FIG. 1

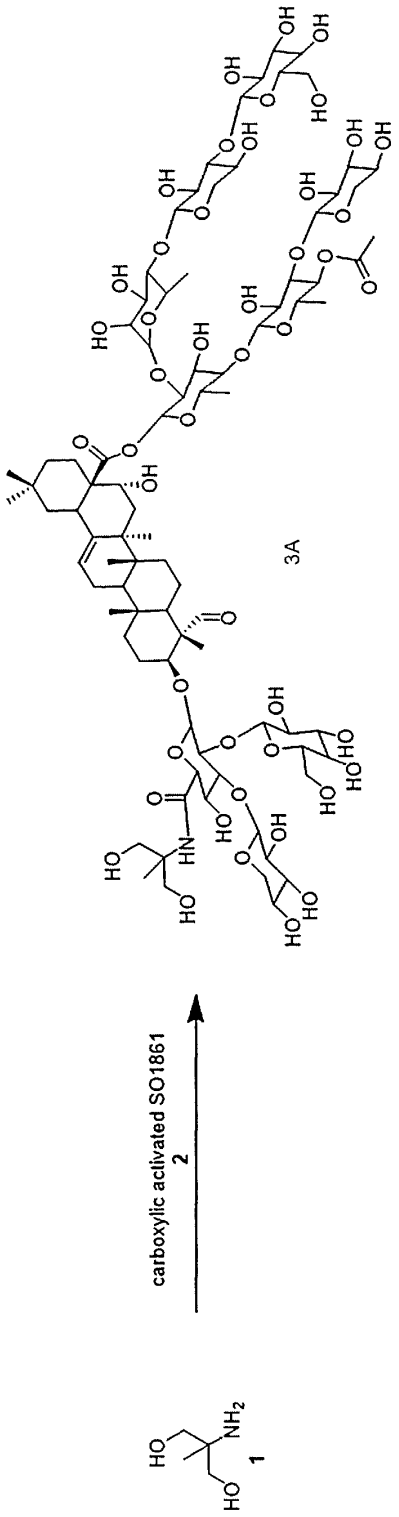


FIG. 2

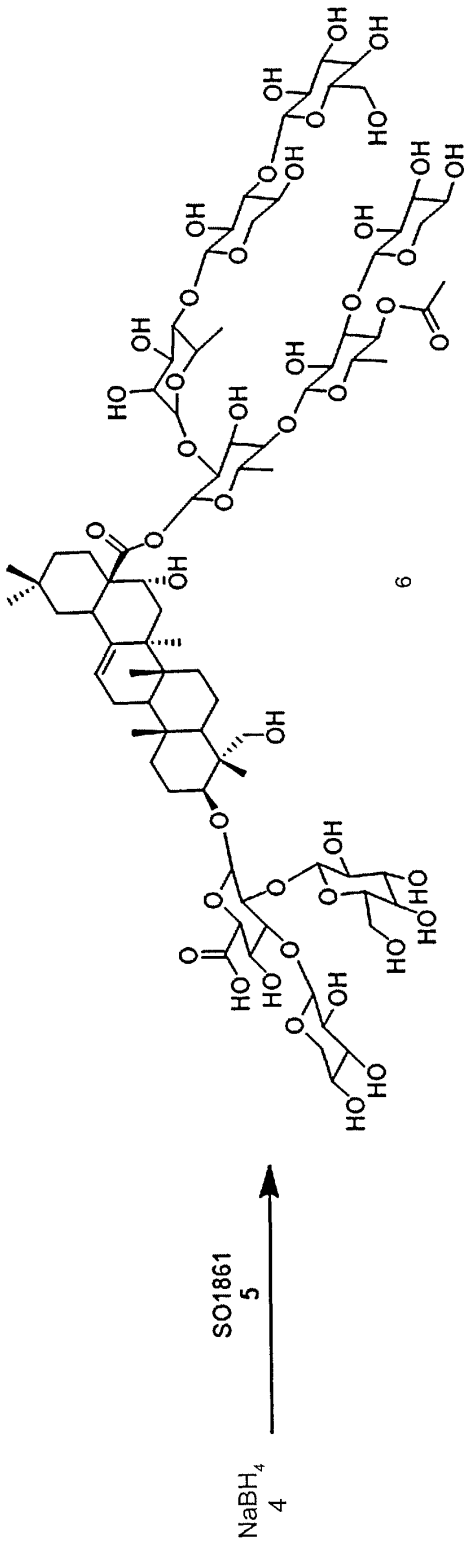


FIG. 3

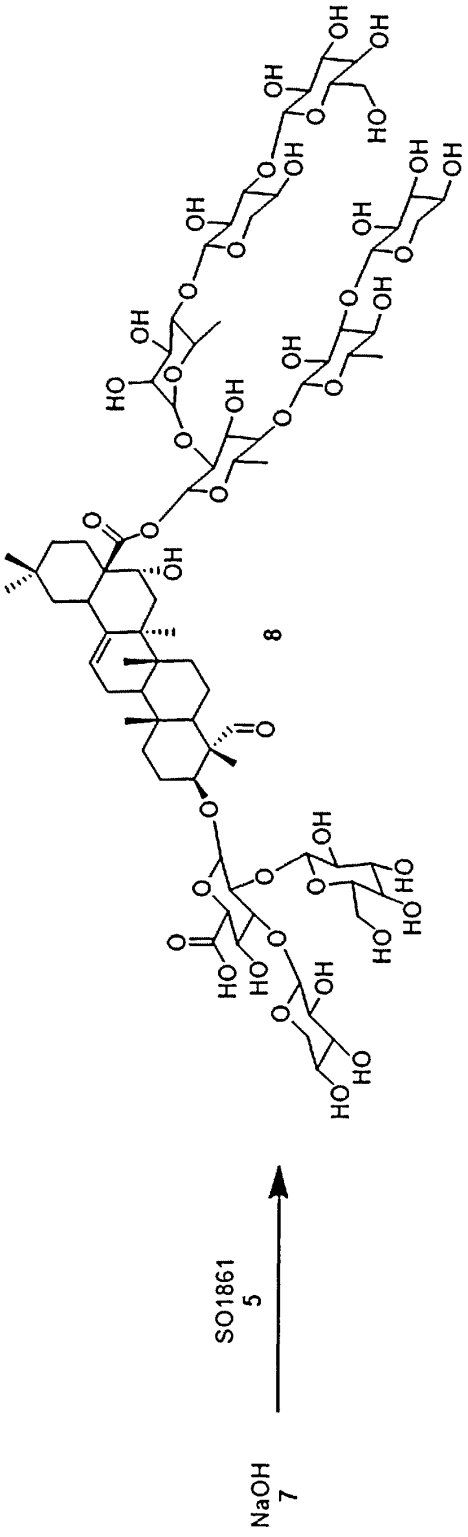


FIG. 4

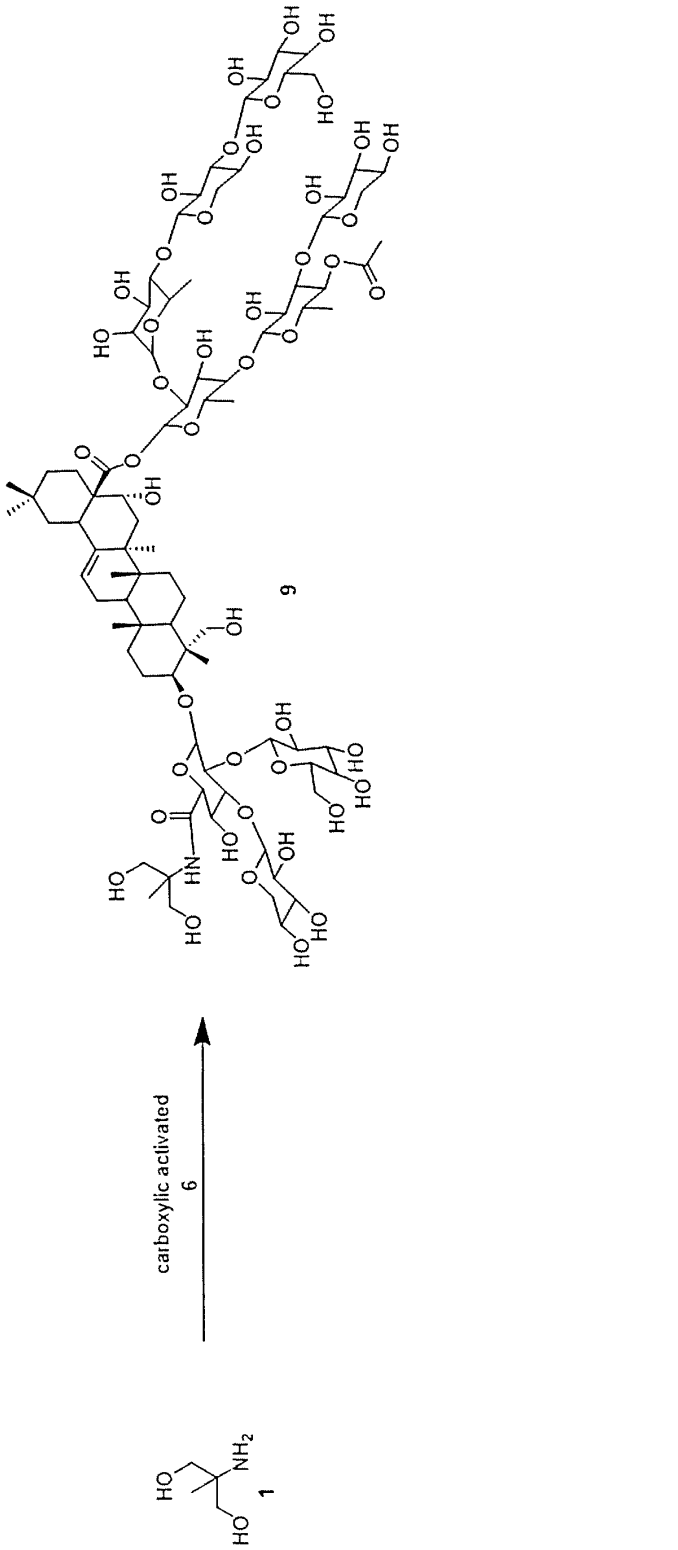


FIG. 5

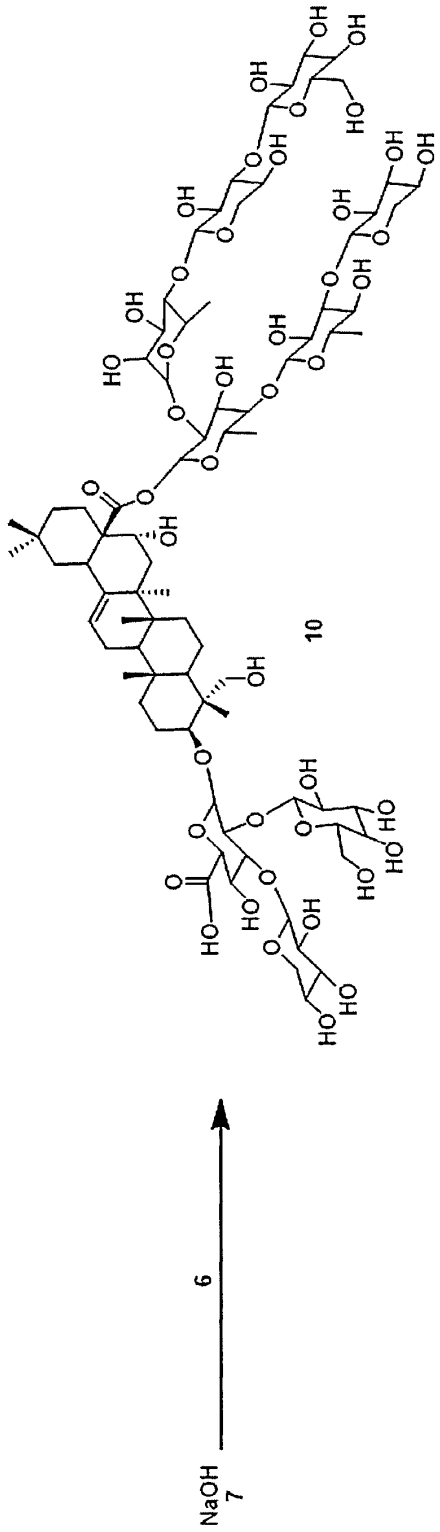


FIG. 6

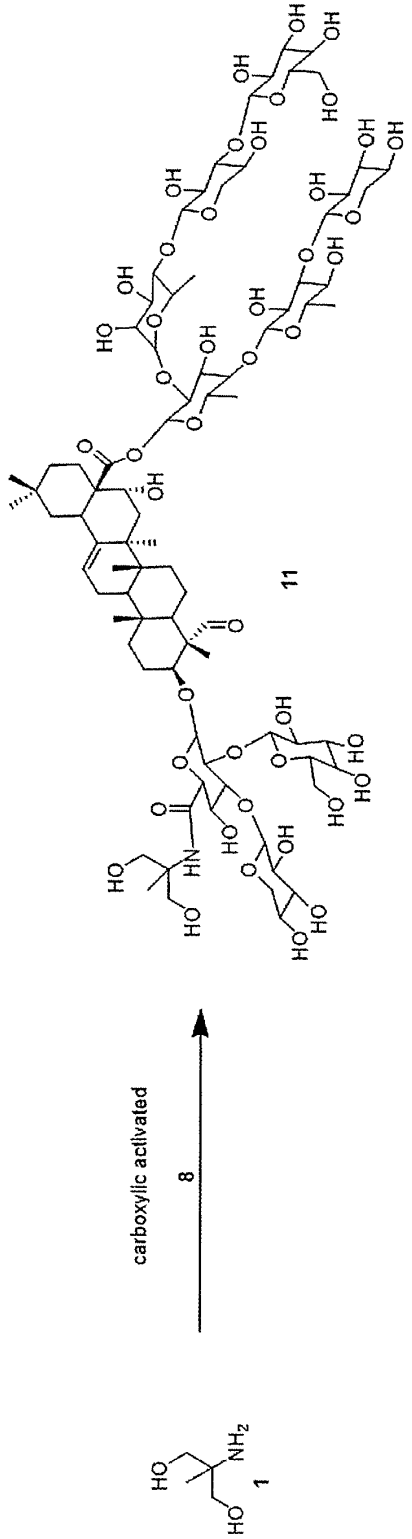


FIG. 7

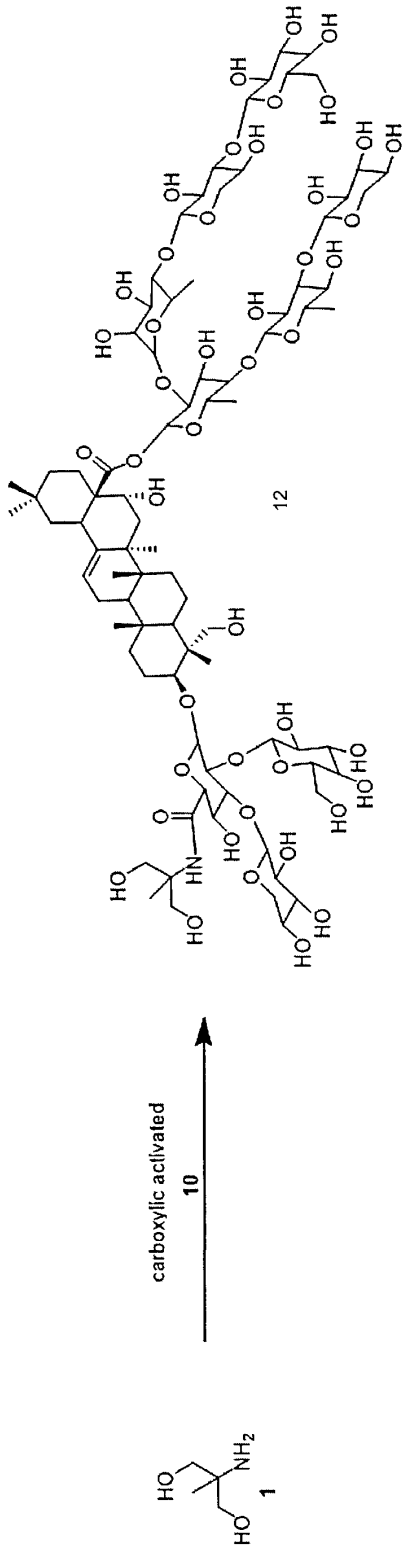


FIG. 8

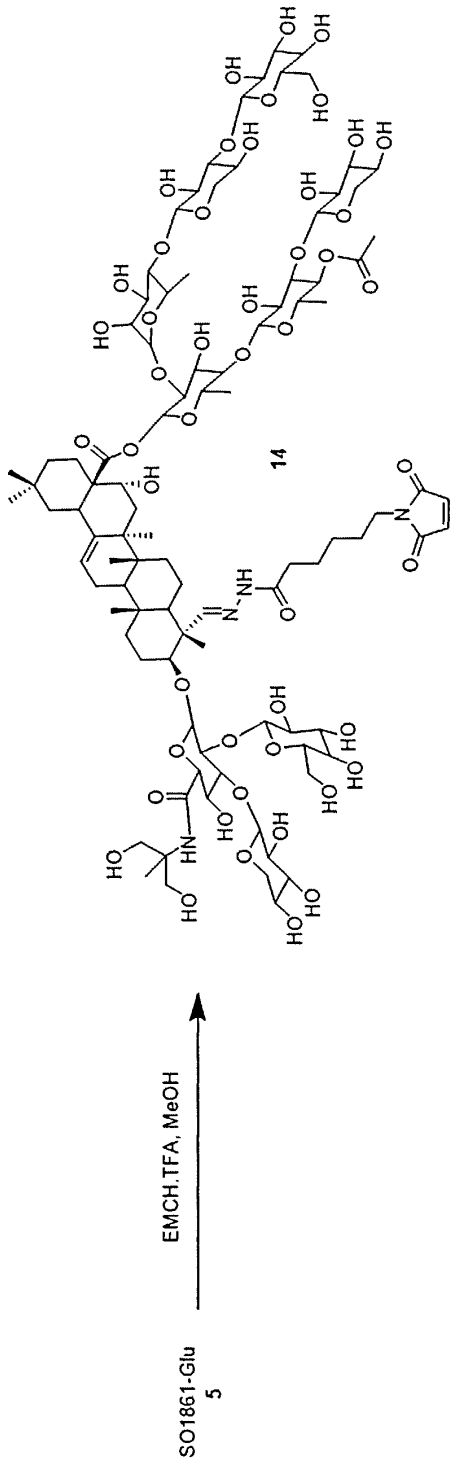


FIG. 9

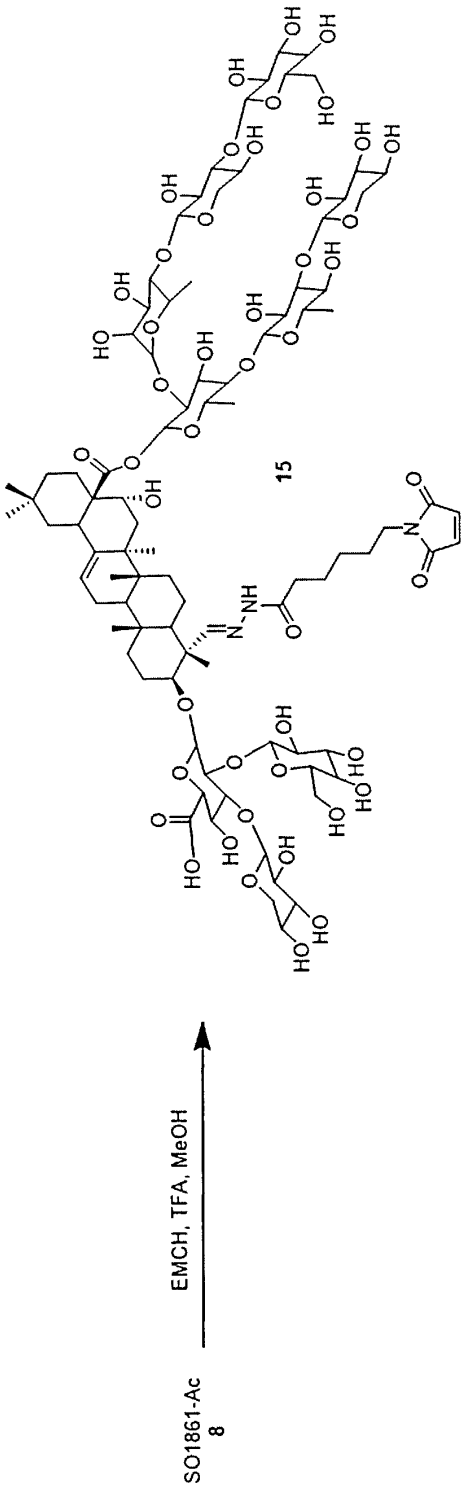


FIG. 10

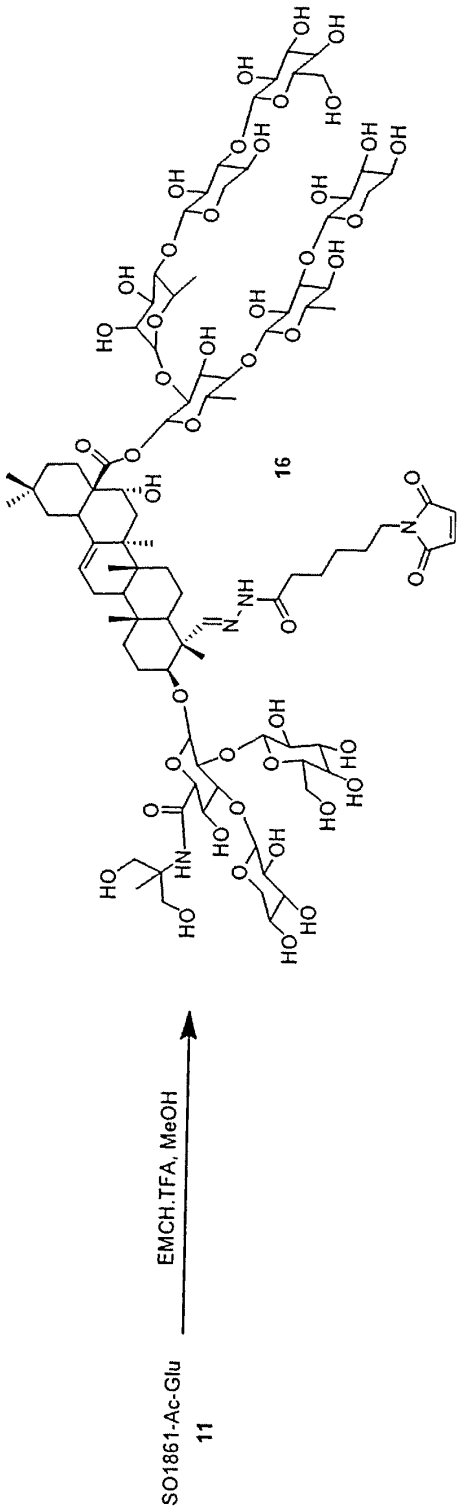


FIG. 11

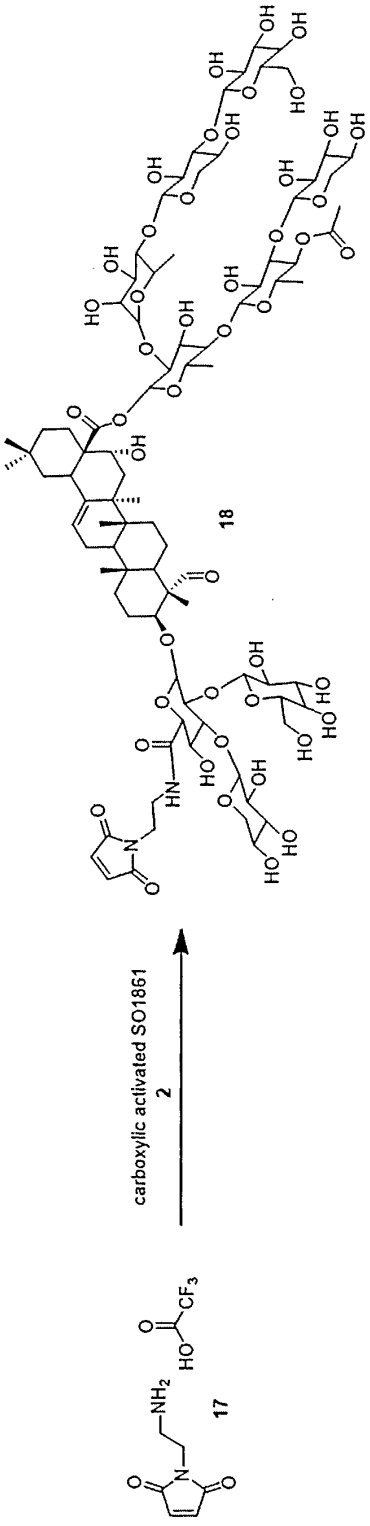


FIG. 12

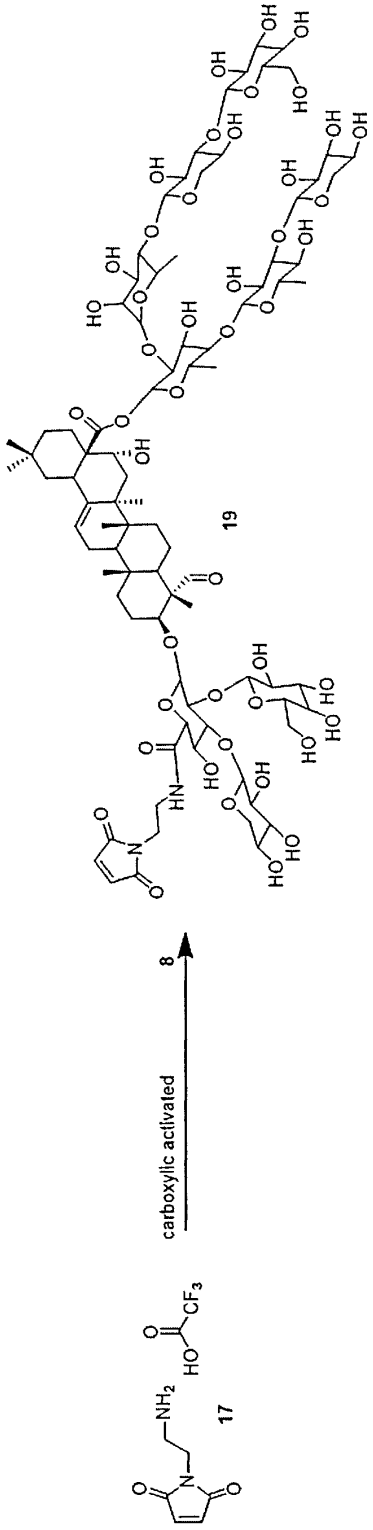


FIG. 13

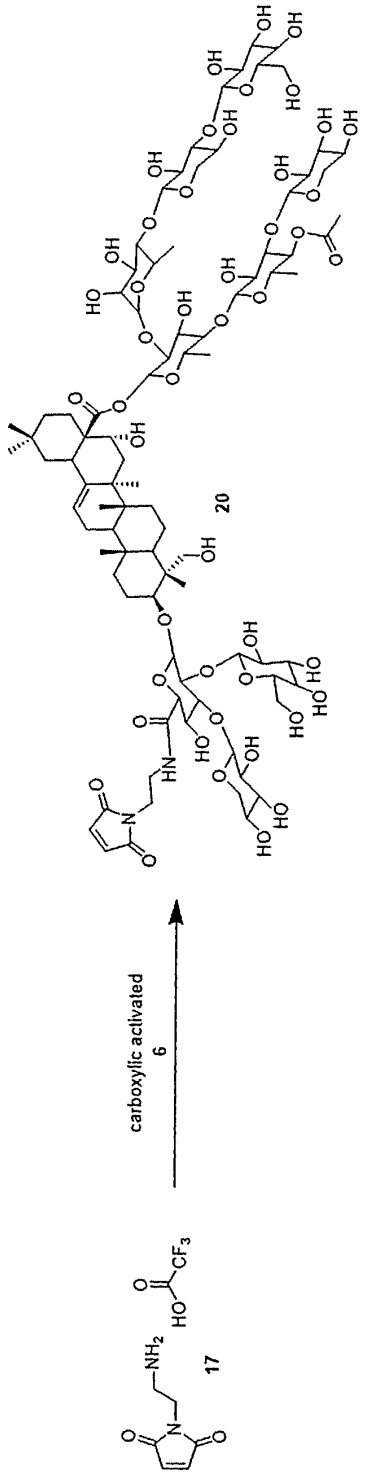


FIG. 14

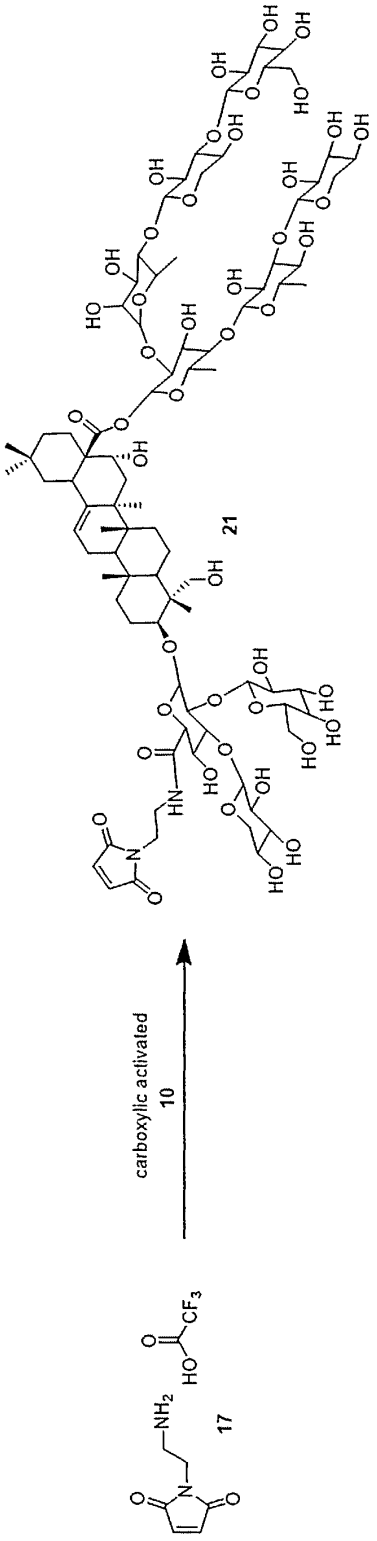


FIG. 15

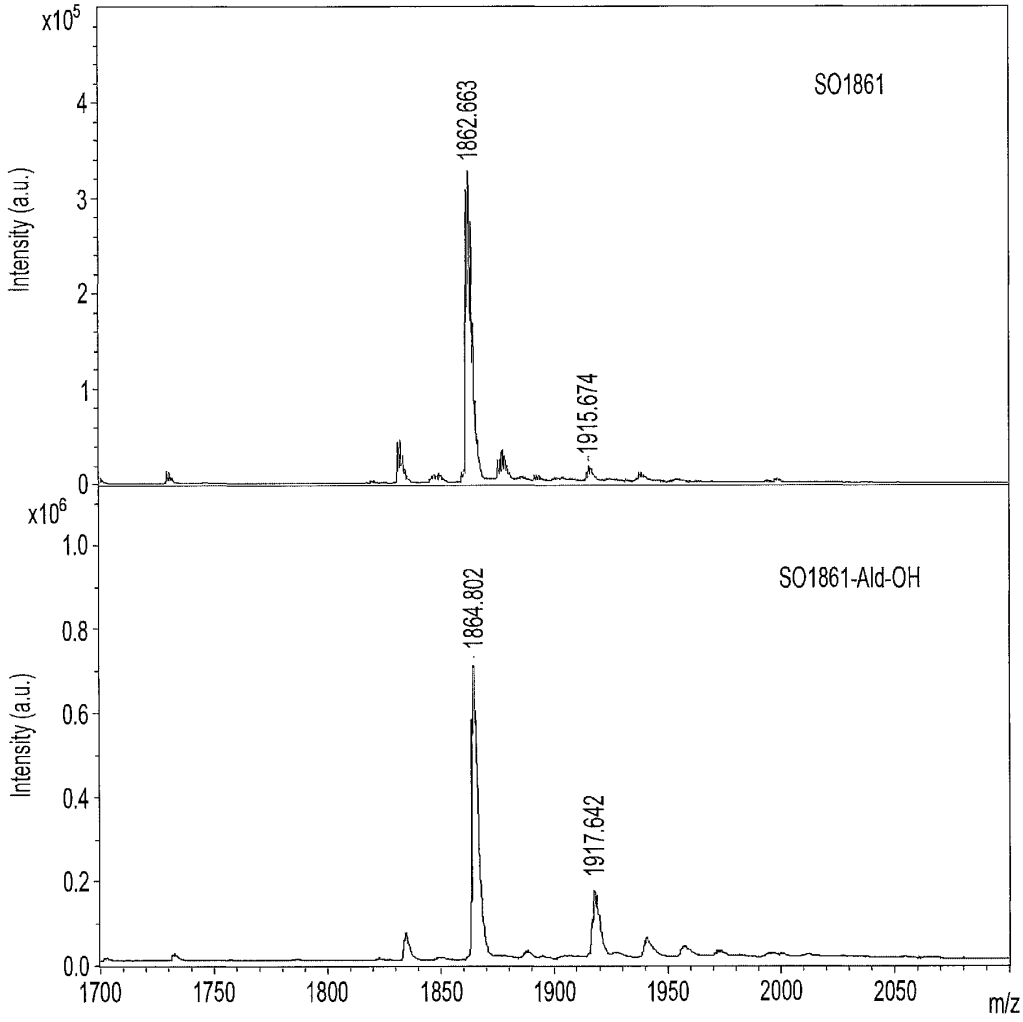

x10^5
Intensity (a.u.)
SO1861
