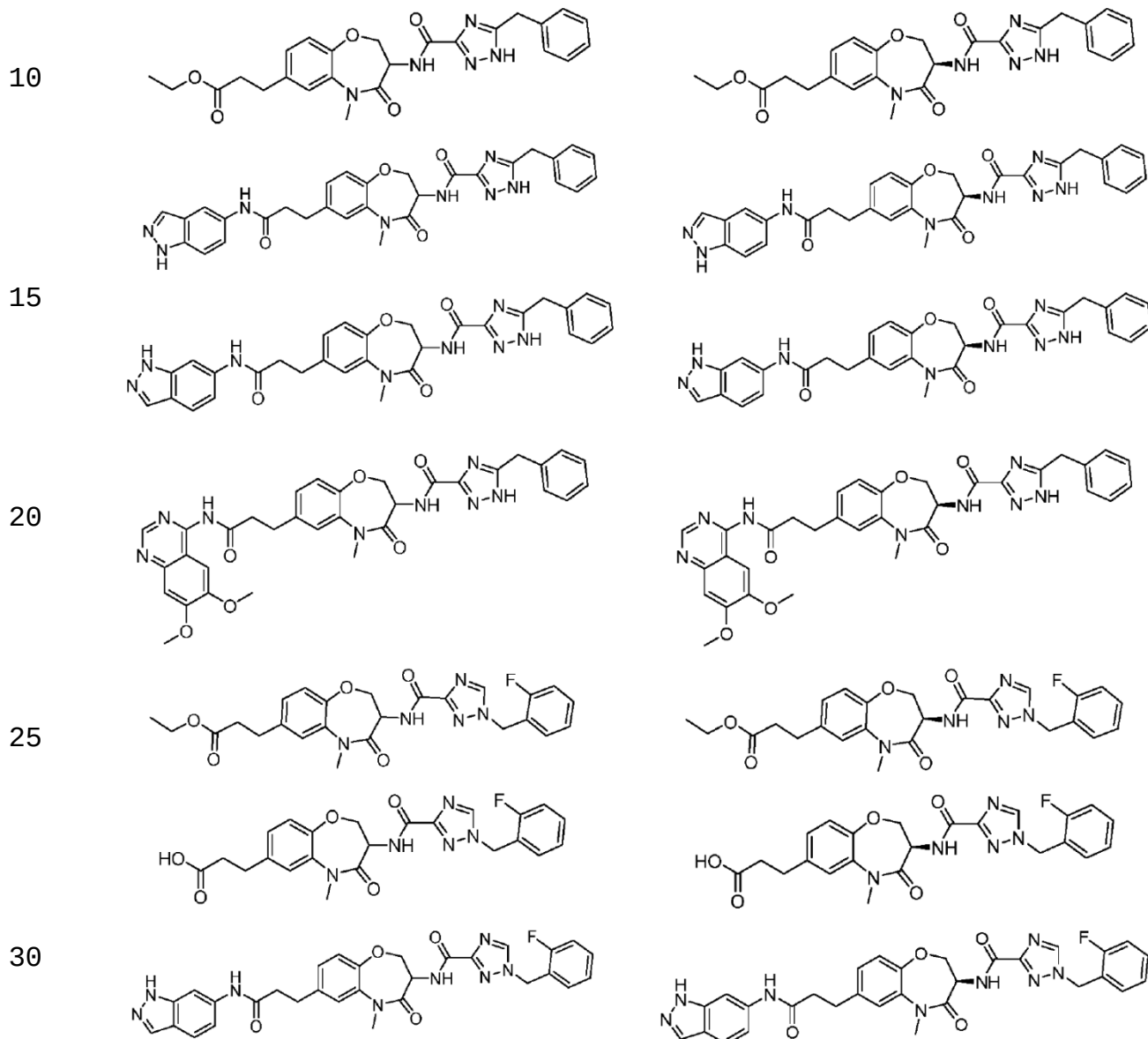


W02019/213447

PCT/US2019/030476

I-96: (S)-5-bencil-N-(5-metil-7-(3-morfolinoprop-1-il-1-il)-4-oxo-2,3,4,5-tetrahidrobenzo[b][1,4]oxazepin-3-il)-1H-1,2,4-triazol-3-carboxamida; o

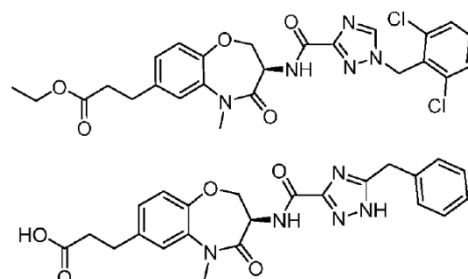
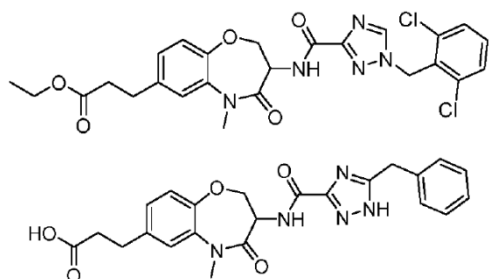
I-97: (S)-5-bencil-N-(5-metil-4-oxo-7-
 5 ((trimetilsilil)etnil)-2,3,4,5-tetrahidrobenzo[b][1,4]oxazepin-3-il)-1H-1,2,4-triazol-3-carboxamida.



