), 3.17 (t, *J* = 7.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{16}H_{17}N_2O_3S^+$: 316.08; found: 317.22.



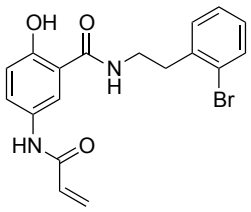
RA5

3-chloro-4-fluorophenyl 5-acrylamido-2-hydroxybenzoate(RA5) Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA5** in a 15% over all yield. R_f = 0.28 (PE/EtOAc 6:4);^{1}H (MeOD, 400 MHz, 25 °C) δ = 10.2 (s, 1H), 8.38 (s, 1H), 7.63 (d, *J* = 8.8 Hz, 1H), 7.31 (d, *J* = 6.0 Hz, 1H), 7.19-7.09 (m, 2H), 7.04 (d, *J* = 8.8 Hz, 1H), 6.46 (d, *J* = 16.8 Hz, 1H), 6.24 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.81 (d, *J* = 10.0 Hz, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{16}H_{12}ClFNO_4^+$: 335.03; found: 336.18.



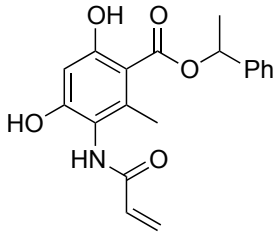
RA6

5-Acrylamido-2-hydroxy-*N*-(4-phenylbutan-2-yl)benzamide (RA6) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA6** in a 44% over all yield. R_f = 0.45 (PE/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.47 (br s, 1H), 8.03 (s, 1H), 7.39 (s, 1H), 7.28-7.14 (m, 6H), 6.93 (d, *J* = 8.8 Hz, 1H), 6.45-6.37 (m, 2H), 6.28 (dd, *J* = 17.2, 10.4 Hz, 1H), 5.80 (d, *J* = 10.4 Hz, 1H), 4.24 (quin, *J* = 7.6 Hz, 1H), 2.69 (t, *J* = 8.0 Hz, 2H), 1.96-1.83 (m, 2 H), 1.28 (d, *J* = 5.2 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{20}H_{23}N_2O_3^+$: 339.17; found: 339.28.



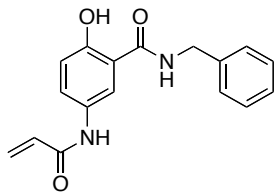
RA7

5-acrylamido-*N*-(2-bromophenethyl)-2-hydroxybenzamide (RA7): Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA7** in a 35% over all yield. R_f = 0.37 (PE/EtOAc 2:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 12.3 (s, 1H), 8.19 (s, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.43(s, 1H), 7.28 (s, 2H), 7.18-7.10 (m, 2H), 6.95 (d, J = 8.4 Hz, 1H), 6.80 (s, 1H), 6.44 (d, J = 16.4 Hz, 1H), 6.25 (dd, J = 16.8, 10.0 Hz, 1H), 5.80 (d, J = 10.4 Hz, 1H), 3.71 (t, J = 7.2 Hz, 2H), 3.10 (t, J = 7.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{18}H_{18}BrN_2O_3^+$:390.04; found: 390.16.



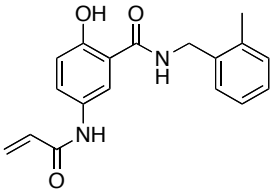
RA8

1-phenylethyl 3-acrylamido-4,6-dihydroxy-2-methylbenzoate (RA8): Starting from compound **1b**[2] and using procedure J followed by G, K and finally E yielded **RA8** in a 32% over all yield. R_f = 0.3 (PE/EtOAc 2:3); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.46 (d, J = 7.6 Hz, 2H), 7.39 (t, J = 8.4 Hz, 2H), 7.32 (d, J = 7.2 Hz, 1H), 6.51 (dd, J = 17.2, 10.4 Hz, 1H), 6.37-6.32 (m, 2H), 6.16 (q, J = 6.4 Hz, 1H), 5.79 (dd, J = 10.0, 1.6 Hz, 1H), 1.69 (d, J = 6.8 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{19}H_{20}NO_5^+$: 342.13; found: 342.14.



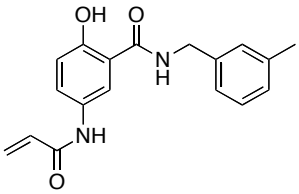
RA9

5-Acrylamido-*N*-benzyl-2-hydroxybenzamide (RA9) Starting from compound **1a** and using procedure D followed by F and finally G yielded RA9 in a 30% over all yield. R_f = 0.25 (PE/EtOAc 3:2); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.32 (s, 1H), 8.24 (d, J = 2.4 Hz, 1H), 7.35-7.28 (m, 5 H), 7.24 (br s, 1H), 7.13 (dd, J = 8.8, 2.4 Hz, 1H), 7.00 (brs, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.42 (d, J = 16.8 Hz, 1H), 6.24 (dd, J = 16.8, 10.0 Hz, 1H), 5.79 (d, 10.0 Hz, 1H) 4.63 (d, J = 5.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For : $C_{17}H_{17}N_2O_3^+$: 297.13; found: 297.24.



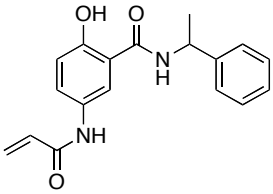
RA10

5-Acrylamido-2-hydroxy-*N*-(2-methylbenzyl)benzamide (RA10) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA10** in a 45% over all yield. R_f = 0.35 (PE/EtOAc 3:2); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.31 (br s, 1H), 8.16 (d, *J* = 2.4 Hz, 1H), 7.28-7.14 (m, 5H), 6.96 (d, *J* = 9.2 Hz, 2H), 6.66 (br s, 1H), 6.40 (d, *J* = 16.8 Hz, 1H), 6.20 (dd, *J* = 16.8, 10.0 Hz), 5.77 (d, *J* = 10 Hz, 1H), 2.36-2.34 (m, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For : $C_{18}H_{19}N_2O_3^+$: 311.14; found: 311.25.



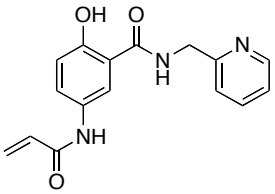
RA11

5-Acrylamido-2-hydroxy-*N*-(3-methylbenzyl)benzamide (RA11) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA11** in a 39% over all yield. R_f = 0.25 (PE/EtOAc 3:2); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.31 (br s, 1H), 8.22 (d, 3.2 Hz, 1H), 7.25-7.23 (m, 1H), 7.15-7.12 (m, 4H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.73 (br s, 1H), 6.39 (d, *J* = 16.8 Hz, 1H), 6.43 (dd, *J* = 16.8, 10.0 Hz, 1H), 5.80 (d, *J* = 10.0 Hz, 1H), 4.6 (d, *J* = 5.2 Hz), 2.35 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For : $C_{18}H_{19}N_2O_3^+$: 311.14; found: 311.27.



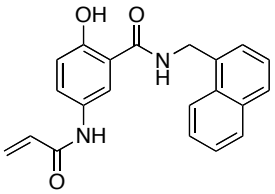
RA12

5-Acrylamido-2-hydroxy-*N*-(1-phenylethyl)benzamide (RA12) Starting from compound **1a** and using procedure E followed by F and finally G yielded **RA12** in a 43% over all yield. R_f = 0.35 (PE/EtOAc 3:2); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.27 (br s, 1H), 8.19 (d, *J* = 2.4 Hz, 1H), 7.39-7.25 (m, 5H), 7.15 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 6.87 (br s, 1H), 6.43 (d, *J* = 16.8 Hz, 1H), 6.25 (dd, *J* = 16.8, 10.0 Hz, 1H), 5.78 (d, *J* = 10.0 Hz, 1H), 5.30 (quin, *J* = 7.2 Hz, 1H), 1.60 (d, *J* = 7.2 Hz, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For : $C_{18}H_{19}N_2O_3^+$: 311.14; found: 311.26.



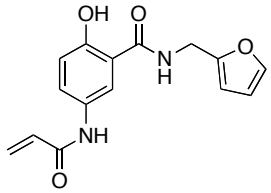
RA13

5-Acrylamido-2-hydroxy-*N*-(pyridin-2-ylmethyl)benzamide (RA13) Starting from compound **1a** and using procedure E followed by F and finally G yielded **RA13** in a 25% over all yield. R_f = 0.10 (PE/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.23 (br s, 1H), 8.99 (br s, 1H), 8.21-8.18 (m, 1H), 8.12 (s, 1H), 7.70-7.63 (m, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.12 (dd, *J* = 15.8, 9.6 Hz, 1H), 6.89 (d, *J* = 9.6 Hz, 1H), 6.58 (d, *J* = 15.8 Hz, 1H), 6.42-6.22 (m, 1H), 6.01 (d, *J* = 9.6 Hz, 1H), 5.70-5.66 (m, 2H), 4.72-4.68 (m, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{16}H_{16}N_3O_3^+$: 298.12; found: 298.21.



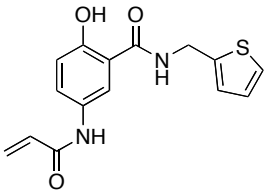
RA14

5-Acrylamido-2-hydroxy-*N*-(naphthalen-1-ylmethyl)benzamide (RA14) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA14** in a 44% over all yield. R_f = 0.25 (PE/EtOAc 3:2); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 12.37 (br s, 1H), 8.05 (d, *J* = 2.8 Hz, 1H), 7.90-7.83 (m, 2H), 7.57-7.42 (m, 4 H), 7.18 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.97 (d, *J* = 8.8 Hz, 1H), 6.80 (br s, 1H), 6.36 (d, *J* = 16.8 Hz, 1H), 6.18 (dd, *J* = 16.8, 10.0 Hz, 1H), 5.74 (d, *J* = 10.0 Hz, 1H), 5.09 (d, *J* = 5.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{21}H_{19}N_2O_3^+$:347.14; found: 347.25.



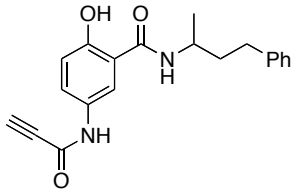
RA15

5-Acrylamido-*N*-(furan-2-ylmethyl)-2-hydroxybenzamide (RA15) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA15** in a 31% over all yield. R_f = 0.25 (PE/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 8.03 (d, *J* = 2.4 Hz, 1H), 7.56 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.44 (s, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 6.44-6.31 (m, 4H), 5.76 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.58 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{15}H_{15}N_2O_4^+$: 287.11; found: 287.22.



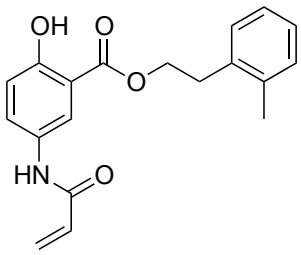
RA16

5-Acrylamido-2-hydroxy-*N*-(thiophen-2-ylmethyl)benzamide (RA16) Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA16** in a 44% over all yield. R_f = 0.20 (PE/EtOAc 1:1); ^{1}H NMR (400 MHz, CD_3OD, 23 °C) δ 8.03 (d, *J* = 2.4 Hz, 1H), 7.56 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.30 (d, *J* = 4.8 Hz, 1H), 7.06 (d, *J* = 3.2 Hz, 1H), 6.96 (dd, *J* = 5.2, 3.6 Hz, 1H), 6.89 (d, *J* = 9.2 Hz, 1H), 6.44-6.32 (m, 2H), 5.75 (dd, *J* = 9.2, 2.4 Hz, 1H) 4.76 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{15}H_{16}N_2O_3S^+$: 303.08; found: 303.18.



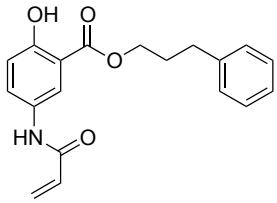
RA17

2-Hydroxy-*N*-(4-phenylbutan-2-yl)-5-propiolamidobenzamide (RA17): Starting from compound **1a** and using procedure D followed by F and finally GI yielded RA17 in a 15% over all yield. R_f = 0.40 (PE/EtOAc 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, 23 °C) δ 7.69 (d, *J* = 2.4 Hz, 1H), 7.54 (s, 1H), 6.94 (d, *J* = 8.8 Hz, 1H), 6.19 (d, *J* = 8.0 Hz, 1H), 2.95 (s, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{21}N_2O_3^+$:337.16; found: 337.25.