1862.663
1915.674
x10^6
Intensity (a.u.)
SO1861-Ald-OH
1864.802
1917.642
m/z

FIG. 16

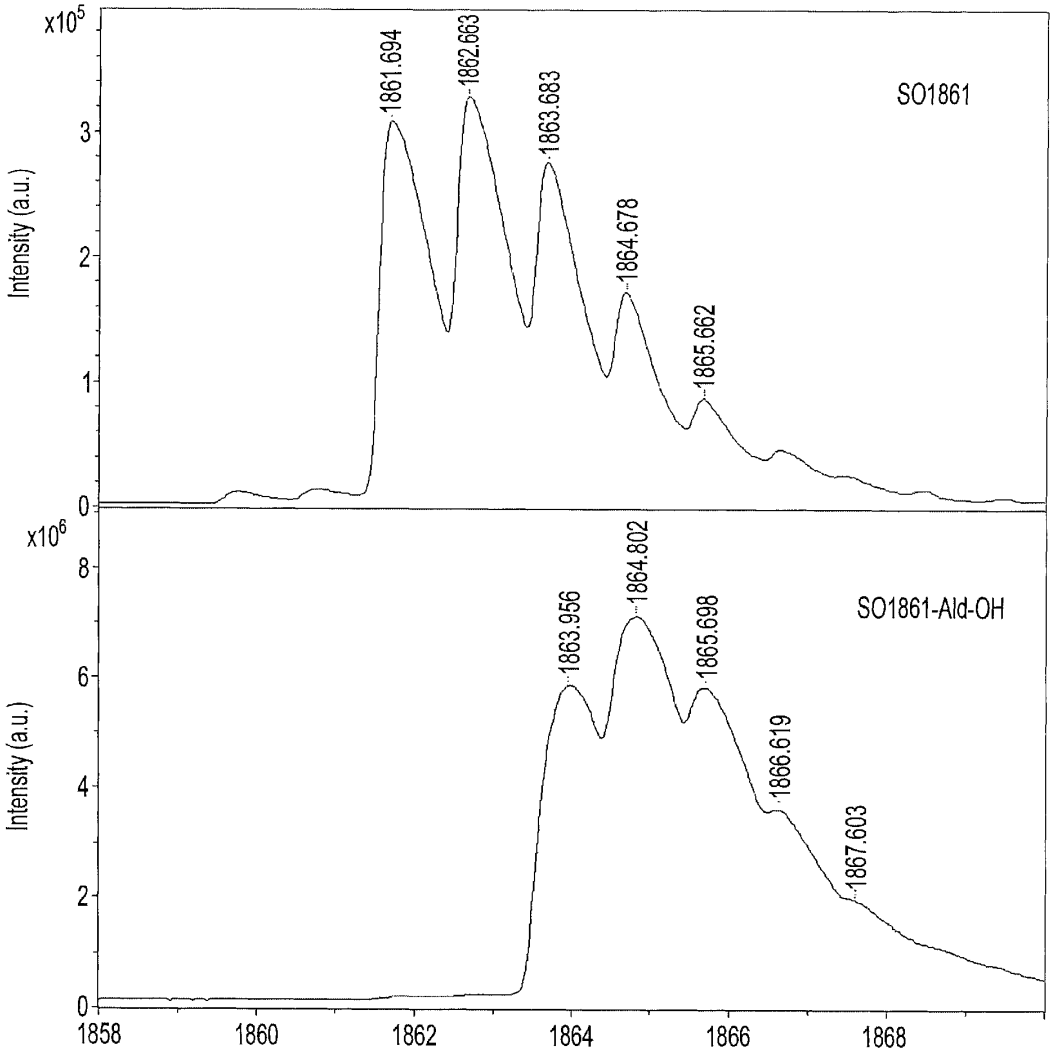

x10^5
Intensity (a.u.)
1861.694
1862.663
1863.683
1864.678
1865.662
SO1861
x10^6
Intensity (a.u.)
1863.956
1864.802
1865.698
1866.619
1867.603
SO1861-Ald-OH
1858
1860
1862
1864
1866
1868

FIG. 17

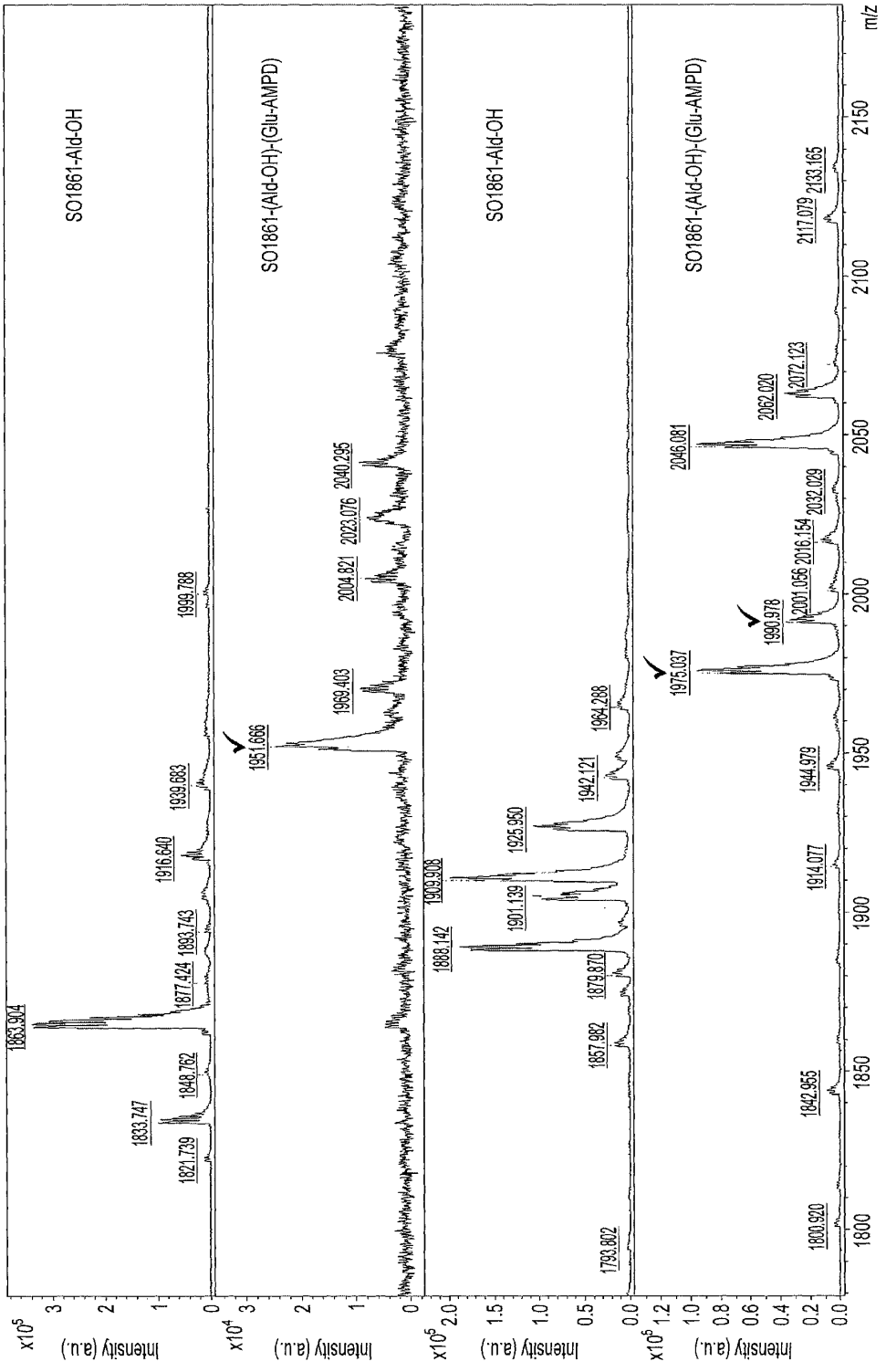

SO1861-Ald-OH
SO1861-(Ald-OH)-(Glu-AMPD)
SO1861-Ald-OH
SO1861-(Ald-OH)-(Glu-AMPD)
Intensity (a.u.)
m/z
1867.904
1833.747
1848.762
1821.739
1877.424
1893.743
1916.640
1939.683
1999.788
1951.666
1969.403
2004.821
2023.076
2040.295
1888.142
1903.908
1901.139
1925.950
1942.121
1964.288
1857.982
1879.870
1793.802
1975.037
1990.978
2001.056
2016.154
2032.029
2046.081
2062.020
2072.123
2117.079
2133.165
1800.920
1842.955
1914.077
1944.979