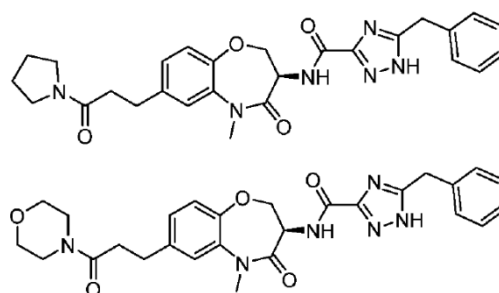
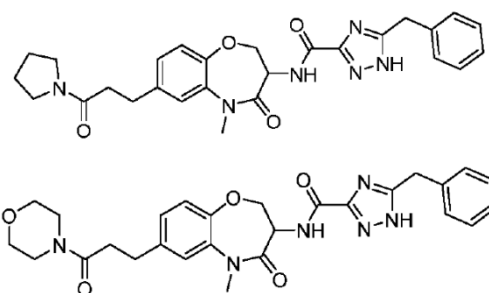
W02019/213447

PCT/US2019/030476

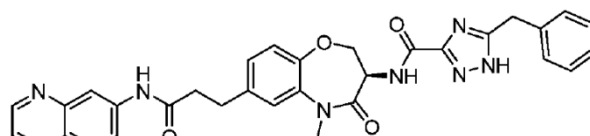
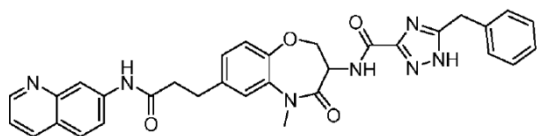
5



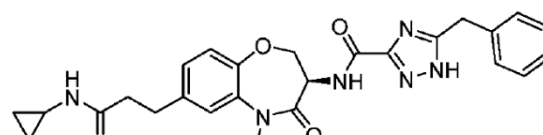
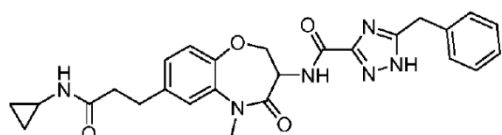
10



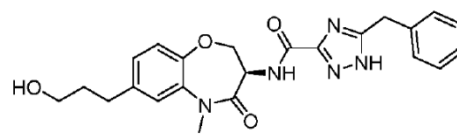
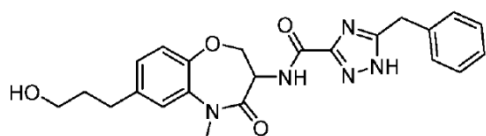
15



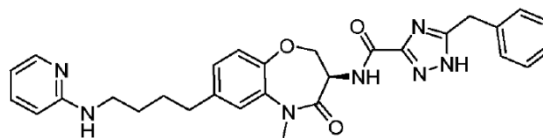
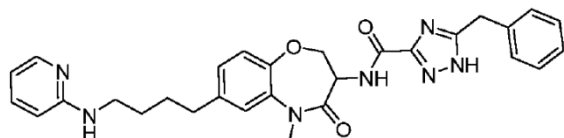
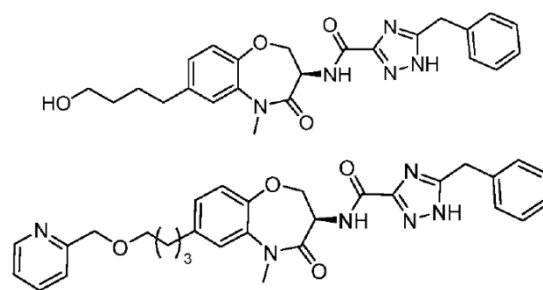
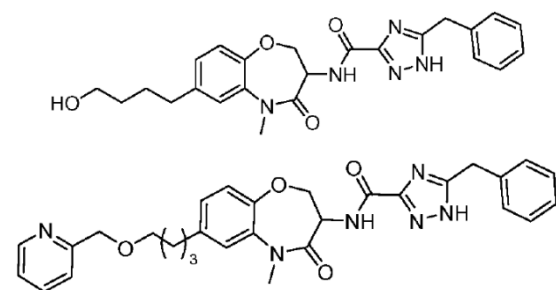
20



25



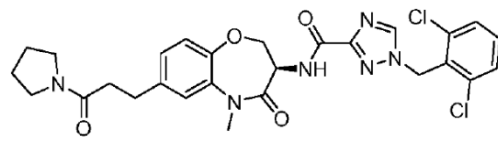
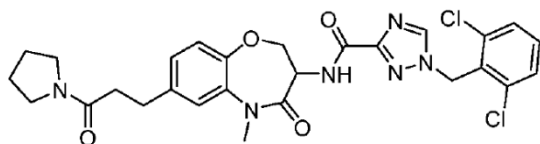
30



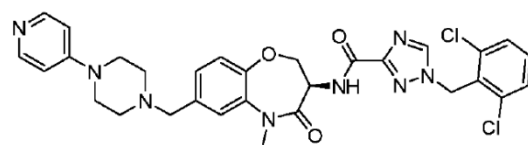
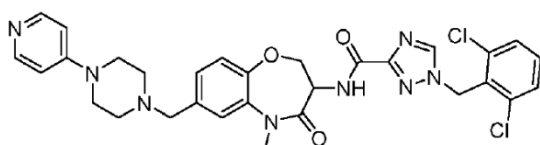
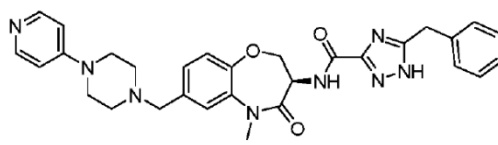
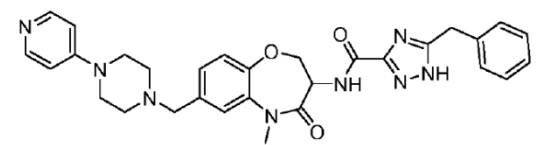
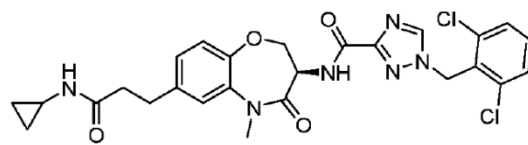
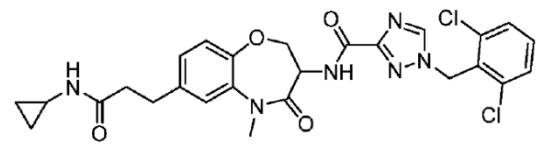
W02019/213447