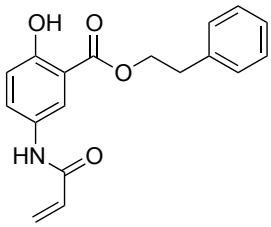
RA18

2-methylphenethyl 5-acrylamido-2-hydroxybenzoate (**RA18**): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA18** in a 30% over all yield. R_f = 0.3 (PE/EtOAc 2:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) *δ* = 10.6 (s, 1H), 8.14 (d, *J* = 2.4 Hz, 1H), 7.62 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.27-7.18 (m, 4H), 6.99 (d, *J* = 8.8 Hz, 1H), 6.48 (d, *J* = 16.4 Hz, 1H), 6.26 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.81 (d, *J* = 10.4 Hz, 1H), 4.54 (t, *J* = 7.6 Hz, 2H), 3.13 (t, *J* = 7.6 Hz, 2H), 2.42 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{20}NO_4^+$: 326.16; found: 326.29.



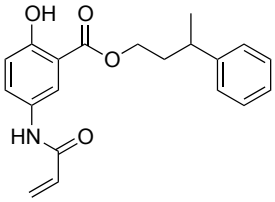
RA19

3-phenylpropyl 5-acrylamido-2-hydroxybenzoate (RA19): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA19** in a 32% over all yield. R_f = 0.31 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 7.99 (s, 1H), 7.67 (dd, J = 8.8, 2.4 Hz, 1H), 7.35-7.28 (m, 3H), 7.25-7.21 (m, 2H), 6.99 (d, J = 8.8 Hz, 1H), 6.47 (d, J = 17.2 Hz, 1H), 6.27 (dd, J = 16.8, 10.4 Hz, 1H), 5.81 (d, J = 10.4 Hz, 1H), 4.39 (t, J = 6.4 Hz, 2H), 2.81 (t, J = 7.6 Hz, 2H), 2.19-2.12 (m, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{20}NO_4^+$: 326.16; found: 326.28.



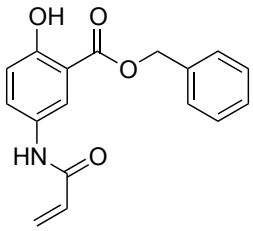
RA20

Phenethyl 5-acrylamido-2-hydroxybenzoate (RA20): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA20** in a 35% over all yield. R_f = 0.31 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.12 (d, J = 2.4 Hz, 1H), 7.62 (dd, J = 8.8, 2.4 Hz, 1H), 7.39-7.26 (m, 5H), 7.17 (s, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.48 (d, J = 16.8 Hz, 1H), 6.26 (dd, J = 16.8, 10.0 Hz, 1H), 5.82 (d, J = 10.0 Hz, 1H), 4.58 (t, J = 7.2 Hz, 2H), 3.13 (t, J = 7.6 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{18}NO_4^+$: 312.12; found: 312.25.



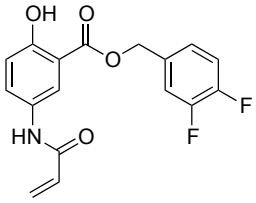
RA21

3-phenylbutyl 5-acrylamido-2-hydroxybenzoate (RA21): Starting from compund **1a** and using procedure A followed by F and finally G yielded **RA21** in a 30% over all yield. R_f = 0.3 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 7.88 (d, J = 2.4 Hz, 1H), 7.69 (dd, J = 8.8, 2.4 Hz, 1H), 7.36-7.32 (m, 2H), 7.25-7.21 (m, 3H), 7.17 (s, 1H), 6.98 (d, J = 8.8 Hz, 1H), 6.48 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 16.8, 10.4 Hz, 1H), 5.82 (d, J = 10.0 Hz, 1H), 4.58 (td, J = 7.2, 1.6 Hz, 2H), 2.98-2.92 (m, 1H), 2.14-2.09 (m, 2H), 1.36 (d, J = 7.2 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{22}NO_4^+$: 340.15; found: 340.29.



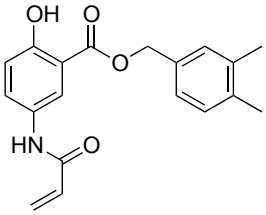
RA22

Benzyl 5-acrylamido-2-hydroxybenzoate (RA22): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA22** in a 40% over all yield. R_f = 0.3 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.04 (d, J = 2.4 Hz, 1H), 7.70 (dd, J = 8.8, 2.0 Hz, 1H), 7.47-7.36 (m, 5H), 7.34 (s, 1H), 6.99 (d, J = 8.8 Hz, 1H), 6.44 (d, J = 17.2 Hz, 1H), 6.23 (dd, J = 16.8, 10.4 Hz, 1H), 5.77 (d, J = 10.0 Hz, 1H), 5.40 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{17}H_{16}NO_4^+$: 298.10; found: 298.23.



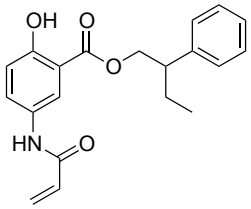
RA23

3,4-difluorobenzyl 5-acrylamido-2-hydroxybenzoate (RA23): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA23** in a 32% over all yield. R_f = 0.37 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.5 (s, 1H), 8.14 (d, J = 2.8 Hz, 1H), 7.66 (dd, J = 9.2, 2.8 Hz, 1H), 7.37-7.17 (m, 3H), 7.01 (d, J = 9.2 Hz, 1H), 6.46 (d, J = 17.2 Hz, 1H), 6.24 (dd, J = 16.8, 10.4 Hz, 1H), 5.81 (d, J = 10.0 Hz, 1H), 5.35 (s, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{17}H_{14}F_2NO_4^+$: 334.08; found: 334.23.



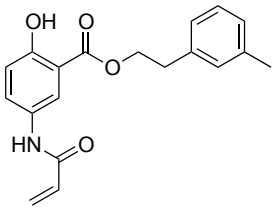
RA24

3,4-dimethylbenzyl 5-acrylamido-2-hydroxybenzoate (RA24): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA24** in a 36% over all yield. R_f = 0.4 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 7.99 (d, J = 2.4 Hz, 1H), 7.70 (dd, J = 8.8, 2.4 Hz, 1H), 7.45 (s, 1H), 7.21-7.16 (m, 3H), 6.96 (d, J = 8.8 Hz, 1H), 6.43 (d, J = 16.8 Hz, 1H), 6.22 (dd, J = 17.2, 10.4 Hz, 1H), 5.76 (d, J = 10.4 Hz, 1H), 5.32 (s, 2H), 2.30 (s, 3H), 2.29 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{20}NO_4^+$: 326.13; found: 326.29.



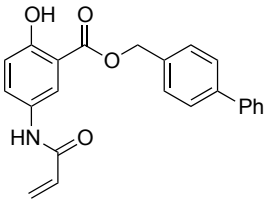
RA25

2-phenylbutyl 5-acrylamido-2-hydroxybenzoate (RA25): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA25** in a 27 % over all yield. R_f = 0.41 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 7.97 (d, J = 2.4 Hz, 1H), 7.67 (dd, J = 8.8, 2.0 Hz, 1H), 7.38-7.25 (m, 7H), 6.97 (d, J = 9.2 Hz, 1H), 6.47 (d, J = 16.8 Hz, 1H), 6.26 (dd, J = 16.8, 10.4 Hz, 1H), 5.81 (d, J = 10.0 Hz, 1H), 4.49 (d, J = 6.8 Hz, 2H), 3.10-3.02 (m, 1H), 1.96-1.89 (m, 1H), 1.79-1.71 (m, 1H), 0.92 (t, J = 7.6 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{22}NO_4^+$: 340.15; found: 340.28.



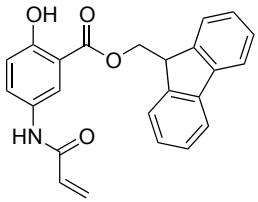
RA26

3-methylphenethyl 5-acrylamido-2-hydroxybenzoate (RA26): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA26** in a 32% over all yield. R_f = 0.4 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.13 (d, J = 2.0 Hz, 1H), 7.63 (dd, J = 8.8, 2.0 Hz, 1H), 7.26-7.21 (m, 1H), 7.13-7.10 (m, 3H), 6.99 (d, J = 8.8 Hz, 1H), 6.48 (d, J = 16.8 Hz, 1H), 6.26 (dd, J = 16.8, 10.4 Hz, 1H), 5.82 (d, J = 10.0 Hz, 1H), 4.56 (t, J = 7.2 Hz, 2H), 3.10 (t, J = 7.2 Hz, 2H), 2.37 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{20}NO_4^+$: 326.13; found: 326.29.



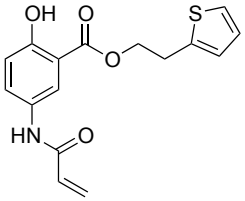
RA27

[1,1'-biphenyl]-4-ylmethyl 5-acrylamido-2-hydroxybenzoate (RA27): Starting from compund **1a** and using procedure A followed by F and finally G yielded **RA27** in a 38% over all yield. R_f = 0.41 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.66 (s, 1H), 8.09 (d, J = 2.8 Hz, 1H), 7.72 (d, J = 9.2 Hz, 1H), 7.65-7.62 (m, 4H), 7.60-7.49 (m, 4H), 7.33-7.32 (m, 1H), 7.00 (d, J = 9.2 Hz, 1H), 6.44 (d, J = 16.8 Hz, 1H), 6.26 (dd, J = 16.8, 10.4 Hz, 1H), 5.78(d, J = 10.4 Hz, 1H), 5.44 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{23}H_{20}NO_4^+$: 374.13; found: 374.29.



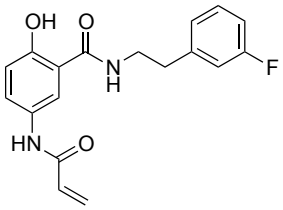
RA28

(9*H*-fluoren-9-yl)methyl 5-acrylamido-2-hydroxybenzoate (RA28): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA28** in a 25% over all yield. R_f = 0.36 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.66 (s, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.94-7.90 (m, 3H), 7.87-7.77 (m, 2H), 7.68-7.48 (m, 4H), 7.26 (s, 1H), 6.98 (d, J = 8.8 Hz, 1H), 6.36 (d, J = 16.8 Hz, 1H), 6.09 (dd, J = 16.8, 10.0 Hz, 1H), 5.83 (s, 2H), 5.69(d, J = 10.4 Hz, 1H), 5.33(s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{24}H_{20}NO_4^+$: 386.13; found: 386.28.



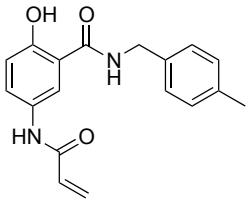
RA29

2-(thiophen-2-yl)ethyl 5-acrylamido-2-hydroxybenzoate (RA29): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA29** in a 28% over all yield. R_f = 0.36(PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.66 (s, 1H), 8.12 (d, J = 2.4 Hz, 1H), 7.66 (dd, J = 9.2, 2.0 Hz, 1H),.26 (s, 1H), 7.20 (dd, J = 5.6, 1.2 Hz, 1H),6.99-6.94 (m, 3H), 6.46 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 17.2, 10.4 Hz, 1H), 5.79(d, J = 10.4 Hz, 1H), 4.57 (t, J = 7.2 Hz, 2H), 3.32 (t, J = 7.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{16}H_{16}NO_4S^+$: 318.08; found: 318.21.



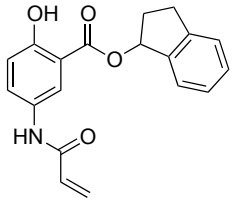
RA30

5-acrylamido-*N*-(3-fluorophenethyl)-2-hydroxybenzamide (RA30): Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA30** in a 35% over all yield. R_f = 0.34 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 12.2 (s, 1H), 8.21 (s, 1H), 7.33-7.29 (m, 1H), 7.18-7.04 (m, 3H), 6.98 (d, J = 16.8 Hz, 1H), 6.70 (s, 1H), 6.46 (d, J = 16.8 Hz, 1H), 6.25 (dd, J = 16.8, 10.0 Hz, 1H), 5.83 (d, J = 10.4 Hz, 1H), 3.71 (t, J = 7.2 Hz, 2H), 3.10 (t, J = 7.2 Hz, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{18}FN_2O_3^+$: 329.13; found: 329.25.