FIG. 18A

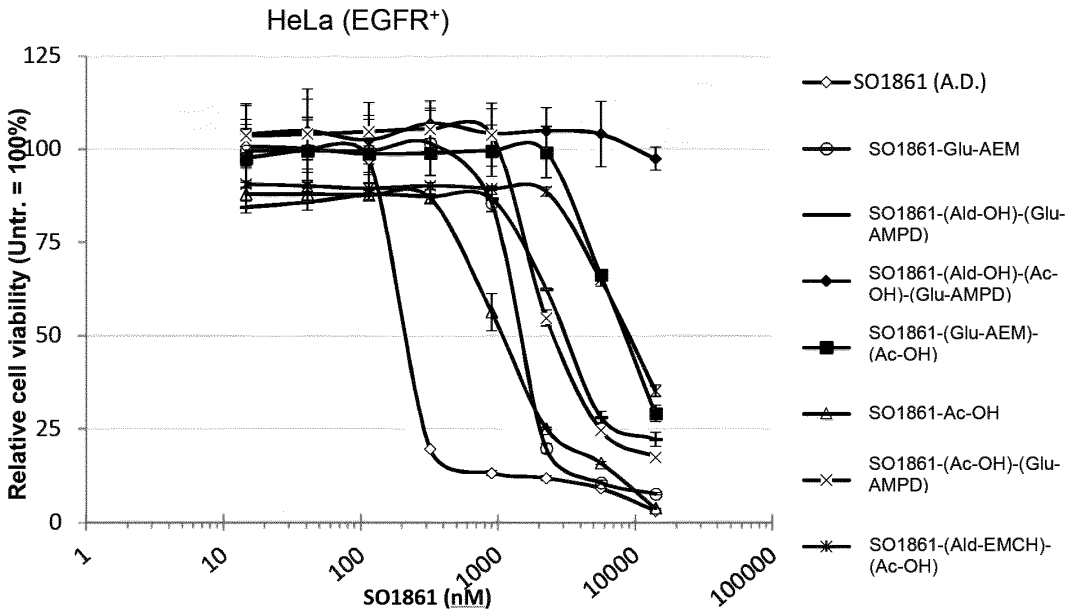


FIG. 18B

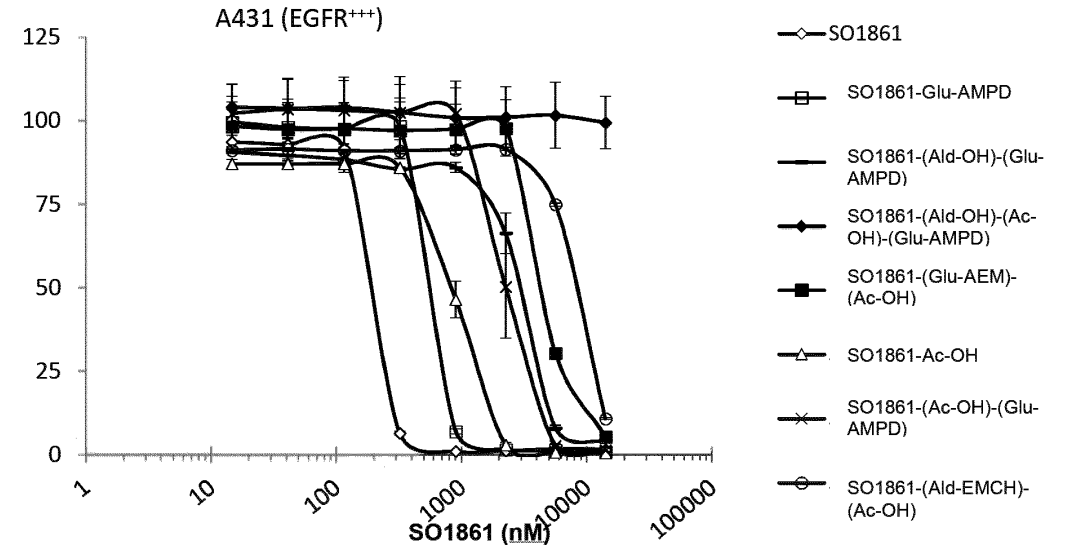


FIG. 19A

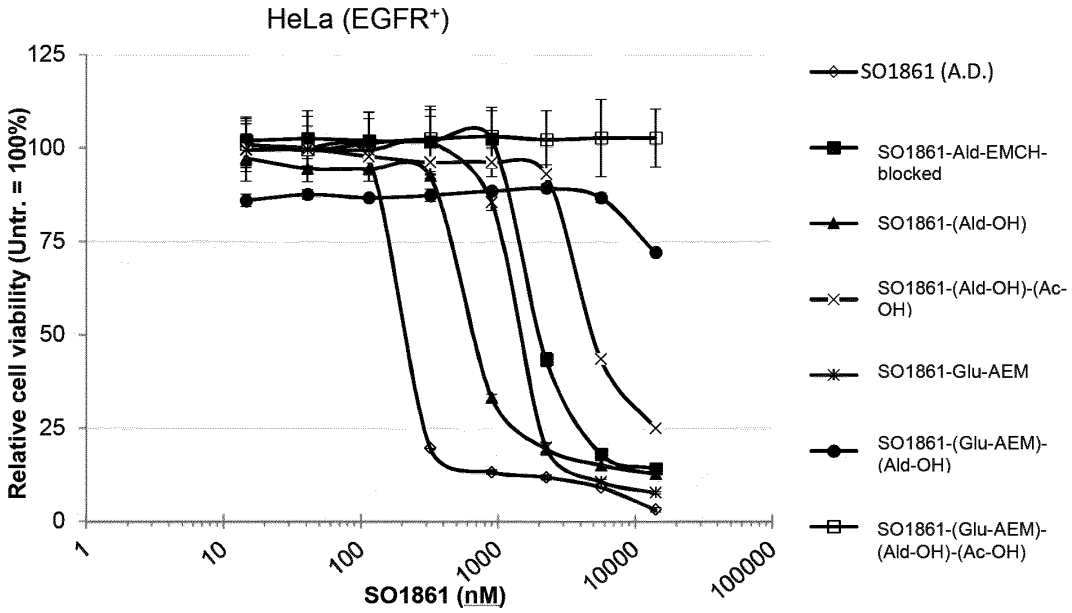


FIG. 19B

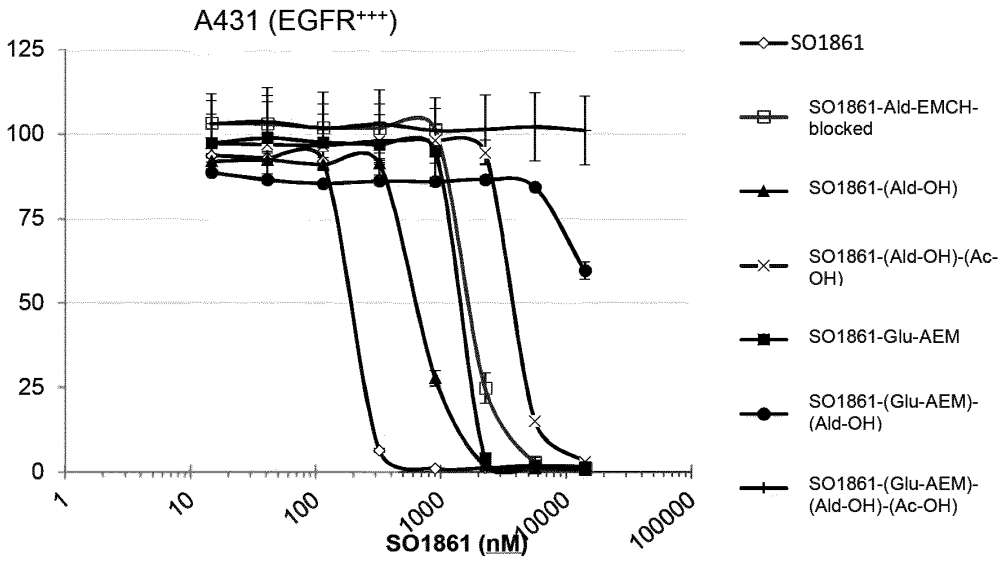


FIG. 20A

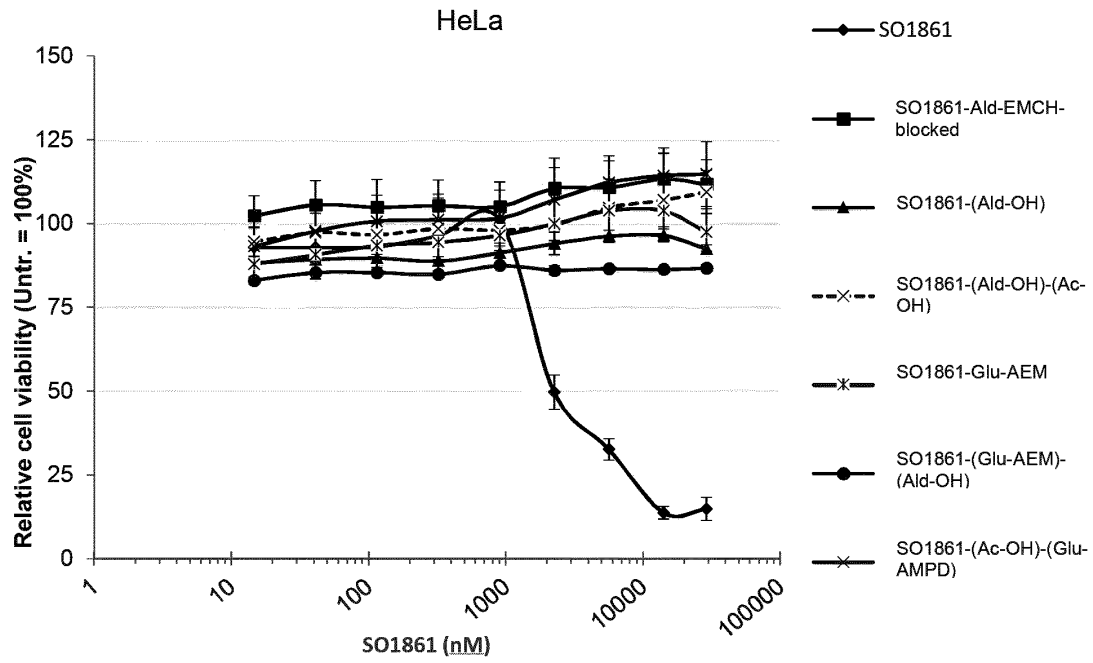


FIG. 20B

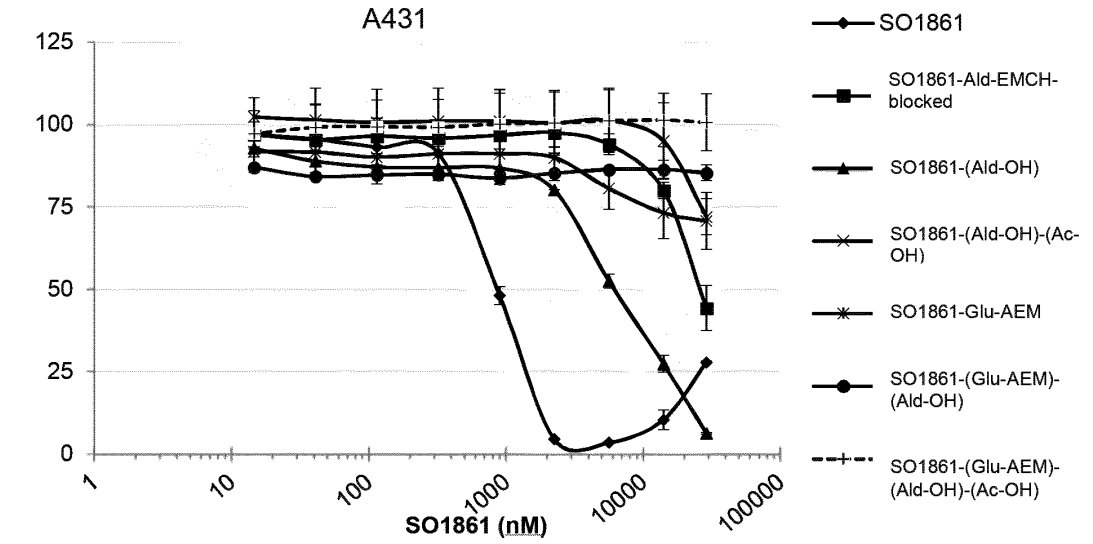


FIG. 21A

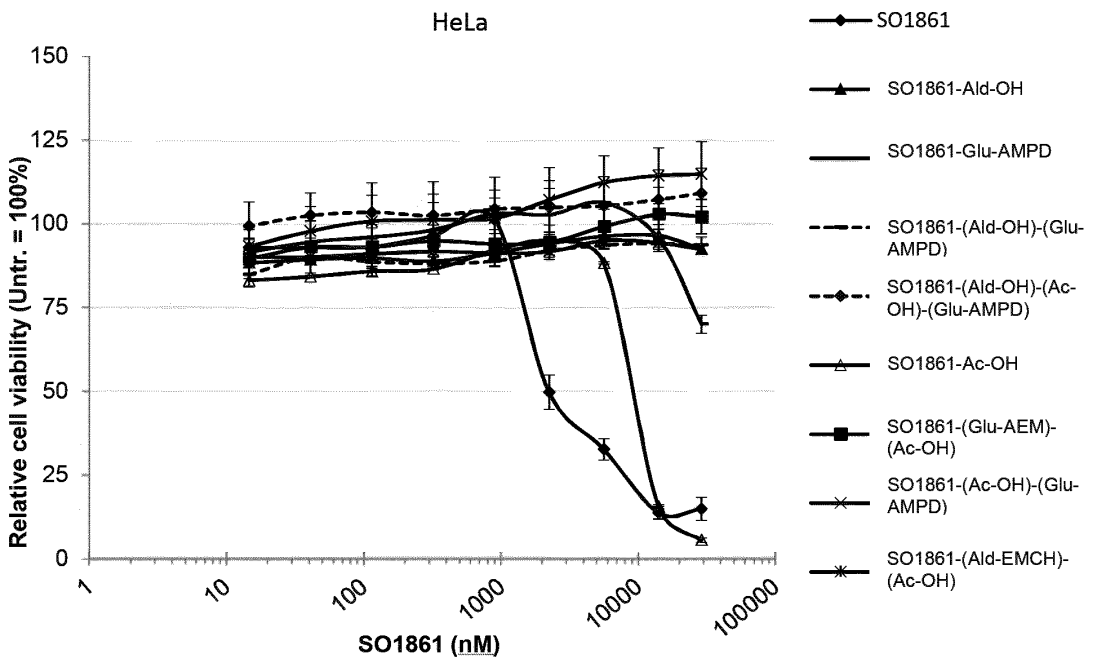


FIG. 21B

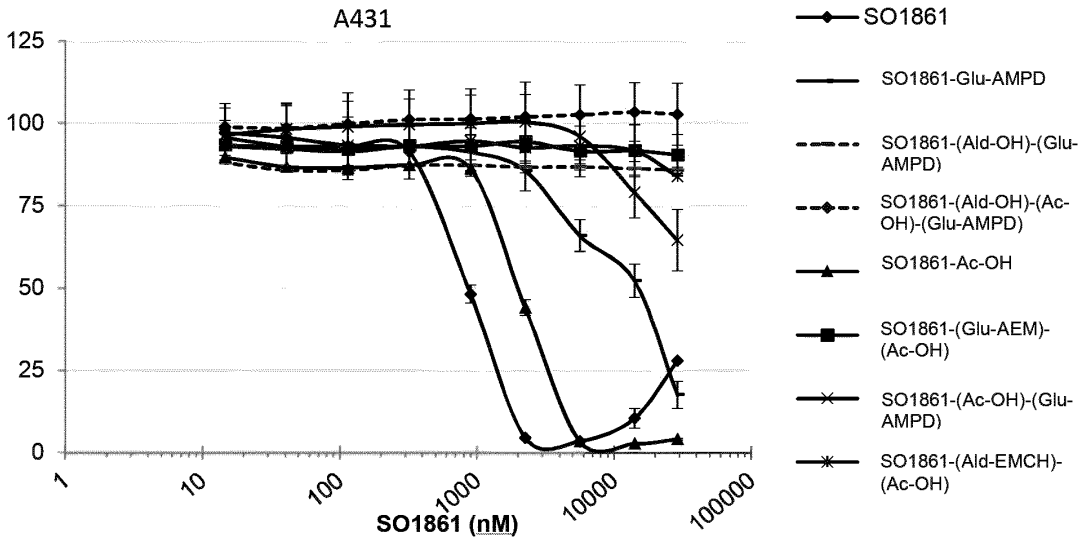


FIG. 22

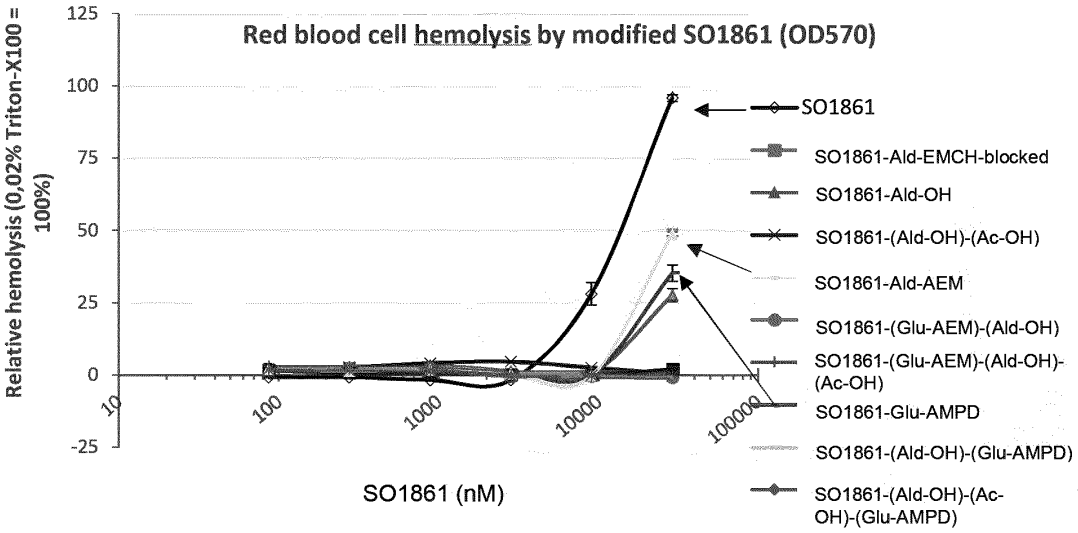

Red blood cell hemolysis by modified SO1861 (OD570)
Relative hemolysis (0,02% Triton-X100 = 100%)
125
100
75
50
25
0
-25
10
100
1000
10000
100000
SO1861 (nM)
SO1861
SO1861-Ald-EMCH-blocked
SO1861-Ald-OH
SO1861-(Ald-OH)-(Ac-OH)
SO1861-Ald-AEM
SO1861-(Glu-AEM)-(Ald-OH)
SO1861-(Glu-AEM)-(Ald-OH)-(Ac-OH)
SO1861-Glu-AMPD
SO1861-(Ald-OH)-(Glu-AMPD)
SO1861-(Ald-OH)-(Ac-OH)-(Glu-AMPD)