PCT/US2019/030476

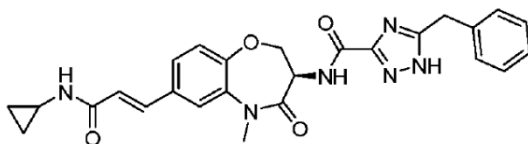
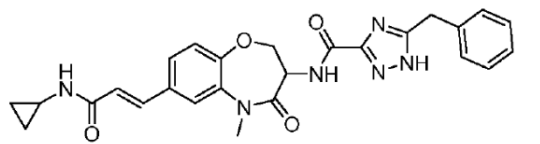
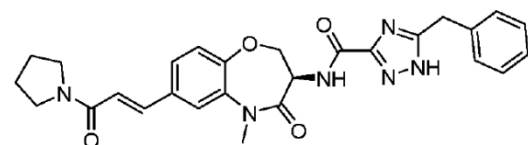
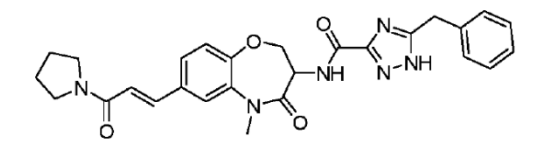
5



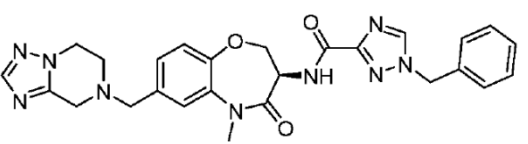
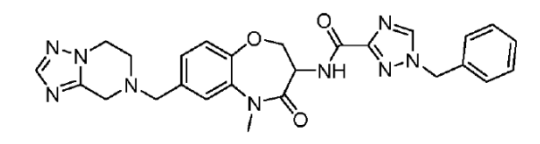
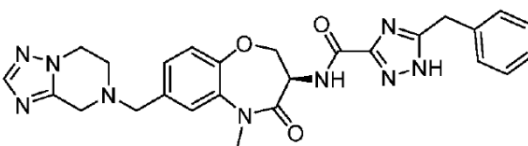
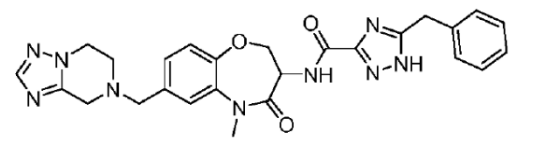
10



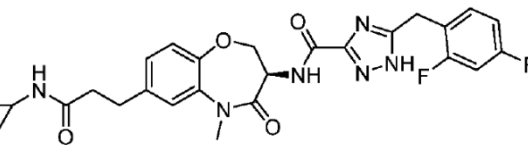
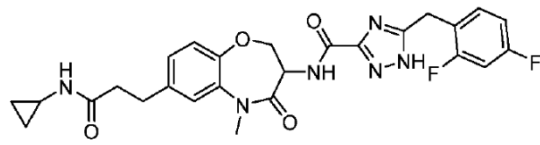
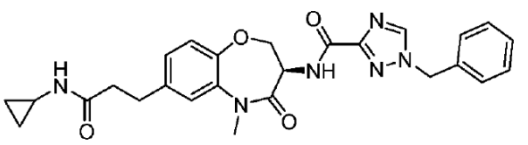
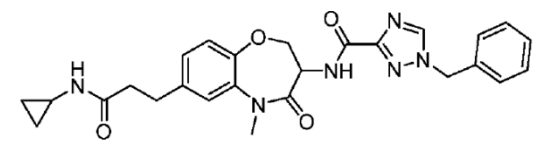
15



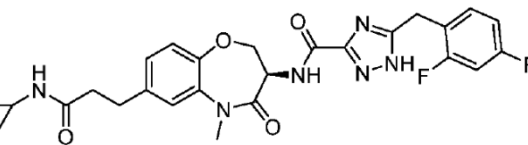
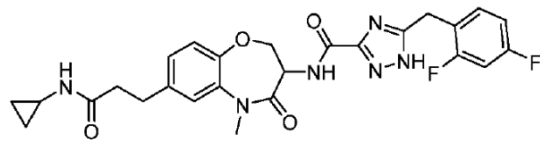
20



25



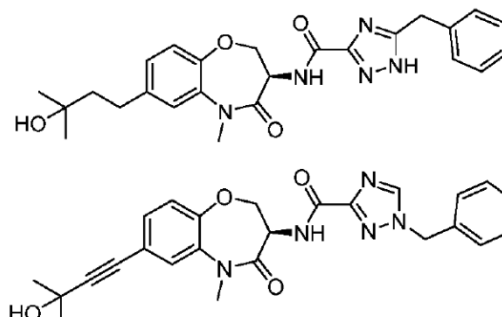
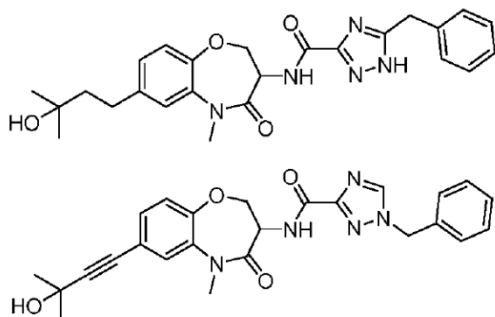
30



