

## Supporting Information

***N*-(2-{3-[3,5-bis(trifluoromethyl)phenyl]ureido}ethyl)-  
glycyrrhetinamide (6b): A Novel Anticancer Glycyrrhetic Acid  
Derivative that Targets the Proteasome and Displays Anti-Kinase  
Activity**

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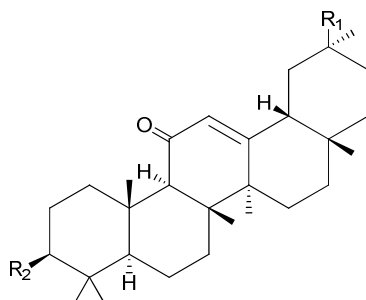
*Organique ;* <sup>¶</sup>*Laboratoire de Structure et Fonction des Membranes Biologiques ;*

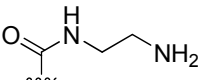
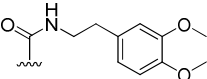
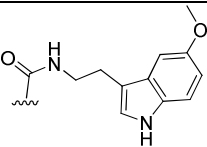
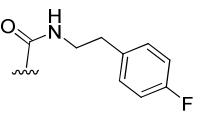
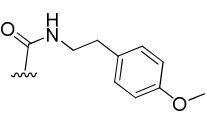
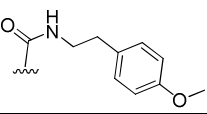
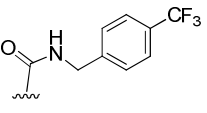
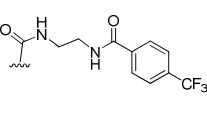
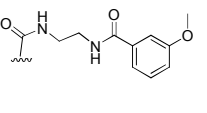
*Faculté des Sciences, ULB ; Brussels, Belgium.*

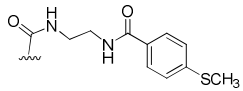
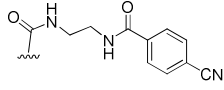
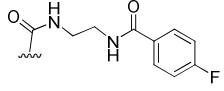
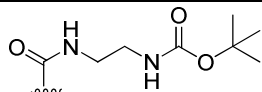
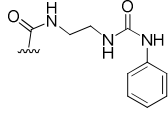
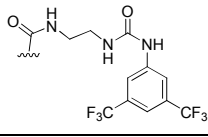
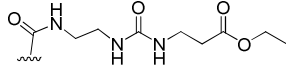
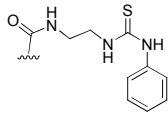
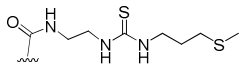
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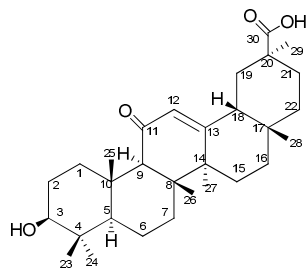


Compound #	R <sup>1</sup> =	R <sup>2</sup> =
1	COOH	OH
2		OH
<b>Monoamides</b>		
3a		OH
3b		OH
3c		OH
3d		OH
3e		= O
3f		OH
<b>Diamides</b>		
4a		OH
4b		OH

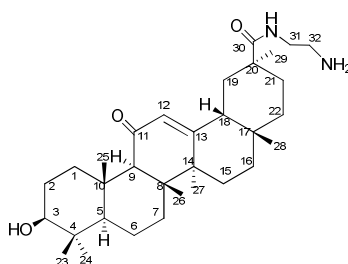
Compound #	R <sup>1</sup> =	R <sup>2</sup> =
4c		OH
4d		OH
4e		OH
<b>Carbamate</b>		
5		OH
<b>Ureides</b>		
6a		OH
6b		OH
6c		OH
<b>Thioureides</b>		
7a		OH
7b		OH

## Compound Characterization Data

### 18 $\beta$ -glycyrrhetic acid (1)

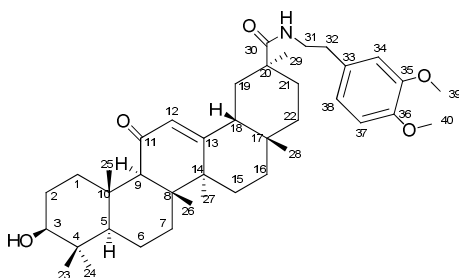


### *N*-(2-aminoethyl)-glycyrrhetinamide (2)<sup>36</sup>



**Yield:** 97% of white powder. **RP-HPLC:** purity = 96%,  $t_R$  = 5.15 min. **HRMS (ESI-QTOF)** calcd for (2)  $C_{32}H_{53}N_2O_3$  ( $MH^+$ ) 513.4051, obsd 513.4057, error: 1.18 ppm. The  $^1H$  NMR and  $^{13}C$  NMR spectra are consistent with those reported in the literature.<sup>26</sup>

### *N*-[2-(3,4-dimethoxyphenyl)ethyl]-glycyrrhetinamide (3a)



**Yield:** 86%. **R<sub>f</sub>** = 0.21 (cyclohexane; AcOEt 4:6). **RP-HPLC:** purity = 96%,  $t_R$  = 6.39 min  **$^1H$ -NMR** characteristic protons (HSQC, 300MHz,  $CDCl_3$ ):  $\delta$  (ppm) = 6.82 (d, 1H,  $J$  = 7.8 Hz, Ar-H37), 6.73 (dd, 1H,  $J$  = 7.8 Hz, 1.8 Hz, Ar-H38), 6.71 (s, 1H, Ar-H34), 5.65 (t, 1H,  $J$  = 5.5 Hz, NH), 5.54 (s, 1H, CH-12), 3.84 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.60-3.46 (m, 2H, NH- $CH_2$ ), 3.20 (dd, 1H,  $J$  = 10.5 Hz, 5.8 Hz, CH-3), 2.79-2.74 (t, 2H,  $J$  = 6.9 Hz, NH- $CH_2CH_2$ ; m, 1H, CH-1), 2.29 (s, 1H, CH-9), 1.32 (s, 3H, Me-H27), 1.11 (s, 3H, Me-H25), 1.09 (s, 3H, Me-H26), 1.04 (s, 3H, Me-H29),

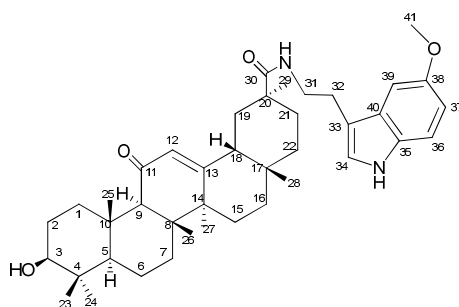
0.98 (s, 3H, Me-H23), 0.78 (s, 3H, Me-H24), 0.74 (s, 3H, Me-H28), 0.67 (d, 1H,  $J = 11.1$  Hz, CH-5).  $^{13}\text{C}$  NMR (DEPT 135, HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 200.2 (C11), 175.8 (C30), 169.3 (C13), 149.2 (C35), 147.9 (C36), 131.2 (C33), 128.6 (C12), 120.7 (C38), 111.9 (C37), 111.6 (C34), 78.9 (C3), 62.0 (C9), 56.1 (OMe), 56.0 (OMe), 55.1 (C5), 48.1 (C18), 45.5 (C14), 43.7 (C20), 43.3 (C8), 42.0 (C19), 40.5 (C31), 39.3 (C1/C4), 37.6 (C22), 37.3 (C10), 35.4 (C32), 32.9 (C7), 32.0 (C17), 31.6 (C21), 29.9 (C29), 28.5 (C28), 28.3 (C23), 27.4 (C2), 26.6 (C15), 26.5 (C16), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

IR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3400 (OH), 2949 (CH aliphatic), 1662 (C=O), 1654 (C=O), 1517, 1465, 1262, 1236, 1029, 731.

Mp: 123 °C.

HRMS (ESI-QTOF) calcd for (3a)  $\text{C}_{40}\text{H}_{59}\text{NO}_5$  ( $\text{MH}^+$ ) 634.4466, obsd 634.4463, error: 0.53 ppm.

### N-[2-(5-methoxy-1*H*-indol-3-yl)ethyl]-glycyrrhethinamide (3b)



**Yield:** 81%. **R<sub>f</sub>** = 0.28 (cyclohexane; AcOEt 4:6). **RP-HPLC:** purity = 97%,  $t_R$  = 5.99 min  $^1\text{H-NMR}$  characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 8.67 (br s, 1H, NH), 7.20 (d, 1H,  $J = 9.0$  Hz, Ar-H36), 7.10 (br s, 1H, Ar-H34), 7.01 (d, 1H,  $J = 3.0$  Hz, Ar-H39), 6.83 (dd, 1H,  $J = 9.0$  Hz, 3.0 Hz, Ar-H37), 5.66 (br t, 1H,  $J = 4.8$  Hz, NH), 4.94 (s, 1H, CH-12), 3.84 (s, 3H, OMe), 3.71-3.57 (m, 2H,  $\text{NHCH}_2$ ), 3.24 (dd, 1H,  $J = 10.5$  Hz, 5.8 Hz, CH-3), 2.90-3.10 (m, 2H,  $\text{NHCH}_2\text{CH}_2$ ), 2.84 (dt, 1H,  $J = 13.0$  Hz, 3.0 Hz, CH-1), 2.27 (s, 1H, CH-9), 1.24 (s, 3H, Me-H27), 1.16 (s, 3H, Me-H25), 1.06 (s, 3H, Me-H29), 1.04 (s, 3H, Me-H26), 1.01 (s, 3H, Me-H23), 0.82 (s, 3H, Me-H24), 0.69 (d, 1H,  $J = 12.0$  Hz, CH-5), 0.63 (s, 3H, Me-H28).  $^{13}\text{C}$  NMR (DEPT 135, HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 201.0 (C11), 175.8 (C30), 169.9 (C13), 154.3 (C38), 132.1 (C35), 127.9 (C40), 127.6 (C12), 122.9 (C34), 112.8 (C33), 112.7 (C36), 112.5 (C37), 100.0 (C39), 78.9 (C3), 62.0 (C9), 56.0 (OMe), 55.1 (C5), 48.4 (C18), 45.6 (C14), 43.8 (C20), 43.2 (C8), 41.9 (C19), 39.5 (C31), 39.4 (C1), 39.3 (C4), 37.5

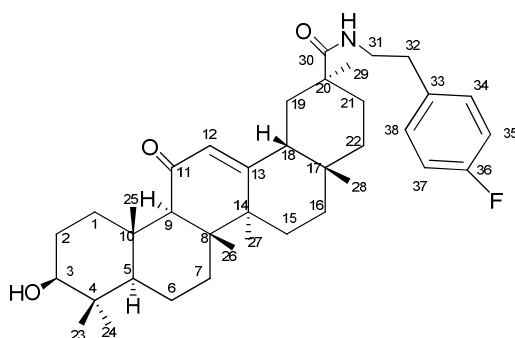
(C22), 37.2 (C10), 32.8 (C7), 31.9 (C17), 31.4 (C21), 29.8 (C29), 28.5 (C28), 28.3 (C23), 27.4 (C2), 26.5 (C15), 26.4 (C16), 24.7 (C32), 23.4 (C27), 18.7 (C26), 17.6 (C6), 16.6 (C25), 15.7 (C24).