FIG. 23A

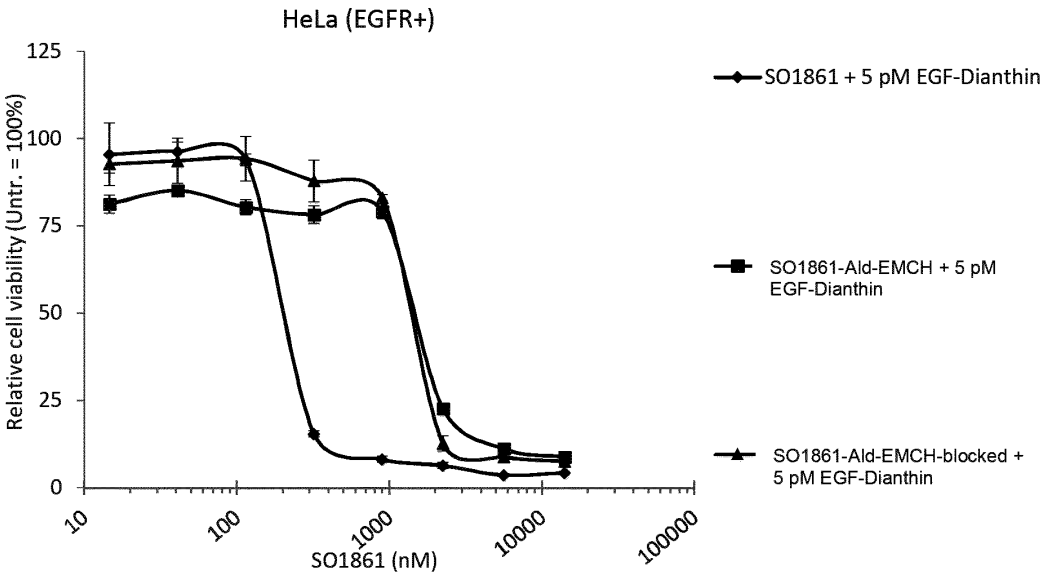


FIG. 23B

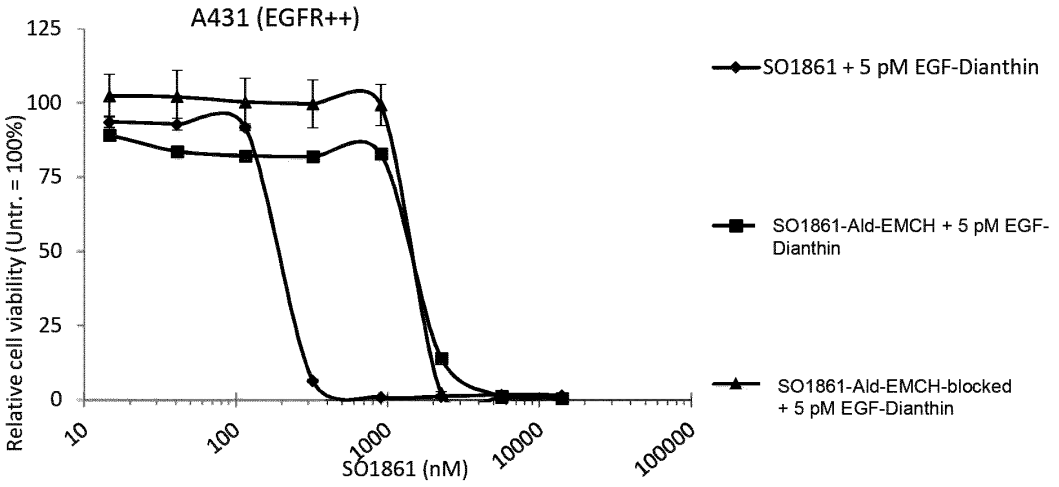


FIG. 24A

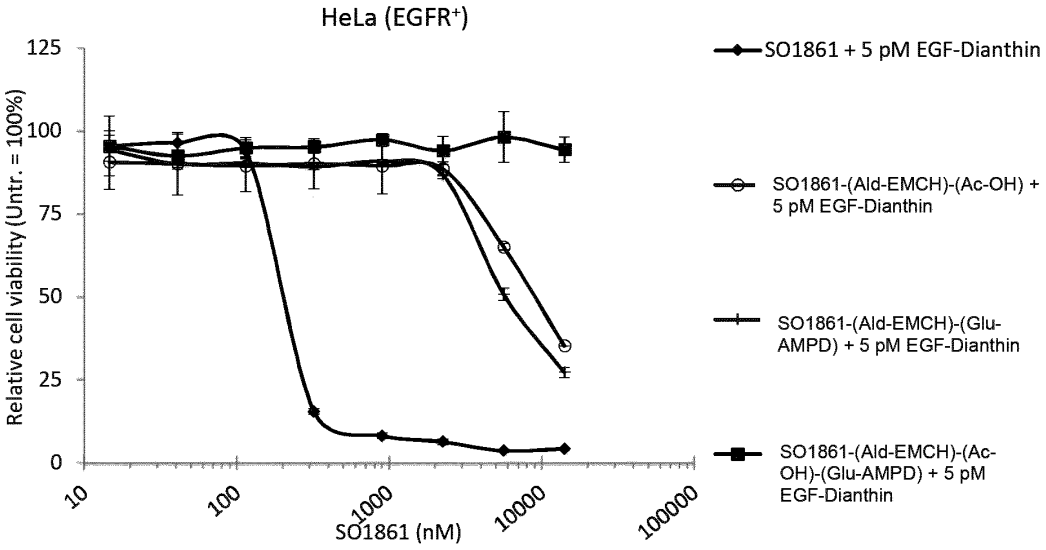


FIG. 24B

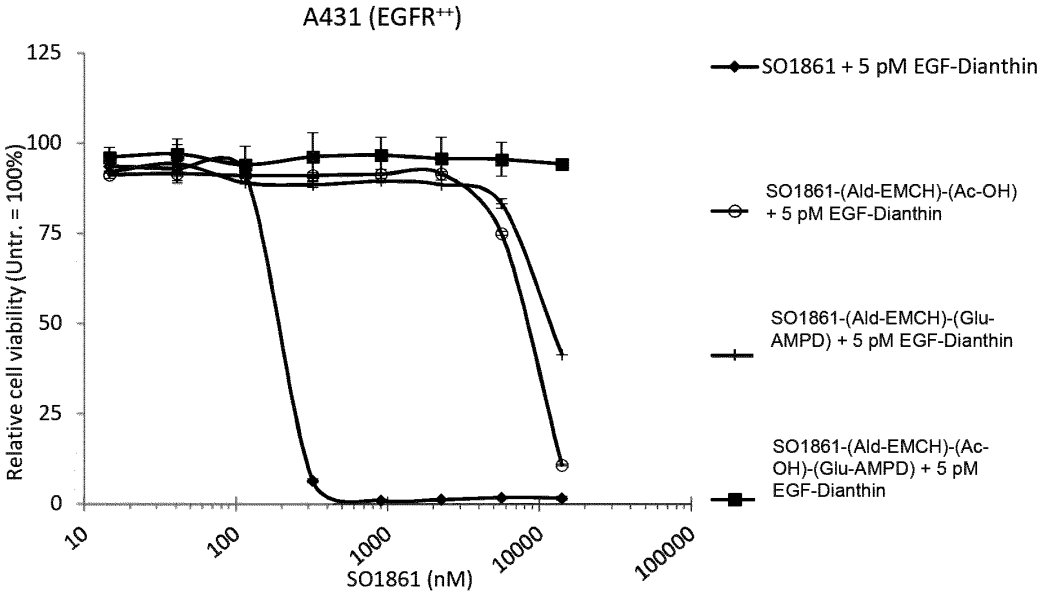


FIG. 25A

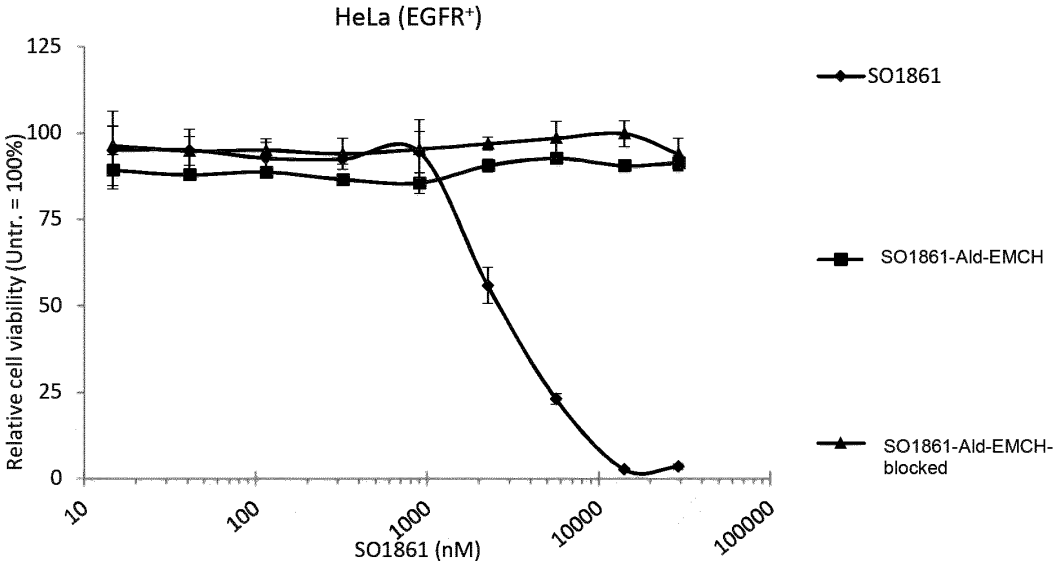


FIG. 25B

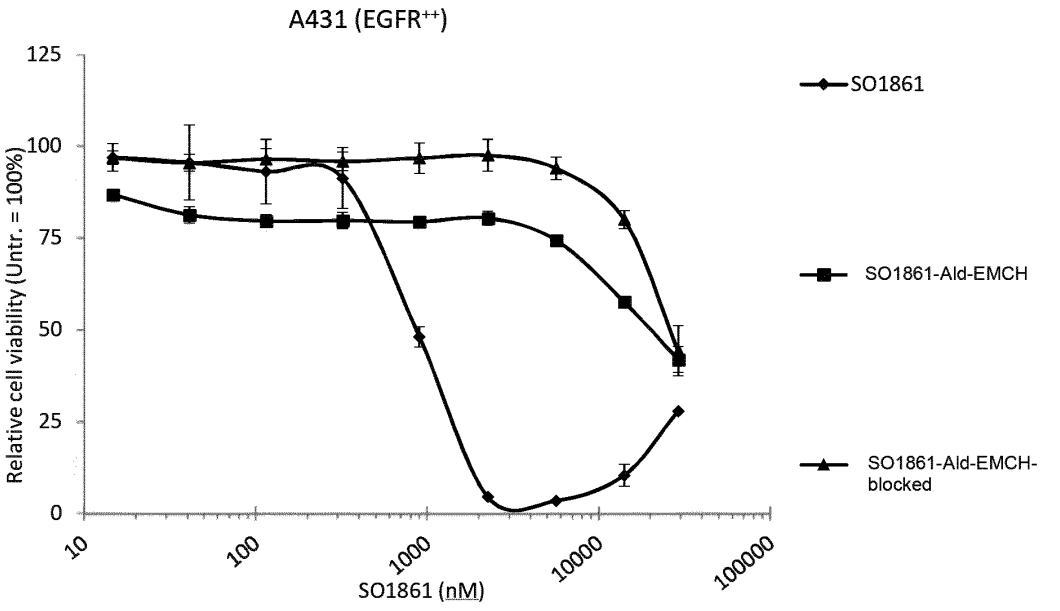


FIG. 26A

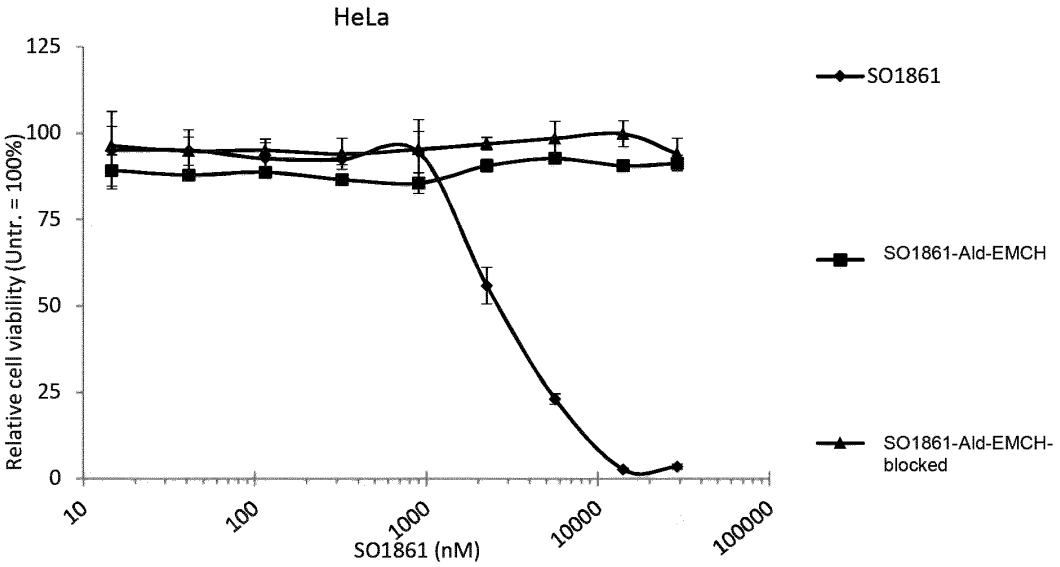


FIG. 26B

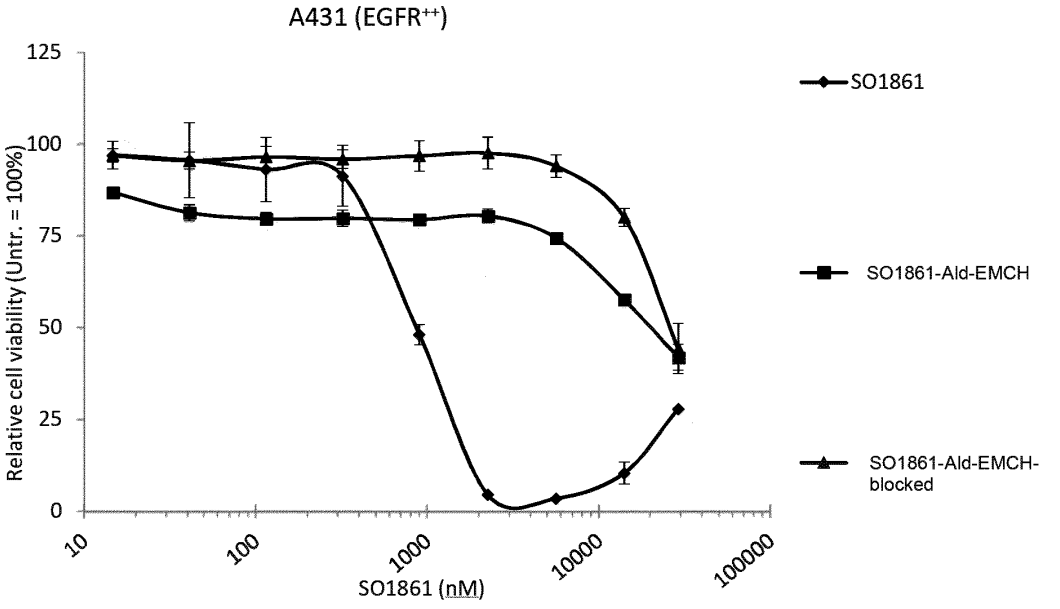


FIG. 27

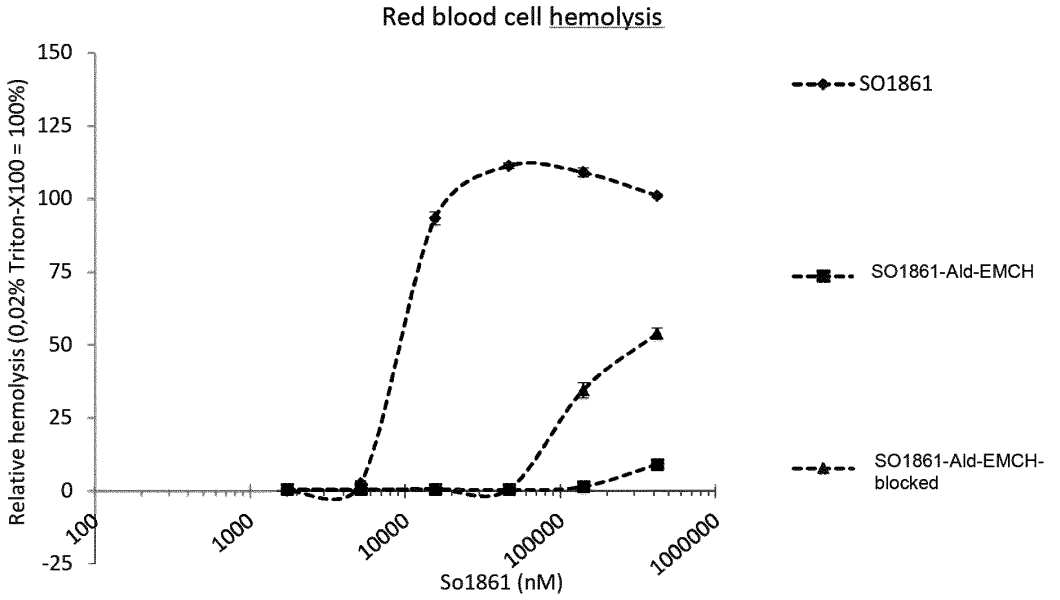

Red blood cell hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
150
125
100
75
50
25
0
-25
100
1000
10000
100000
1000000
So1861 (nM)
SO1861
SO1861-Ald-EMCH
SO1861-Ald-EMCH-blocked

FIG. 28

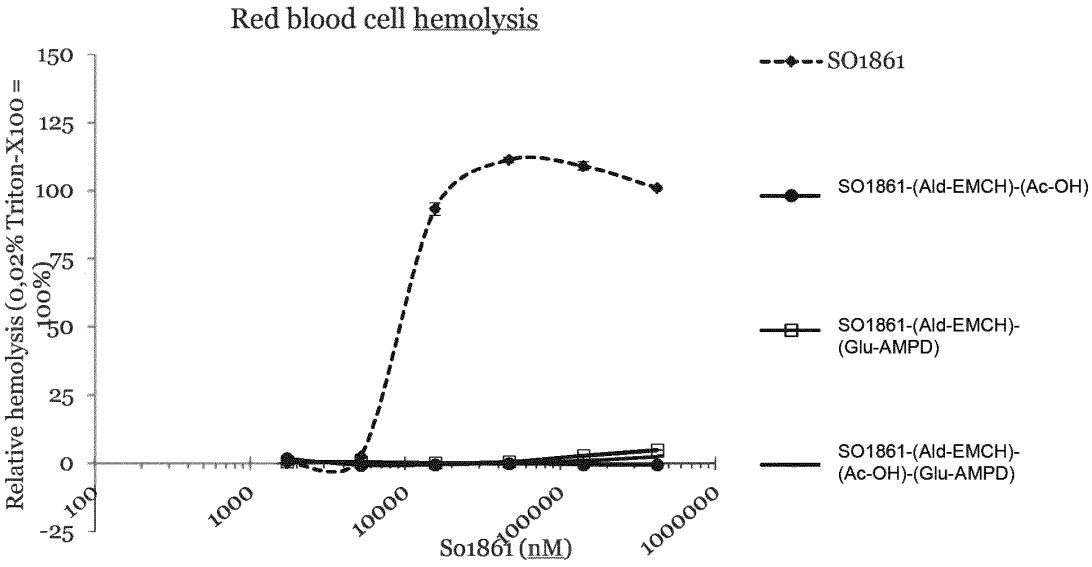

Red blood cell hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
150
125
100
75
50
25
0
-25
100
1000
10000
100000
1000000
So1861 (nM)
SO1861
SO1861-(Ald-EMCH)-(Ac-OH)
SO1861-(Ald-EMCH)-(Glu-AMPD)
SO1861-(Ald-EMCH)-(Ac-OH)-(Glu-AMPD)

FIG. 29

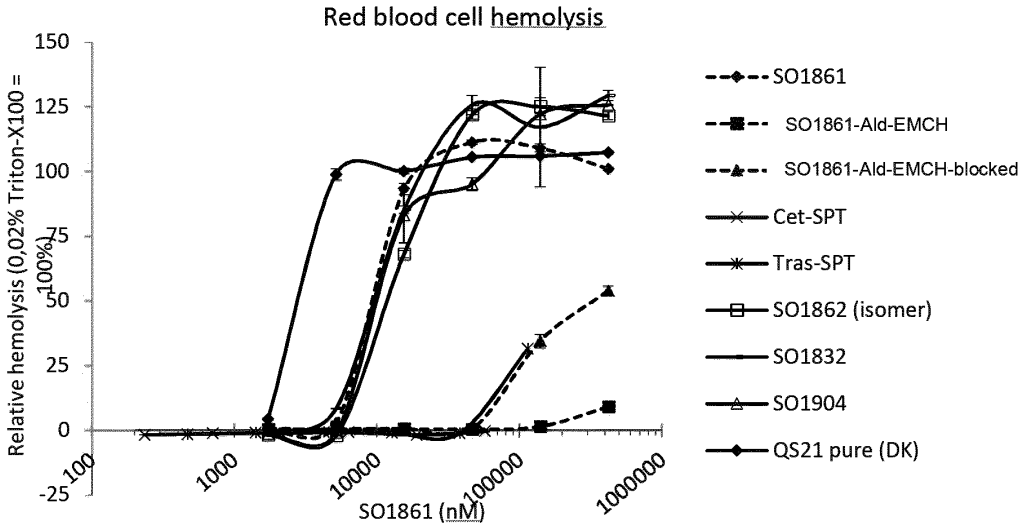

Red blood cell hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
150
125
100
75
50
25
0
-25
100
1000
10000
100000
1000000
SO1861 (nM)
SO1861
SO1861-Ald-EMCH
SO1861-Ald-EMCH-blocked
Cet-SPT
Tras-SPT
SO1862 (isomer)
SO1832
SO1904
QS21 pure (DK)