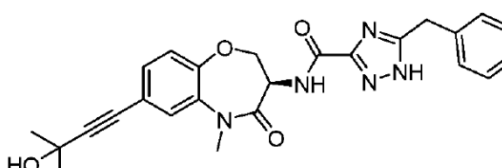
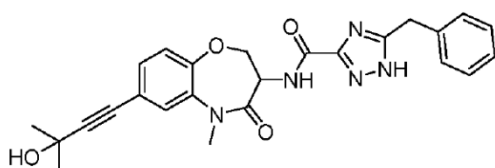
W02019/213447

PCT/US2019/030476

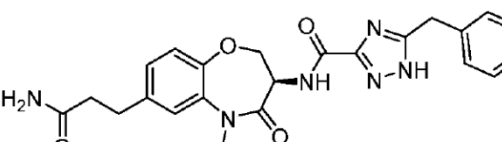
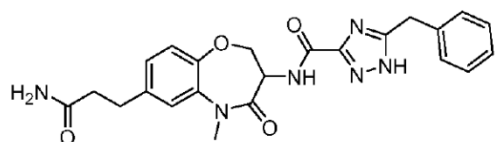
5



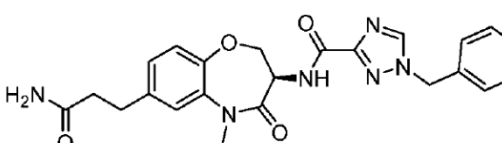
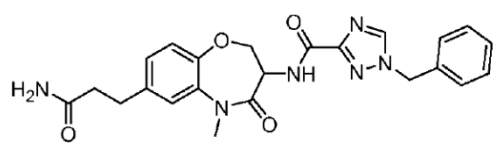
10



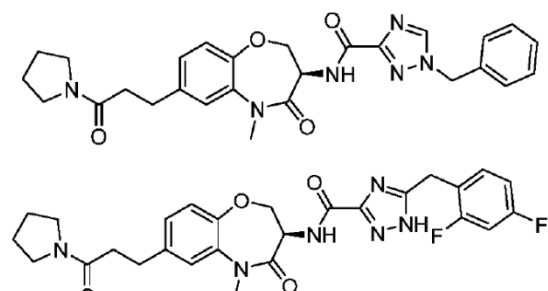
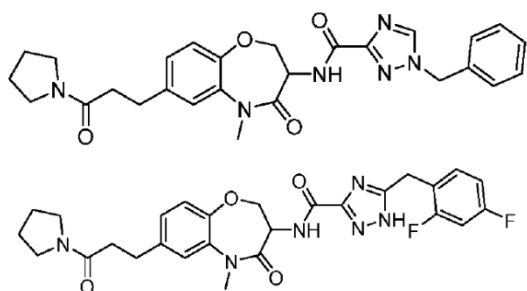
15



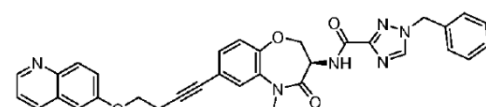
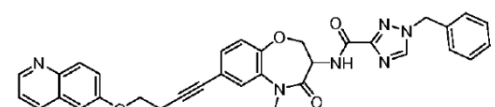
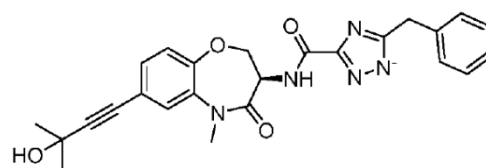
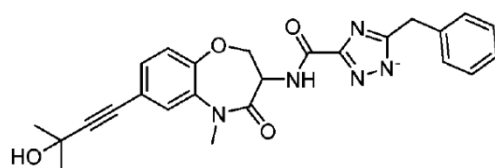
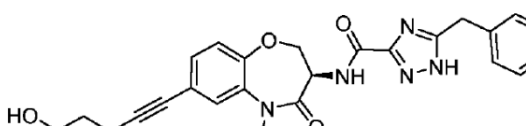
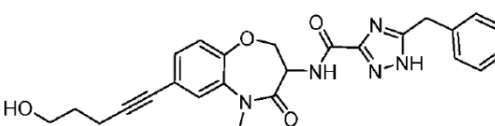
20