**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3380 (OH), 2928 (CH aliphatic), 1735 (C=O), 1654 (C=O), 1522, 1486, 1388, 1213, 1173, 1040, 994.

**Mp**: 171 °C.

**HRMS (ESI-QTOF)** calcd for **(3b)**  $\text{C}_{41}\text{H}_{58}\text{N}_2\text{O}_4$  ( $\text{MH}^+$ ) 643.4469, obsd 643.4472, error: 0.41 ppm.

### ***N***-[2-(4-fluorophenyl)ethyl]-glycyrrhettinamide (**3c**)



**Yield**: 76%. **R<sub>f</sub>** = 0.27 (cyclohexane; AcOEt 5:5). **RP-HPLC**: purity = 95%, **t<sub>R</sub>** = 7.44 min. **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.16 (dd, 2H,  $J$  = 8.7 Hz, 5.4 Hz, Ar-H34, H38), 6.99 (t, 2H,  $J$  = 8.7 Hz, Ar-H35, H37), 5.56 (t, 1H,  $J$  = 5.4 Hz, NH), 5.51 (s, 1H, CH-12), 3.50-3.57 (m, 2H,  $\text{NHCH}_2\text{CH}_2$ ), 3.22 (dd, 1H,  $J$  = 10.5 Hz, 5.8 Hz, CH-3), 2.83-2.75 (t, 2H,  $J$  = 6.6 Hz,  $\text{NHCH}_2\text{CH}_2$ ; m, 1H, CH-1), 2.31 (s, 1H, CH-9), 1.34 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H25), 1.11 (s, 3H, Me-H26), 1.05 (s, 3H, Me-H29), 0.98 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.77 (s, 3H, Me-H28), 0.69 (d, 1H,  $J$  = 11.4 Hz, CH-5). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 200.8 (C11), 176.4 (C30), 169.8 (C13), 162.9 (d,  $J$  = 242.3 Hz, C36), 135.1 (d,  $J$  = 3.0 Hz, C33), 130.9 (d,  $J$  = 8.3 Hz, C34/C38), 129.2 (C12), 116.4 (d,  $J$  = 21.0 Hz, C37/C35), 62.6 (C9), 55.2 (C5), 48.2 (C18), 45.6 (C14), 43.8 (C20), 43.4 (C8), 42.0 (C19), 40.7 (C31/C32\*), 39.4 (C1/C4), 37.6 (C22), 37.3 (C10), 35.1 (C31/C32\*), 33.0 (C7), 32.1 (C17), 31.7 (C21), 29.9 (C29), 28.5 (C28), 28.3 (C23), 27.4 (C2), 26.7 (C15), 26.6 (C16), 23.6 (C27), 18.9 (C26), 17.7 (C6), 16.6 (C25), 15.8 (C24).

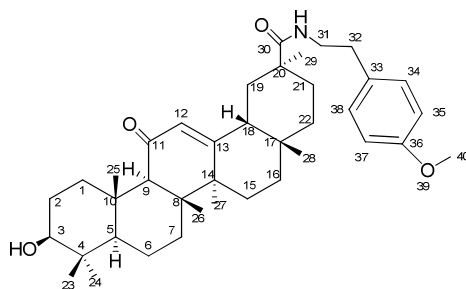
\*attribution may be reversed

**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3387 (OH), 2928 (CH aliphatic), 1654 (C=O), 1651 (C=O), 1510, 1452, 1223, 1048, 994, 823.

**Mp:** 155 °C.

**HRMS (ESI-QTOF)** calcd for **(3c)** C<sub>38</sub>H<sub>54</sub>FNO<sub>3</sub> (MH<sup>+</sup>) 592.4160, obsd 592.4163, error: 0.35 ppm.

***N*-[2-(4-methoxyphenyl)ethyl]-glycyrrhettinamide (3d)**



**Yield:** 71%. **R<sub>f</sub>** = 0.24 (cyclohexane; AcOEt 4:6). **RP-HPLC:** purity = 95%, **t<sub>R</sub>** = 7.51 min. **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.12 (d, 2H, *J* = 8.4 Hz, Ar-H34, H38), 6.85 (d, 2H, *J* = 8.4 Hz, Ar-H35, H37), 5.54 (t, 1H, *J* = 5.7 Hz, NH), 5.44 (s, 1H, CH-12), 3.78 (s, 3H, OMe), 3.59-3.47 (m, 2H, NHCH<sub>2</sub>), 3.22 (dd, 1H, *J* = 10.5 Hz, 5.8 Hz, CH-3), 2.80-2.76 (t, 2H, *J* = 6.6 Hz, NHCH<sub>2</sub>CH<sub>2</sub>; m, 1H, CH-1), 2.30 (s, 1H, CH-9), 1.33 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H25), 1.10 (s, 3H, Me-H26), 1.05 (s, 3H, Me-H29), 0.99 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.77 (s, 3H, Me-H28), 0.69 (d, 1H, *J* = 11.4 Hz, CH-5). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz, CDCl<sub>3</sub>): δ (ppm) = 200.4 (C11), 176.0 (C30), 169.4 (C13), 158.7 (C36), 131.0 (C33), 130.0 (C34/C38), 128.9 (C12), 114.6 (C35/C37), 79.2 (C3), 62.2 (C9), 55.6 (OMe), 55.4 (C5), 48.3 (C18), 45.7 (C14), 43.7 (C20), 43.3 (C8), 42.0 (C19), 40.6 (C31), 39.3 (C1/C4), 37.6 (C22), 37.3 (C10), 34.7 (C32), 32.9 (C7), 32.0 (C17), 31.6 (C21), 29.9 (C29), 28.6 (C28), 28.2 (C23), 27.4 (C2), 26.6 (C15), 26.5 (C16), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

**IR** (KBr): ν (cm<sup>-1</sup>): 3392 (OH), 2929 (CH aliphatic), 1653, 1649 (C=O), 1513, 1457, 1387, 1247, 1040, 995.

**Mp:** 156 °C.

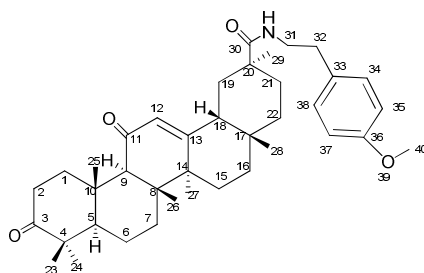
**HRMS (ESI-QTOF)** calcd for **(3d)** C<sub>39</sub>H<sub>57</sub>NO<sub>4</sub> (MH<sup>+</sup>) 604.4360, obsd 604.4360, error: 0.01 ppm.

***N*-[2-(4-methoxyphenyl)ethyl]-3-oxoglycyrrhettinamide (3e)**

**Yield:** 98%. **R<sub>f</sub>** = 0.55 (cyclohexane; AcOEt 4:6). **RP-HPLC:** purity = 98%, **t<sub>R</sub>** = 7.93 min. **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.12 (



d, 2H,  $J = 8.7$  Hz, Ar-H34, H38), 6.85 (d, 2H,  $J = 8.7$  Hz, Ar-H35, H37), 5.53 (t, 1H,  $J = 5.7$  Hz, NH), 5.48 (s, 1H, CH-12), 3.78 (s, 3H, OMe), 3.65-3.45 (m, 2H, NHCH<sub>2</sub>), 2.97 (ddd, 1H,  $J = 13.5$  Hz, 6.9 Hz, 4.2 Hz, CH-1), 2.78 (t, 2H,  $J = 6.9$  Hz NHCH<sub>2</sub>CH<sub>2</sub>), 2.64 (ddd, 1H,  $J = 16.2$  Hz, 11.0 Hz, 6.9 Hz, CH-2), 2.40 (s, 1H, CH-9), 2.41-2.31 (ddd, 1H,  $J = 16.2$  Hz, 6.3 Hz, 3.9 Hz, CH-2), 1.34 (s, 3H, Me-H27), 1.26 (s, 3H, Me-H25), 1.15 (s, 3H, Me-H26), 1.09 (s, 3H, Me-H23), 1.07 (s, 3H, Me-H24), 1.06 (s, 3H, Me-H29), 0.79 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 217.3 (C3), 199.4 (C11), 175.7 (C30), 169.7 (C13), 158.5 (C36), 130.7 (C33), 129.8 (C34/C38), 128.5 (C12), 114.3 (C35/C37), 61.2 (C9), 55.6 (C5), 55.4 (C39), 48.0 (C18), 47.9 (C4), 45.3 (C14), 43.7 (C20), 43.4 (C8), 41.9 (C19), 40.6 (C31), 39.9 (C1), 37.6 (C22), 36.9 (C10), 34.8 (C32), 34.4 (C2), 32.3 (C7), 32.0 (C17), 31.6 (C21), 29.8 (C29), 28.6 (C28), 26.6 (C15), 26.6 (C23), 26.5 (C16), 23.5 (C27), 21.6 (C24), 18.9 (C6), 18.7 (C26), 15.8 (C25).

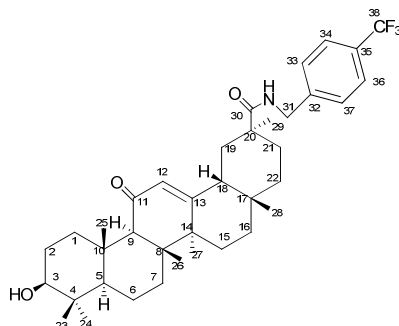


**IR** (KBr):  $\nu$  (cm<sup>-1</sup>): 2929 (CH aliphatic), 1653, 1649 (C=O), 1513, 1457, 1387, 1247, 1040, 995.

**Mp**: 134 °C.

**HRMS (ESI-QTOF)** calcd for **(3e)** C<sub>39</sub>H<sub>55</sub>NO<sub>4</sub> (MH<sup>+</sup>) 602.4204, obsd 602.4211, error: 1.15 ppm.