FIG. 30A

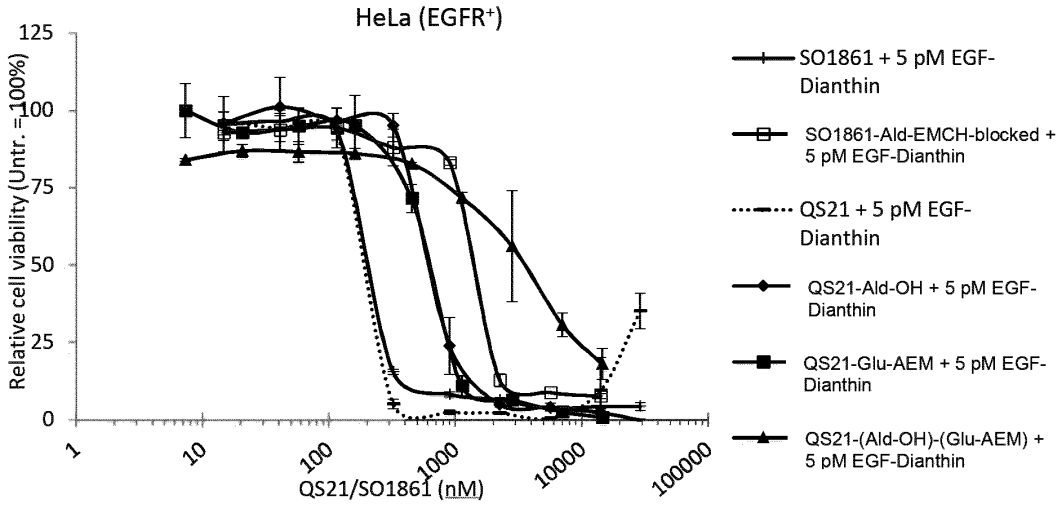


FIG. 30B

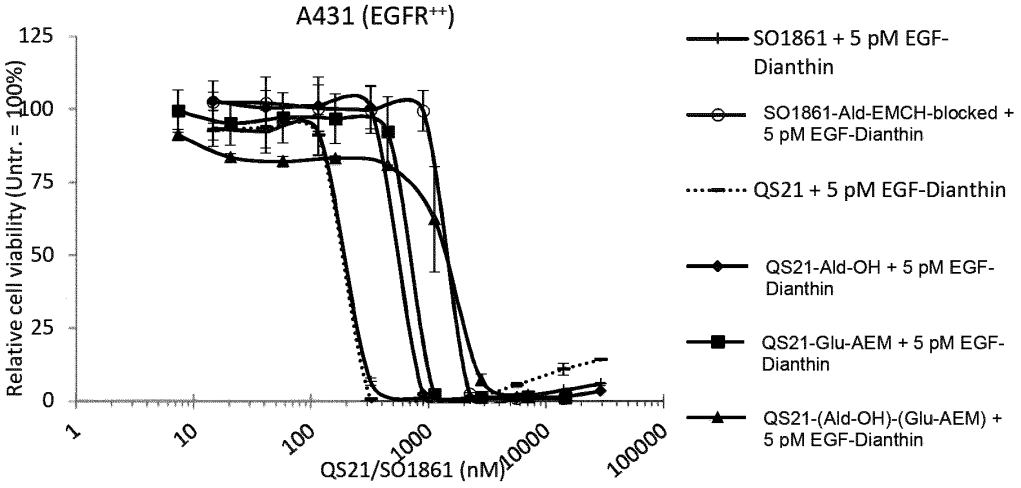


FIG. 31A

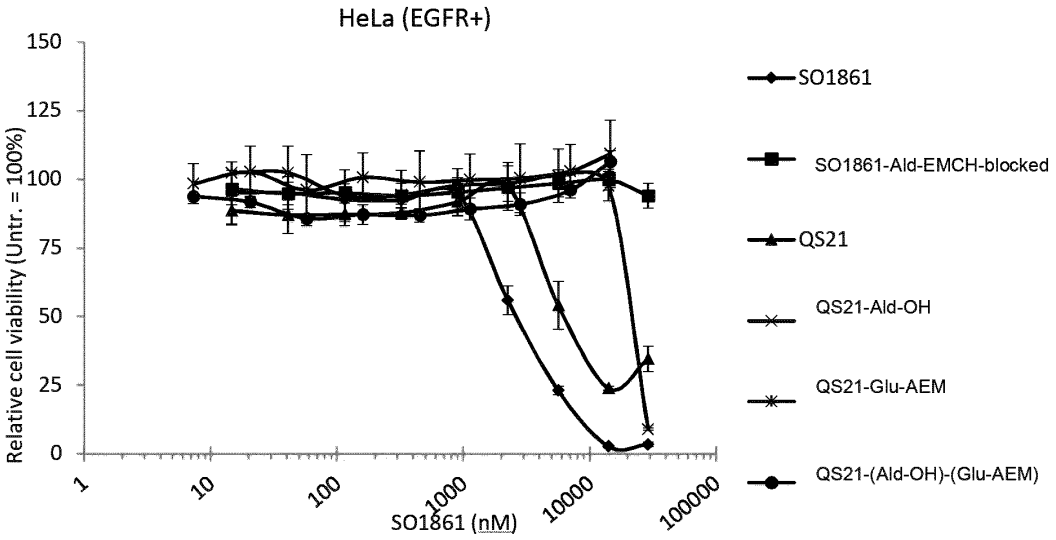


FIG. 31B

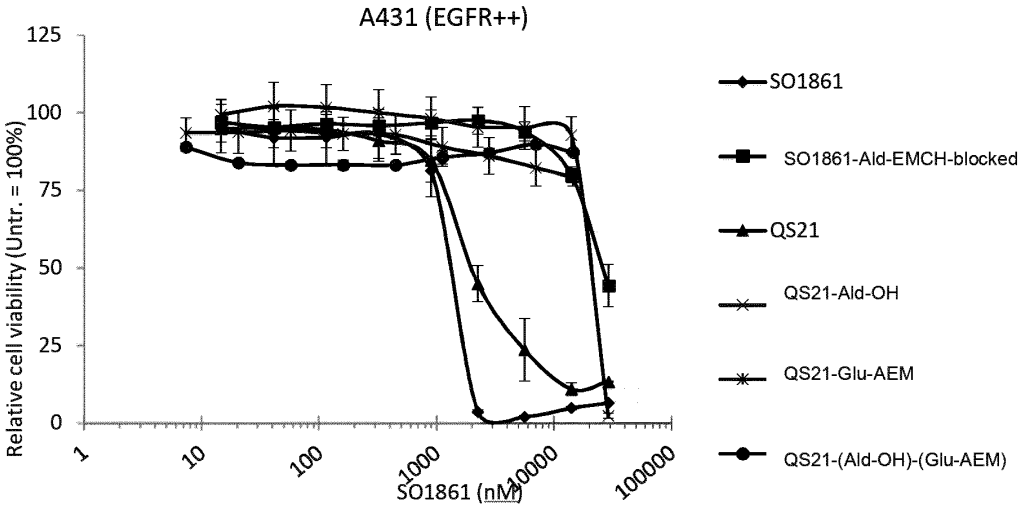


FIG. 32

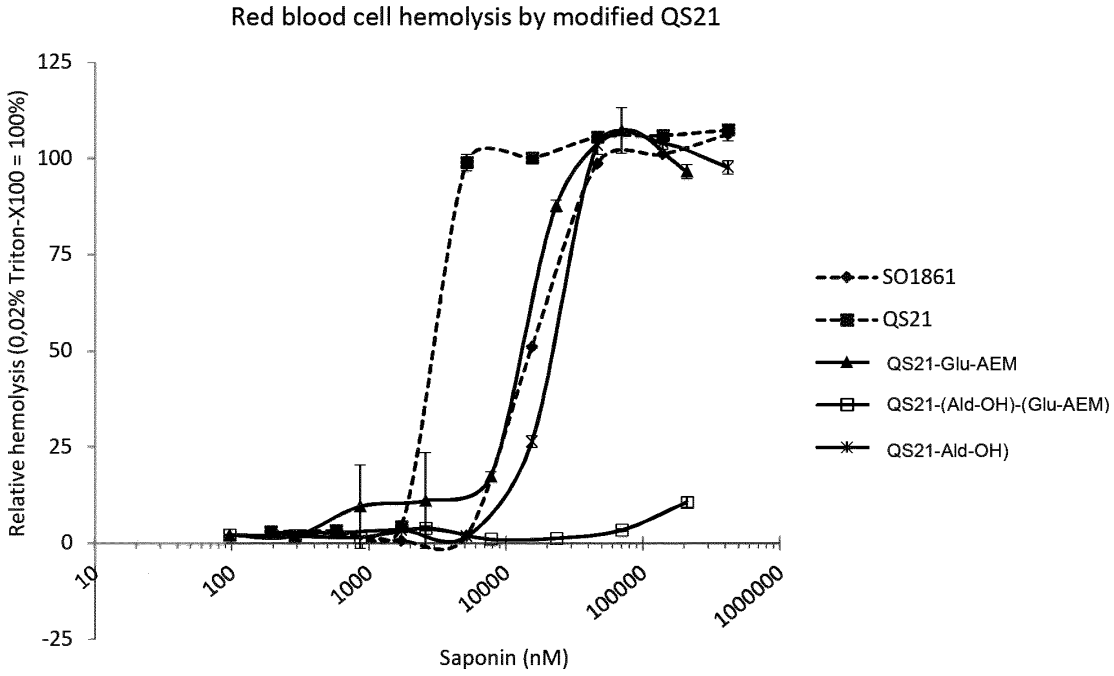

Red blood cell hemolysis by modified QS21
Relative hemolysis (0,02% Triton-X100 = 100%)
125
100
75
50
25
0
-25
10
100
1000
10000
100000
1000000
Saponin (nM)
SO1861
QS21
QS21-Glu-AEM
QS21-(Ald-OH)-(Glu-AEM)
QS21-Ald-OH)

FIG. 33A

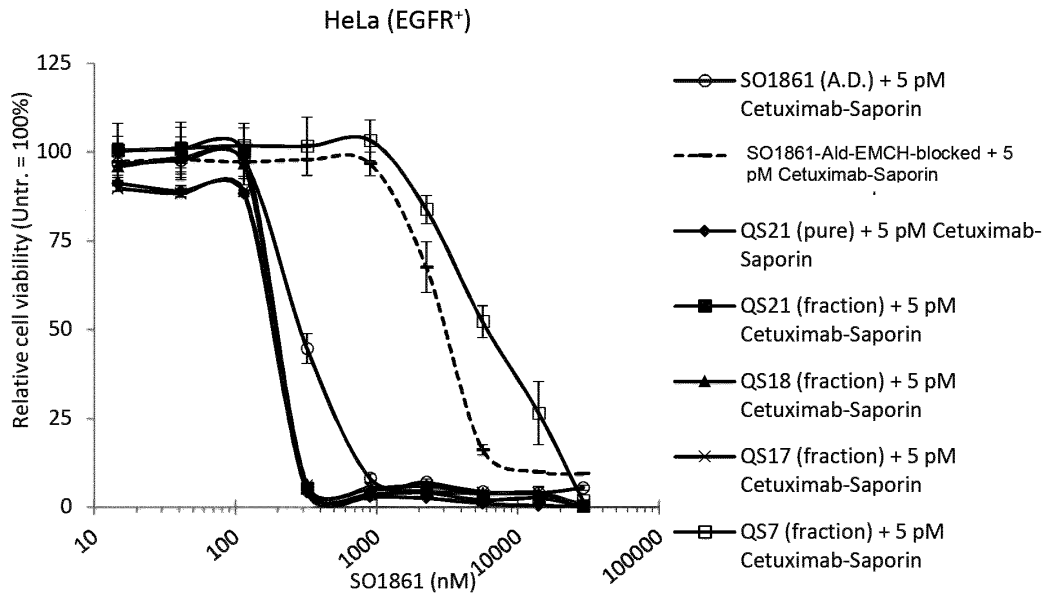


FIG. 33B

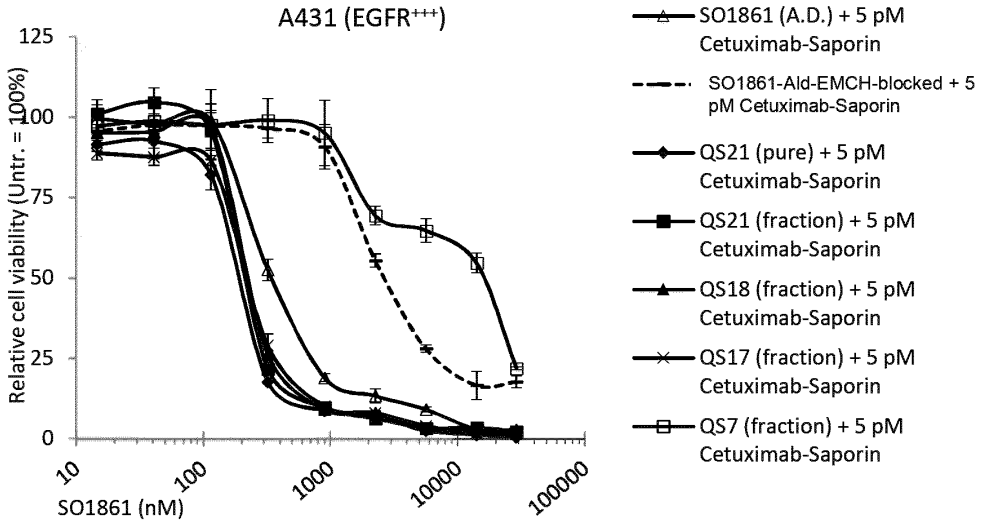


FIG. 34A

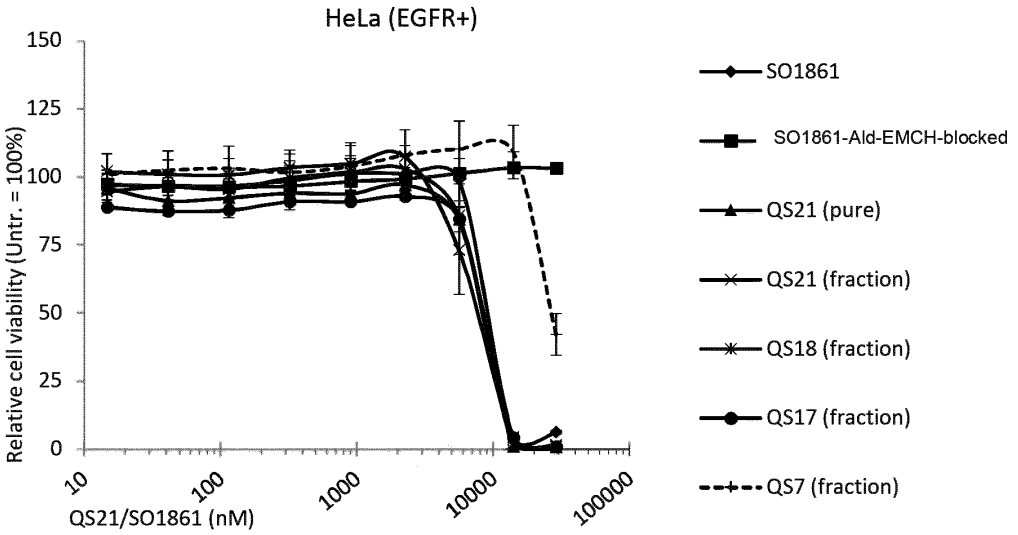


FIG. 34B

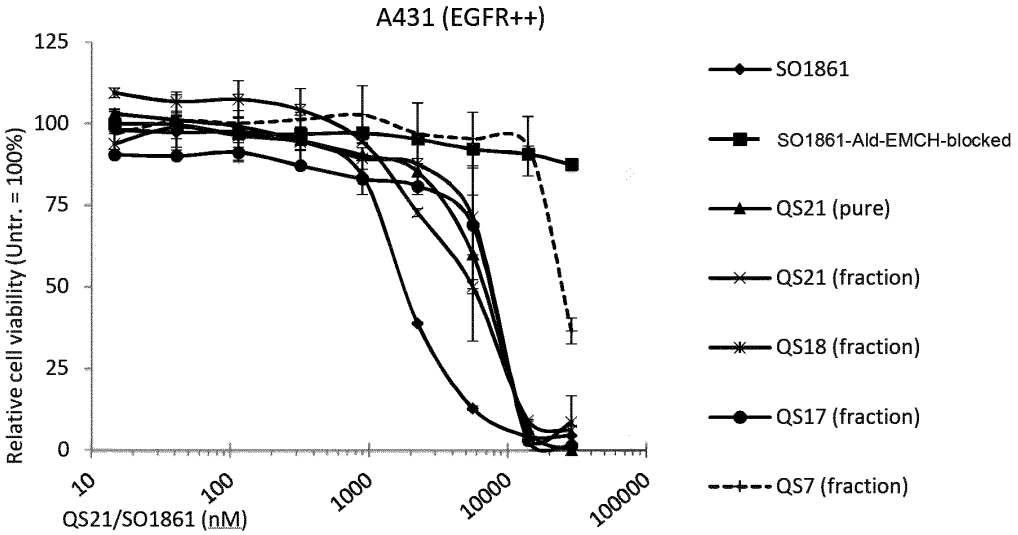


FIG. 35

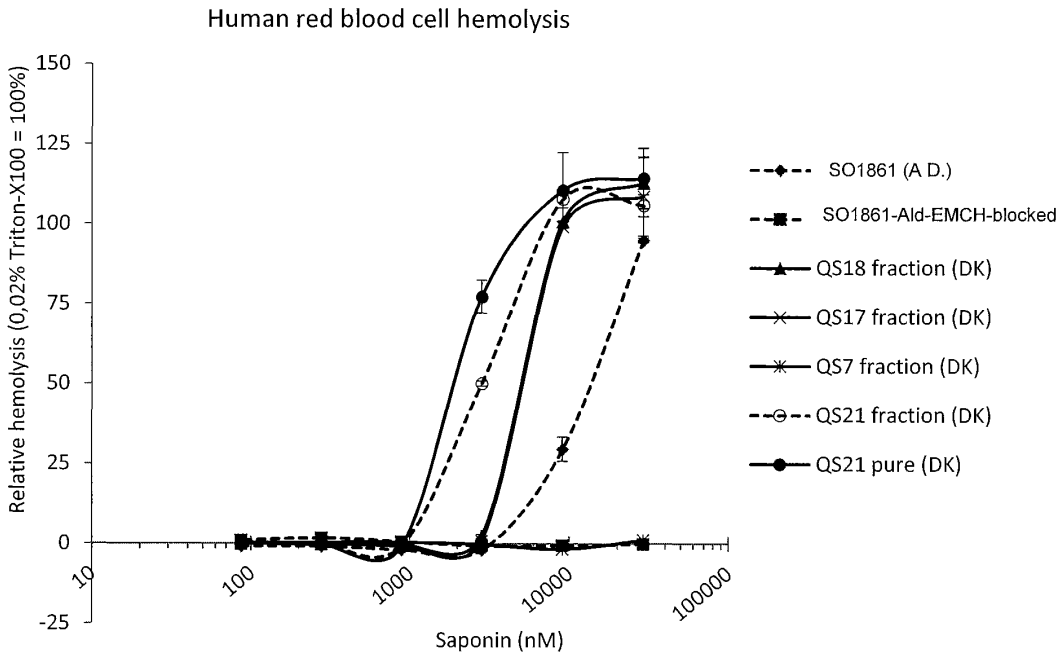

Human red blood cell hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
150
125
100
75
50
25
0
-25
10
100
1000
10000
100000
Saponin (nM)
SO1861 (A D.)
SO1861-Ald-EMCH-blocked
QS18 fraction (DK)
QS17 fraction (DK)
QS7 fraction (DK)
QS21 fraction (DK)
QS21 pure (DK)