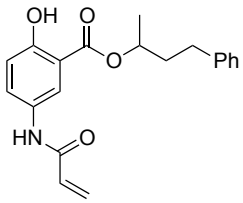
RA31

5-acrylamido-2-hydroxy-*N*-(4-methylbenzyl)benzamide (RA31): Starting from compound **1a** and using procedure D followed by F and finally G yielded **RA31** in a 30% over all yield. R_f = 0.38 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 12.3 (s, 1H), 8.21 (d, J = 2.8 Hz, 1H), 7.30-7.14 (m, 5H), 6.98 (d, J = 8.8 Hz, 1H), 6.43 (d, J = 16.8 Hz, 1H), 6.23 (dd, J = 16.8, 10.0 Hz, 1H), 5.82 (d, J = 10.0 Hz, 1H), 4.61 (d, J = 5.6 Hz, 2H), 2.39 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{19}N_2O_3^+$: 311.14; found: 311.27.



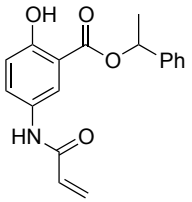
RA32

2,3-dihydro-1*H*-inden-1-yl 5-acrylamido-2-hydroxybenzoate RA32: Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA32** in a 26% over all yield. R_f = 0.53 (PE/EtOAc 7:3);^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.8 (s, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.76 (dd, J = 8.4, 2.0 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.38-7.33 (m, 2H), 7.27-7.25 (m, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.49 (dd, J = 6.4, 3.2 Hz, 1H), 6.39 (d, J = 16.8 Hz, 1H), 6.18 (dd, J = 17.2, 10.0 Hz, 1H), 5.73 (d, J = 10.0 Hz, 1H), 3.25-3.17 (m, 1H), 3.01-2.93 (m, 1H), 2.67-2.58 (m, 1H), 2.32-2.24 (m, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{17}NO_4^+$: 324.12; found: 324.26.



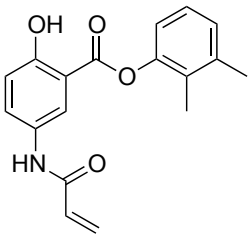
RA33

4-phenylbutan-2-yl 5-acrylamido-2-hydroxybenzoate RA33: Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA33** in a 28% over all yield. R_f = 0.32 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.8 (s, 1H), 7.99 (d, J = 2.4 Hz, 1H), 7.69 (dd, J = 8.8, 2.8 Hz, 1H), 7.45 (s, 1H), 7.31-7.20 (m, 5H), 6.98 (d, J = 8.8 Hz, 1H), 6.47 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 16.8, 10.4 Hz, 1H), 5.79 (d, J = 10.0 Hz, 1H), 5.25-5.21 (m, 1H), 2.77-2.72 (m, 2H), 2.19-2.08 (m, 1H), 2.01-1.95 (m, 1H), 1.41 (d, J = 6.4 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{22}NO_4^+$: 340.15; found: 340.28.



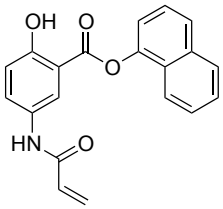
RA34

1-phenylethyl 5-acrylamido-2-hydroxybenzoate (RA34): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA34** in a 28% over all yield. R_f = 0.45 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.14 (d, J = 2.4 Hz, 1H), 7.69 (dd, J = 8.8, 2.8 Hz, 1H), 7.51 (s, 1H), 7.46-7.32 (m, 5H), 6.96 (d, J = 9.2 Hz, 1H), 6.46 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 17.2, 10.4 Hz, 1H), 6.16 (q, J = 6.8 Hz, 1H), 5.79 (d, J = 10.0 Hz, 1H), 1.71 (d, J = 6.8 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{18}NO_4^+$: 312.12; found: 312.26.



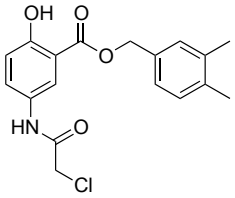
RA35

2,3-dimethylphenyl 5-acrylamido-2-hydroxybenzoate (RA35): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA35** in a 22% over all yield. R_f = 0.22 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.46 (s, 1H), 8.39 (d, J = 2.4 Hz, 1H), 7.75 (dd, J = 8.8, 2.4 Hz, 1H), 7.48 (s, 1H), 7.21-7.13 (m, 2H), 7.05 (d, J = 9.2 Hz, 1H), 6.97 (d, J = 8.0 Hz, 1H), 6.47 (d, J = 17.2 Hz, 1H), 6.27 (dd, J = 17.2, 10.4 Hz, 1H), 5.80 (d, J = 10.0 Hz, 1H), 2.35 (s, 3H), 2.14 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{17}NO_4^+$: 312.12; found: 312.26.



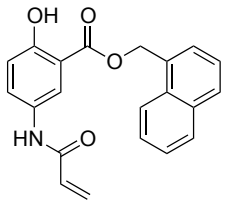
RA36

Naphthalen-1-yl 5-acrylamido-2-hydroxybenzoate (RA36): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA36** in a 24% over all yield. R_f = 0.18 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.3 (s, 1H), 8.53 (d, J = 2.0 Hz, 1H), 7.95(d, J = 1.6, Hz, 1H), 7.89 (dd, J = 18.0, 10.0 Hz, 2H), 7.79 (dd, J = 8.8, 2.4 Hz, 1H), 7.64 (s, 1H), 7.58-7.51 (m, 4H), 7.35 (d, J = 7.2 Hz, 1H), 7.06 (d, J = 9.2 Hz, 1H), 6.47 (d, J = 16.8 Hz, 1H), 6.28 (dd, J = 16.8, 10.4 Hz, 1H), 5.78 (d, J = 10.4 Hz, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{15}NO_4^+$: 334.10 found: 334.21.



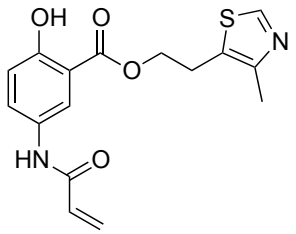
RA37

3,4-dimethylbenzyl 5-(2-chloroacetamido)-2-hydroxybenzoate (RA37): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA37** in a 30% over all yield. R_f = 0.45 (PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.61 (s, 1H), 7.94 (d, J = 2.4 Hz, 1H), 7.72 (dd, J = 8.8, 2.8 Hz, 2H), 7.24-7.20 (m, 3H), 7.01 (d, J = 8.8 Hz, 1H), 5.35 (s, 2H), 4.23 (s, 2H), 2.32 (s, 3H), 2.31 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{19}ClNO_4^+$: 348.08 found: 348.18.



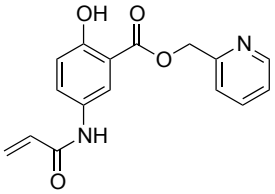
RA38

Naphthalen-1-ylmethyl 5-acrylamido-2-hydroxybenzoate (RA38): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA38** in a 32% over all yield. R_f = 0.47 (PE/EtOAc 3:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.44 (d, J = 8.0 Hz, 1H), 7.91 (t, J = 8.0 Hz, 2H), 7.81-7.75 (m, 2H), 7.62-7.47 (m, 4H), 7.35 (s, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.35 (d, J = 17.2 Hz, 1H), 6.07 (dd, J = 17.2, 10.4 Hz, 1H), 5.82 (s, 2H), 5.67 (d, J = 10.0 Hz, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{21}H_{18}NO_4^+$: 348.08 found: 348.18.



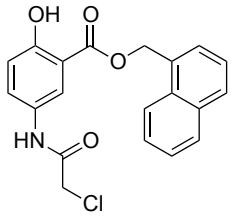
RA39

2-(4-methylthiazol-5-yl) ethyl 5-acrylamido-2-hydroxybenzoate (RA39): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA39** in a 35% over all yield. R_f = 0.15 (PE/EtOAc 2:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.5 (s, 1H), 8.63 (s, 1H), 8.14 (d, J = 1.6 Hz, 1H), 7.83 (s, 1H), 7.58 (dd, J = 8.8, 2.0 Hz, 1H), 6.96 (d, J = 8.8 Hz, 1H), 6.46 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 16.8, 10.0 Hz, 1H), 5.77 (d, J = 10.0 Hz, 1H), 4.51 (t, J = 6.8 Hz, 2H), 3.26 (t, J = 6.8 Hz, 2H), 2.46 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{16}H_{17}N_2O_4S^+$: 333.09 found: 333.18.



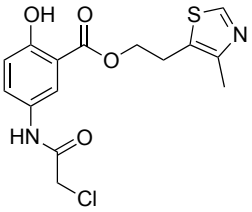
RA40

Pyridin-2-ylmethyl 5-acrylamido-2-hydroxybenzoate (RA40): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA40** in a 30% over all yield. R_f = 0.21 (PE/EtOAc 2:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.5 (s, 1H), 8.65 (d, J = 4.0 Hz, 1H), 8.14 (s, 1H), 7.79-7.72 (m, 2H), 7.46 (d, J = 7.6, Hz, 1H), 7.39 (s,1H), 7.30 (s,1H), 7.01 (d, J = 8.8 Hz, 1H), 6.45 (d, J = 16.8, Hz, 1H), 5.79 (d, J = 10.0 Hz, 1H), 5.53 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{16}H_{15}N_2O_4^+$: 299.10 found: 299.21.



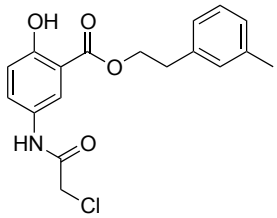
RA41

Naphthalen-1-ylmethyl 5-(2-chloroacetamido)-2-hydroxybenzoate (RA41): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA41** in a 32% over all yield. R_f = 0.41 (PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.11 (d, J = 7.6 Hz, 1H), 8.09 (s, 1H), 7.96-7.92 (m, 2H), 7.84 (d, J = 2.8, Hz, 1H), 7.72 (dd, J = 8.8, 2.8 Hz, 1H), 7.67-7.51 (m, 4H), 7.02 (d, J = 9.2, Hz, 1H), 5.87 (s, 2H), 4.15 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{17}ClNO_4^+$: 371.07 found: 371.19.



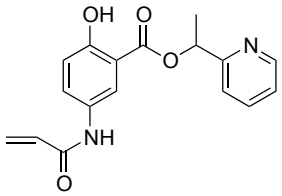
RA42

2-(4-methylthiazol-5-yl)ethyl 5-(2-chloroacetamido)-2-hydroxybenzoate (RA42): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA42** in a 30% over all yield. R_f = 0.11(PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.65 (s, 1H), 8.23 (s, 1H), 8.10 (d, J = 2.8 7.58 (dd, J = 8.8, 2.4 Hz, 1H), 7.01 (d, J = 8.8, Hz, 1H), 4.54 (t, J = 6.4 Hz, 1H), 4.22 (s, 2H), 3.29 (t, J = 6.8 Hz, 1H), 2.48 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{15}H_{16}ClN_2O_4S^+$: 355.05 found: 355.17.



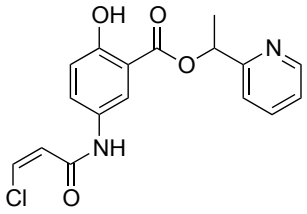
RA43

3-methylphenethyl 5-(2-chloroacetamido)-2-hydroxybenzoate (RA43): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA43** in a 36% over all yield. R_f = 0.5(PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.17 (s, 1H), 8.06 (d, J = 2.8, Hz, 1H), 7.64 (dd, J = 8.8, 2.8 Hz, 1H), 7.25(d, J = 6.4, Hz, 1H), 7.13-7.09 (m, 3H), 7.01(d, J = 8.8, Hz, 1H), 4.56 (t, J = 7.6 Hz, 1H), 4.24 (s, 2H), 3.09 (t, J = 7.6 Hz, 1H), 2.83 (s, 3H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{19}ClNO_4^+$: 348.09 found: 348.79.



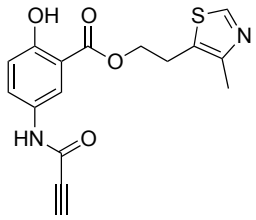
RA44

1-(pyridin-2-yl)ethyl 5-acrylamido-2-hydroxybenzoate (RA44): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA44** in a 30% over all yield. R_f = 0.31 (PE/EtOAc 2:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.63 (d, J = 4.4, Hz, 1H), 8.15 (d, J = 2.4, Hz, 1H), 7.77-7.72 (m, 2H), 7.53 (s, 1H), 7.44 (d, J = 8.0, Hz, 1H), 7.27-7.25 (m, 1H), 6.97(d, J = 8.8, Hz, 1H), 6.46 (d, J = 16.8 Hz, 1H), 6.30-6.18 (m, 2H), 5.80 (d, J = 11.2 Hz, 1H), 1.77 (d, J = 6.8 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{17}H_{17}N_2O_4^+$: 313.11 found: 313.22.