25

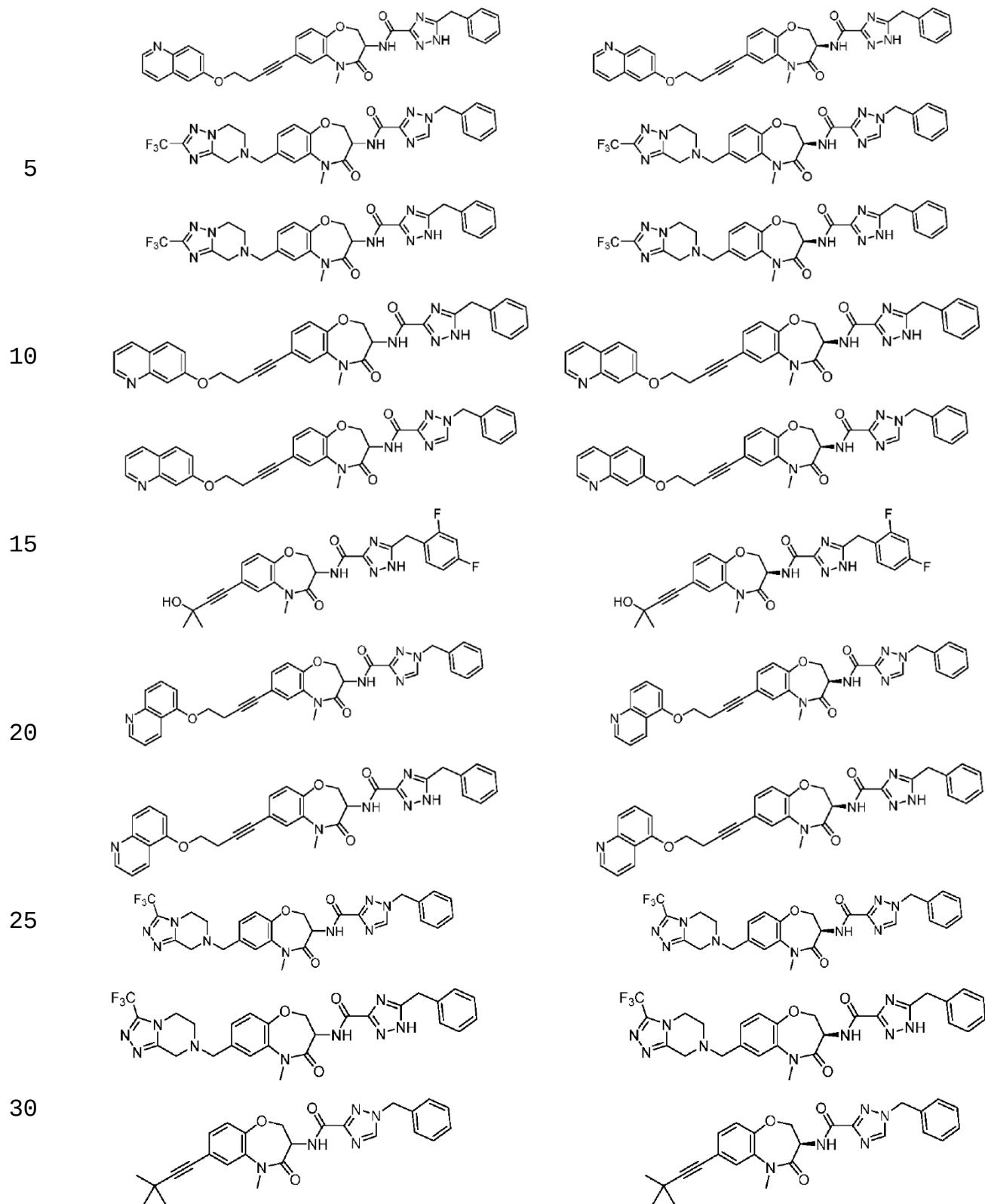


30



W02019/213447

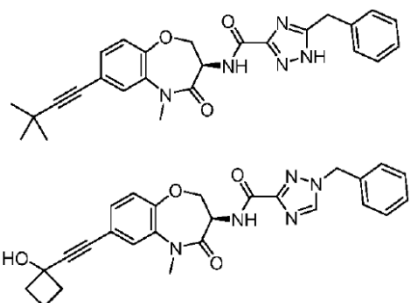
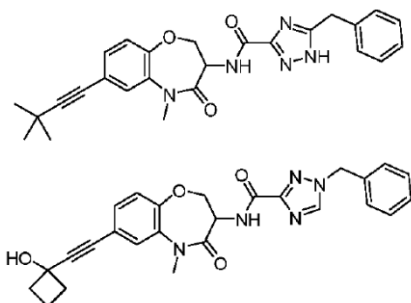
PCT/US2019/030476



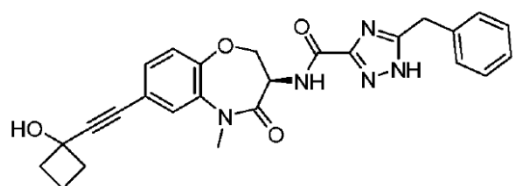
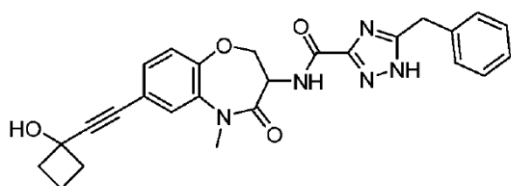
W02019/213447

PCT/US2019/030476

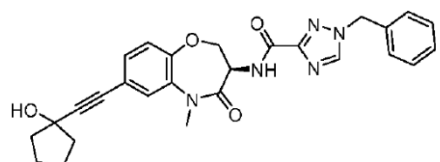
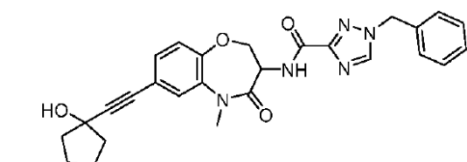
5



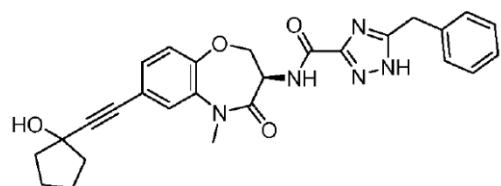
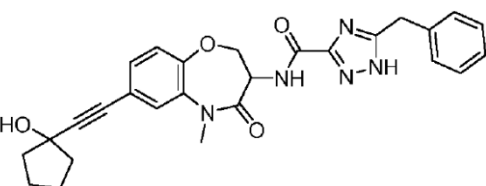
10



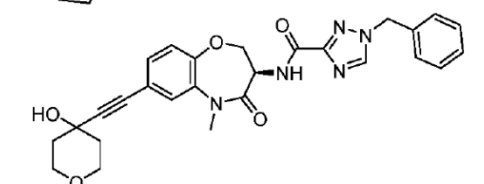
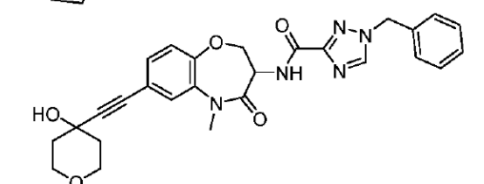
15