FIG. 36

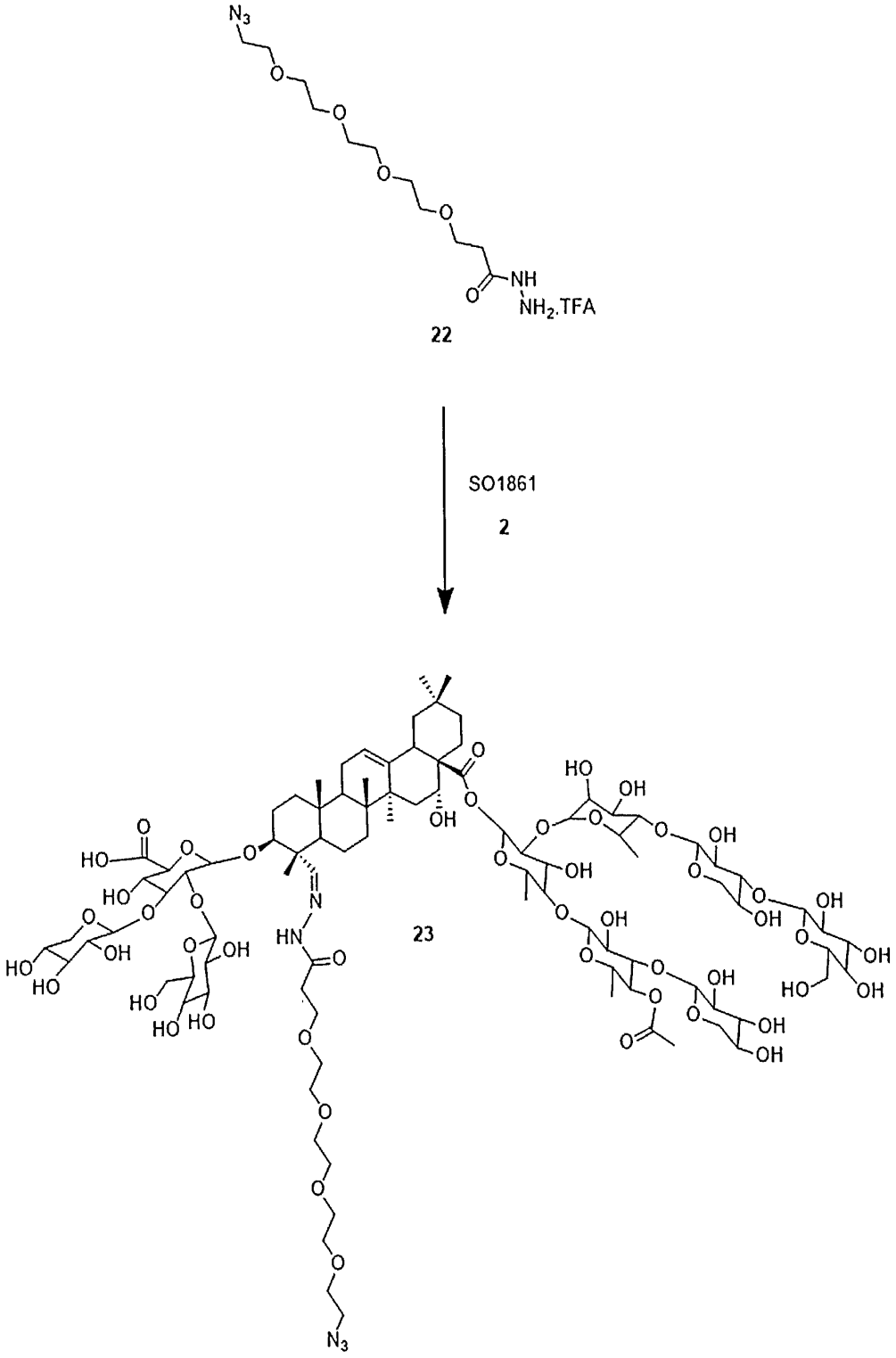


FIG. 37

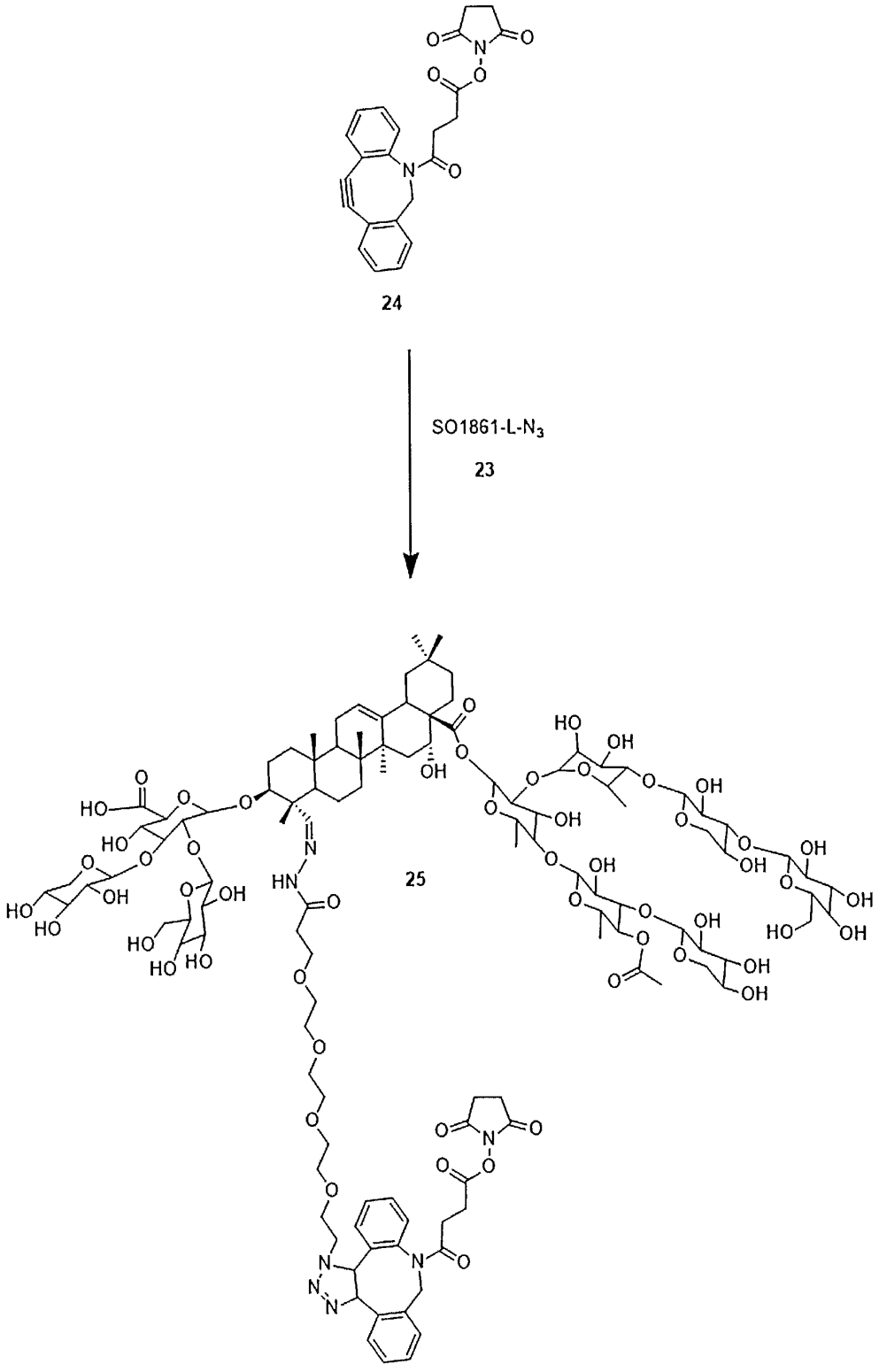


FIG. 38

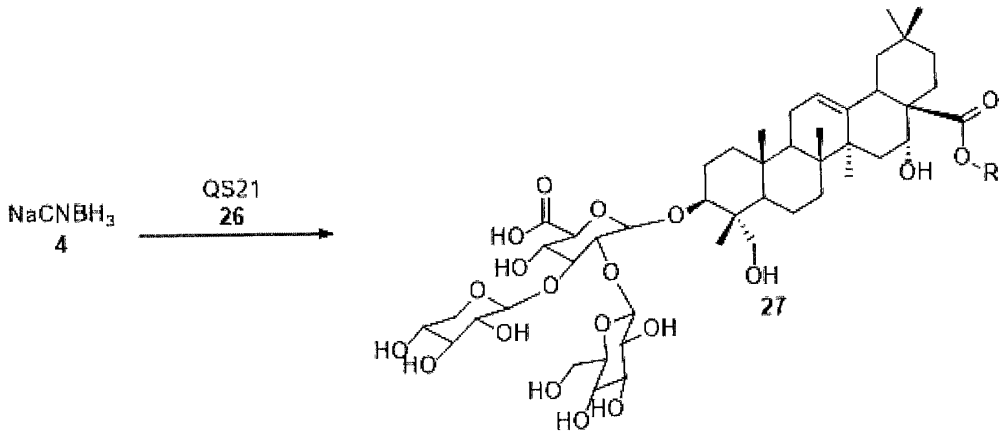


FIG. 39

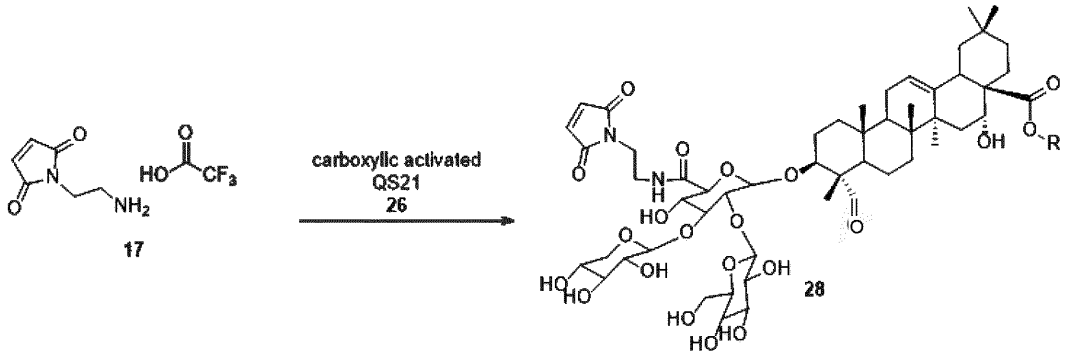


FIG. 40A

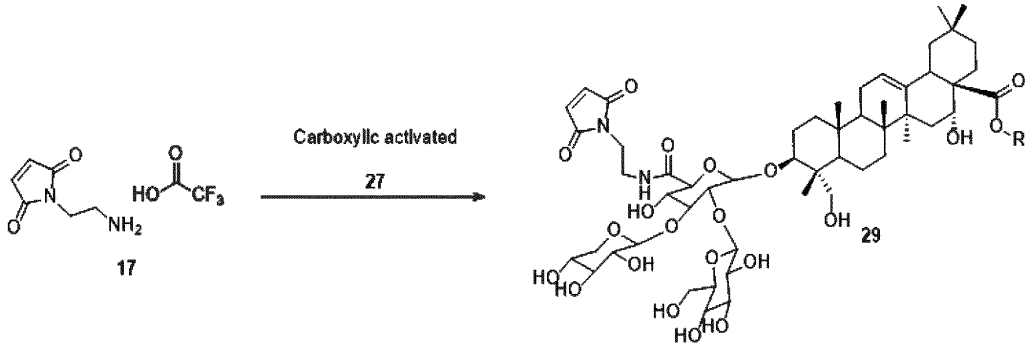
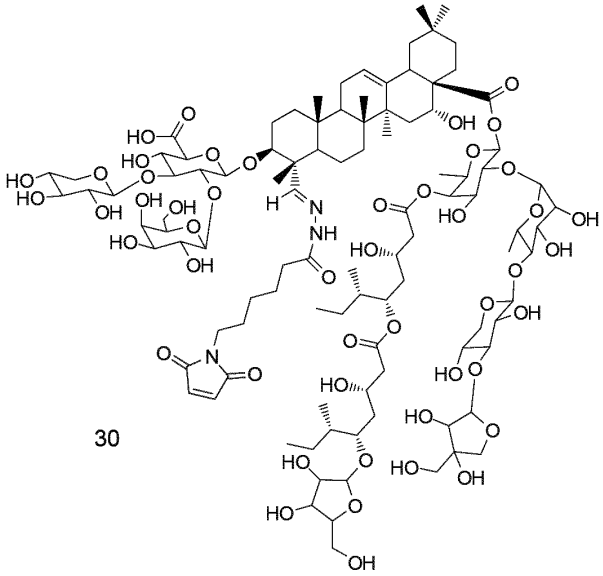
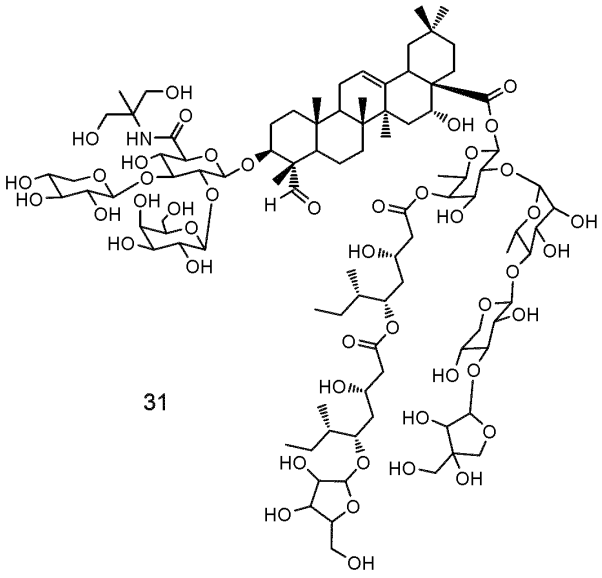


FIG. 40B



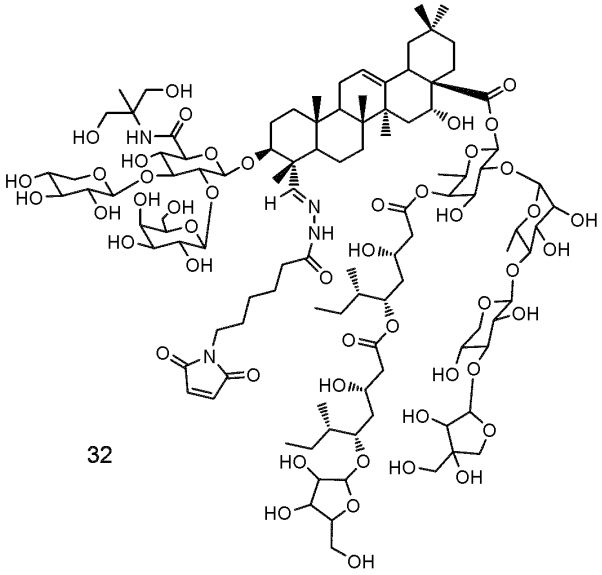
30

FIG. 40C



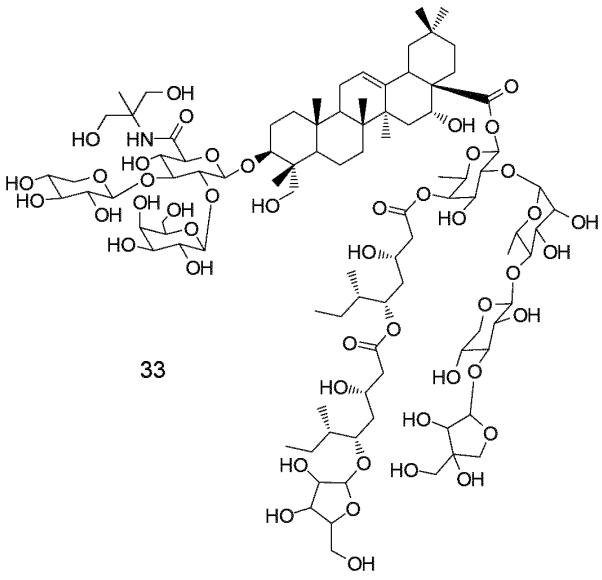
31

FIG. 40D



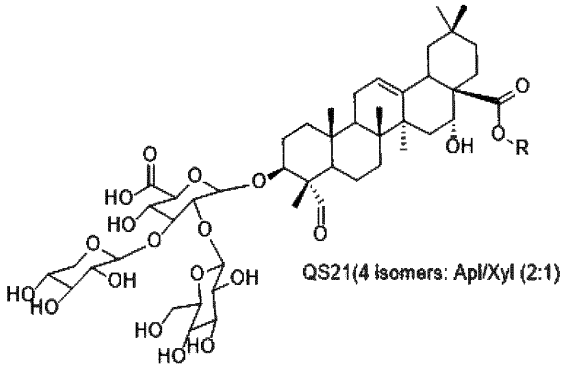
32

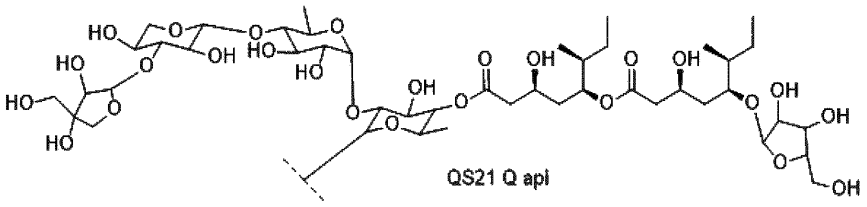
FIG. 40E

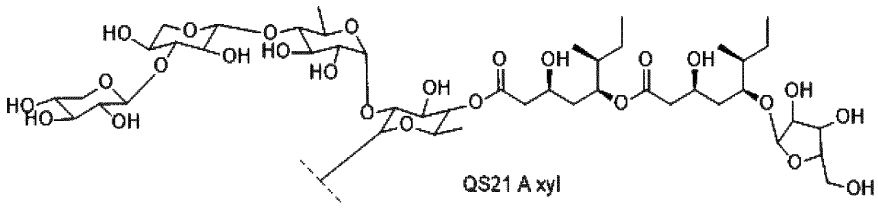


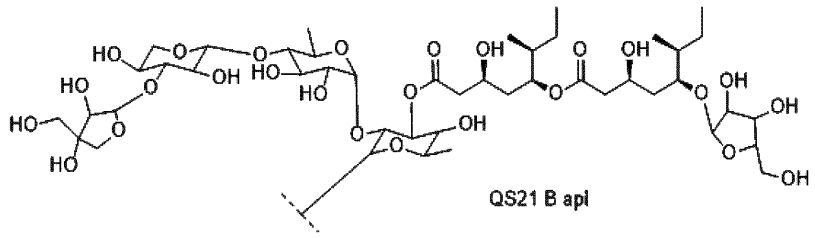
33

FIG. 41


QS21(4 isomers: Api/Xyl (2:1)


QS21 Q api


QS21 A xyl


QS21 B api

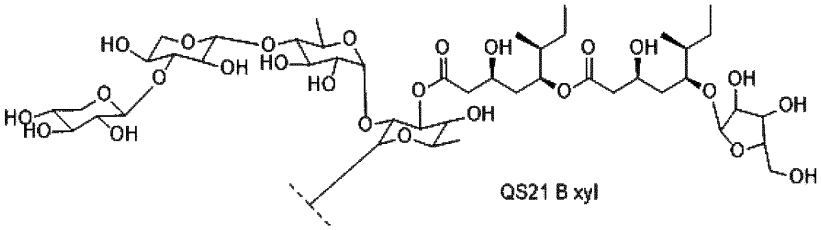

QS21 B xyl

FIG. 42

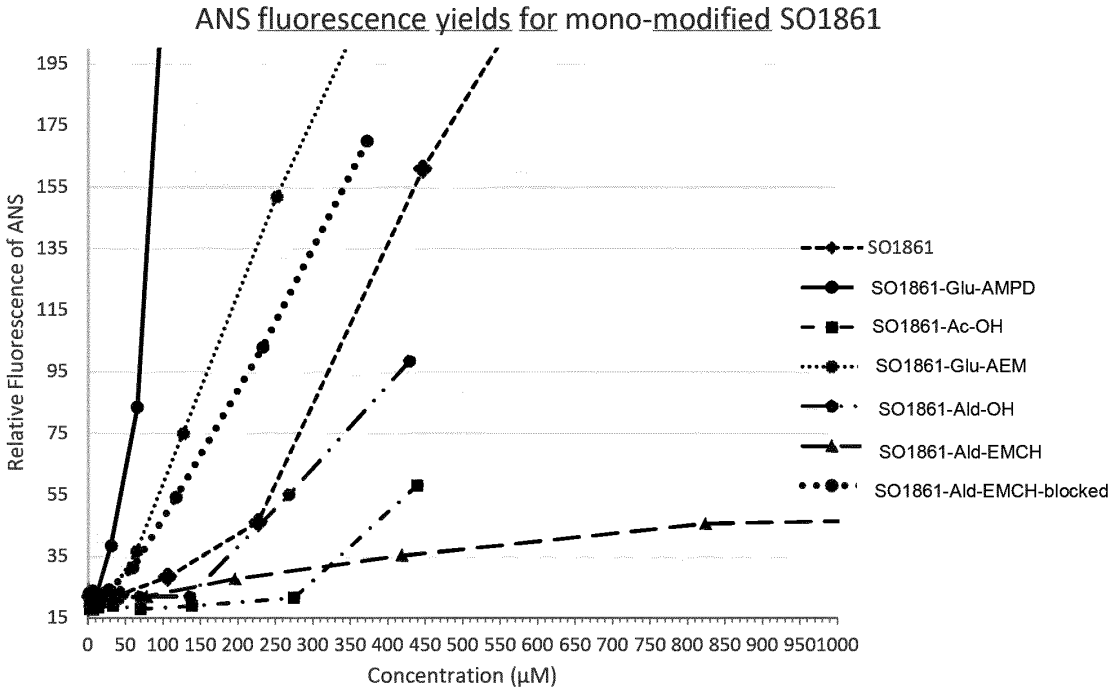

ANS fluorescence yields for mono-modified SO1861
Relative Fluorescence of ANS
195
175
155
135
115
95
75
55
35
15
0 50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 9501000
Concentration (µM)
SO1861
SO1861-Glu-AMPD
SO1861-Ac-OH
SO1861-Glu-AEM
SO1861-Ald-OH
SO1861-Ald-EMCH
SO1861-Ald-EMCH-blocked

FIG. 43

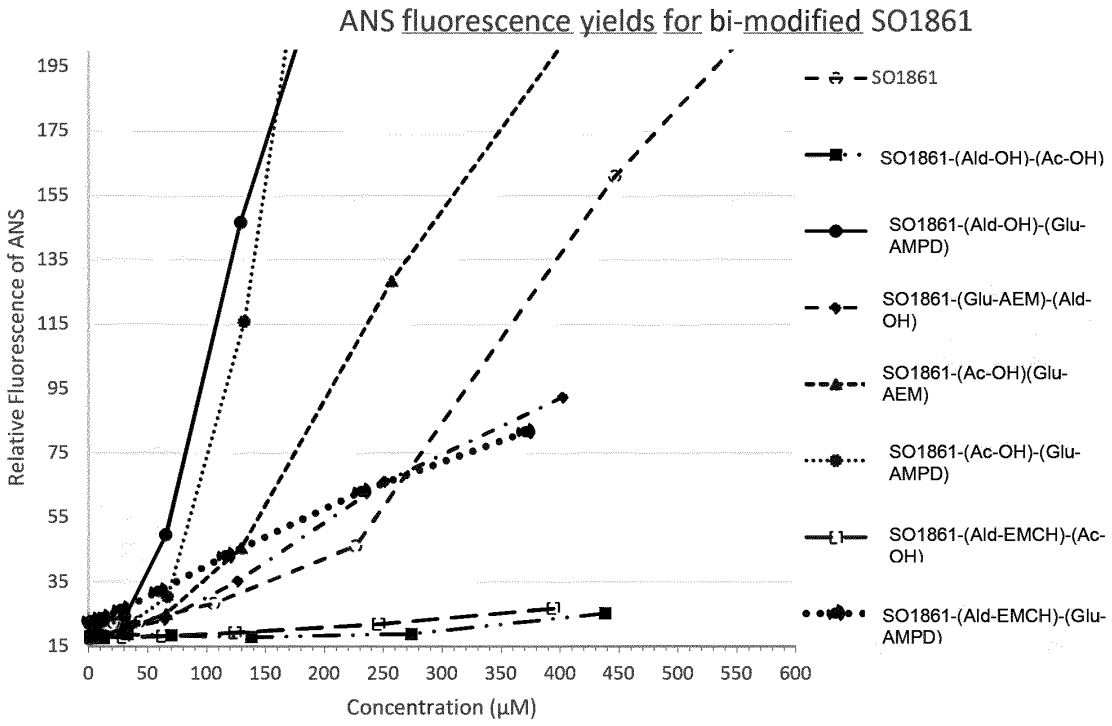

ANS fluorescence yields for bi-modified SO1861
Relative Fluorescence of ANS
195
175
155
135
115
95
75
55
35
15
0
50
100
150
200
250
300
350
400
450
500
550
600
Concentration (µM)
SO1861
SO1861-(Ald-OH)-(Ac-OH)
SO1861-(Ald-OH)-(Glu-AMPD)
SO1861-(Glu-AEM)-(Ald-OH)
SO1861-(Ac-OH)(Glu-AEM)
SO1861-(Ac-OH)-(Glu-AMPD)
SO1861-(Ald-EMCH)-(Ac-OH)
SO1861-(Ald-EMCH)-(Glu-AMPD)