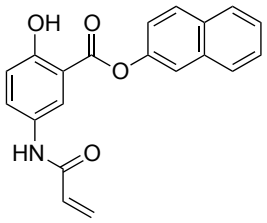
RA45

(*Z*)-1-(pyridin-2-yl)ethyl 5-(3-chloroacrylamido)-2-hydroxybenzoate (RA45): Starting from compound **1a** and using procedure B followed by F and finally E yielded **RA45** in a 18% over all yield. R_f = 0.24 (PE/EtOAc 4:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.12 (d, J = 2.4, Hz, 1H), 7.93 (s, 1H), 7.68 (dd, J = 9.2, 2.8, Hz, 1H), 7.48-7.33 (m, 5H), 6.99 (d, J = 8.8, Hz, 1H), 6.66(d, J = 8.8, Hz, 1H), 6.35 (d, J = 8.8 Hz, 1H), 6.17 (q, J = 6.8 Hz, 1H), 1.73 (d, J = 6.4 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{17}H_{16}ClN_2O_4^+$: 347.08 found: 347.17.



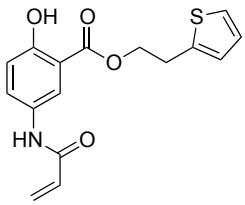
RA46

2-(4-methylthiazol-5-yl) ethyl 2-hydroxy-5-propiolamidobenzoate (RA46): Starting from compound **1a** and using procedure B followed by F and finally GI yielded **RA46** in a 25% over all yield. R_f = 0.16 (PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.66 (s, 1H), 8.10 (d, J = 2.8, Hz, 1H), 7.79 (s, 1H), 7.56 (dd, J = 8.8, 2.8 Hz, 1H), 6.99(d, J = 8.8, Hz, 1H), 4.54 (t, J = 6.4 Hz, 1H), 3.28 (t, J = 7.6 Hz, 1H), 2.96 (s, 1H), 2.47 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{16}H_{15}N_2O_4S^+$: 331.107 found: 331.20.



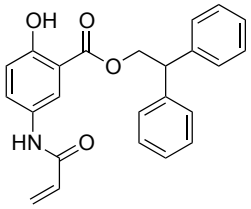
RA47

Naphthalen-2-yl 5-acrylamido-2-hydroxybenzoate (RA47): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA47** in a 28% over all yield. R_f = 0.19 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.4 (s, 1H), 8.46 (d, J = 2.8, Hz, 1H), 7.96-7.86 (m, 3H), 7.75 (dd, J = 8.8, 2.0 Hz, 1H), 7.70 (d, J = 2.0, Hz, 1H), 7.58-7.52 (m, 2H), 7.37 (dd, J = 8.8, 2.4 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 6.50 (d, J = 16.8 Hz, 1H), 6.29 (dd, J = 16.8, 10.0 Hz, 1H), 5.83 (d, J = 10.0 Hz, 1H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{20}H_{16}NO_4^+$: 334.10 found: 334.22.



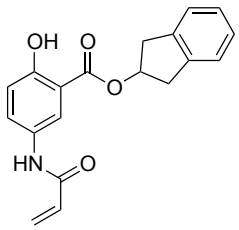
RA48

2-(thiophen-2-yl)ethyl 5-acrylamido-2-hydroxybenzoate (RA48): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA48** in a 40% over all yield. R_f = 0.46 (PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.46 (d, J = 2.8, Hz, 1H), 7.61 (dd, J = 8.8, 2.4 Hz, 1H), 7.45 (s, 1H), 7.33-7.31 (m,1H), 7.12 (d, J = 2.0 Hz, 1H), 7.05 (dd, J = 4.0, 1.2 Hz, 1H), 6.97 (d, J = 8.8 Hz, 1H), 6.47 (dd, J = 16.8, 0.8 Hz, 1H), 6.27 (dd, J = 16.8, 10.0 Hz, 1H), 5.79 (dd, J = 10.0, 0.8 Hz, 1H),4.56 (t, J = 6.8, Hz, 2H), 3.14 (t, J = 6.8 Hz, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{16}H_{16}NO_4S^+$: 317.07 found: 318.20.



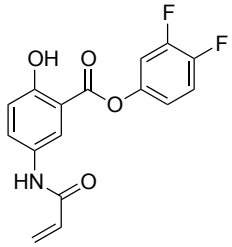
RA49

2,2-diphenylethyl 5-acrylamido-2-hydroxybenzoate (RA49): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA49** in a 40% over all yield. R_f = 0.48 (PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.5 (s, 1H), 7.81 (s, 1H), 7.71 (d, J = 9.2, Hz, 1H), 7.38-7.25 (m,11H), 6.96 (d, J = 9.2 Hz, 1H), 6.45 (d, J = 16.0 Hz, 1H), 6.27 (dd, J = 16.8, 10.0 Hz, 1H),5.79 (d, J = 10.0 Hz, 1H), 4.91 (d, J = 7.6 Hz, 1H), 4.56 (t, J = 7.2, Hz, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{24}H_{22}NO_4^+$: 387.14 found: 388.27.



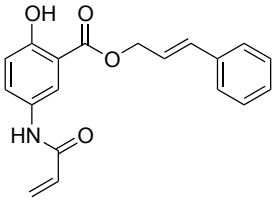
RA50

2,3-dihydro-1*H*-inden-2-yl 5-acrylamido-2-hydroxybenzoate (RA50): Starting from compound **1a** and using procedure E followed by F and finally G yielded **RA50** in a 15% over all yield. R_f = 0.44 (PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 7.82 (s, 1H), 7.80 (s, 1H), 7.32-7.24 (m, 4H), 7.21 (s, 1H), 7.00 (d, J = 9.2 Hz, 1H), 6.43 (d, J = 16.8 Hz, 1H), 6.27 (dd, J = 16.8, 10.0 Hz, 1H), 5.84- 5.83 (m, 1H), 3.48 (dd, J = 17.2, 6.8 Hz, 2H), 3.23 (dd, J = 17.2, 3.2 Hz, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{18}NO_4^+$: 323.11 found: 324.21.



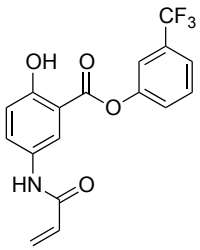
RA51

3,4-difluorophenyl 5-acrylamido-2-hydroxybenzoate (RA51): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA51** in a 10% over all yield. R_f = 0.37 (PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.0 (s, 1H), 8.42 (d, J = 2.4 Hz, 1H), 7.73 (dd, J = 9.2 2.0 Hz, 1H), 7.19-7.14 (m, 2H), 7.07-7.03 (m, 2H), 6.47 (d, J = 16.0 Hz, 1H), 6.28 (dd, J = 16.8, 10.0 Hz, 1H), 5.79 (d, J = 10.0 Hz, 1H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{16}H_{12}F_2NO_4^+$: 319.06 found: 320.21.



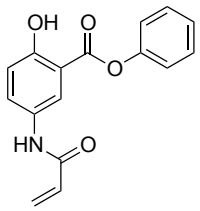
RA52

Cinnamyl 5-acrylamido-2-hydroxybenzoate (RA52): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA52** in a 40% over all yield. R_f = 0.41 (PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.10 (d, J = 2.0 1H), 7.62 (dd, J = 8.8, 2.4 Hz, 2H), 7.41 (d, J = 6.8 Hz, 1H), 7.33 (t, J = 5.2 Hz, 1H), 7.26-7.24 (m, 1H), 7.19-7.16 (m, 1H), 6.95 (d, J = 9.2 Hz, 1H), 6.73 (d, J = 16.0 Hz, 1H), 6.39- 6.32 (m, 2H), 5.74 (d, J = 10.4 Hz, 1H), 4.99 (d, J = 15.2 Hz, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{19}H_{18}NO_4^+$: 323.11 found: 324.23.



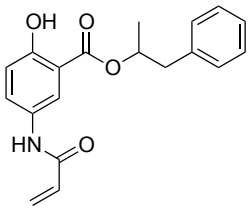
RA53

3-(trifluoromethyl)phenyl 5-acrylamido-2-hydroxybenzoate (RA53): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA53** in a 10% over all yield. Rf = 0.48 x(PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.2 (s, 1H), 8.45 (d, J = 2.4 Hz, 1H), 7.68 (dd, J = 8.8, 2.4 Hz, 1H), 7.61 (d, J = 5.4 Hz, 1H), 7.52 (s, 1H), 7.46-7.43 (m, 2H), 7.05 (d, J = 9.2 Hz, 1H), 6.49 (d, J = 16.8 Hz, 1H), 6.28 (dd, J = 16.8, 10.4 Hz, 1H), 5.82 (d, J = 10.4 Hz, 1H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{17}H_{13}F_3NO_4^+$: 351.07 found: 352.18.



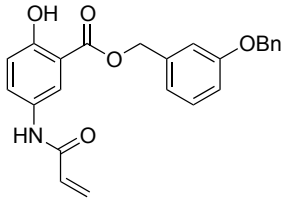
RA54

Phenyl 5-acrylamido-2-hydroxybenzoate(RA54): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA54** in a 10% over all yield. Rf = 0.33 (PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.4 (s, 1H), 8.38 (s, 1H), 7.74-7.73 (m, 1H), 7.51-7.46(m, 2H), 7.36-7.32 (m, 2H), 7.24-7.22 (m, 2H), 7.08-7.05 (m, 1H), 6.49 (d, J = 16.8 Hz, 1H), 6.31-6.24 (m, 1H), 5.83 (dd, J = 9.2, 2.8 Hz, 1H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{16}H_{14}NO_4^+$: 283.08 found: 284.20.



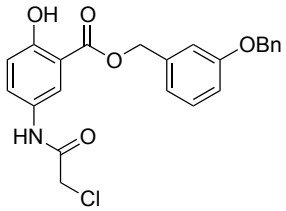
RA55

1-phenylpropan-2-yl 5-acrylamido-2-hydroxybenzoate (RA55): Starting from compound **1a** and using procedure C followed by F and finally G yielded **RA55** in 40% over all yield. R*f* = 0.42 (PE/EtOAc 6:4); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.15 (s, 1H), 7.62 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.47 (s, 1H), 7.34-7.23(m, 5H), 6.96 (dd, *J* = 8.8, 2.8 Hz, 1H), 6.48 (d, *J* = 16.8 Hz, 1H), 6.28 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.80 (d, *J* = 10.0 Hz, 1H), 5.44-5.38 (m, 1H), 3.14-3.09 (m, 1H), 2.94-2.89 (m, 1H), 1.38 (d, *J* = 6.4 Hz, 3H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For $C_{19}H_{20}NO_4^+$: 325.13 found: 326.27.



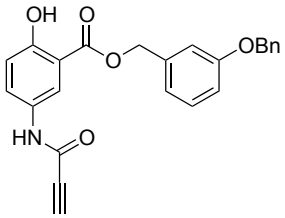
RA56

3-(benzyloxy)benzyl 5-acrylamido-2-hydroxybenzoate (RA56): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA56** in 40% over all yield. R*f* = 0.58 (PE/EtOAc 8:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.04 (d, *J* = 2.4 Hz, 1H), 7.73 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.47-7.30 (m, 7H), 7.09-6.98 (m, 4H), 6.42 (d, *J* = 16.8 Hz, 1H), 6.21 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.77 (d, *J* = 10.4 Hz, 1H), 5.37 (s, 2H), 5.12 (s, 1H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{24}H_{22}NO_5^+$: 403.13 found: 403.91.



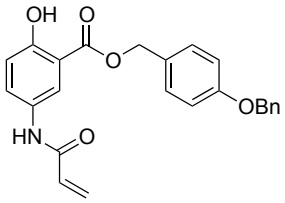
RA57

3-(benzyloxy)benzyl 5-(2-chloroacetamido)-2-hydroxybenzoate(RA57): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA57** in a 40% over all yield. R*f* = 0.44 (PE/EtOAc 8:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.16 (s, 1H), 7.99 (d, *J* = 2.8 Hz, 1H), 7.70 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.48-7.33 (m, 6H), 7.10-6.99 (m, 4H), 5.39 (s, 2H), 5.12 (s, 2H), 4.20 (s, 2H). LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{23}H_{21}ClNO_5^+$: 425.10 found: 426.12.



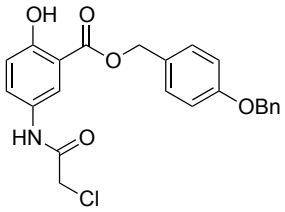
RA58

3-(benzyloxy)benzyl 2-hydroxy-5-propiolamidobenzoate (RA58): Starting from compound **1a** and using procedure B followed by F and finally GI yielded **RA58** in a 10% over all yield. R*f* = 0.45 (PE/EtOAc 1:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 7.97 (d, *J* = 2.8 Hz, 1H), 7.48 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.41-7.35 (m, 6H), 7.09-7.00 (m, 4H), 5.38 (s, 2H), 5.13 (s, 2H), 2.95 (s, 1H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{24}H_{20}NO_5^+$: 401.12 found: 402.19.