### ***N*-{[(4-trifluoromethyl)phenyl]methyl}-glycyrrhettinamide (3f)**



**Yield**: 70%. **R<sub>f</sub>** = 0.4 (cyclohexane ; AcOEt 4:6). **RP-HPLC**: purity = 96%, **t<sub>R</sub>** = 7.42 min. **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 7.59 (d, 2H,  $J = 8.0$  Hz, Ar-H34, H36), 7.38 (d, 2H,  $J = 8.0$  Hz, Ar-H33, H37), 6.00 (t, 1H,  $J =$

5.7 Hz, NH), 5.59 (s, 1H, CH-12), 4.59-4.45 (m, 2H, NHCH<sub>2</sub>), 3.21 (dd, 1H, *J* = 10.5 Hz, 5.8 Hz, CH-3), 2.78 (dt, 1H, *J* = 13.0 Hz, 3.0 Hz, CH-1), 2.31 (s, 1H, CH-9), 1.36 (s, 3H, Me-H27), 1.16 (s, 3H, Me-H29), 1.12 (s, 6H, Me-H25, H26), 0.99 (s, 3H, Me-H23), 0.80 (s, 6H, Me-H24, H28), 0.69 (d, 1H, *J* = 11.4 Hz, CH-5). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz, CDCl<sub>3</sub>): δ (ppm) = 200.2 (C11), 175.9 (C30), 169.0 (C13), 142.9 (C32), 129.9 (q, *J* = 32.3 Hz, C35), 128.7 (C12), 127.9 (C33/C37), 125.8 (q, *J* = 3.7 Hz, C34/C36), 122.3 (q, *J* = 251.3 Hz, C38), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.3 (C18), 45.5 (C14), 43.8 (C20), 43.4 (C8), 43.2 (C31), 41.9 (C19), 39.3 (C1/C4), 37.6 (C22), 37.3 (C10), 32.9 (C7), 32.1 (C17), 31.7 (C21), 29.7 (C29), 28.6 (C28), 28.3 (C23), 27.5 (C2), 26.7 (C15), 26.6 (C16), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

**IR** (KBr): ν (cm<sup>-1</sup>): 3349 (OH), 2929 (CH aliphatic), 1654, 1651 (C=O), 1528, 1457, 1326, 1164, 1125, 1067, 994.

**Mp**: 165 °C.

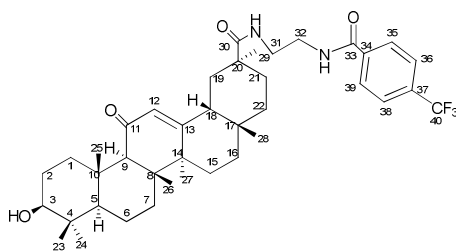
**HRMS (ESI-QTOF)** calcd for **(3f)** C<sub>38</sub>H<sub>52</sub>F<sub>3</sub>NO<sub>3</sub> (MH<sup>+</sup>) 628.3972, obsd 628.3971, error: 0.14 ppm.

#### ***N*-(2-[[4-(trifluoromethyl)benzamido]ethyl])-glycyrrhetinamide (4a)**

**Yield**: 36%. **R<sub>f</sub>** = 0.34 (DCM; MeOH 95:5). **RP-HPLC**: purity = 95%, *t<sub>R</sub>* = 6.21 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.94 (d, 2 H, *J* = 9.0 Hz, ArH), 7.74 (d, 2 H, *J* = 8.4 Hz ArH and CH<sub>2</sub>NHCOArH), 6.48 (s, 1H, CONHCH<sub>2</sub>CH<sub>2</sub>), 5.59 (s, 1H, CH-12), 3.74-3.42 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH), 3.21 (dd, 1H, *J* = 6.0 Hz, 9.9 Hz, CH-3), 2.79 (dt, 1H, *J* = 13.2 Hz, 3.3 Hz, CH-1), 2.32 (s, 1H, CH-9), 1.34 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H25), 1.09 (s, 3H, Me-H29), 1.06 (s, 3H, Me-H26), 1.00 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.68 (d, 1H, *J* = 11.4 Hz, CH-5), 0.61 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (HSQC, 75 MHz, CDCl<sub>3</sub>): δ (ppm) = 200.0 (C11), 175.9 (C30), 168.7 (C13), 166.4 (C33), 136.7 (C34), 129.5 (q, *J* = 32.5 Hz, C37), 128.7 (C12), 127.6 (C35/C39), 125.8 (q, *J* = 3.7 Hz, C36/C38), 125.5 (q, *J* = 271.5 Hz, C40), 78.9 (C3), 61.9 (C9), 55.1 (C5), 48.1 (C18), 45.4 (C14), 43.8 (C20), 43.3 (C8), 42.6 (C31/C32\*), 41.6 (C19), 39.6 (C31/C32\*), 39.2 (C1/C4), 37.6 (C22), 37.2 (C10), 33.1 (C7), 31.8 (C17), 31.4 (C21), 29.6 (C29), 28.4 (C23), 28.2 (C28), 27.4 (C2), 26.5 (C15/C16), 23.5 (C27), 18.6 (C26), 17.6 (C6), 16.4 (C25), 15.6 (C24).

\*attribution may be reversed



**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3348 (OH), 2938 (CH aliphatic), 1647 (C=O), 1541, 1458, 1380.

**Mp**: 156°C.

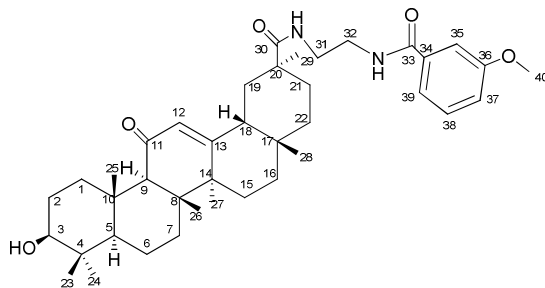
**HRMS (ESI-QTOF)** calcd for (**4a**)  $\text{C}_{40}\text{H}_{55}\text{F}_3\text{N}_2\text{O}_4$  ( $\text{M}+\text{H}^+$ ) 685.4184, obsd 685.4187, error: 0.42 ppm.

### ***N***-{2-[(3-methoxy)benzamido]ethyl}-glycyrrhetinamide (**4b**)

**Yield**: 75%. **R<sub>f</sub>** = 0.32 (DCM; MeOH 95:5). **RP-HPLC**: purity = 95%, **t<sub>R</sub>** = 6.09 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) = 7.41-7.37 (m, 1H, Ar-H35), 7.35-7.31 (m, 2 H, Ar-H38, H37/H39\*), 7.27 (br s, 1H,  $\text{NHCOArH}$ ), 7.05-6.97 (m, 1H, Ar-H37/H39\*), 6.62 (br s, 1 H,  $\text{CONHCH}_2\text{CH}_2$ ), 5.64 (s, 1H, CH-12), 3.83 (s, 3H, OMe), 3.70-3.40 (m, 4H,  $\text{NHCH}_2\text{CH}_2\text{NH}$ ), 3.23 (dd, 1H,  $J$  = 10.2 Hz, 6.3 Hz, CH-3), 2.79 (dt, 1 H,  $J$  = 13.2 Hz, 3.0 Hz, CH-1), 2.31 (s, 1 H, CH-9), 1.34 (s, 3H, Me-H27), 1.12 (s, 3H, Me-H25), 1.09 (s, 3H, Me-H29), 1.07 (s, 3H, Me-H26), 1.00 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.68 (d, 1H,  $J$  = 11.2 Hz, CH-5), 0.65 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 200.1 (C11), 178.1 (C30), 169.1 (C13), 168.3 (C33), 159.9 (C36), 136.3 (C34), 129.8 (C38), 128.7 (C12), 119.0 (C37/C39\*), 118.1 (C37/C39\*), 112.4 (C35), 78.9 (C3), 61.9 (C9), 56.6 (OMe), 55.1 (C5), 48.0 (C18), 45.5 (C14), 43.8 (C20), 43.3 (C8), 41.9 (C31/C32\*), 41.6 (C19), 39.9 (C31/C32\*), 39.3 (C1/C4), 37.7 (C22), 37.2 (C10), 32.9 (C7), 31.9 (C17), 31.7 (C21), 29.7 (C29), 28.5 (C28), 28.3 (C23), 27.4 (C2), 26.6 (C15/C16), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

\*attribution may be reversed

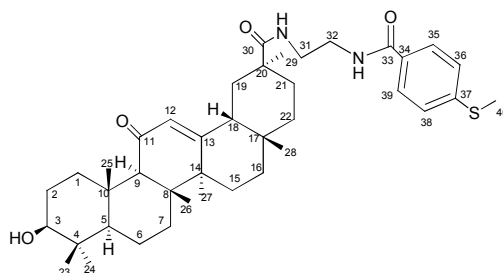


**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3360 (OH), 2964 (CH aliphatic), 1650 (C=O), 1586, 1541, 1469, 1389, 1262, 1047.

**Mp**: 240°C.

**HRMS (ESI-QTOF)** calcd for **(4b)** C<sub>40</sub>H<sub>58</sub>N<sub>2</sub>O<sub>5</sub> (MH<sup>+</sup>) 647.4418, obsd 647.4418, error: 0.03 ppm.

***N***-2-[[**(4-methylthio)benzamido**]ethyl]-glycyrrhetinamide (**4c**)



**Yield:** 54%. **R<sub>f</sub>** = 0.32 (DCM; MeOH 95:5). **RP-HPLC:** purity = 96%, **t<sub>R</sub>** = 6.53 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.73 (d, 2H, *J* = 8.4 Hz, Ar-H35, H-39), 7.32 (br s, 1H, NHCOArH), 7.25 (d, 2H, *J* = 8.4 Hz, Ar-H36, H-38), 6.55 (s, 1H, CONHCH<sub>2</sub>), 5.67 (s, 1H, CH-12), 3.76-3.40 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH), 3.23 (dd, 1H, *J* = 9.9 Hz, 5.7 Hz, CH-3), 2.80 (dt, 1H, *J* = 13.2 Hz, 3.0 Hz, CH-1), 2.50 (s, 3H, SCH<sub>3</sub>), 2.32 (s, 1H, CH-9), 1.34 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H25), 1.09 (s, 3H, Me-H29), 1.07 (s, 3, H, Me-H26), 1.00 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.69 (d, 1H, *J* = 10.2 Hz, CH-5), 0.59 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (HSQC, 75 MHz, CDCl<sub>3</sub>): δ (ppm) = 200.1 (C11), 178.3 (C30), 169.1 (C13), 167.8 (C33), 143.9 (C37), 129.8 (C34), 128.8 (C12), 127.6 (C35/C39), 125.5 (C36/C38), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.0 (C18), 45.5 (C14), 43.8 (C20), 43.3 (C8), 42.2 (C31/C32\*), 41.6 (C19), 39.9 (C31/C32\*), 39.8 (C1/C4), 37.9 (C), 37.7 (C22), 37.3 (C10), 32.9 (C7), 31.9 (C17), 31.8 (C21), 29.6 (C29), 28.5 (C28), 28.3 (C23), 27.5 (C2), 26.6 (C15/C16), 23.6 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24), 15.1 (SMe).