FIG. 44

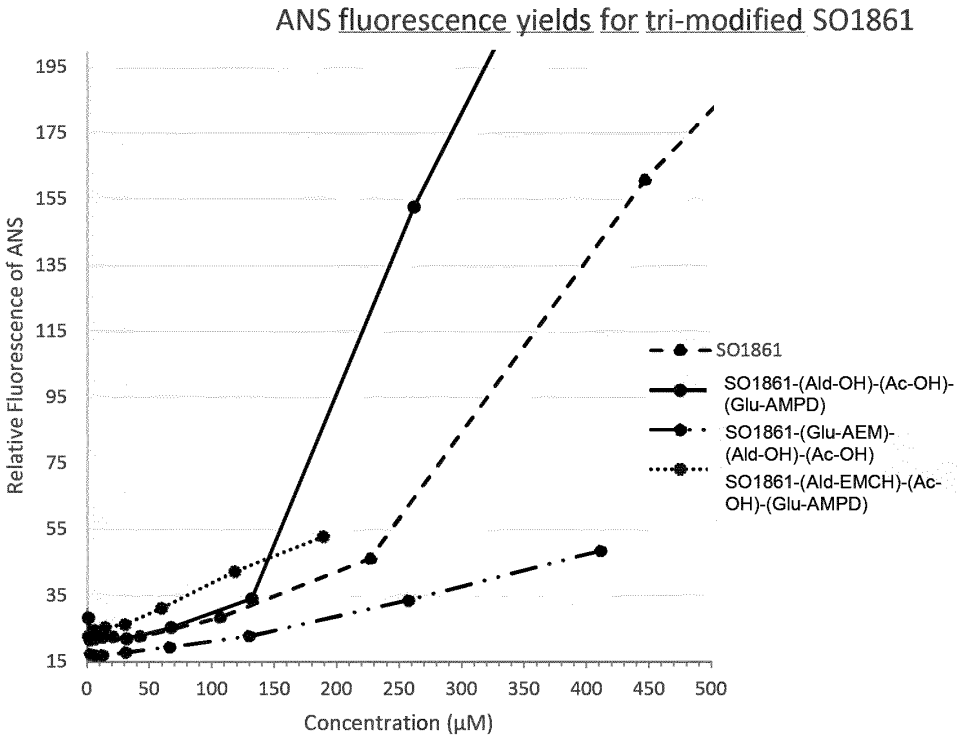

ANS fluorescence yields for tri-modified SO1861
Relative Fluorescence of ANS
195
175
155
135
115
95
75
55
35
15
0
50
100
150
200
250
300
350
400
450
500
Concentration (μM)
SO1861
SO1861-(Ald-OH)-(Ac-OH)-(Glu-AMPD)
SO1861-(Glu-AEM)-(Ald-OH)-(Ac-OH)
SO1861-(Ald-EMCH)-(Ac-OH)-(Glu-AMPD)

FIG. 45

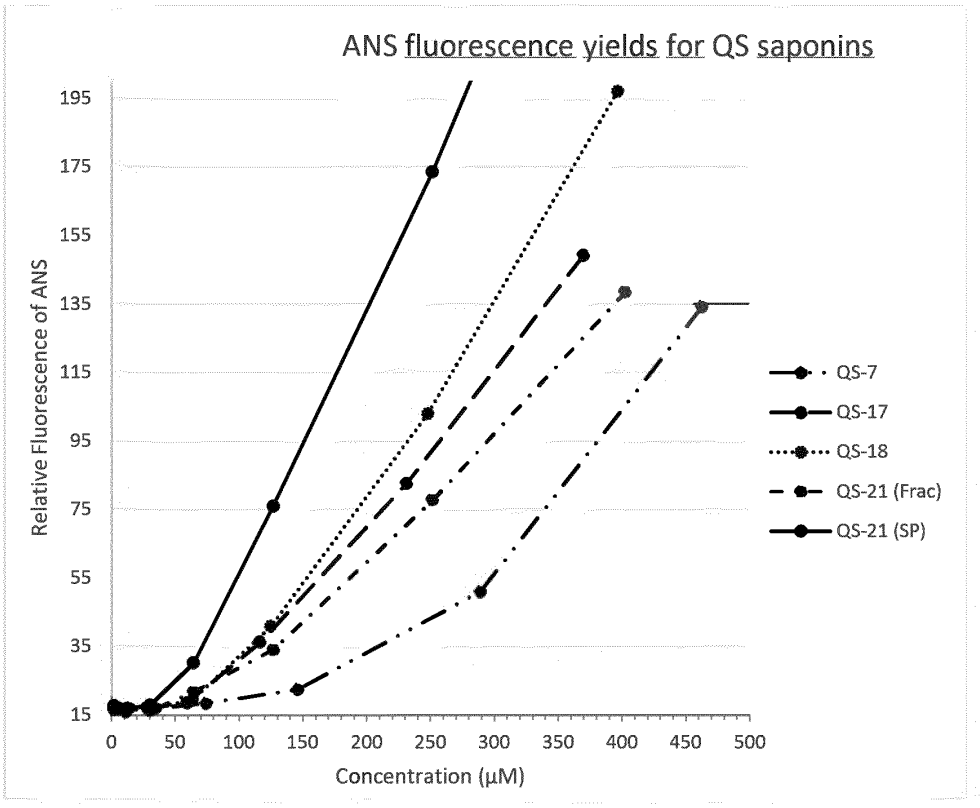

ANS fluorescence yields for QS saponins
Relative Fluorescence of ANS
195
175
155
135
115
95
75
55
35
15
0
50
100
150
200
250
300
350
400
450
500
Concentration (µM)
QS-7
QS-17
QS-18
QS-21 (Frac)
QS-21 (SP)

FIG. 46

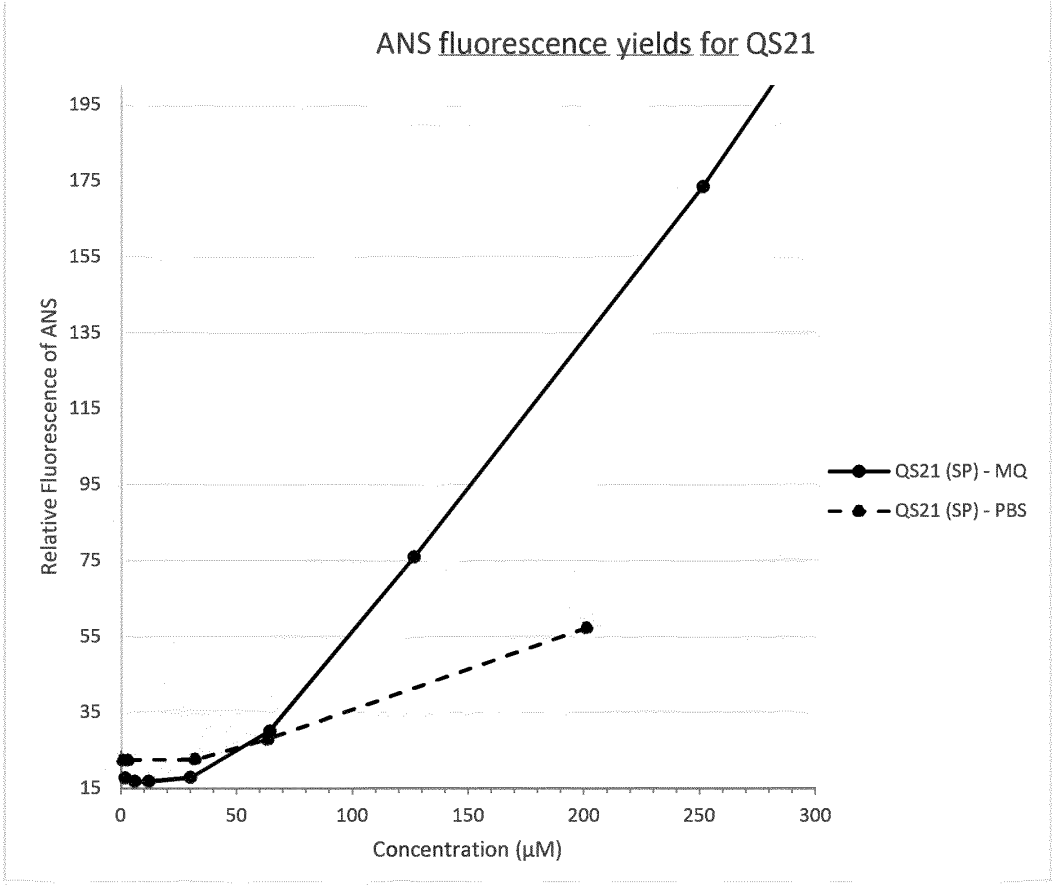

ANS fluorescence yields for QS21
Relative Fluorescence of ANS
195
175
155
135
115
95
75
55
35
15
0
50
100
150
200
250
300
Concentration (µM)
QS21 (SP) - MQ
QS21 (SP) - PBS

FIG. 47A

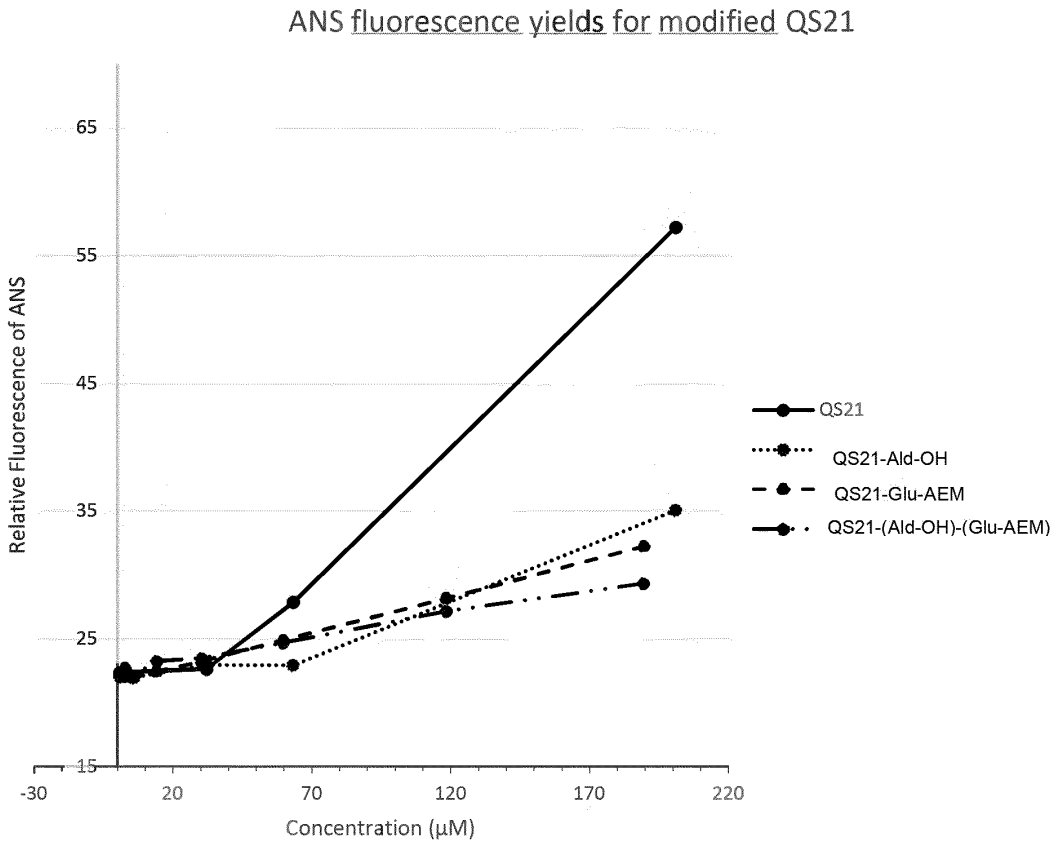

ANS fluorescence yields for modified QS21
Relative Fluorescence of ANS
65
55
45
35
25
15
-30
20
70
120
170
220
Concentration (µM)
QS21
QS21-Ald-OH
QS21-Glu-AEM
QS21-(Ald-OH)-(Glu-AEM)

FIG. 47B

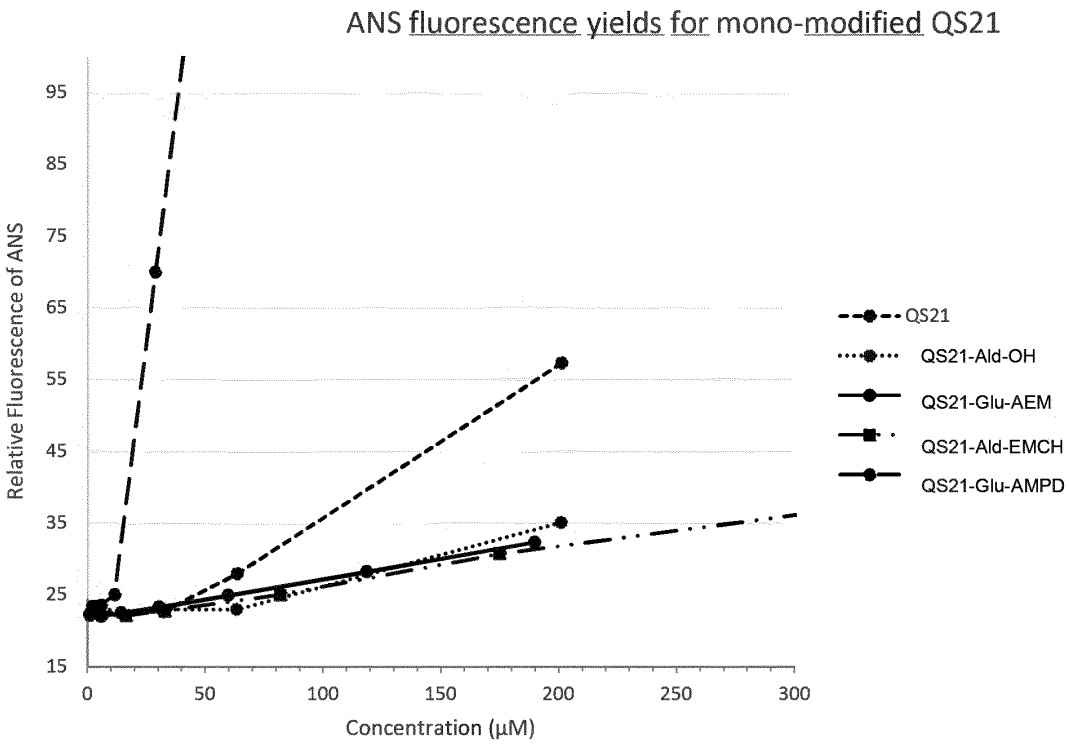

ANS fluorescence yields for mono-modified QS21
Relative Fluorescence of ANS
95
85
75
65
55
45
35
25
15
0
50
100
150
200
250
300
Concentration (μM)
QS21
QS21-Ald-OH
QS21-Glu-AEM
QS21-Ald-EMCH
QS21-Glu-AMPD

FIG. 47C

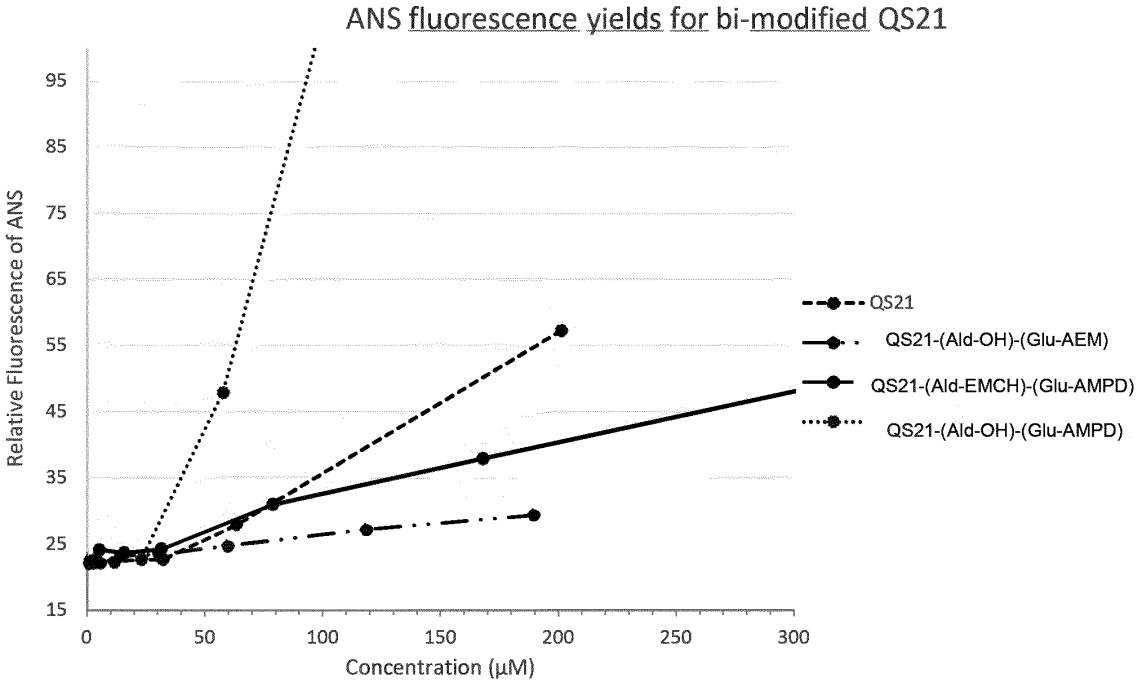

ANS fluorescence yields for bi-modified QS21
Relative Fluorescence of ANS
95
85
75
65
55
45
35
25
15
0
50
100
150
200
250
300
Concentration (µM)
QS21
QS21-(Ald-OH)-(Glu-AEM)
QS21-(Ald-EMCH)-(Glu-AMPD)
QS21-(Ald-OH)-(Glu-AMPD)

FIG. 48

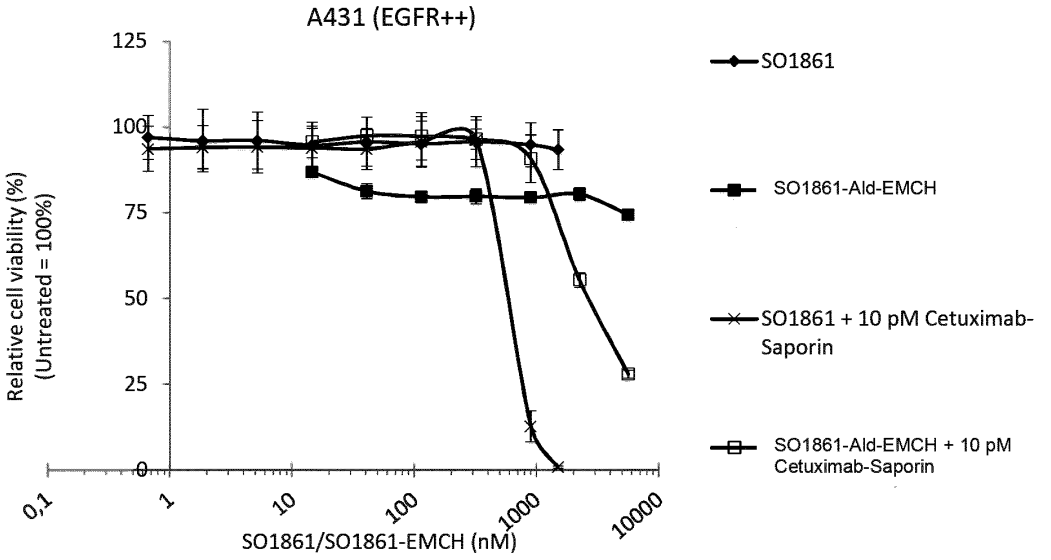

A431 (EGFR++)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
SO1861/SO1861-EMCH (nM)
SO1861
SO1861-Ald-EMCH
SO1861 + 10 pM Cetuximab-Saporin
SO1861-Ald-EMCH + 10 pM Cetuximab-Saporin

FIG. 49

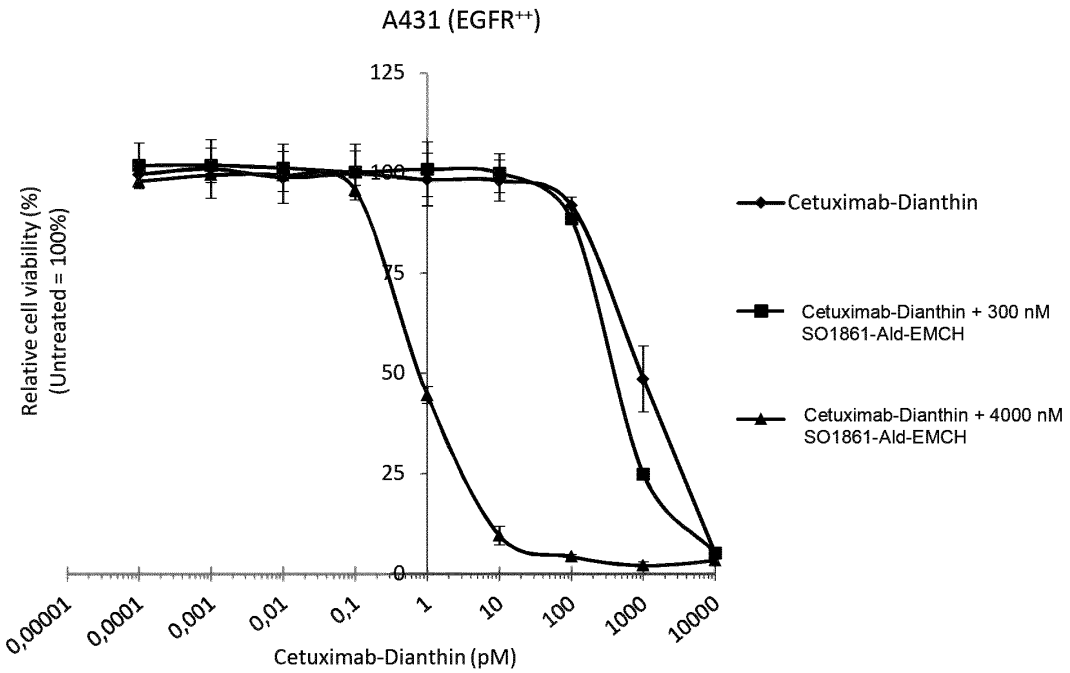

A431 (EGFR++)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab-Dianthin (pM)
Cetuximab-Dianthin
Cetuximab-Dianthin + 300 nM SO1861-Ald-EMCH
Cetuximab-Dianthin + 4000 nM SO1861-Ald-EMCH