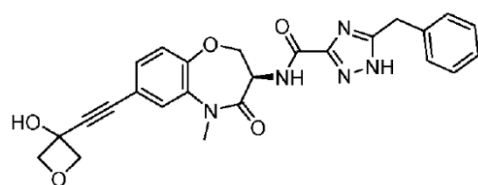
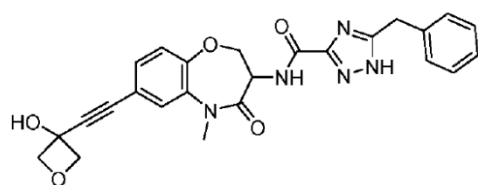
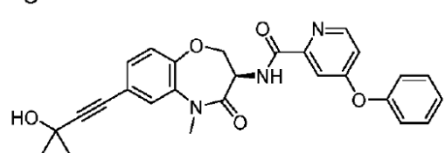
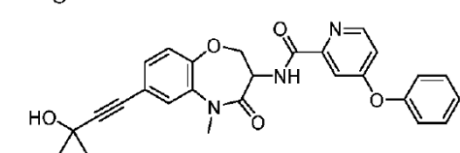
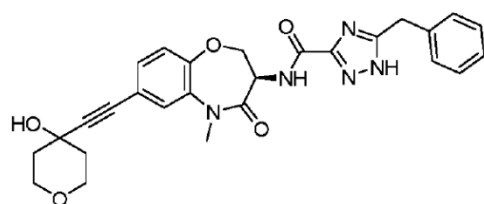
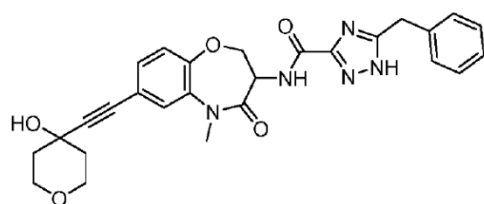
20



25



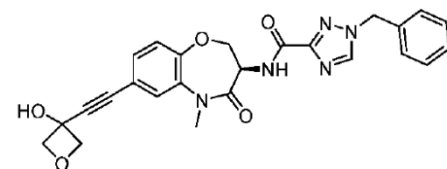
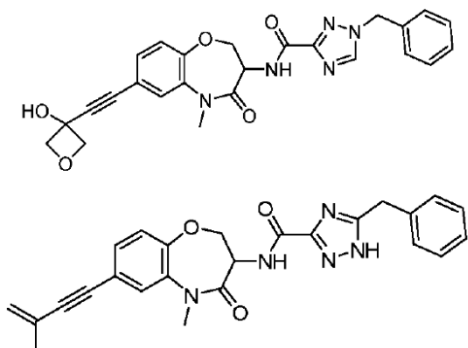
30



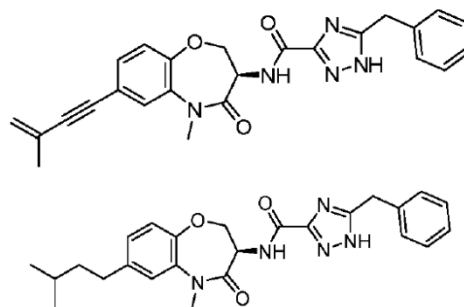
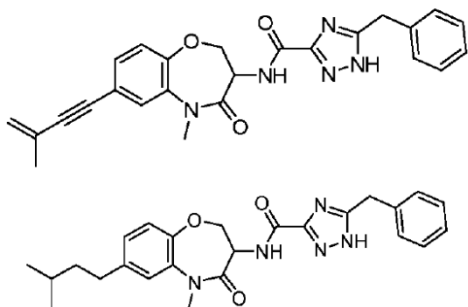
W02019/213447

PCT/US2019/030476

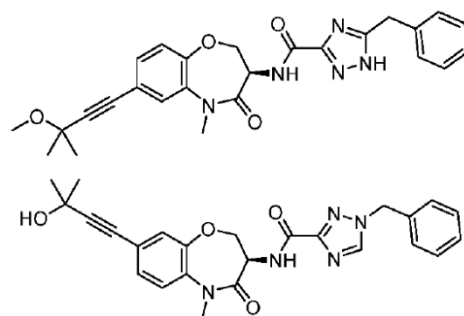
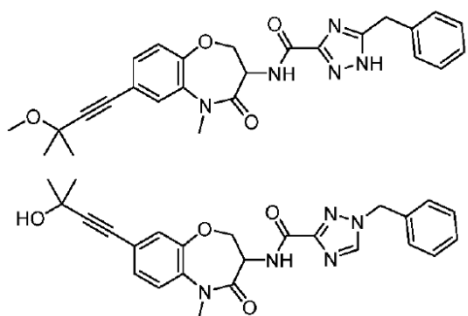
5



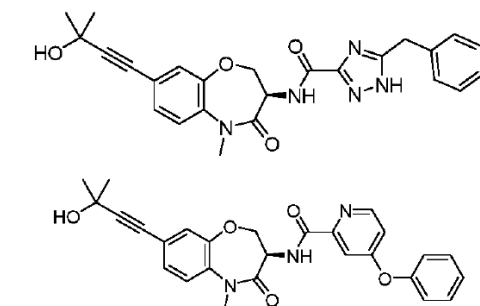
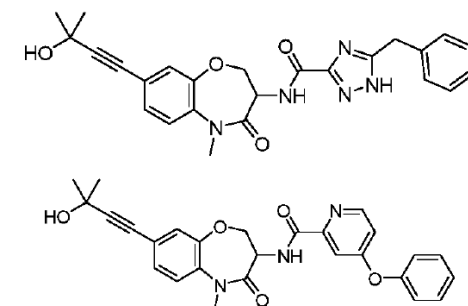
10