\*attribution may be reversed

**IR** (KBr): ν (cm<sup>-1</sup>): 3344 (OH), 2932 (CH aliphatic), 1643 (C=O), 1537, 1468, 1312, 1200, 1094.

**Mp:** 202 °C.

**HRMS (ESI-QTOF)** calcd for **(4c)** (MH<sup>+</sup>) 663.4190, obsd 663.4187, error: 0.54 ppm.

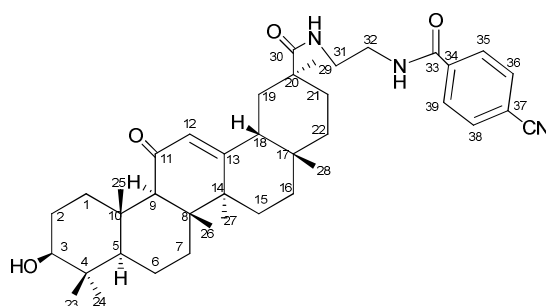
***N***-2-[[**(4-cyano)benzamido**]ethyl]-glycyrrhetinamide (**4d**)

**Yield:** 57%. **R<sub>f</sub>** = 0.19 (DCM; MeOH 97:3). **RP-HPLC:** purity = 98%, **t<sub>R</sub>** = 5.37 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.94 (d, 2H, *J* = 9.0 Hz, Ar-H35, H-39), 7.74 (d, 2H, *J* = 9.0 Hz, Ar-H36, H-38 ; br s, 1H, NHCOArH),

6.48 (br s, 1H, CONHCH<sub>2</sub>CH<sub>2</sub>), 5.59 (s, 1H, CH-12), 3.72-3.44 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH), 3.21 (dd, 1H, *J* = 9.9 Hz, 6.6 Hz, CH-3), 2.79 (dt, 1H, *J* = 13.5 Hz, 3.6 Hz, CH-1), 2.32 (s, 1H, CH-9), 1.34 (s, 3H, Me-H27), 1.14 (s, 3H, Me-H25), 1.09 (s, 3H, Me-H29), 1.07 (s, 3H, Me-H26), 1.00 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.68 (d, 1H, *J* = 9.9 Hz, CH-5), 0.61 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (HSQC, 75 MHz, CDCl<sub>3</sub>): δ (ppm) = 200.1 (C11), 178.1 (C30), 169.0 (C13), 166.0 (C33), 137.7 (C34), 132.6 (C36/C38), 128.7 (C12), 127.9 (C35/39), 118.2 (CN), 115.3 (C37), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.2 (C18), 45.5 (C14), 43.8 (C20), 43.3 (C8), 42.9 (C31/C32\*), 41.7 (C19), 39.4 (C31/C32\*), 39.3 (C1/C4), 37.6 (C22), 37.2 (C10), 32.8 (C7), 31.8 (C17), 31.7 (C21), 29.5 (C29), 28.5 (C28), 28.2 (C23), 27.4 (C2), 26.5 (C15/C16), 23.5 (C27), 18.7 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

\*attribution may be reversed



**IR** (KBr):  $\nu$  (cm<sup>-1</sup>): 3350 (OH), 2940 (CH aliphatic), 2232 (CN), 1647 (C=O), 1539, 1459, 1039, 993.

**Mp**: 231 °C.

**HRMS (ESI-QTOF)** calcd for **(4d)** C<sub>40</sub>H<sub>55</sub>N<sub>3</sub>O<sub>4</sub> (MH<sup>+</sup>) 642.4265, obsd 642.4265, error: 0.09 ppm.

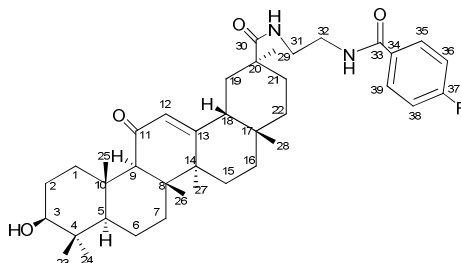
### ***N***-{2-[(4-fluoro)benzamido]ethyl}-glycyrrhetinamide (**4e**)

**Yield**: 69%. **R<sub>f</sub>** = 0.19 (DCM; MeOH 97:3). **RP-HPLC**: purity = 98%, **t<sub>R</sub>** = 6.16 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.83 (dd, 2H, *J* = 9.0 Hz, 5.3 Hz, Ar-H35, H-39), 7.33 (br s, 1 H, NHCOAr), 7.11 (t, 2H, *J* = 9.0 Hz, Ar-H36, H38), 6.55 (br s, 1H, CONHCH<sub>2</sub>), 5.63 (s, 1H, CH-12), 3.72-3.42 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH), 3.23 (dd, 1H, *J* = 10.0 Hz, 5.9 Hz, CH-3), 2.80 (dt, 1H, *J* = 13.0 Hz, 4.0 Hz, CH-1), 2.32 (s, 1H, CH-9), 1.35 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H25), 1.09 (s, 3H, Me-H29), 1.07 (s, 3H, Me-H26), 1.00 (s, 3H, Me-H23), 0.81 (s, 3H, Me-H24), 0.69 (d, 1H, *J* = 11.4 Hz, CH-5), 0.64 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (HSQC, 75 MHz, CDCl<sub>3</sub>): δ (ppm) = 200.1 (C11), 178.3 (C30), 169.1 (C13), 167.2 (C33), 130.0 (d, *J* =

3.0 Hz, C34), 129.5 (d,  $J = 9.0$  Hz, C35/C39), 115.8 (d,  $J = 21.8$  Hz, C36/C38), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.1 (C18), 45.5 (C14), 43.8 (C20), 43.3 (C8), 42.3 (C31/C32\*), 41.7 (C19), 39.9 (C31/C32\*), 39.3 (C1/C4), 37.7 (C22), 37.3 (C10), 32.9 (C7), 31.9 (C17), 31.7 (C21), 29.6 (C29), 28.5 (C28), 28.3 (C23), 27.5 (C2), 26.6 (C15/C16), 23.6 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

\*attribution may be reversed

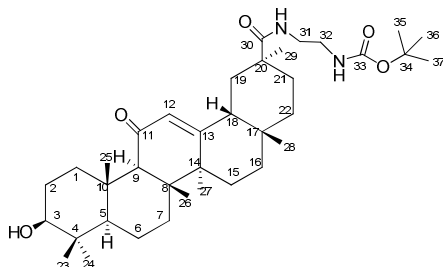


**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3336 (OH), 2936 (CH aliphatic), 2872, 1646 (C=O), 1537, 1508, 1463, 1235, 854.

**Mp**: 256 °C.

**HRMS (ESI-QTOF)** calcd for (**4e**)  $\text{C}_{39}\text{H}_{55}\text{FN}_2\text{O}_4$  ( $\text{MH}^+$ ) 635.4219, obsd 635.4216, error: 0.46 ppm.

### ***N***-[2-(*tert*butoxycarbonylamino)ethyl]-glycyrrhettinamide (**5**)



**Yield**: 64%. **R<sub>f</sub>** = 0.18 (cyclohexane; AcOEt 5:5). **RP-HPLC**: purity = 96%, **t<sub>R</sub>** = 7.19 min **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 6.56 (br s, 1H,  $\text{NHCH}_2\text{CH}_2\text{NH}$ ), 5.72 (s, 1H, CH-12), 4.94 (br s, 1H,  $\text{NHCH}_2\text{CH}_2\text{NH}$ ), 3.43-3.26 (m, 4H,  $\text{NHCH}_2\text{CH}_2\text{NH}$ ), 3.22 (dd, 1H,  $J = 10.2$  Hz, 6.0 Hz, CH-3), 2.77 (dt, 1H,  $J = 13.0$  Hz, 3.0 Hz, CH-1), 2.33 (s, 1H, CH-9), 1.42 (s, 9H, *t*-Bu), 1.37 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H25), 1.12 (s, 6H, Me-H26,H29), 1.00 (s, 3H, Me-H23), 0.81 (s, 3H, Me-H28), 0.80 (s, 3H, Me-H24), 0.70 (d, 1H,  $J = 11.4$  Hz, CH-5). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 200.3 (C11), 176.8 (C30), 171.4 (C13), 157.4 (C33), 128.7 (C12), 78.9 (C3), 61.9 (C9), 55.1 (C5), 53.6 (C34), 48.1 (C18), 45.5 (C14), 43.6 (C20), 43.4 (C8), 41.8 (C19), 41.5 (C31/C32\*), 40.6 (C31/C32\*), 39.3 (C1/C4), 37.7 (C22), 37.2 (C10), 32.9 (C7), 32.0 (C17), 31.6 (C21), 29.7 (C29),

28.7 (C28), 28.5 (C35/C36/C37), 28.3 (C23), 27.5 (C2), 26.6 (C15/C16), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

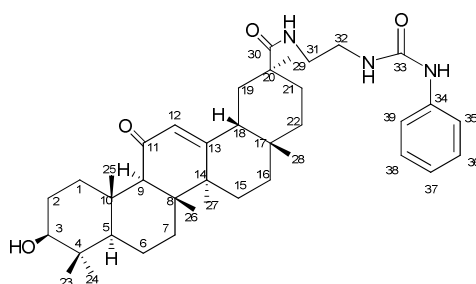
\*attribution may be reversed

**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3480 (OH), 2970 (CH aliphatic), 1700 (C=O), 1654 (C=O), 1648 (C=O), 1540, 1508, 1366, 1244, 1168, 923, 730.

**Mp**: 223 °C.

**HRMS (ESI-QTOF)** calcd for (**5**)  $\text{C}_{37}\text{H}_{60}\text{N}_2\text{O}_5$  ( $\text{MH}^+$ ) 613.4575, obsd 613.4571, error: 0.59 ppm.