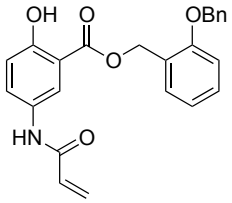
RA59

4-(benzyloxy)benzyl 5-acrylamido-2-hydroxybenzoate (RA59): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA59** in a 40% over all yield. R*f* = 0.61 (PE/EtOAc 8:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 7.73 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.47-7.38 (m, 7H), 7.18 (s, 1H), 7.03-6.98 (m, 3H), 6.44 (d, *J* = 16.8 Hz, 1H), 6.20 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.79 (d, *J* = 10.4 Hz, 1H), 5.34 (s, 2H), 5.11 (s, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{24}H_{22}NO_5^+$: 403.14 found: 403.76.



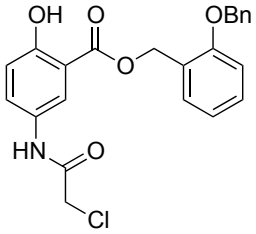
RA60

4-(benzyloxy)benzyl 5-(2-chloroacetamido)-2-hydroxybenzoate (RA60): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA60** in a 40% over all yield. R*f* = 0.50 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.14 (s, 1H), 7.94 (d, *J* = 2.8 Hz, 1H), 7.70 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.47-7.34 (m, 7H), 7.04-7.01 (m, 3H), 5.35 (s, 2H), 5.11 (s, 2H), 4.21 (s, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{23}H_{21}ClNO_5^+$: 425.10 found: 426.12.



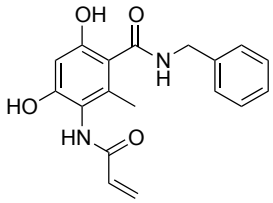
RA61

2-(benzyloxy)benzyl 5-acrylamido-2-hydroxybenzoate (RA61): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA61** in a 40% over all yield. R*f* = 0.61 (PE/EtOAc 8:2); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.6 (s, 1H), 7.89 (s, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.45-7.31 (m, 7H), 7.16 (m, 1H), 7.04-6.98 (m, 3H), 6.43 (d, *J* = 16.8 Hz, 1H), 6.27 (dd, *J* = 16.8, 10.0 Hz, 1H), 5.77 (d, *J* = 10.4 Hz, 1H), 5.52 (s, 2H), 5.16 (s, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{24}H_{22}NO_5^+$: 403.14 found: 403.76.



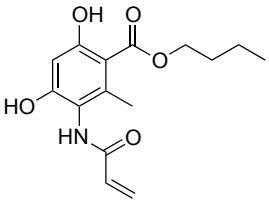
RA62

2-(benzyloxy)benzyl 5-(2-chloroacetamido)-2-hydroxybenzoate (RA62): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA62** in a 40% over all yield. R*f* = 0.50 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.13 (s, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.75 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.46-7.33 (m, 7H), 7.05-7.01 (m, 3H), 5.53 (s, 2H), 5.17 (s, 2H), 4.19 (s, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{23}H_{21}ClNO_5^+$: 425.10 found: 426.12.



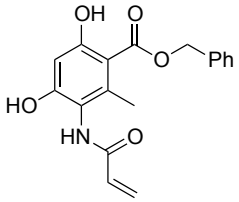
RA63

3-acrylamido-N-benzyl-4,6-dihydroxy-2-methylbenzamide (RA63): Starting from compound **1b** and using procedure J, followed by G, K, and finally D(with HOBt & NMM – 3equiv.) yielded **RA63** in a 24% over all yield. R*f* = 0.2 (PE/EtOAc 1:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.44 (d, *J* = 7.2 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.26 (t, *J* = 7.6 Hz, 1H), 6.51 (dd, *J* = 16.8, 10.4 Hz, 1H), 6.35 (m, 1H), 6.34 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.78 (dd, *J* = 10.4, 1.6 Hz, 1H), 4.57 (s, 2H), 2.10 (s, 2H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{19}N_2O_4^+$: 327.13 found: 327.20



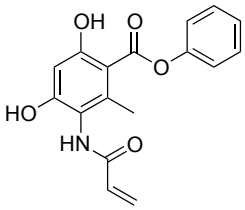
RA64

Butyl 3-acrylamido-4,6-dihydroxy-2-methylbenzoate (RA64): Starting from compound **1b** and using procedure J followed by G, K and finally B yielded **RA64** in a 22% over all yield. R*f* = 0.28(PE/EtOAc 1:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 6.51 (dd, *J* = 16.8, 10.0 Hz, 1H), 6.37 (d, *J* = 1.2 Hz, 1H), 6.33 (s, 1H), 5.80 (dd, *J* = 10.0, 1.2 Hz, 1H), 4.37 (t, *J* = 6.4 Hz, 2H), 2.36 (s, 3H), 1.81-1.74 (m, 2H), 1.54-1.46 (m, 2H), 1.01 (t, *J* = 7.6 Hz, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{15}H_{20}NO_5^+$: 294.13 found: 294.21.



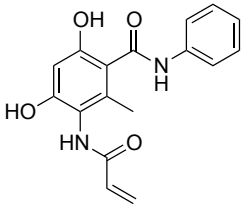
RA65

Benzyl 3-acrylamido-4,6-dihydroxy-2-methylbenzoate (RA65): Starting from compound **1b** and using procedure J followed by G, K and finally B yielded **RA65** in a 24% over all yield. R*f* = 0.18(PE/EtOAc 1:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.48 (d, *J* = 6.4 Hz, 2H), 7.42-7.35 (m, 3H), 6.50 (dd, *J* = 17.2, 10.0 Hz, 1H), 6.34 (s, 1H), 6.33 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.77 (dd, *J* = 10.4, 1.6 Hz, 1H), 5.40 (s, 2H), 2.36 (m, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{18}H_{18}NO_5^+$: 328.11 found: 328.16.



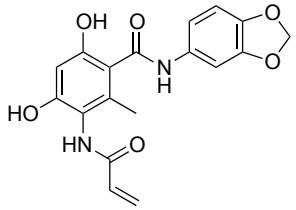
RA66

Phenyl 3-acrylamido-4,6-dihydroxy-2-methylbenzoate (RA66): Starting from compound **1b** and using procedure J followed by G, K and finally C yielded **RA66** in a 18 % over all yield. R*f* = 0.31(PE/EtOAc 2:3); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.49-7.45 (m, 2H), 7.33 (t, *J* = 6.8 Hz, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 6.55 (dd, *J* = 17.2, 10.0 Hz, 1H), 6.40 (s, 1H), 6.37 (dd, *J* = 17.2, 1.6 Hz, 1H), 5.81 (dd, *J* = 10.8, 2.0 Hz, 1H), 2.18 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{17}H_{16}NO_5^+$: 314.10 found: 314.13.



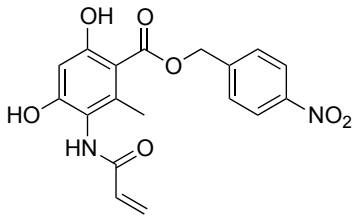
RA67

3-acrylamido-4,6-dihydroxy-2-methyl-N-phenylbenzamide (RA67): Starting from compound **1b** and using procedure J followed by G, K and finally D(with HOBt & NMM -3 equiv.) yielded **RA67** in a 20 % over all yield. R*f* = 0.24 (DCM/MeOH 9:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.67 (d, *J* = 7.6 Hz, 2H), 7.35 (t, *J* = 8.0 Hz, 2H), 7.13 (t, *J* = 7.6 Hz, 1H), 6.53 (dd, *J* = 17.2, 10.4 Hz, 1H), 6.39 (s, 1H), 6.34 (d, *J* = .6 Hz, 1H), 5.79 (dd, *J* = 10.8, 1.6 Hz, 1H), 2.18 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^{+}$calcd. For: $C_{17}H_{17}N_2O_4^{+}$: 312.11 found: 313.22.



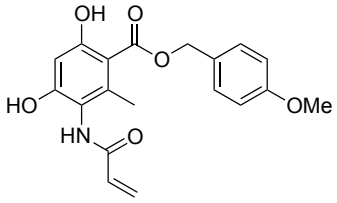
RA68

3-acrylamido-N-(benzo[d][1,3]dioxol-5-yl)-4,6-dihydroxy-2-methyl Benzamide (RA68): Starting from compound **1b** and using procedure J followed by G, K and finally D (with HOBt & NMM – 3equiv.) yielded **RA68** in a 21 % over all yield. R*f* = 0.22 (DCM/MeOH 9:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.37 (d, *J* = 1.6 Hz, 1H), 7.02 (dd, *J* = 8.0, 1.6 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.53 (dd, *J* = 17.2, 10.0 Hz, 1H), 6.37 (t, *J* = .15.6 Hz, 1H), 5.96(s, 2H), 5.79 (dd, *J* = 10.0, 1.2 Hz, 1H), 2.17 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^{+}$calcd. For: $C_{18}H_{17}N_2O_6^{+}$: 357.10 found: 357.14.



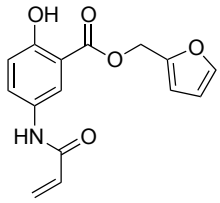
RA69

4-nitrobenzyl 3-acrylamido-4,6-dihydroxy-2-methylbenzoate (RA69): Starting from compound **1b** and using procedure J followed by G, K and finally B yielded **RA69** in a 21% over all yield. R*f* = 0.2 (PE/EtOAc 1:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 8.28 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 8.8 Hz, 1H), 6.51 (dd, *J* = 16.8, 10.4 Hz, 1H), 6.34(s, 1H), 6.32 (dd, *J* = .17.2, 1.6 Hz, 1H), 5.78 (dd, *J* = 10.4, 2.0 Hz, 1H), 5.53 (s, 2H), 2.33 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^{+}$calcd. For: $C_{18}H_{17}N_2O_7^{+}$: 373.10 found: 373.14.



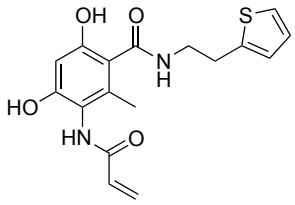
RA70

4-methoxybenzyl 3-acrylamido-4,6-dihydroxy-2-methylbenzoate (RA70): Starting from compound **1b** and using procedure J followed by G, K and finally B yielded **RA70** in a 24% over all yield. R*f* = 0.2(PE/EtOAc 1:1); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.41 (d, *J* = 8.4 Hz, 2H), 6.95 (dd, *J* = 6.8, 2.0 Hz, 2H), 6.48 (dd, *J* = .16.8, 10.4 Hz, 1H), 6.33(s, 1H), 6.32 (dd, *J* = .17.2, 1.6 Hz, 1H), 5.77 (dd, *J* = 10.0, 1.6 Hz, 1H), 5.33 (s, 2H), 3.82 (s, 3H), 2.28 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{19}H_{20}NO_6^+$: 358.12 found: 358.19.



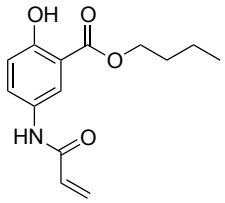
RA71

Furan-2-ylmethyl 5-acrylamido-2-hydroxybenzoate (RA71): Starting from compound **1a** and using procedure A followed by F and finally G yielded **RA71** in a 40% over all yield. R*f* = 0.22 (PE/EtOAc 7:3); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.5 (s, 1H), 7.98 (d, *J* = 2.4 Hz, 1H), 7.74 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.48 (s, 1H), 6.99 (d, *J* = .9.2 Hz, 1H), 6.54 (d, *J* = 3.2 Hz, 1H), 6.46 (s, 1H), 6.42 (s, 1H), 6.22 (dd, *J* = 16.0, 10.4 Hz, 1H), 5.78 (d, *J* = 10.4 Hz, 1H), 5.36 (s, 2H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{15}H_{14}NO_5^+$: 287.07 found: 288.21.