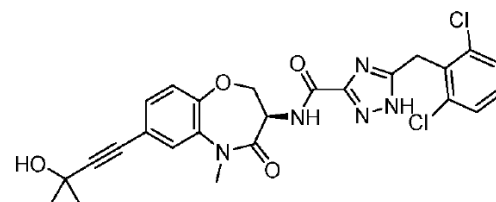
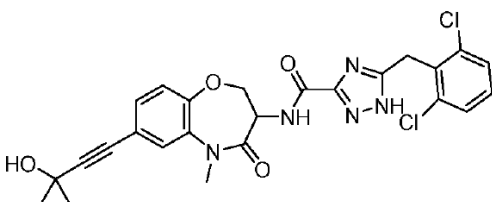
15



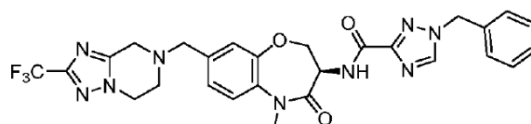
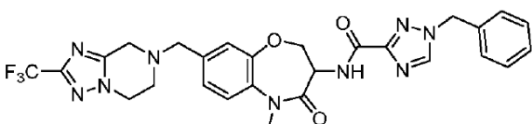
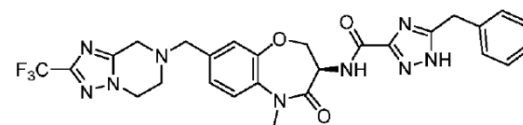
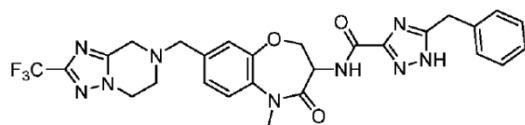
20



25



30



W02019/213447

PCT/US2019/030476

Los compuestos se diluyeron en serie en DMSO a partir de 250 μM en diluciones de factor de dilución de 4, y luego se diluyeron 1:5 en tampón de reacción x2 en una placa de 96 pocillos. Se añadió 1.0 μl de compuesto diluido a una placa de 384 pocillos por duplicado. Se añadieron 2 μl de RIPK1 activa diluida a una placa de 384 pocillos (no añadir a la columna 1), añadiendo tampón de reacción x2 a la columna 1. Se combinó AKT (Anaspec, Fremont, CA, EE. UU.) a 150 nM con solución madre de trabajo de ATP a igual volumen y se añadieron 2 μl /pocillo a la placa de 384 pocillos. El volumen de reacción final fue de 5.0 μl .

La placa se centrifugó rápidamente y la reacción se incubó a 30 °C durante 30 minutos. La adición de 5 μl de ADP-GloTM terminó la reacción. La placa se centrifugó rápidamente y la reacción se incubó a temperatura ambiente durante 40 minutos. A continuación, se añadió el reactivo de detección de quinasa y se incubó a temperatura ambiente durante 30 minutos. Se determinó la unidad de luz relativa (ULR) de la reacción de la quinasa mediante luminiscencia (luminiscencia 0.1 s) usando un luminómetro Wallac Victor2 (PerkinElmer, Waltham, MA, EE. UU.). Los valores de CI_{50} obtenidos de este ejemplo se proporcionan en la Tabla 1.

Tabla 1	
Compuesto	RIPK1 ADP-Glo quinasa (CI_{50}) μM
I-1	0.019
I-2	0.0157
I-3	0.0402
I-4	0.0568
I-5	0.0197
I-6	0.0724
I-7	0.021
I-8	0.0529

W02019/213447

PCT/US2019/030476

5

10

15

20

25

30

Tabla 1	
Compuesto	RIPK1 ADP-Glo quinasa (CI ₅₀) μM
I-9	0.1892
I-10	0.0429
I-11	0.0571
I-12	0.052
I-13	0.0539
I-14	0.073
I-15	0.1359
I-16	1.633
I-17	0.4906
I-18	0.1645
I-19	0.1455
I-20	0.1528
I-21	0.077
I-22	0.048
I-23	0.0352
I-24	0.0592
I-25	0.051
I-26	0.0656
I-27	0.1583
I-28	0.0375
I-29	0.0124
I-30	0.0402
I-31	0.0334
I-32	0.0219
I-33	0.0145
I-34	0.0492
I-35	0.0223
I-36	0.0338
I-37	0.0565
I-38	0.068

W02019/213447

PCT/US2019/030476

5

10

15

20

25

30

Tabla 1	
Compuesto	RIPK1 ADP-Glo quinasa (CI ₅₀) μM
I - 39	0.0364
I - 40	0.0887
I - 41	0.08
I - 42	0.0477
I - 43	0.0854
I - 44	0.034
I - 45	0.0825
I - 46	0.0437
I - 47	0.0683
I - 48	0.0624
I - 49	0.0604
I - 50	0.0234
I - 51	0.0317
I - 52	0.0249
I - 53	0.034
I - 54	0.0269
I - 55	0.0337
I - 57	0.0215
I - 58	0.011
I - 59	0.0579
I - 60	0.0644
I - 61	0.0654
I - 62	0.0355
I - 63	0.0303
I - 64	0.019
I - 65	0.0639
I - 66	0.0794
I - 67	0.0415
I - 68	0.0222
I - 69	0.0286