### ***N***-[2-(3-phenylureido)ethyl]-glycyrrhetinamide (**6a**)



**Yield**: 88%. **R<sub>f</sub>** = 0.32 (DCM; MeOH 95:5). **RP-HPLC**: purity = 99%, **t<sub>R</sub>** = 5.87 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.34 (d, 2H,  $J$  = 7.8 Hz, Ar-H35, H39), 7.26 (t, 2H,  $J$  = 7.7 Hz, Ar-H36, H38), 7.03 (t, 1H,  $J$  = 7.1 Hz, Ar-H37), 5.69 (s, 1H, CH-12), 3.57-3.30 (m, 4H,  $\text{NHCH}_2\text{CH}_2\text{NH}$ ), 3.22 (dd, 1H,  $J$  = 9.9 Hz, 7.1 Hz, CH-3), 2.72 (d, 1H,  $J$  = 13.0 Hz, CH-1), 2.30 (s, 1 H, CH-9), 1.34 (s, 3H, Me-H27), 1.13 (s, 3H, Me-H29), 1.11 (s, 3H, Me-H25), 1.07 (s, 3H, Me-H26), 1.00 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.76 (s, 3H, Me-H28), 0.68 (d, 1H,  $J$  = 11.4 Hz, CH-5). **<sup>13</sup>C NMR** (HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 200.8 (C11), 177.9 (C30), 170.3 (C13), 157.8 (C33), 138.5 (C34), 129.3 (C36/C38), 128.5 (C12), 124.1 (C37), 121.2 (C35/C39), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.5 (C18), 45.7 (C14), 43.9 (C20), 43.5 (C8), 41.8 (C19), 41.6 (C31/C32\*), 40.1 (C31/C32\*), 39.4 (C1/C4), 37.7 (C22), 37.3 (C10), 32.9 (C7), 32.1 (C17), 31.4 (C21), 29.7 (C29), 28.8 (C28), 28.3 (C23), 27.5 (C2), 26.7 (C15/C16), 23.5 (C27), 18.9 (C26), 17.7 (C6), 16.7 (C25), 15.8 (C24).

\*attribution may be reversed

**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3383 (OH), 2928 (CH aliphatic), 1649 (C=O), 1554, 1500, 1443, 1388, 1313, 1249, 1042, 996.

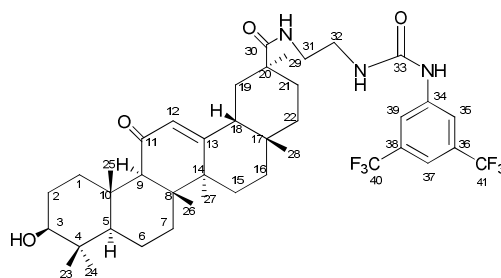
**Mp**: 261 °C.

**HRMS (ESI-QTOF)** calcd for **(6a)** C<sub>39</sub>H<sub>57</sub>N<sub>3</sub>O<sub>4</sub> (MH<sup>+</sup>) 632.4422, obsd 632.4416, error: 0.90 ppm.

***N*-(2-{3-[3,5-bis(trifluoromethyl)phenyl]ureido}ethyl)-glycyrrhettinamide (6b)**

**Yield:** 36%. **R<sub>f</sub>** = 0.30 (DCM; MeOH 95:5). **RP-HPLC:** purity = 99%, **t<sub>R</sub>** = 8.40 min **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CD<sub>3</sub>OD): δ (ppm) = 8.02 (s, 2H, Ar-H35, H39), 7.42 (s, 1H, Ar-H37), 5.61 (s, 1H, CH-12), 3.62-3.22 (m, 4H, NHCH<sub>2</sub>CH<sub>2</sub>NH), 3.15 (dd, 1H, *J* = 7.0 Hz, 5.1 Hz, CH-3), 2.66 (dt, 1H, *J* = 13.0 Hz, 3.0 Hz CH-1), 2.37 (s, 1H, CH-9), 1.38 (s, 3H, Me-H27), 1.10 (s, 3H, Me-H29), 1.05 (s, 3H, Me-H25), 0.98 (s, 3H, Me-H23), 0.94 (s, 3H, Me-H26), 0.78 (s, 3H, Me-H24), 0.74 (s, 3H, Me-H28). **<sup>13</sup>C NMR** (HSQC, 75 MHz, CD<sub>3</sub>OD): δ (ppm) = 202.4 (C11), 179.4 (C30), 172.6 (C13), 157.6 (C33), 143.4 (C34), 133.0 (q, *J* = 33.0 Hz, C36/C38), 128.9 (C12), 124.8 (q, *J* = 270.1 Hz, CF<sub>3</sub>), 118.8 (q, *J* = 3.0 Hz, C35/C39), 115.3 (q, *J* = 7.0 Hz, C37), 79.4 (C3), 63.1 (C9), 56.2 (C5), 49.3 (C18), 46.6 (C14), 44.9 (C20), 44.5 (C8), 42.6 (C19), 41.1 (C31/C32\*), 40.6 (C31/C32\*), 40.3 (C1), 40.2 (C4), 38.7 (C22), 38.3 (C10), 33.7 (C7), 32.9 (C17), 31.9 (C21), 29.6 (C29), 29.1 (C28), 28.7(C23), 27.8 (C2), 27.6 (C15/C16\*), 27.4 (C15/C16\*), 23.7 (C27), 19.1 (C26), 18.6 (C6), 16.8 (C25), 16.3 (C24).

\*attribution may be reversed



**IR** (KBr):  $\nu$  (cm<sup>-1</sup>): 3360 (OH), 2932 (CH aliphatic), 1643 (C=O), 1544, 1467, 1386.

**Mp:** 201 °C.

**HRMS (ESI-QTOF)** calcd for **(6b)** C<sub>41</sub>H<sub>55</sub>F<sub>6</sub>N<sub>3</sub>O<sub>4</sub> (M+H<sup>+</sup>) 768.4170, obsd 768.4167, error: 0.29 ppm.

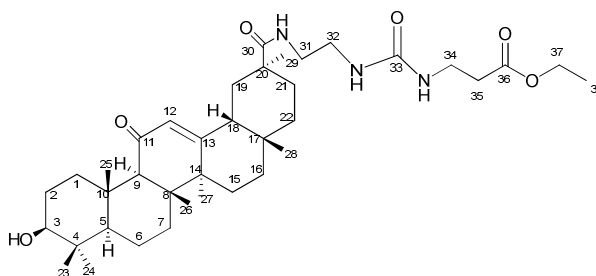
***N*-(2-{3-[2-(ethoxycarbonyl)ethyl]ureido}ethyl)-glycyrrhettinamide (6c)**

**Yield:** 71%. **R<sub>f</sub>** = 0.28 (DCM; MeOH 96:4). **RP-HPLC:** purity = 98%, **t<sub>R</sub>** = 6.73 min. **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>): δ (ppm) = 7.27 (br s, 1H, NH), 7.16 (br s, 1H, NH), 5.67 (s, 1H, CH-12), 5.47 (br s, 1H, NH), 4.12 (q, 2H, *J* =



7.1 Hz,  $\text{OCH}_2\text{CH}_3$ ), 3.58-3.31 (m, 6 H, H-31, H-32, H-34), 3.23 (dd, 1H,  $J = 10.5$  Hz, 5.7 Hz, CH-3), 2.75 (d, 1H,  $J = 13.0$  Hz, CH-1), 2.52 (t, 2H,  $J = 5.9$  Hz, H-35), 2.33 (s, 1H, CH-9), 1.37 (s, 3H, Me-H27), 1.24 (t, 3H,  $J = 7.0$  Hz, H-38), 1.12 (s, 9H, Me-H25,H29, H26), 1.00 (s, 3H, Me-H23), 0.80 (s, 6H, Me-H24, H28), 0.69 (d, 1H,  $J = 11.4$  Hz, CH-5).  $^{13}\text{C}$  NMR (HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 200.5 (C11), 177.2 (C30), 172.9 (C36), 169.7 (C13), 161.9 (C33), 128.5 (C12), 78.9 (C3), 61.9 (C9), 60.8 (C37), 55.1 (C5), 48.3 (C18), 45.5 (C14), 43.7 (C20), 43.4 (C8), 42.0 (C31/C32\*), 41.8 (C19), 40.1 (C31/C32\*), 39.3 (C1/C4), 37.7 (C22), 37.3 (C10), 36.3 (C34), 35.1 (C35), 32.9 (C7), 32.0 (C17), 31.5 (C21), 29.6 (C29), 28.7 (C28), 28.3 (C23), 27.4 (C2), 26.7 (C15/C16), 23.5 (C27), 18.8 (C26), 17.6 (C6), 16.5 (C25), 15.8 (C24), 14.3 (C38).

\*attribution may be reversed

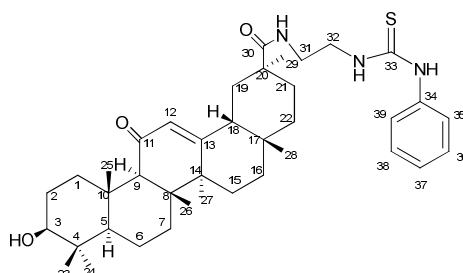


**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3346 (OH), 2935 (CH aliphatic), 2873, 1726 (C=O), 1648 (C=O), 1551, 1459, 1379, 1260, 1041.

**Mp**: 216°C.

**HRMS (ESI-QTOF)** calcd for (6c)  $\text{C}_{38}\text{H}_{61}\text{N}_3\text{O}_6$  ( $\text{MH}^+$ ), 656.4633, obsd 656.4642, error: 1.31 ppm.

### ***N*-[2-(3-phenylthioureido)ethyl]-glycyrrhetinamide (7a)**



**Yield**: 73%. **R<sub>f</sub>** = 0.34 (DCM; MeOH 95:5). **RP-HPLC**: purity = 97%, **t<sub>R</sub>** = 5.94 min.

$^1\text{H-NMR}$  characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 8.09 (br s, 1H,  $\text{NHArH}$ ), 7.43 (t, 2H,  $J = 7.2$  Hz, Ar-H36, H38), 7.36-7.16 (m, 3H, Ar-H35, H37, H39),

6.89 (t, 1H, CONHCH<sub>2</sub>CH<sub>2</sub>,  $J = 3.9$  Hz), 6.66 (t, 1H, CH<sub>2</sub>NH,  $J = 4.0$  Hz), 5.71 (s, 1H, CH-12), 4.16- 3.96 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>NHCS), 3.84-3.66 (m, 1H, CH<sub>2</sub>CH<sub>2</sub>NHCS), 3.53-3.33 (m, 2H, NHCH<sub>2</sub>CH<sub>2</sub>NH), 3.22 (dd, 1H,  $J = 10.0$  Hz, 6.9 Hz, CH-3), 2.72 (dt, 1H,  $J = 10.0$  Hz, 4.0 Hz, CH-1), 2.35 (s, 1 H, CH-9), 1.36 (s, 3H, Me-H27), 1.12 (s, 6H, Me-H25, H26), 1.10 (s, 3H, Me-H29), 1.00 (s, 3H, Me-H23), 0.80 (s, 3H, Me-H24), 0.78 (s, 3H, Me-H28), 0.70 (d, 1H,  $J = 11.4$  Hz, CH-5). **<sup>13</sup>C NMR** (HSQC, 75 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 200.6 (C11), 181.7 (C33), 177.4 (C30), 169.4 (C13), 136.1 (C34), 130.4 (C36/C38), 128.6 (C12), 127.6 (C37), 125.4 (C35/C39), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.3 (C18), 45.5 (C14), 44.8 (C32), 43.7 (C20), 43.3 (C8), 41.6 (C19), 41.1 (C31), 39.3 (C1/C4), 37.7 (C22), 37.3 (C10), 32.9 (C7), 32.0 (C17), 31.6 (C21), 29.6 (C29), 28.7 (C28), 28.3 (C23), 27.4 (C2), 26.7 (C15), 26.6 (C16), 23.5 (C27), 18.9 (C26), 17.6 (C6), 16.6 (C25), 15.7 (C24).