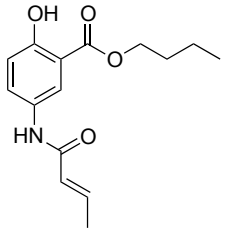
RA72

3-acrylamido-4,6-dihydroxy-2-methyl-N-(2-(thiophen-2-yl)ethyl)benzamide) (RA72): Starting from compound **1b** and using procedure J followed by G, K and finally D (with HOBt & NMM-3equiv.) yielded **RA72** in a 24% over all yield. R*f* = 0.23 (PE/EtOAc 2:3); ^{1}H (MeOD, 400 MHz, 25 °C) δ = 7.24-7.22 (m, 1H), 6.95-6.93 (m, 2H), 6.51 (dd, *J* = 17.2, 10.0 Hz, 1H), 6.38-6.32 (m, 2H), 5.78 (dd, *J* = .10.4, 2.4 Hz, 1H), 3.63 (t, *J* = 7.2 Hz, 1H), 3.14 (t, *J* = 7.2 Hz, 1H), 2.05 (s, 3H) ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{17}H_{19}N_2O_4S^+$: 347.10 found: 347.15.



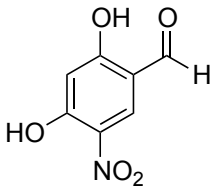
RA73

Butyl 5-acrylamido-2-hydroxybenzoate (RA73): Starting from compound **1a** and using procedure B followed by F and finally G yielded **RA73** in a 35% over all yield. R*f* = 0.2 (PE/EtOAc 4:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.09 (d, *J* = 2.4 Hz, 1H), 7.66 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.24 (s, 1H), 6.99 (d, *J* = 9.2 Hz, 1H), 6.47 (d, *J* = 16.4 Hz, 1H), 6.26(dd, *J* = 17.2, 10.4 Hz, 1H), 5.80 (d, *J* = .10.4 Hz, 1H), 4.38 (t, *J* = 6.4 Hz, 2H),1.81-1.76 (m, 2H), 1.53-1.45 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 3H)ppm; LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{14}H_{18}NO_4^+$: 264.12 found: 264.19.



RA74

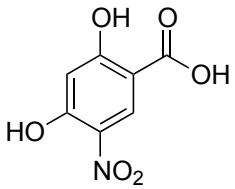
(E)-butyl 5-(but-2-enamido)-2-hydroxybenzoate (RA74): Starting from compound **2a** and using procedure B followed by F and finally G yielded **RA74** in a 30% over all yield. R*f* = 0.2 (PE/EtOAc 4:1); ^{1}H ($CDCl_3$, 400 MHz, 25 °C) δ = 10.7 (s, 1H), 8.05 (s, 1H), 7.99(d, *J* = 2.8 Hz, 1H), 7.58 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.07-7.02 (m, 1H), 7.01-6.97 (m, 1H), 5.95 (d, *J* = 16.4 Hz, 1H), 5.37 (dd, *J* = 16.4, 10.0 Hz, 1H), 4.38 (t, *J* = 7.2 Hz, 2H),1.94 (d, *J* = 7.2 Hz, 3H), 1.81-1.76 (m, 2H), 1.53-1.47 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 3H) ppm. LC-MS (ESI): m/z $[M+H]^+$calcd. For: $C_{15}H_{19}NO_4^+$: 277.13 found: 278.25.



12

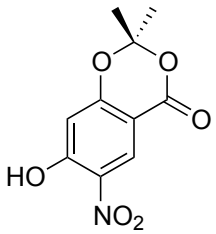
***Para*-nitro 2-4 dihydroxybenzaldehyde 12.** Glacial acetic acid (8 mL, 139 mmol) and fuming nitric acid (7.2 mL, 169 mmol) were mixed together in a 100 mL round bottomed flask and the mixture was cooled to 0 °C using an ice water bath. After 10 minutes of stirring, 2,4-dihydroxybenzaldehyde (2.0 g, 14.5 mmol, 1.0 equiv) was added portion wise over 15 minutes to the solution. The reaction was brought to room temperature (after 1 hour at room temperature there was a strong gas evolution) and was stirred for additional 24 hours. The pink solid precipitate was filtered on a fritted funnel and washed with a small amount of ice-cold water to obtain, the desired compound **12** as a pink solid in 61% yield (1.6 g). ^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C): δ = 11.66 (s, 1H); 11.22 (s, 1H); 9.86 (s, 1H);

8.51 (s, 1H); 6.65 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 193.9, 168.0, 161.4, 133.5, 124.7, 115.2, 106.1, ppm.



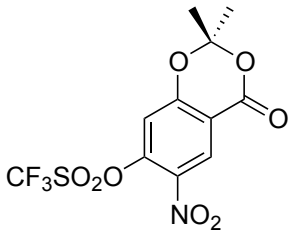
2c

Nitro acid 2c. Nitro aldehyde **12** (1.6 g, 8.74 mmol, 1.0 equiv), was dissolved in DMSO and cooled to 0 °C using an ice water bath. To this solution NaH_2PO_4 (2.9 g, 21.8 mmol, 2.5 equiv) in 7 mL of distilled water, and $NaClO_2$ (1.9 g, 21 mmol, 2.4 equiv) in 7 mL of distilled water, were added during a period of 10 minutes. The deep yellow mixture was warmed slowly to room temperature and kept stirred over night. The mixture was diluted slowly with saturated aqueous Na_2CO_3 (15 mL), and washed with EtOAc (5 mL). The aqueous layer was acidified to pH 1 by adding HCl 1M at 0 °C and the resulting solution was stored at 0°C for 12 hours. The precipitate was filtered on a fritted funnel and washed with a small amount of ice-cold water to obtain, the desired compound **4** as a yellow solid in 86% yield (1.5 g). ^{1}H NMR (CD_3OD, 400 MHz, 23 °C): δ = 8.71 (s, 1H); 6.57 (s, 1H); 9.86 (s, 1H). ^{13}C NMR (CD_3OD, 100 MHz, 25 °C): δ = 171.9, 169.2, 161.0, 131.0, (x2) 108.1, 106.1 ppm.



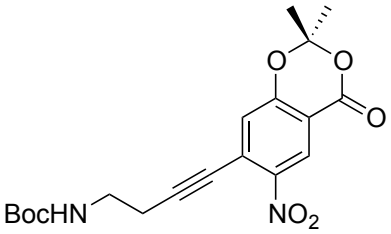
13

Acetonide protected phenol 13. TFAA (7.5 mL, 52.5 mmol, 7.0 equiv) was added slowly to a solution of acid **2c** (1.5 g, 7.5 mmol, 1.0 equiv) dissolved in TFA (9.4 mL, 122.2 mmol, 16.3 equiv). Acetone (4.0 mL, 52.5 mmol, 7.0 equiv) was then added and the reaction heated to reflux for 12 hours. The reaction was then allowed to reach room temperature and the volatile were removed under vacuum. The resulting brown solid was dissolved in EtOAc (30 mL), the organic layer was washed with saturated aqueous $NaHCO_3$ (2 x 15 mL) and the combined organic phases were dried over Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure afforded the desired compound **13** as a brown solid in 83% yield (1.5 g). The desired compound was used without further purification in the next step. ^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C): δ = 11.17 (s, 1H); 8.88 (s, 1H); 6.70 (s, 1H): 1.80 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 161.9, 160.1, 158.9, 129.9, 129.3, 107.8, 106.7, 106.4, 26.1 (x2) ppm.



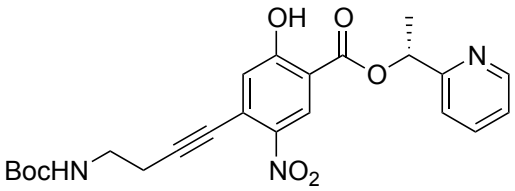
14

Synthesis of the trifluoro methansulphonic ester 14. TEA (665 µl, 4.8 mmol, 3.0 equiv) was added to a solution containing phenol **13** (380 mg, 1.6 mmol, 1.0 equiv) in dry CH_2Cl_2 (8 mL) and the mixture was cooled down to 0 °C using an ice water bath. After stirring for 10 minutes, Tf_2O (296 µl, 1.76 mmol, 1.1 equiv) was added dropwise. After complete consumption of the starting phenol **13**, as judged by TLC analysis, the reaction was quenched with saturated aqueous $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried over Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 80/20 petroleum ether/EtOAc) afforded the desired compound **14** as a yellow solid in 80% yield (470 mg). ^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C): δ = 8.88 (s, 1H); 7.11 (s, 1H); 1.85 (s, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 160.0, 157.3, 146.8, 129.3 (x2), 113.5 (x2), 112.9, 109.0, 26.1 (x2) ppm.



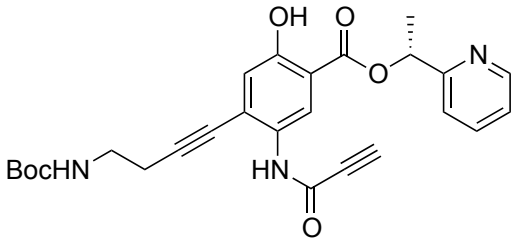
3

Alkyne-branched amine 3. A flame dried 100 mL two necked round bottomed flask was charged with $Pd(PPh_3)_2Cl_2$ (63 mg, 0.09 mmol, 4%), TEA (933 µl, 6.7 mmol, 3.0 equiv) trifluoro methansulphonic ester **14** (830 mg, 2.23 mmol, 1.0 equiv.) and 20 mL of dry THF. Boc-protected alkyne **4** (415 mg, 2.45 mmol, 1.1 equiv) in THF (5 mL) was added over 10 minutes, then a catalytic amount of CuI was added and the mixture heated to reflux. After complete consumption of the starting material **14**, as judged by TLC analysis, the reaction was quenched with saturated aqueous NH_4Cl (15 mL) extracted with Et_2O (3 x 30 mL) and the combined organic phases were dried over Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 75/25 petroleum ether/EtOAc) afforded the desired compound **3** as a yellow solid in 81% yield (700 mg). ^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C): δ = 8.72 (s, 1H); 7.19 (s, 1H); 5.10 (bs, 1H); 3.44 (m, 2H); 2.74 (t, J = 6.4 Hz, 2H); 1.79 (s, 6H); 1.47 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 157.4, 157.2, 154.7, 143.4, 125.9, 122.1, 111.2, 106.8 (x2), 100.5, 78.5, 37.9, 27.2 (x3), 24.8 (x2), 20.6 ppm. The quaternary carbon of tert-butyl group is not visible.



15

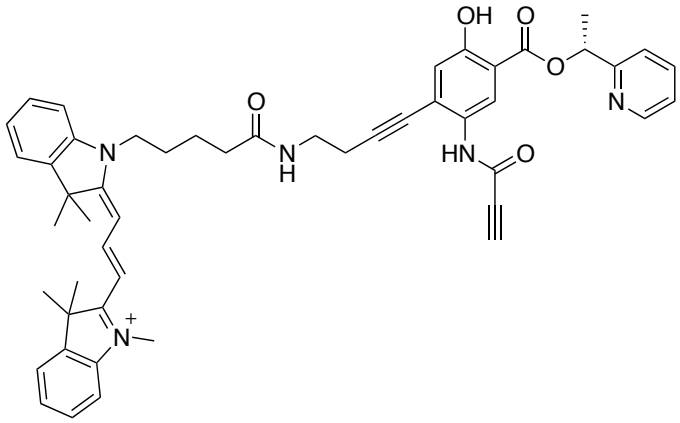
Synthesis of the (*R*)-Pyridil ester 15. (*R*)-α-Methyl-2-pyridinemethanol **5** (64 mg, 0.56 mmol, 1.1 equiv) was added to a solution of NaH (23 mg, 0.56 mmol, 1.1 equiv) in dry THF (5 mL) at 0 °C and the mixture stirred for 10 minutes. Compound **3** (200 mg, 0.513 mmol, 1.0 equiv) was then added as a solid portion wise to the cold mixture, and a deep green coloration appeared. After complete consumption of the starting material **3**, as judged by TLC analysis, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) extracted with Et_2O (3 x 20 mL) and the combined organic layers were dried over Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure followed by flash chromatography (silica gel, 65/35 petroleum ether/EtOAc) afforded the desired compound **15** as a light yellow solid in 65% yield (150 mg). ^{1}H NMR ($CDCl_3$, 400 MHz, 23 °C): δ = 8.76 (s, 1H); 8.66 (m, 1H); 7.81 (m, 1H); 7.46 (d, J = 8 Hz, 1H); 7.35 (m, 1H); 7.17 (s, 1H); 6.27 (q, J = 6.8 Hz, 1H); 3.45 (bd, J = 6 Hz, 2H); 2.73 (t, J = 6.4 Hz, 2H); 1.83 (d, J = 6.8 Hz, 3H); 1.49 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 166.4, 163.0, 157.5, 147.9, 136.5, 126.8 (x2), 122.5 (x2), 122.3, 119.4, 110.6, 74.1, 37.9, 28.5, 20.6, 19.5 (x2) ppm. The quaternary carbon of tert-butyl group is not visible. Two carbons are hidden under the $CDCl_3$ signal.