W02019/213447

PCT/US2019/030476

Tabla 1	
Compuesto	RIPK1 ADP-Glo quinasa (CI ₅₀) μM
I-70	1.155
I-71	0.0217
I-73	0.0429
I-74	0.0518
I-75	0.0546
I-76	0.0449
I-77	0.0327
I-78	0.0334
I-79	0.0347
I-80	0.0206
I-81	0.019
I-82	0.0255
I-83	0.0664
I-84	0.0441
I-85	0.0423
I-86	0.6161
I-87	0.0187
I-88	0.0738
I-89	0.0527
I-90	0.3649
I-91	0.3879
I-92	0.9325
I-93	0.0641
I-94	0.0333
I-95	0.0271
I-96	0.0317
I-97	0.0858

Ejemplo 12

En este ejemplo, se expusieron las células U937 y L929 a compuestos de la presente divulgación, y se realizó un

W02019/213447

PCT/US2019/030476

5 %. Los resultados de este ejemplo se resumen en la Tabla 2. Este ejemplo establece que las realizaciones de los compuestos desvelados en la presente tienen una actividad inesperadamente potente contra RIP1 humana y RIP1 murina, lo que permite su evaluación en modelos de enfermedad de ratón in vivo. Estos resultados son útiles para determinar dosis seguras y eficaces para seres humanos.

Tabla 2		
Compuesto	Recuperación de CTG de L929, L929, TNFa+zVAD (CI ₅₀) µM	Recuperación de CTG de TNF U937 Zvad, U937, TNFa+zVAD (CI ₅₀) µM
I-1	2.356	0.0027
I-2		0.0319
I-3	0.4278	0.0049
I-4		0.0293
I-5	0.0188	0.0005
I-6	97.54	0.0438
I-7	1.959	0.0009
I-8	0.0092	0.0014
I-9		0.3623
I-10	11.23	0.0079
I-11	14.1	0.0081
I-12	41.95	0.0053
I-13		0.0138
I-14	5.878	0.0114
I-15	2.2	0.0048
I-16	5.669	0.0517
I-17	0.3683	0.0024
I-18	0.2797	0.0013
I-19	1.302	0.0027
I-20	53.37	0.0136

W02019/213447

PCT/US2019/030476

5

10

15

20

25

30

Tabla 2		
Compuesto	Recuperación de CTG de L929, L929, TNFa+zVAD (CI ₅₀) µM	Recuperación de CTG de TNF U937 Zvad, U937, TNFa+zVAD (CI ₅₀) µM
I - 21	1.306	0.0025
I - 22	3.383	0.0048
I - 23	14.5	0.0067
I - 24	4.608	0.0036
I - 25	0.4895	0.0027
I - 26	0.6131	0.0044
I - 27		0.0697
I - 28	0.6735	0.0054
I - 29	0.0088	0.0023
I - 30	0.0723	0.0036
I - 31		0.0337
I - 32	17.74	0.0027
I - 33	1.227	0.0015
I - 34	9.592	0.0222
I - 35	0.0178	0.0031
I - 36	0.0014	0.0022
I - 37	0.0002	0.001
I - 38	0.0005	0.0036
I - 39	0.0004	0.0027
I - 40	0.0003	0.0023
I - 41	0.0016	0.0052
I - 42	0.0012	0.0033
I - 43	0.0009	0.0038
I - 44	0.0002	0.0011
I - 45	0.0003	0.0014
I - 46	0.0004	0.0012
I - 47	0.0015	0.0046

W02019/213447

PCT/US2019/030476

Tabla 2		
Compuesto	Recuperación de CTG de L929, L929, TNFa+zVAD (CI ₅₀) µM	Recuperación de CTG de TNF U937 Zvad, U937, TNFa+zVAD (CI ₅₀) µM
I-48	0.0005	0.0016
I-49	0.0012	0.005
I-50	0.0003	0.0011
I-51	0.0005	0.0017
I-52	0.0003	0.0009
I-53	0.0005	0.0018
I-54	0.0003	0.0009
I-55	0.0006	0.002
I-57	0.0009	0.003
I-58	0.0002	0.0008
I-59	0.0007	0.0019
I-60	0.0024	0.0048
I-61	0.0013	0.0034
I-62	0.0007	0.002
I-63	0.0003	0.001
I-64	0.0001	0.0005
I-65	0.0415	0.042
I-66	0.0949	0.1886
I-67	0.0085	0.0152
I-68	0.0003	0.0007
I-69	0.0009	0.0022
I-70	0.0195	0.1876
I-71	0.0002	0.0009
I-73	0.0027	0.0117
I-74	0.0002	0.0014
I-75	0.0014	0.0083
I-76	0.0024	0.0181

W02019/213447

PCT/US2019/030476

Tabla 2		
Compuesto	Recuperación de CTG de L929, L929, TNFa+zVAD (CI ₅₀) µM	Recuperación de CTG de TNF U937 Zvad, U937, TNFa+zVAD (CI ₅₀) µM
I-77	0.0003	0.0028
I-78	0.0009	0.0025
I-79	0.0008	0.0051
I-80	0.0007	0.0027
I-81	0.0012	0.0042
I-82	0.0003	0.0019
I-83	0.0001	0.001
I-84	0.0122	0.0563
I-85	0.0006	0.0033
I-86	0.0006	0.0041
I-87	0.0292	0.1385
I-88	0.00009757	0.0007
I-89	0.0007	0.0045
I-90	0.0046	0.0091
I-91	0.0037	0.0198
I-92	0.4305	4.835
I-93	0.9207	3.238
I-94	0.0046	0.022
I-95	0.0005	0.0014
I-96	0.0008	0.0031
I-97	0.0009	0.0017

Ejemplo 13

En este ejemplo, se usó un ensayo de modelo de ratón de hipotermia aguda para evaluar la capacidad de los compuestos desvelados en la presente para inhibir la hipotermia inducida por TNF-alfa.