**IR** (KBr):  $\nu$  (cm<sup>-1</sup>): 3359 (OH), 2954 (CH aliphatic), 1649 (C=O), 1536, 1515, 1456, 1388, 1320, 1261, 1210.

**Mp**: 206 °C.

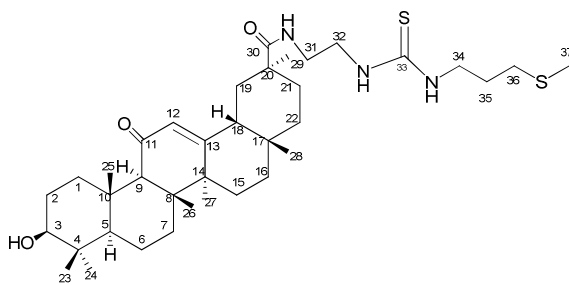
**HRMS (ESI-QTOF)** calcd for (7a) C<sub>39</sub>H<sub>57</sub>N<sub>3</sub>O<sub>3</sub>S (MH<sup>+</sup>) 648.4193, obsd 648.4198, error: 0.65 ppm.

### ***N*-(2-{3-[3-(methylthio)propyl]thioureido}ethyl)- glycyrrhetinamide (7b)**

**Yield**: 73%. **R<sub>f</sub>** = 0.21 (DCM; MeOH 96:4). **RP-HPLC**: purity = 97%, **t<sub>R</sub>** = 6.43 min.

**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 7.27 (br s, 1H, NH), 7.16 (br s, 1 H, NH), 5.62 (br s, 1H, NH), 5.61 (s, 1H, CH-12), 3.96-3.31 (m, 6H, H-31, H-32, H34), 3.22 (dd, 1H,  $J = 9.6$  Hz, 5.7 Hz, CH-3), 2.76 (d, 1H,  $J = 13.2$  Hz, 2.7 Hz, CH-1), 2.56 (t, 2H,  $J = 6.9$  Hz, H-36), 2.33 (s, 1H, CH-9), 2.09 (s, 3H, SMe), 1.37 (s, 3H, Me-H27), 1.12 (s, 9H, Me-H25, H-29, H-26), 1.00 (s, 3H, Me-H23), 0.80 (s, 6H, Me-H24, H-28), 0.69 (d, 1H,  $J = 11.1$  Hz, CH-5). **<sup>13</sup>C NMR** (DEPT 135, HSQC, 75 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 200.5 (C11), 182.6 (C33), 178.2 (C30), 170.0 (C13), 128.5 (C12), 78.9 (C3), 62.0 (C9), 55.1 (C5), 48.4 (C18), 45.6 (C14), 43.8 (C20), 43.4 (C8), 43.4 (C31/C32\*), 41.7 (C19), 40.5 (C34), 39.4 (C31/C32\*), 39.3 (C1/C4), 37.7 (C22), 37.3 (C10), 32.9 (C7), 32.0 (C17), 31.6 (C21/C36), 29.5 (C29), 28.8 (C28), 28.3 (C23/C35), 27.4 (C2), 26.6 (C15/C16), 23.5 (C27), 18.9 (C26), 17.6 (C6), 16.6 (C25), 15.7 (C24), 15.6 (SMe).

\*attribution may be reversed

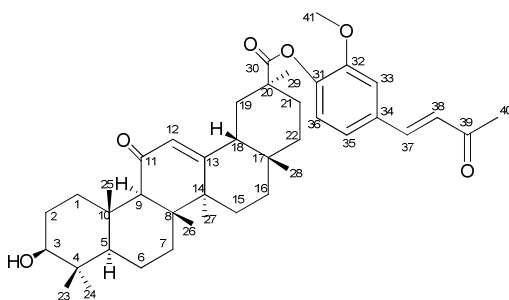


**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 3346 (OH), 2955 (CH aliphatic), 1647 (C=O), 1548, 1543, 1468, 1388, 1262, 1092.

**Mp**: 176 °C.

**HRMS (ESI-QTOF)** calcd for **(7b)**  $\text{C}_{37}\text{H}_{61}\text{N}_3\text{O}_3\text{S}_2$  ( $\text{MH}^+$ ) 660.4227, obsd 660.4242, error: 2.25 ppm.

### DZ-GA conjugate. **8**<sup>27</sup>



#### Synthesis protocol:

18  $\beta$ -glycyrrhetic acid **1** (255 mg, 0.542 mmol) was dissolved in 20 mL of anhydrous DCM. Dimethylaminopyridine (139 mg, 1.136 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (214 mg, 1.118 mmol) were then added and the mixture was stirred at room temperature for 30 minutes. Vanillylideneacetone (DZ) (105 mg, 0.549 mmol) was then added to the reaction mixture which was then stirred for 19 hours. After concentration under vacuum, the residual oil was chromatographed on silica (DCM; MeOH 95:5).

**Yield**: 46%. **R<sub>f</sub>** = 0.23 (DCM; MeOH 95:5). **RP-HPLC**: purity = 99%,  $t_R$  = 14.4 min.

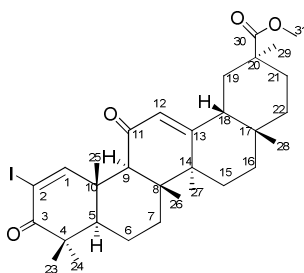
**<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 7.47 (d, 1H,  $J$  = 16.2 Hz, H-37), 7.15 (d, 1 H,  $J$  = 1.8 Hz, H-35), 7.13 (d, 1H,  $J$  = 8.4 Hz, H-33), 7.01 (d, 1H,  $J$  = 8.4 Hz, H-36), 6.65 (d, 1H,  $J$  = 16.2 Hz, H-38), 5.69 (s, 1H, CH-12), 3.86 (s, 3H, OMe), 3.22 (dd, 1H,  $J$  = 9.6 Hz, 5.7 Hz, CH-3), 2.76 (d, 1H,  $J$  = 13.2 Hz, 2.7 Hz, CH-1), 2.39 (s, 1H, H-40), 2.34 (s, 1H, CH-9), 1.39 (s, 3H, Me-H27), 1.35 (s, 3H, Me-H28), 1.15 (s, 3H, Me-H26), 1.14 (s, 3H, Me-H25), 1.01 (s, 3H, Me-H23), 0.87 (s, 3H, Me-H29), 0.81 (s, 3H, Me-H24), 0.70 (d, 1H,  $J$  = 11.1 Hz, CH-5). **<sup>13</sup>C NMR**

(DEPT 135, HSQC, 75 MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 200.4 (C11), 198.4 (C39), 174.5 (C30), 169.3 (C13), 151.7 (C33), 142.9 (C37), 141.8 (C34), 133.4 (C31), 128.7 (C12), 127.4 (C38), 123.4 (C36), 121.6 (C33), 111.4 (C35), 78.9 (C3), 61.9 (C9), 55.9 (C41), 55.1 (C5), 48.3 (C18), 45.6 (C14), 44.6 (C20), 43.4 (C8), 41.4 (C19), 39.3 (C1/C4), 37.6 (C22), 37.2 (C10), 32.9 (C7), 32.0 (C17), 31.4 (C21), 28.7 (C29), 28.4 (C28), 28.3 (C23), 27.6 (C40), 27.5 (C2), 26.6 (C15/C16), 23.5 (C27), 18.9 (C26), 17.6 (C6), 16.5 (C25), 15.7 (C24).

**IR** (KBr):  $\nu$  (cm<sup>-1</sup>): 3469 (OH), 2931 (CH aliphatic), 1752, 1654, 1617 (C=O), 1508, 1458, 1388, 1256, 1070.

**HRMS (ESI-QTOF)** calcd for **(8)** C<sub>41</sub>H<sub>56</sub>O<sub>6</sub> (M+H<sup>+</sup>) 645.4150, obsd 645.4153, error: 0.45 ppm.

### 1,2 dehydro-2-iodo-3-oxo-glycyrrheticin methyl ester. **9**<sup>23</sup>



#### Synthesis protocol:

Methyl glycyrrhetinate<sup>23</sup> (170 mg, 0.351 mmol) was dissolved in 10 mL of DMSO. 2-iodoxybenzoic acid (IBX, 45 % m/m, 902.0 mg, 3.221 mmol) was added to the reaction mixture which was then heated at 85°C for 7 days. The intermediate product was then precipitated in water and filtered. The precipitate was dissolved in 10 mL DCM. After drying on Na<sub>2</sub>SO<sub>4</sub>, the solution was filtered and evaporated. The residue (96 mg, 0.199 mmol) was dissolved in 8 mL of anhydrous THF. Iodine (115 mg, 0.453 mmol) and pyridine (54  $\mu$ L, 0.669 mmol) were added to the reaction mixture which was stirred overnight. The reaction mixture was concentrated, then dissolved in 10 mL DCM and washed with water, dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product was chromatographed on silica (cyclohexane/AcOEt: 8/2) leading to **9**.

**Yield:** 38%. **R<sub>f</sub>** = 0.29 (cyclohexane; AcOEt 8:2). **RP-HPLC:** purity = 98%, **t<sub>R</sub>** = 12.1 min. **<sup>1</sup>H-NMR** characteristic protons (HSQC, 300MHz; HMBC, 400MHz, CDCl<sub>3</sub>):  $\delta$  (ppm) = 8.53 (s, 1H, CH-1), 5.78 (s, 1 H, CH-12), 3.71 (s, 3H, OMe), 2.72 (s, 1H,

CH-9), 1.43 (s, 3H, Me-H25), 1.39 (s, 3H, Me-H27), 1.20 (s, 3H, Me-H29), 1.16 (s, 9H, Me-H23, H-24, H-26), 0.91 (d, 1H,  $J = 7.5$  Hz, CH-5), 0.83 (s, 3H, Me-H28).  $^{13}\text{C}$

**NMR** (HMBC, 400 MHz; DEPT 135, HSQC, 75 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) = 198.6 (C11), 197.6 (C3), 177.0 (C30), 171.2 (C13), 169.7 (C1), 128.2 (C12), 101.0 (C2), 55.4 (C9), 52.7 (C5), 52.0 (OMe), 48.7 (C18), 45.7 (C10/C8), 44.2 (C4), 43.7 (C14/C20), 41.3 (C19), 37.9 (C22), 32.0 (C7/C17), 31.3 (C21), 28.8 (C29), 28.7 (C28), 28.4 (C23), 26.7 (C15/C16), 23.6 (C27), 22.3 (C24), 20.0 (C25), 18.1 (C26), 18.5 (C6).