16

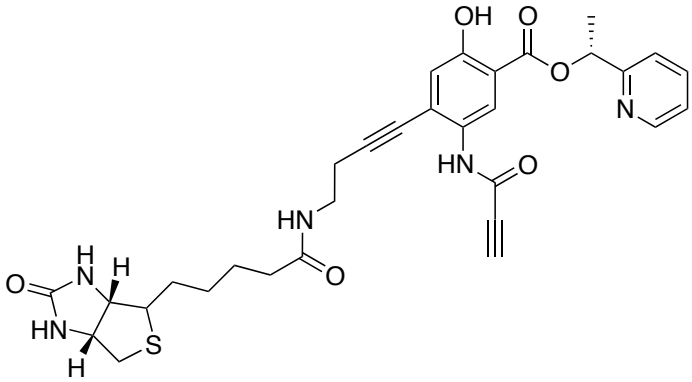
Nitro group reduction and Michael acceptor introduction. An oven dried 100 mL round bottomed flask, was charged with Zn dust (261 mg, 3.99 mmol, 14 equiv), the starting (*R*)-pyridil ester **15** (130 mg, 0.285 mmol, 1.0 equiv) and CH_2Cl_2 (8 mL). This slurry was brought to 0°C using an ice water bath, subsequently glacial acetic acid (2.7 mL, 47.46 mmol, 164 equiv) was added slowly, and the mixture warmed to room temperature. After complete consumption of the starting material **15**, as judged by TLC analysis, the reaction was filtered on a celite pad, neutralized with saturated aqueous $NaHCO_3$ (50 mL) extracted with CH_2Cl_2 (3 x 30 mL) and the combined organic phases were dried over Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure afforded green fluorescent oil that was used directly in the next step. LC-MS: m/z $[M]^+$calcd. for $C_{23}H_{27}N_3O_5^+$: 425.20; found: 425.95. The crude material was dissolved in dry CH_2Cl_2 (3 mL), added to a 50 mL two necked round bottomed flask containing a solution in dry CH_2Cl_2 (3 mL) of DCC (97 mg, 0.47 mmol, 2.0 equiv), propiolic acid (29 µl, 0.47 mmol, 2.0 equiv) and DMAP (catalytic amount) at 0 °C. After complete consumption of the starting material, as judged by TLC analysis, the reaction was quenched with saturated aqueous NH_4Cl (10 mL) extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic phases were dried with Na_2SO_4. Filtration and evaporation of the solvents under reduced pressure

followed by flash chromatography (silica gel, 55/45 petroleum ether/EtOAc) afforded the desired compound **16** as a light white solid in 52% yield (70 mg) over two steps. LC-MS: m/z $[M]^+$calcd. for $C_{26}H_{27}N_3O_6^+$: 477.19; found: 477.90. ^{1}H ($CDCl_3$, 400 MHz, 23 °C): δ = 10.66 (bs, 1H); 8.86 (s, 1H); 8.63 (d, J = 4.4 Hz, 1H); 8.46 (s, 1H); 7.83-7.79 (m, 1H); 7.55 (d, J = 7.2 Hz, 1H); 7.04 (s, 1H); 6.24 (q, J = 6.8 Hz, 1H); 3.59-3.47 (m, 2H); 3.01 (s, 1H); 2.75 (t, J = 6.4 Hz, 2H); 1.79 (d, J = 6.8 Hz, 3H); 1.45 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 168.5, 159.4, 157.9, 148.0, 138.4, 129.8, 123.3, 121.9, 121.3, 120.6 (x2), 120.4, 98.2, 74.4 (x2), 49.3, 39.4, 33.9, 30.9, 29.7, 28.4 (x3), 25.6, 24.9, 21.8, 21.1 (x2) ppm.



Cy3-RA1 (8)

Cy3-RA1 (8): Boc-group removal and coupling to the fluorogenic probe. An oven dried 25 mL round bottomed flask containing the Michael acceptor **16** (70 mg, 0.146 mmol, 1.0 equiv) was charged with dry CH_2Cl_2 (3 mL) and brought to 0°C using an ice water bath. To the cold solution was added TFA (3 mL) slowly, over 5 minutes. After complete consumption of the starting material **16**, as judged by TLC analysis, the reaction was allowed to reach room temperature and the volatiles were removed under vacuum. The crude material **(7)** was not isolated but used directly in the next step. LC-MS: m/z $[M]^+$calcd. for $C_{21}H_{19}N_3O_4^+$: 377.19; found: 377.89. The crude amine **7** (35 mg, 0.092 mmol, 1.0 equiv) was taken up in dry DMF (3 mL) and DIPEA (36 µl, 0.20 mmol, 2.2 equiv) was added. This mixture was transferred via syringe to a solution in dry DFM (3 mL) of HATU (46 mg, 0.120 mmol, 1.2 equiv) and the cyanine-based fluorophore $Cy3CO_2H$ **6** (64 mg, 0.110 mmol, 1.2 equiv), the reaction was then stirred over night. The reaction was followed by LC-MS and after complete consumption of the starting amine, the solvent was removed under vacuum and the residue directly purified by HPLC (20-80% CH_3CN in water gradient in 50 min, flow: 2 mL/min, DiscoveryR HS C18, 5µm, 5 cm x 10.0 mm). ^{1}H NMR (Acetone-d6, 400 MHz, 23 °C): δ = 8.48 (d, J = 4 Hz, 1H); 8.45 (t, J = 13.6 Hz, 1H); 8.32 (d, J = 6.4 Hz, 1H); 7.75 (t, J = 7.6 Hz, 1H); 7.48 (m, 3H); 7.34-7.17 (m, 8H); 6.80- 6.77 (m, 1H); 6.72 (d, J = 14 Hz, 1H); 6.07 (q, J = 6.4 Hz, 1H); 4.14-4.03 (m, 2H); 3.67 (m, 4H); 3.42 (m, 3H); 3.07-3.02 (m, 2H); 2.68 (s, 1H); 2.61 (m, 2H); 2.34 (m, 2H); 1.69 (s, 10H); 1.60 (d, J = 6.4 Hz, 3H); 0.73 (s, 6H) ppm. The signals corresponding to OH and NH are not visible. ^{13}C NMR (Acetone-d6, 100 MHz, 23 °C): δ = 175.9, 175.2, 173.5, 160.4, 151.7, 149.9, 143.9, 142.9, 141.8, 138.2 (x2), 129.6 (x 2), 126.2 (x 2), 125.8, 124.0 (x2), 123.2 (x2), 121.3 (x2), 121.0, 112.1 (x2), 104.5, 104.0, 76.3, 75.3, 50.1, 50.1, 46.3, 44.6, 38.6, 35.7, 34.2, 32.7, 32.2, 31.7, 27.4 (x6), 25.7, 23.3, 21.8, 20.9 (x2) ppm. LC-MS: m/z $[M]^+$calcd. for $C_{50}H_{52}N_5O_5^+$: 802.40; found: 802.21; 401.80 $[M/2]^{+2}$



Biotin-RA1 (9)

Biotin-RA1 (9): Boc-group removal and coupling to Biotin. An oven dried 25 mL round bottomed flask containing the Michael acceptor **16** (70 mg, 0.146 mmol, 1.0 equiv) was charged with dry CH_2Cl_2 (3 mL) and brought to 0°C using an ice water bath. To the cold solution was added TFA (3 mL) slowly, over 5 minutes. After complete consumption of the starting material **16**, as judged by TLC analysis, the reaction was allowed to reach room temperature and the volatiles were removed under vacuum. The crude material (**7**) was not isolated but used directly in the next step. LC-MS: m/z $[M]^+$calcd. for $C_{21}H_{19}N_3O_4^+$: 377.19; found: 377.89. The crude amine **7** (20 mg, 0.053 mmol, 1.0 equiv) was taken up in dry DMF (3 mL) DIPEA (11 µl, 0.063 mmol, 1.2 equiv) and then BiotinOSu (22 mg, 0.063 mmol, 2.0 equiv) was added, the reaction was then stirred over night. The reaction was followed by LC-MS and after complete consumption of the starting amine, the solvent was removed under vacuum and the residue directly purified by HPLC (20-80% CH_3CN in water gradient in 50 min, flow: 2 mL/min, DiscoveryR HS C18, 5µm, 5 cm x 10.0 mm). ^{1}H NMR (Acetone-d^6, 400 MHz, 23 °C) δ = 8.56 (d, *J* = 5.2 Hz, 1H); 8.47 (s, 1H); 7.93 (m, 1H); 7.62 (d, *J* = 6.8 Hz, 1H); 7.39 (m, 1H); 6.92 (s, 1H); 6.19 (q, *J* = 6.4 Hz, 1H); 4.41 (dd, J = 7.6, 4.8 Hz, 1H); 4.19 (dd, J = 7.6, 4.4 Hz, 1H); 3.43 (m, 2H); 2.94 (m, 1H); 2.85-2.77 (m, 2H); 2.61-2.57 (m, 3H); 1.68 (d, *J* = 7.2 Hz, 3H); 1.59-1.51 (m, 4H); 1.34-1.30 (m, 4H) ppm. The signals corresponding to OH and NH are not visible. ^{13}C NMR (Acetone-d^6, 100 MHz, 23 °C) δ = 173.9, 169.1, 159.6, 158.6, 148.7, 139.8, 125.3, 124.8 (x 2), 121.9 (x 2), 121.1, 113.0, 99.6, 78.6, 76.4, 74.6, 62.5 (x 2), 60.9 (x 2), 56.3 (x 2), 40.8 (x 2), 38.5, 36.3, 26.4, 21.8, 20.9, 14.3 ppm. LC-MS: *m/z* $[M]^+$calcd. for $C_{31}H_{33}N_5O_6S^+$: 603.69; found: 604.08.

Enzymatic inhibition of EGFR. EGFR was expressed in Sf 9 insect cells as human recombinant GST-fusion protein by means of the baculovirus expression system and was then purified by affinity chromatography using either GSH-agarose (Sigma). The purity was checked by SDS-PAGE/silver staining. For measuring the enzymatic activity of the protein kinase a proprietary protein kinase assay (^{33}PanQinase® Activity Assay) was used. All kinase assays were performed in 96-well FlashPlates from Perkin–Elmer/NEN (Boston, MA, USA) in a 50 µL reaction volume by using a BeckmanCoulter/Sagian robotic system. The reaction cocktail was pipetted in four steps in the following order: 1) 20 µL of assay buffer, 2) 5 µL of ATP solution (in H_2O), 3) 5 µL of test compound (in 10 % DMSO), and 4) 10 µL of substrate/10 µL of enzyme solution (premixed). The assay for all kinases contained HEPES-NaOH (60 mM), $MgCl_2$ (pH 7.5, 3 mM), $MnCl_2$ (3 mM), Na-orthovanadate (3 µM), DTT (1.2 mM), PEG_{20000} (50 µg mL^{-1}), [γ-^{33}P]-ATP (1 µM, ca. 5×10^5 cpm per well). The final DMSO concentration was 1 % in all assays. The reaction cocktails were incubated at 30 °C for 80 minutes. The reaction was stopped with H_3PO_4 (50 µL, 2 % v/v). Plates were aspirated and washed two times with H_2O (200 µL) and NaCl (200 µL, 0.9 % w/v). Incorporation of $^{33}P_i$ was determined

with a microplate scintillation counter (Microbeta, Wallac).
For each concentration of the test compounds residual activities (in %) were calculated relative to control values without test compounds. With the set of residual activities (in %) obtained for each test compound, IC_{50} values were calculated by using Quattro Workflow V2.0.1.3 (Quattro Research GmbH, Munich, Germany; www.quattro-research.com). The mathematical model used was "sigmoidal response (variable slope)" with parameters "top" fixed at 100 % and "bottom" at 0 %.

Cellular inhibitions of EGFR auto phosphorylation in epidermoid carcinoma A431 following stimulation with EGFR. Human epidermoid carcinoma cell line A431 was used, which expresses endogenously a high level of EGF-R. Stimulation of these cells with human epidermal growth factor (EGF) results in receptor tyrosine autophosphorylation. A431 cells were plated in RPMI supplemented with 10% FCS in multiwell cell culture plates. After serum-starvation overnight, cells were incubated with compounds in serum-free medium. Prediluted test samples (1,0E-02 M) were added 1:100 to the cell culture medium resulting in a final DMSO concentration of 1%. After 90min incubation at 37°C, cells were stimulated with EGF-R EGF 50 ng/ml for 3 min. Tested range concentration of the compounds was between 1.0E-05 – 3.0E-09 M and Lapatinib was used as the reference compound. Quantification of substrate phosphorylation was assessed in 96well plates via sandwich ELISA using a substrate specific capture antibody and an anti-phosphotyrosine detection antibody. Raw data were converted into percent substrate phosphorylation relative to High controls, which were set to 100%. IC_{50} values were determined using GraphPad Prism 5.01 software with constrain of bottom to 0 and top to 100 using a nonlinear regression curve fit with variable hill slope. The equation is a four-parameter logistic equation. The average of three experiments was used to calculate the IC_{50}.

Protein kinase covalent labeling with Cy3-RA1 (8): Protein kinases human EGFR +strep-tag (Genway GWB-297EF6), ERBB2-HIS tag (Life Technologies, PV3366), JAK3 his tag (Millipore, 14629), CHK2 his tag (Calbiochem, cat: 220487) and Bovine Carbonic Anhydrase (Sigma) were diluted to 300 nM in the following buffer: 50 mM HEPES, pH 7.4, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na- orthovanadate, 5 mM DTT.

The Cy3 labeled inhibitor **Cy3-RA1 (8)** was added to a final concentration of 200 nM and the labeling reaction was incubated 30 min at 37 °C on a final volume of 25 µL.

After incubation was completed, denaturing sample buffer was added to the reaction and the samples were heat denatured at 95 °C for 5 minutes. Then the samples were loaded on SDS NuPAGE, 4-12% Acrylamide Bis (Life Technologies) and separated by electrophoresis.

Once the electrophoresis ended, the gel was dismounted, fixed and scanned on Ettan DIGE Imager (GE Healthcare) using appropriate light filters for Cy3. Finally the gel was stained with silver nitrate using Plus one Silver staining kit for Proteins (GE Healthcare).

Cellular imaging of kinases

Experimental Procedures with HEK293T cells

Background labeling in HEK cells with Cy3-RA1 (8) probe. HEK293T cells maintained in 10% FBS in DMEM (Invitrogen) at 37 ºC under 5% CO_2 were incubated at 37 ºC for 48 h. Then, the cells were washed three times with DMEM and incubated with 100 nM of **Cy3-RA1 (8)** for 30 min in a CO_2 incubator. After the culture medium was replaced, microscopic images were acquired.

Labeling of cell surface protein (BL-EGFR) with FA and Cy3-RA1 (8). HEK293T cells maintained in 10% FBS in DMEM (Invitrogen) at 37 ºC under 5% CO_2 were transfected with the pcDNA3.1(+)-BL-EGFR plasmids using Lipofectamine 2000 (Invitrogen).[2] After 5–6 h, the culture medium was replaced with DMEM (without phenol red), and the cells were incubated at 37 ºC for 24 hours. Then, the cells were washed three times with DMEM and incubated with 500 nM **FA**[3, 4] and 100 nM of **Cy3-RA1 (8)** for 30 min in a CO_2 incubator. After the culture medium was replaced, microscopic images were acquired.

Experimental Procedures with BT474 cells

Labeling of Erbb2 protein in live cells with Cy3-RA1 (8). BT474 cells maintained in 10% FBS in DMEM (Invitrogen) at 37 ºC under 5% CO_2 were incubated at 37 ºC for 48 h. Then, the cells were washed three times with DMEM and incubated with 100 nM of **Cy3-RA1 (8)** for 30 min in a CO_2 incubator. After the culture medium was replaced, microscopic images were acquired. For control experiment, lapatinib (1.0 µM) was introduced in the cell culture and microscopic images were acquired after 16 hours.
Lapatinib: *N*-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-[5-[(2-methylsulfonylethylamino)methyl]-2-furyl]

[2] S. Mizukami, S. Watanabe, Y. Hori and K. Kikuchi, *J. Am. Chem. Soc.* **2009**, *131*, 5016.
[3] S. Watanabe, S. Mizukami, Y. Hori and K. Kikuchi, *Bioconjugate Chem.* **2010**, *21*, 2320.
[4] S. Mizukami, S. Watanabe, Y. Akimoto and K. Kikuchi, *J. Am. Chem. Soc.* **2012**, *134*, 1623.

quinazolin-4-amine

Labeling of Erbb2 protein in fixed cells with Cy3-RA1. BT474 cells maintained in 10% FBS in DMEM (Invitrogen) at 37 ºC under 5% CO_2 were incubated at 37 ºC for 48 hours. Then the cells were washed with PBS and fixed with 3.7% formaldehyde for 20 min. After washing with DMEM they were treated with 100 nM of **Cy3-RA1 (8)** and 1 mM of DAPI in DMEM for 30 min in a CO_2 incubator. After the culture medium was replaced, microscopic images were acquired. For control experiment, lapatinib (1.0 mM) was introduced in the cell culture and microscopic images were acquired after 16 hours.

List of kinase with less than 50% residual binding at 1 µM (For assay conditions, see ref 12 in the main manuscript).

KINOMEscan Gene Symbol	Entrez Gene Symbol	Percent Control	inhibition
EGFR(L747-E749del, A750P)	EGFR	0.65	99.35
EGFR(G719S)	EGFR	2.6	97.4
MEK4	MAP2K4	3.8	96.2
EGFR(G719C)	EGFR	4.4	95.6
EGFR(L858R)	EGFR	5.2	94.8
EGFR(L747-T751del,Sins)	EGFR	5.5	94.5
EGFR(L861Q)	EGFR	8.2	91.8
EGFR(S752-I759del)	EGFR	8.7	91.3
EGFR(L747-S752del, P753S)	EGFR	10	90
JAK3(JH1domain-catalytic)	JAK3	10	90
ERBB2	ERBB2	11	89
EGFR	EGFR	12	88
LKB1	STK11	12	88
EGFR(E746-A750del)	EGFR	16	84
BLK	BLK	47	53

Affinity based capture from crude cell extract

HEK293-T cells were grown on DMEM complemented with 10% Calf Fetal Serum (Sigma), 1% PenStrep, 1% non-essential amino acids, 1% Glutamine. When the cells reached confluence after 48-72 h culture at 37° C, 5% CO_2, the monolayer from a T75 cell culture (10^6 cells approx.) flask was carefully washed with cold, sterile PBS and finally 1 ml of cold lysis buffer was added (20 mM Tris-HCl pH 8, 137 mM NaCl, 10 % glycerol, 1% Triton X100, 2 mM EDTA, 1X HALT Protease inhibitors, Pierce). The flask was gently agitated for 5 min in ice and the cell monolayer scrapped from the flask and recovered on a mini centrifuge tube. The recovered cells were subjected to ultra sonication (three cycled of 30 seconds, on ice) and the protein lysate was centrifuged for 30 min at 14000 rpm, to eliminate cell membranes and cell debris. The supernatant was transferred to a fresh tube, and the soluble cytosolic proteins were quantified by Bradford assay.

A sample of crude cell lysate (10µg, 8 µL) was diluted in buffer Kinase (50 mM HEPES pH 7.4, 3 mM $MnCl_2$, 3 mM $MgCl_2$, 3 µM Na-orthovanadate,, 2 mM DTT) complemented with 1x HALT protease

inhibitor (Pierce) to a final volume of 20 µL. Recombinant ErbB2 (# PV3366, Life Technologies) was added to the cell extract to a final concentration of 100 nM. RA1-biotin **(9)** or biotin were added to the crude extract to a final concentration of 1 µM and the samples were incubated on a rotation wheel for 30 min at room temperature. After incubation, 50 µl of streptavidin coated magnetic beads MyOne ^{Tm} (Invitrogen, Life Technologies) were washed three times with 20 mM Tris-HCl, pH 7.4, NaCl 2 M and the beads were added to the crude cell extracts. The mixture was incubated on the rotation wheel for another 30 min period, to allow streptavidin - biotin capture. Then the beads were washed 5 times with 100 µl of kinase buffer and 5 times with100 µl of 20 mM Tris-HCl, pH 7.4, NaCl 2 M. The magnetic beads were re-suspended in 50 µl of HBS and treated to MS analysis according to the method reported by Fischer et al. Journal of Proteome Resarch. 2010, 9, 806-817. The experiment was also performed without the addition of ErbB2 and with ErbB2 but using biotin instead of biotin-RA1 **(9)**. The MS results were ranked by metascore for the sample with ErbB2 and compared to the other two samples (Tables S1). Results with less than four peptide fragment per hit were removed.

Table S1. Meta score for MS analysis of protein pull-down from HEK cell lystate.

	ERBB2	-	+	+
	Biotin-TA1	+	+	-
Accession	Name	Meta Score	Meta Score	Meta Score
P08107|HSP71_HUMAN	Heat shock 70 kDa protein 1A/1B OS=Homo sapiens GN=HSPA1A PE=1 SV=5	2271	3055	1691
P06733|ENOA_HUMAN	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	772	1265	244
P04264|K2C1_HUMAN	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	1131	1204	56
P68371|TBB4B_HUMAN	Tubulin beta-4B chain OS=Homo sapiens GN=TUBB4B PE=1 SV=1	554	1029	432
P07437|TBB5_HUMAN	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2	0	1022	423
P08670|VIME_HUMAN	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4	631	959	91
P10809|CH60_HUMAN	60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	1067	950	287
P35527|K1C9_HUMAN	Keratin, type I cytoskeletal 9 OS=Homo sapiens GN=KRT9 PE=1 SV=3	781	933	411
P13645|K1C10_HUMAN	Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	430	890	512
P68363|TBA1B_HUMAN	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1	606	797	514
P13639|EF2_HUMAN	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	262	752	138
P07900|HS90A_HUMAN	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5	434	739	293
P63261|ACTG_HUMAN	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1	607	711	441
E9PKE3|E9PKE3_HUMAN	Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=3 SV=1	0	705	269
E7EUT4|E7EUT4_HUMAN	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=3 SV=1	0	644	274
P22314|UBA1_HUMAN	Ubiquitin-like modifier-activating enzyme 1 OS=Homo sapiens GN=UBA1 PE=1 SV=3	152	624	243
P04075|ALDOA_HUMAN	Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2	366	557	118
P08238|HS90B_HUMAN	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	322	528	246
B4DTR1|B4DTR1_HUMAN	Receptor tyrosine-protein kinase erbB-2 OS=Homo sapiens GN=ERBB2 PE=2 SV=1	0	505	0
P14618-2|KPYM_HUMAN	Isoform M1 of Pyruvate kinase isozymes M1/M2 OS=Homo sapiens GN=PKM	0	476	115
P00338|LDHA_HUMAN	L-lactate dehydrogenase A chain OS=Homo sapiens GN=LDHA PE=1 SV=2	0	454	226
P07195|LDHB_HUMAN	L-lactate dehydrogenase B chain OS=Homo sapiens GN=LDHB PE=1 SV=2	185	450	211
O43175|SERA_HUMAN	D-3-phosphoglycerate dehydrogenase OS=Homo sapiens GN=PHGDH PE=1 SV=4	383	379	209
Q00839-2|HNRPU_HUMAN	Isoform Short of Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU	139	344	162
P14625|ENPL_HUMAN	Endoplasmin OS=Homo sapiens GN=HSP90B1 PE=1 SV=1	0	340	0
P12277|KCRB_HUMAN	Creatine kinase B-type OS=Homo sapiens GN=CKB PE=1 SV=1	306	335	91
P62937|PPIA_HUMAN	Peptidyl-prolyl cis-trans isomerase A OS=Homo sapiens GN=PPIA PE=1 SV=2	352	332	197
A8K092|A8K092_HUMAN	ATP synthase subunit alpha OS=Homo sapiens GN=ATP5A1 PE=2 SV=1	47	294	0
E9PBS1|E9PBS1_HUMAN	Phosphoribosylaminoimidazole-succinocarboxamide synthase (Fragment) OS=Homo sapiens GN=PAICS PE=4 SV=1	152	293	126
P55072|TERA_HUMAN	Transitional endoplasmic reticulum ATPase OS=Homo sapiens GN=VCP PE=1 SV=4	146	290	37
P08865|RSSA_HUMAN	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4	0	286	74
B4DEM7|B4DEM7_HUMAN	T-complex protein 1 subunit theta OS=Homo sapiens GN=CCT8 PE=2 SV=1	0	279	71
P52292|IMA2_HUMAN	Importin subunit alpha-2 OS=Homo sapiens GN=KPNA2 PE=1 SV=1	182	255	96
P62826|RAN_HUMAN	GTP-binding nuclear protein Ran OS=Homo sapiens GN=RAN PE=1 SV=3	116	242	91
P40227|TCPZ_HUMAN	T-complex protein 1 subunit zeta OS=Homo sapiens GN=CCT6A PE=1 SV=3	0	215	60