**IR** (KBr):  $\nu$  ( $\text{cm}^{-1}$ ): 2953 (CH aliphatic), 1729 (C=O), 1677, 1654, 1650, 1541.

**HRMS (ESI-QTOF)** calcd for **(9)**  $\text{C}_{31}\text{H}_{43}\text{IO}_4$  ( $\text{M}+\text{H}^+$ ) 607.2315, obsd 607.2287, error: 1.38 ppm.

**List of Kinases and Substrates**

No	Kinase	Lot	Conc. (ng/50µl)	Substrate	Lot	Conc. (µg/50µl)
1	ABL1 T315I	2	50	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.25
2	ABL1 wt	3	25	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
3	ABL2	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
4	ACK1	3	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
5	ACV-R1	1	20	GSK3(14-27)	8	2
6	ACV-R1B	2	5	RBBER-CHKtide	24	4
7	ACV-RL1	1	20	Casein	SIG_83K7430	1
8	AKT1	7	25	GSK3(14-27)	8	2
9	AKT2	3	200	GSK3(14-27)	6	1
10	ALK	2	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
11	AMPK-alpha1 fl	1	200	RBBER-CHKtide	23	2
12	Aurora-A	4	50	tetra(LRRWSLG)	5	0.5
13	Aurora-B	7	100	tetra(LRRWSLG)	5	0.25
14	Aurora-C	8	200	tetra(LRRWSLG)	4	0.25
15	AXL	3	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
16	BLK	1	100	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
17	BMX	3	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
18	B-RAF V600E	1	25	MEK1-KM (kinase-dead)	21	0.5
19	B-RAF wt	1	20	MEK1-KM (kinase-dead)	22	0.5
20	BRK	4	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
21	BRSK1	1	3	RBBER-CHKtide	24	2
22	BTK	2	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
23	CAMK1D	1	100	RBBER-CHKtide	24	1
24	CAMK2A	1	2	RBBER-CHKtide	23	2
25	CAMK2B	1	100	RBBER-CHKtide	022.1	2
26	CAMK2D	1	2	RBBER-CHKtide	022.1	2
27	CAMK4	1	150	JUN	3	0.5

28	CAMKK2	1	10	GSK3(14-27)	6	2
29	CDC42BPA	1	10	S6-Peptide	4	1
30	CDC42BPB	1	25	S6-Peptide	4	1
31	CDK1/CycA	5	10	RBBER-CHKtide	22	2
32	CDK1/CycB1	25	25	RBBER-CHKtide	24	2
33	CDK1/CycE	1	50	RBBER-CHKtide	22	2
34	CDK2/CycA	5	25	RBBER-CHKtide	24	1
35	CDK2/CycE	9	10	RBBER-CHKtide	9	1
36	CDK3/CycE	1	10	RBBER-CHKtide	23	1
37	CDK4/CycD1	7	20	RBBER-CHKtide	24	2
38	CDK4/CycD3	1	10	RBBER-CHKtide	24	1
39	CDK5/p25NCK	1	15	RBBER-CHKtide	24	1
40	CDK5/p35NCK	1	15	RBBER-CHKtide	24	1
41	CDK6/CycD1	4	200	RBBER-CHKtide	24	2
42	CDK7/CycH/MAT1	2	25	RBBER-CHKtide	24	2
43	CDK8/CycC	2	50	RBBER-CHKtide	1	1
44	CDK9/CycT	4	15	RBBER-CHKtide	22	1
45	CHK1	2	50	RBBER-CHKtide	17	2
46	CHK2	2	10	tetra(LRRWSLG)	4	0.5
47	CK1-alpha1	1	100	Casein	SIG_65K7410	1
48	CK1-delta	1	5	Casein	SIG_65K7410	1
49	CK1-epsilon	1	2,5	Casein	SIG_83K7430	0.5
50	CK1-gamma1	1	5	Casein	SIG_65K7410	1
51	CK1-gamma2	1	5	Casein	SIG_83K7430	1
52	CK1-gamma3	1	5	Casein	SIG_83K7430	1
53	CK2-alpha1	3	20	Casein	SIG_83K7430	1
54	CK2-alpha2	1	50	Casein	SIG_65K7410	1
55	CLK1	1	400	H2O (Autophos.)	0	0
56	COT	18	300	RBBER-CHKtide	24	3
57	CSF1-R	4	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
58	CSK	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125

59	DAPK1	2	40	GSK3(14-27)	8	2
60	DAPK2	1	10	S6-Peptide	4	2
61	DAPK3	1	5	GSK3(14-27)	6	2
62	DCAMKL2	1	10	RBER-CHKtide	24	1
63	DDR2	2	50	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.25
64	DMPK	1	200	tetra(LRRWSLG)	5	2
65	DYRK1A	2	5	RBER-CHKtide	24	2
66	DYRK1B	1	2	RBER-CHKtide	23	2
67	EEF2K	1	1	GSK3(14-27)	6	4
68	EGF-R L858R	1	5	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
69	EGF-R T790M	1	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
70	EGF-R T790M/L858R	1	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
71	EGF-R wt	15	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
72	EIF2AK2	2	10	Rb-CTF	13	1
73	EIF2AK3	1	10	MEK1-KM (kinase-dead)	SIG_83K7430	2
74	EPHA1	1	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
75	EPHA2	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
76	EPHA3	1	150	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
77	EPHA4	1	150	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
78	EPHA5	1	5	Poly(Glu,Tyr)4:1	SIG_20K5903	0.5
79	EPHA7	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
80	EPHA8	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
81	EPHB1	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
82	EPHB2	6	100	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
83	EPHB3	1	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
84	EPHB4	7	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
85	ERBB2	12	100	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
86	ERBB4	7	40	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
87	ERK1	2	5	RBER-CHKtide	24	2
88	ERK2	4	10	RBER-CHKtide	24	2



89	FAK	15	40	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
90	FER	2	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
91	FES	2	5	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
92	FGF-R1 V561M	2	10	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.25
93	FGF-R1 wt	11	40	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
94	FGF-R2	1	5	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
95	FGF-R3	4	100	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
96	FGF-R4	6	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
97	FGR	1	50	Poly(Glu,Tyr)4:1	SIG_20K5903	50
98	FLT3 D835Y	3	10	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.5
99	FLT3 ITD	1	100	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
100	FLT3 wt	11	50	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
101	FRK	1	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.5
102	FYN	2	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
103	GRK2	1	50	Casein	SIG_65K7410	0.5
104	GRK3	1	10	Casein	SIG_65K7410	2
105	GRK4	1	5	Casein	SIG_65K7410	0.5
106	GRK5	1	5	Casein	SIG_83K7430	1
107	GRK6	2	10	Casein	SIG_65K7410	1
108	GSK3-alpha	1	50	RBBER-CHKtide	24	4
109	GSK3-beta	3	50	RBBER-CHKtide	24	1
110	HCK	1	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
111	HIPK1	1	20	RBBER-CHKtide	24	2
112	HIPK3	1	20	RBBER-CHKtide	24	2
113	HRI	1	100	Casein	SIG_83K7430	0.5
114	IGF1-R	12	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
115	IKK-alpha	3	50	RBBER-CHKtide	23	2
116	IKK-beta	5	100	RBBER-CHKtide	24	1
117	IKK-epsilon	6	20	GSK3(14-27)	8	1
118	INS-R	6	10	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
119	INSRR	1	150	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.25

120	IRAK1	2	10	RB-CTF	13	2
121	IRAK4	6	20	Histone H2B	SIG_87H7445	0.25
122	ITK	1	100	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
123	JAK2	7	100	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
124	JAK3	2	200	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
125	JNK1	5	5	ATF2	6	0.25
126	JNK2	3	5	ATF2	6	0.25
127	JNK3	4	5	ATF2	6	0.25
128	KIT T670I	2	100	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
129	KIT wt	8	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
130	LCK	3	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
131	LIMK1	1	50	RBER-CHKtide	23	2
132	LTK	1	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
133	LYN	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
134	MAP4K4	1	5	Casein	SIG_83K7430	0.5
135	MAPKAPK3	1	20	tetra(LRRWSLG)	4	0.25
136	MAPKAPK5	5	10	RBER-CHKtide	23	4
137	MARK1	1	100	RBER-CHKtide	23	1
138	MARK2	2	4,5	RBER-CHKtide	23	2
139	MARK3	1	100	RBER-CHKtide	17	1
140	MATK	1	5	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
141	MEK1 wt	2	50	ERK2-KR (kinase-dead)	6	2
142	MELK	1	100	RBER-CHKtide	23	2
143	MERTK	1	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.5
144	MET	12	25	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
145	MET T1250M	1	20	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
146	MINK1	2	10	RBER-CHKtide	24	1
147	MKK6 S207D/T211D	1	50	p38-alpha-KA (kinase-dead)	1	1
148	MST1	2	5	RB-CTF	13	2
149	MST2	3	10	RB-CTF	13	2

150	MST3	1	20	Casein	SIG_65K7410	1
151	MST4	1	100	RB-CTF	13	1
152	mTOR	1	20	Casein	SIG_65K7410	1
153	MUSK	3	150	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
154	MYLK2	1	10	S6-Peptide	4	1
155	NEK1	1	3	RBBER-CHKtide	24	4
156	NEK11	1	25	Myelin Basic Protein	MIL_28053	2
157	NEK2	2	100	RB-CTF	13	1
158	NEK3	1	5	S6-Peptide	4	2
159	NEK6	1	20	GSK3(14-27)	7	2
160	NEK7	2	15	Casein	SIG_83K7430	1
161	NIK	3	200	RBBER-CHKtide	24	2
162	NLK	2	15	GSK3(14-27)	9	2
163	p38-alpha	5	10	ATF2	6	0.5
164	p38-beta	4	3	ATF2	10	1
165	p38-delta	1	2	RBBER-CHKtide	24	2
166	p38-gamma	1	2,5	RB-CTF	13	1
167	PAK1	2	15	tetra(LRRWSLG)	6	1
168	PAK2	1	50	tetra(LRRWSLG)	5	0.25
169	PAK3	1	20	tetra(LRRWSLG)	5	0.5
170	PAK4	3	50	tetra(LRRWSLG)	6	0.5
171	PAK6	1	50	tetra(LRRWSLG)	4	0.125
172	PAK7	1	50	tetra(LRRWSLG)	4	0.25
173	PASK	1	50	RBBER-CHKtide	17	2
174	PBK	3	200	Histone H1	SIG_94H8010	0.5
175	PCTAIRE1	4	400	RBBER-CHKtide	23	2
176	PDGFR-alpha	9	50	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
177	PDGFR-beta	13	50	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
178	PDK1	2	20	tetra(LRRWSLG)	5	0.25
179	PHKG2	1	10	RBBER-CHKtide	24	4
180	PIM1	3	10	GSK3(14-27)	8	1

181	PIM2	2	50	GSK3(14-27)	7	2
182	PKA	2	5	tetra(LRRWSLG)	5	0.5
183	PKC-alpha	5	2,5	PKC(19-31)	2	0.25
184	PKC-beta1	4	2,5	PKC(19-31)	2	0.25
185	PKC-beta2	3	5	PKC(19-31)	2	0.25
186	PKC-delta	4	50	PKC(19-31)	2	0.5
187	PKC-epsilon	6	10	PKC(19-31)	2	0.5
188	PKC-eta	5	20	Histone H2B	SIG_87H7445	0.25
189	PKC-gamma	7	10	MEK1-KM (kinase-dead)	2	0.25
190	PKC-iota	6	100	PKC(19-31)	2	0.5
191	PKC-mu	4	12,5	RBBER-CHKtide	23	2
192	PKC-nu	2	50	tetra(LRRWSLG)	6	0.125
193	PKC-theta	8	2,5	PKC(19-31)	2	0.25
194	PKC-zeta	5	50	PKC(19-31)	2	0.5
195	PLK1	13	50	RBBER-CHKtide	17	2
196	PLK3	1	30	Casein	SIG_65K7410	0.5
197	PRK1	4	25	RBBER-CHKtide	23	2
198	PRK2	1	10	RBBER-CHKtide	24	4
199	PRKD2	1	3	RBBER-CHKtide	24	4
200	PRKG1	1	5	PKC(19-31)	2	1
201	PRKG2	1	1	GSK3(14-27)	6	4
202	PRKX	1	10	GSK3(14-27)	6	4
203	PYK2	1	75	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
204	RAF1 Y340D/Y341D	1	10	MEK1-KM (kinase-dead)	21	0.5
205	RET	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
206	RIPK2	3	50	RBBER-CHKtide	24	2
207	ROCK1	2	4	PKC(19-31)	2	1
208	ROCK2	2	2,5	S6-Peptide	4	2
209	RON	2	2,5	p38-alpha-KA (kinase-dead)	3	1

210	ROS	1	15	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
211	RPS6KA1	2	10	RBBER-CHKtide	23	2
212	RPS6KA2	1	5	tetra(LRRWSLG)	5	0.5
213	RPS6KA3	2	5	MEK1-KM (kinase-dead)	4	0.25
214	RPS6KA4	1	50	RBBER-CHKtide	17	2
215	RPS6KA5	1	25	RBBER-CHKtide	17	2
216	RPS6KA6	1	2	GSK3(14-27)	8	2
217	S6K	6	50	GSK3(14-27)	6	4
218	S6K-beta	1	100	RBBER-CHKtide	24	4
219	SAK	3	100	p38-alpha-KA (kinase-dead)	4	4
220	SGK1	4	200	GSK3(14-27)	7	1
221	SGK2	1	20	GSK3(14-27)	6	1
222	SGK3	4	50	GSK3(14-27)	9	1
223	SNARK	1	100	Histone H2B	SIG_87H7445	0.5
224	SNF1LK2	1	2,5	RBBER-CHKtide	24	2
225	SNK	5	50	GSK3(14-27)	7	2
226	SRC	4	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
227	SRPK1	1	200	MEK1-KM (kinase-dead)	UPS_DAM1475041	1
228	SRPK2	1	200	Myelin Basic Protein	UPS_DAM1475041	1
229	STK17A	1	25	RB-CTF	13	4
230	STK23	2	25	RBBER-CHKtide	24	4
231	STK33	1	50	RBBER-CHKtide	24	2
232	SYK	3	100	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
233	TAOK2	2	10	Casein	SIG_83K7430	1
234	TAOK3	5	50	PKC(19-31)	2	0.5
235	TBK1	2	5	Casein	SIG_65K7410	1
236	TEC	1	200	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
237	TGFB-R1	3	10	GSK3(14-27)	8	1
238	TIE2	7	10	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
239	TRK-A	1	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125

240	TRK-B	4	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
241	TRK-C	5	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
242	TSF1	2	25	Casein	SIG_65K7410	1
243	TSK2	2	25	RBER-CHKtide	21	2
244	TSSK1	1	3	RBER-CHKtide	24	2
245	TTK	3	100	RBER-CHKtide	23	1
246	TYK2	1	80	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
247	TYRO3	1	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.5
248	VEGF-R1	9	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
249	VEGF-R2	15	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
250	VEGF-R3	12	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
251	VRK1	1	50	RBER-CHKtide	21	2
252	WEE1	5	200	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
253	WNK2	1	30	RBER-CHKtide	24	2
254	WNK3	1	10	S6-Peptide	4	2
255	YES	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
256	ZAP70	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
257	PKC-delta	4	20	PKC(19-31)	2	0.5
258	PKC-epsilon	6	10	PKC(19-31)	2	0.125
259	PKC-eta	5	10	Histone H2B	SIG_87H7445	0.25
260	PKC-gamma	7	10	PKC(19-31)	2	0.25
261	PKC-iota	6	50	PKC(19-31)	2	0.5
262	PKC-mu	4	12.5	RBER-CHKtide	24	2
263	PKC-nu	2	50	Tetra(LRRWSLG)	6	0.125
264	PKC-theta	8	5	PKC(19-31)	2	0.25
265	PKC-zeta	5	50	PKC(19-31)	2	0.125
266	PLK1	13	15	RBER-CHKtide	25	2
267	PLK3	1	30	Casein	SIG_83K7430	0.5
268	PRK1	4	25	RBER-CHKtide	25	2
269	PRK2	1	10	RBER-CHKtide	24	4
270	PRKD2	1	3	RBER-CHKtide	2	4

271	PRKG1	1	5	PKC(19-31)	2	1
272	PRKG2	2	1	GSK(14-27)	11	2
273	PRKX	1	5	GSK(14-27)	9	1
274	PYK2	1	75	Poly(Glu,Tyr)4 :1	SIG_20K5903	0.125
275	RAF1 Y340D/Y341D	2	10	MEK1 K97M (kinase-dead)	23	0.5
276	RET V804L	1	10	Poly(Glu, Tyr)4 :1	SIG_20K5903	0.125
277	RET wt	1	20	Poly(Glu, Tyr)4 :1	SIG_20K5903	0.125
278	RET Y791F	1	5	Poly(Glu, Tyr)4 :1	SIG_20K5903	0.125
279	RIPK2	3	75	RBER-CHKtide	24	2
280	RIPK5	1	5	RBER-CHKtide	25	2
281	ROCK1	2	4	PKC(19-31)	2	1
282	ROCK2	2	5	S6-Peptide	6	1
283	RON	2	8	P38-alpha K53A (kinase-dead)	7	1
284	RON	2	8	P38-alpha K53A (kinase-dead)	7	1
285	ROS	2	15	Poly(Ala,Glu, Lys, Tyr)6:2:5:1	SIG_53H5516	0.125
286	RPS6KA1	2	10	RBER-CHKtide	24	2
287	RPS6KA2	1	5	Tetra(LRRWSLG)	6	0.5
288	RPS6KA3	2	3	Tetra(LRRWSLG)	6	0.25
289	RPS6KA4	1	50	RBER-CHKtide	24	2
290	RPS6KA5	1	25	RBER-CHKtide	2	2
291	RPS6KA6	1	2.5	GSK3(14-27)	10	2
292	S6K	6	50	GSK3(14-27)	11	2
293	S6K-beta	1	100	RBER-CHKtide	25	4
294	SAK	3	100	P38-alpha K53A (kinase-dead)	7	2
295	SGK1	5	50	GSK3(14-27)	9	2
296	SGK2	1	20	GSK3(14-27)	9	1

297	SGK3	4	50	GSK3(14-27)	9	1
298	SLK	1	20	S6-peptide	7	4
299	SNARK	1	100	Histone H2B	SIG_87H7445	0.5
300	SNF1LK2	1	2.5	RBER-CHKtide	24	2
301	SNK	5	50	GSK3(14-27)	9	2
302	SRC	4	10	Poly(Glu,Tyr)4 :1	SIG_20K5903	0.125
303	SRMS	2	25	Poly(Glu,Tyr)4 :1	SIG_20K5903	0.125
304	SRPK1	1	25	Myelin Basic Protein	UPS_28053	1
305	SRPK2	1	10	Myelin Basic Protein	UPS_DAM1475041	1
306	STK17A	1	25	RB-CTF	18	4
307	STK23	2	25	RBER-CHKtide	25	4
308	STK25	1	10	Casein	SIG_83K7430	1
309	STK33	1	50	RBER-CHKtide	25	2
310	STK39	1	50	P38-alpha K53A (kinase-dead)	7	4
311	SYK	3	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
312	TAOK2	2	10	Casein	SIG_83K7430	1
313	TAOK3	5	50	PKC(19-31)	2	0.5
314	TBK1	4	5	Casein	SIG_83K7430	1
315	TEC	1	150	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
316	TGFB-R1	3	5	GSK3(14-27)	9	1
317	TGFB-R2	1	10	S6-peptide	5	4
318	TIE2	7	5	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
319	TRK-A	1	50	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
320	TRK-B	4	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.25
321	TRK-C	5	10	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
322	TSF1	2	25	Casein	SIG_83K7430	1
323	TSK2	2	25	RBER-CHKtide	25	2
324	TSSK1	1	4	RBER-CHKtide	25	2
325	TTK	3	100	RBER-CHKtide	24	1
326	TXK	1	2.5	RBER-CHKtide	25	2



327	TYK2	1	80	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125
328	TYRO3	1	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.5
329	VEGF-R1	11	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
330	VEGF-R2	15	25	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
331	VEGF-R3	12	20	Poly(Glu,Tyr)4:1	SIG_20K5903	0.125
332	VRK1	1	50	RBBER-CHKtide	9	2
333	WEE1	5	200	Poly(Ala,Glu,Lys,Tyr)6:2:5:1	SIG_53H5516	0.125