FIG. 50

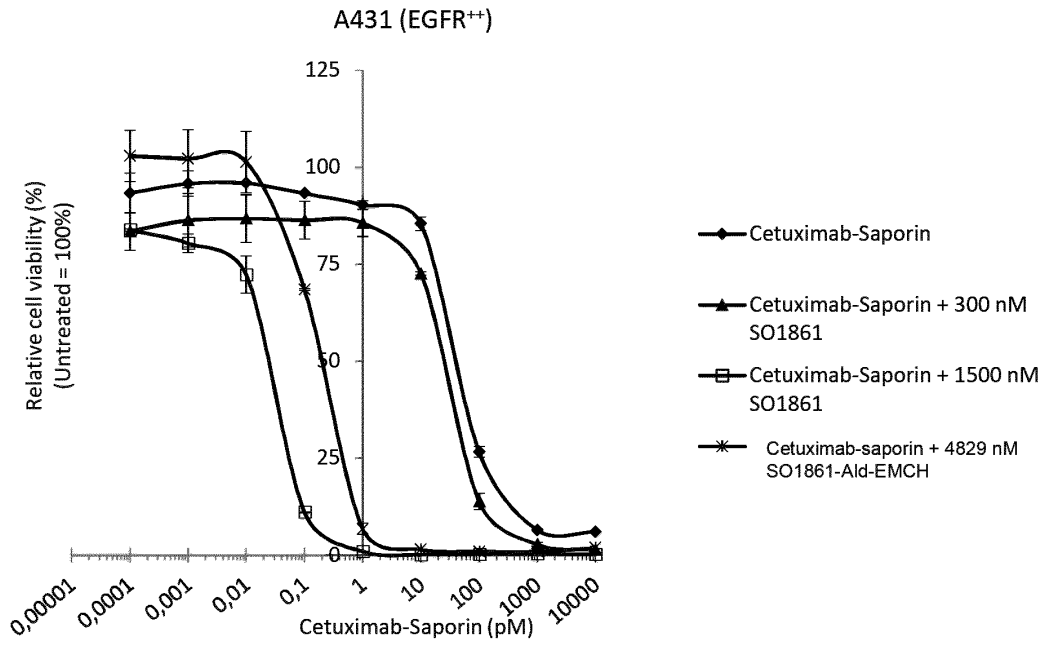

A431 (EGFR++)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,00001
0,0001
0,001
0,01
0,1
1
10
100
1000
10000
Cetuximab-Saporin (pM)
Cetuximab-Saporin
Cetuximab-Saporin + 300 nM SO1861
Cetuximab-Saporin + 1500 nM SO1861
Cetuximab-saporin + 4829 nM SO1861-Ald-EMCH

FIG. 51

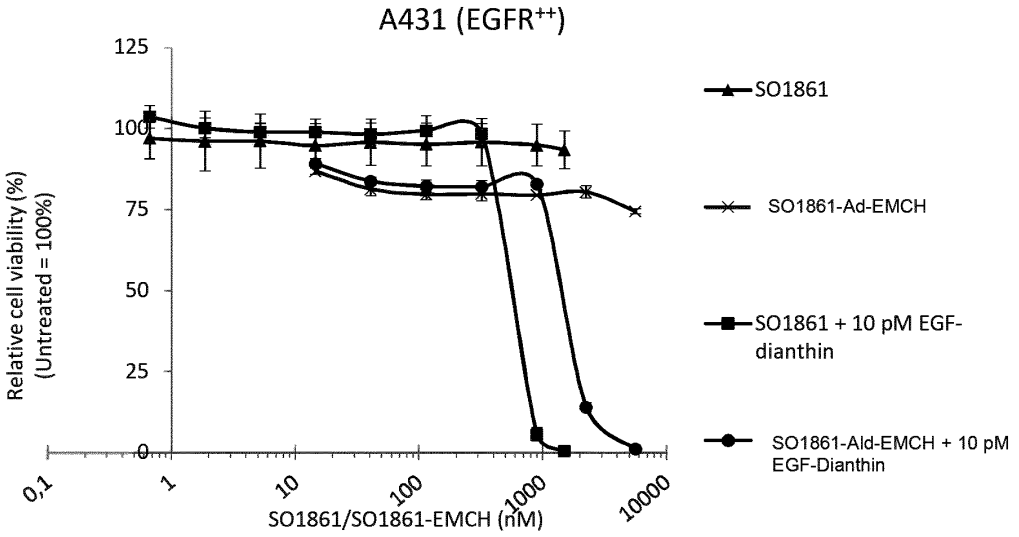

A431 (EGFR++)
Relative cell viability (%)
(Untreated = 100%)
125
100
75
50
25
0
0,1
1
10
100
1000
10000
SO1861/SO1861-EMCH (nM)
SO1861
SO1861-Ad-EMCH
SO1861 + 10 pM EGF-dianthin
SO1861-Ald-EMCH + 10 pM EGF-Dianthin

FIG. 52A

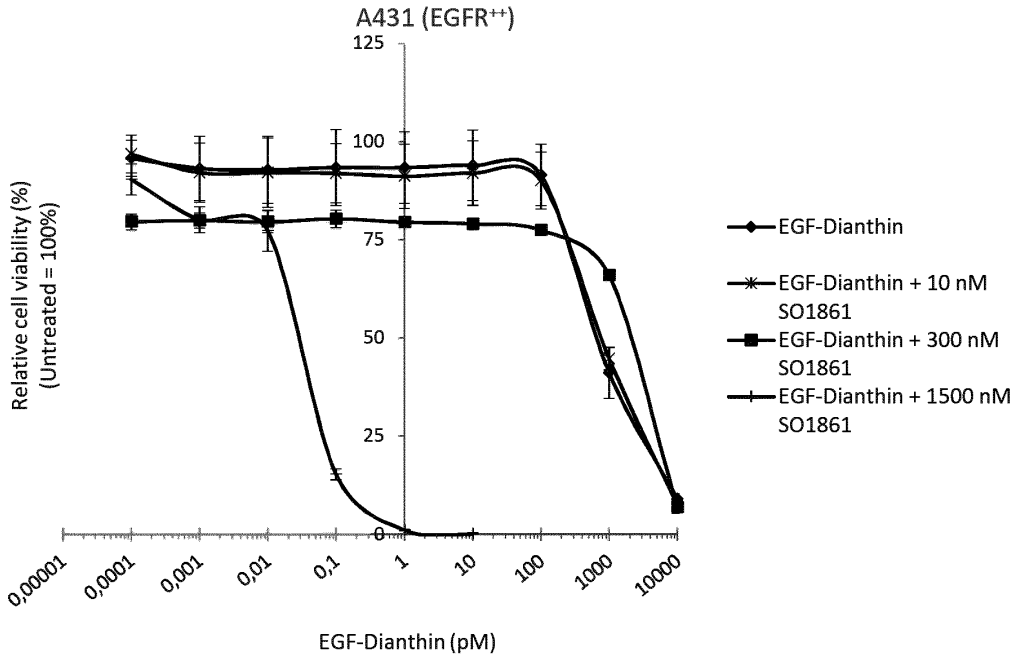


FIG. 52B

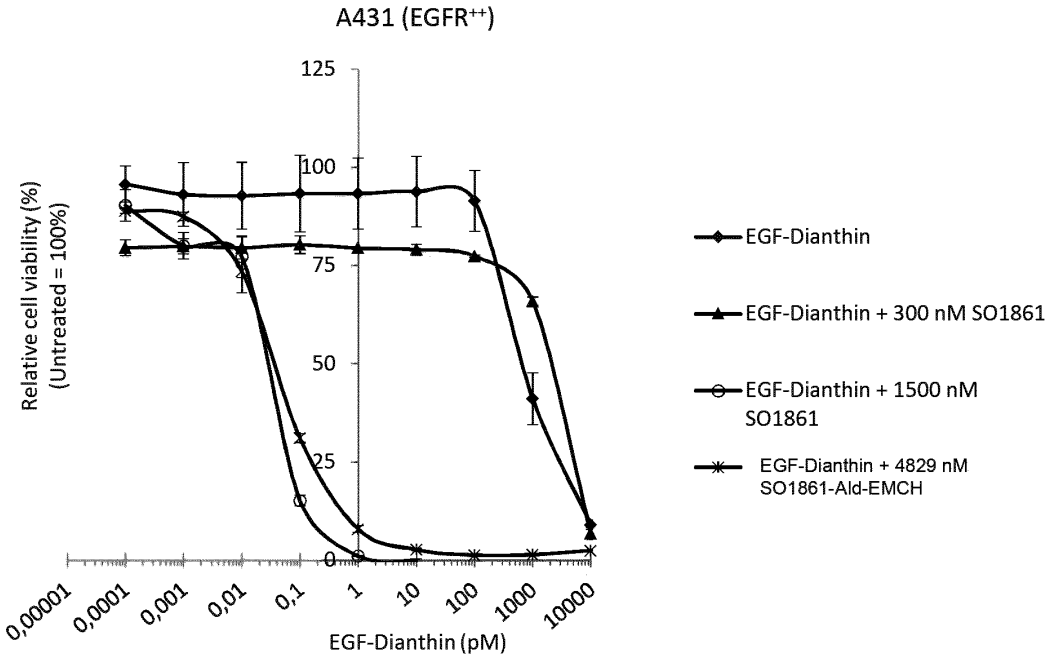


FIG. 53A

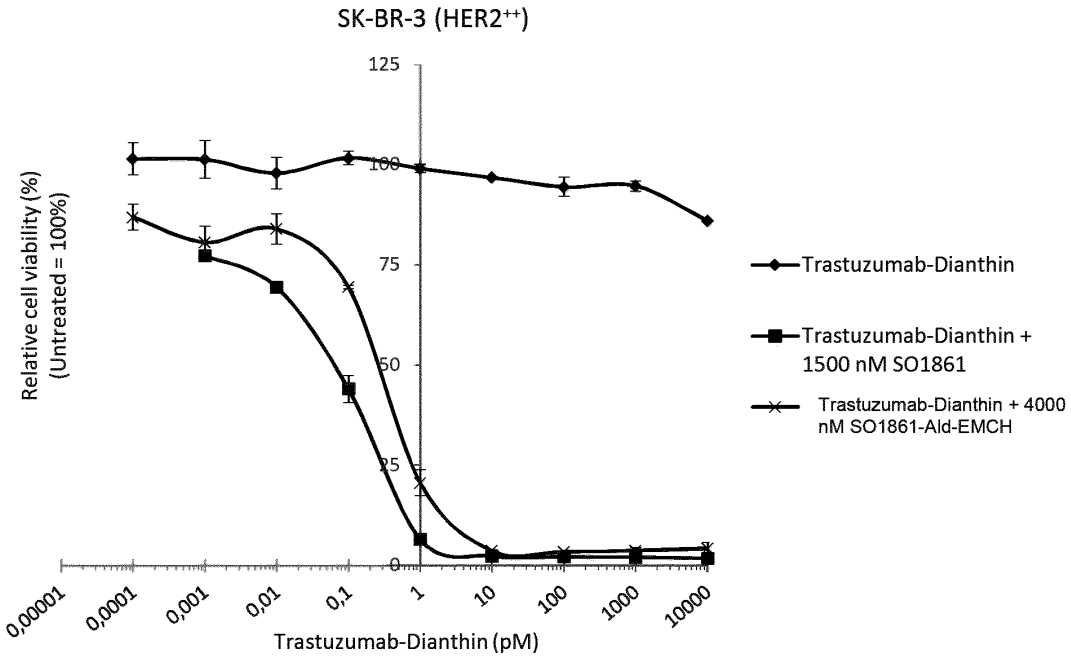


FIG. 53B

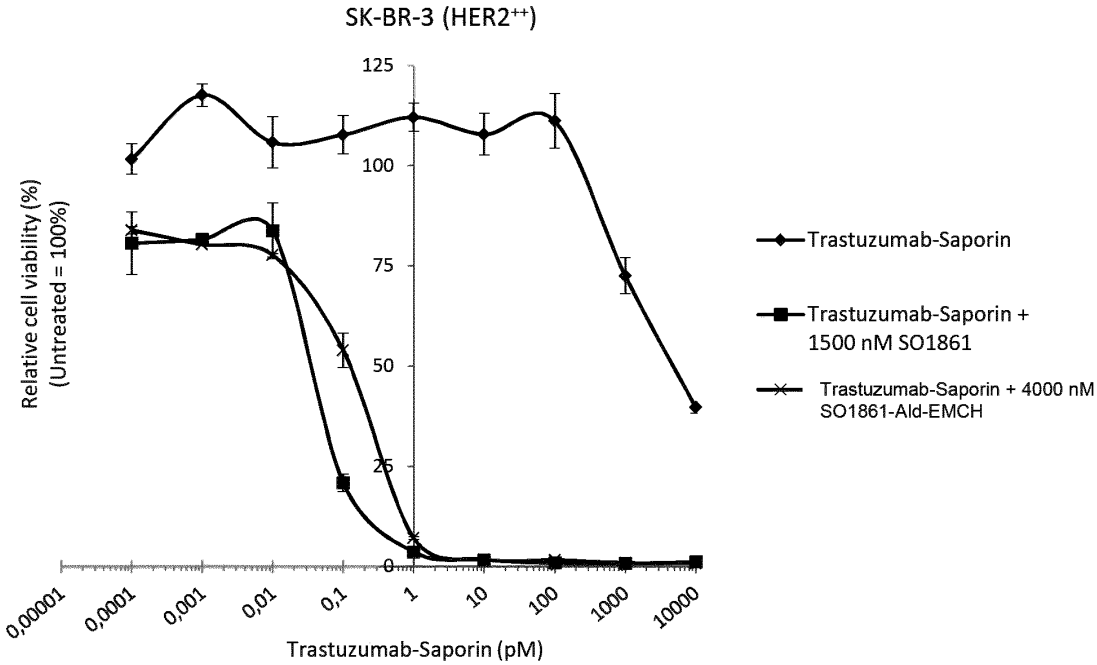


FIG. 54

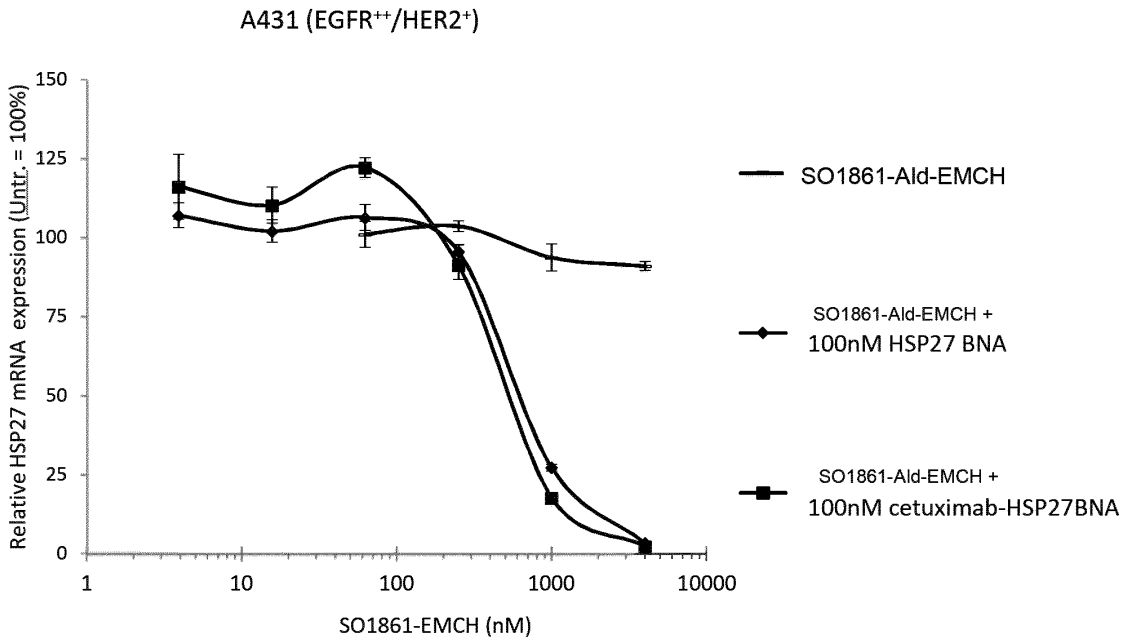

A431 (EGFR++/HER2+)
Relative HSP27 mRNA expression (Untr. = 100%)
150
125
100
75
50
25
0
1
10
100
1000
10000
SO1861-EMCH (nM)
SO1861-Ald-EMCH
SO1861-Ald-EMCH +
100nM HSP27 BNA
SO1861-Ald-EMCH +
100nM cetuximab-HSP27BNA

FIG. 55

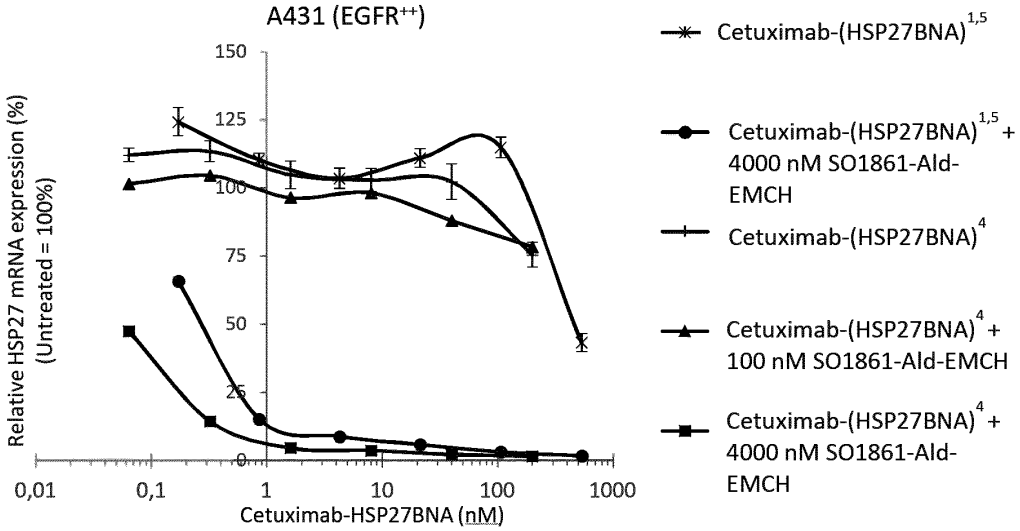

A431 (EGFR++)
Relative HSP27 mRNA expression (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
1000
Cetuximab-HSP27BNA (nM)
Cetuximab-(HSP27BNA)1,5
Cetuximab-(HSP27BNA)1,5 +
4000 nM SO1861-Ald-
EMCH
Cetuximab-(HSP27BNA)4
Cetuximab-(HSP27BNA)4 +
100 nM SO1861-Ald-EMCH
Cetuximab-(HSP27BNA)4 +
4000 nM SO1861-Ald-
EMCH

FIG. 56

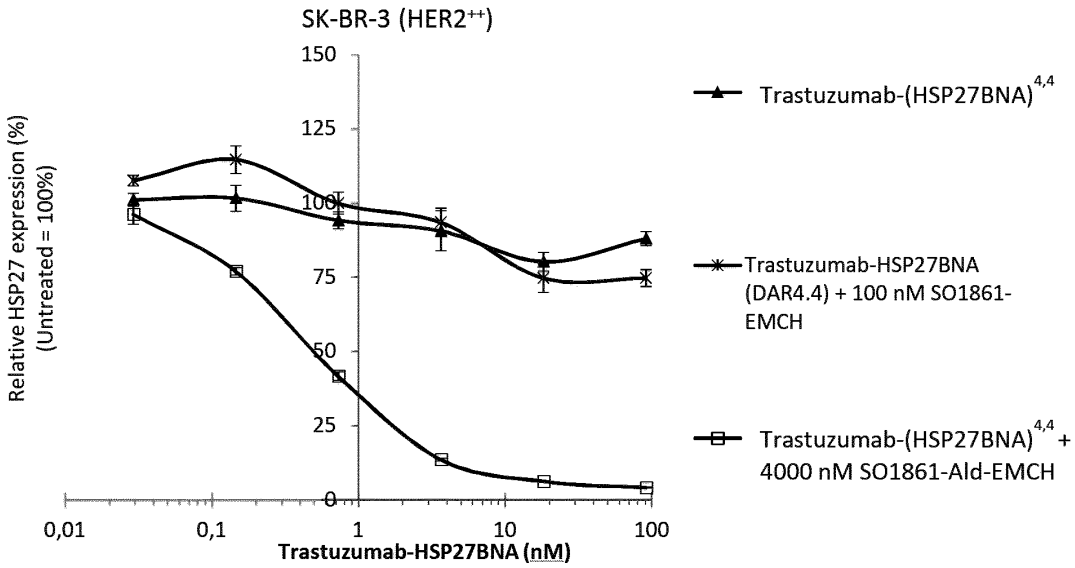

SK-BR-3 (HER2++)
Relative HSP27 expression (%)
(Untreated = 100%)
150
125
100
75
50
25
0
0,01
0,1
1
10
100
Trastuzumab-HSP27BNA (nM)
Trastuzumab-(HSP27BNA)4,4
Trastuzumab-HSP27BNA (DAR4.4) + 100 nM SO1861-EMCH
Trastuzumab-(HSP27BNA)4,4 + 4000 nM SO1861-Ald-EMCH

FIG. 57A

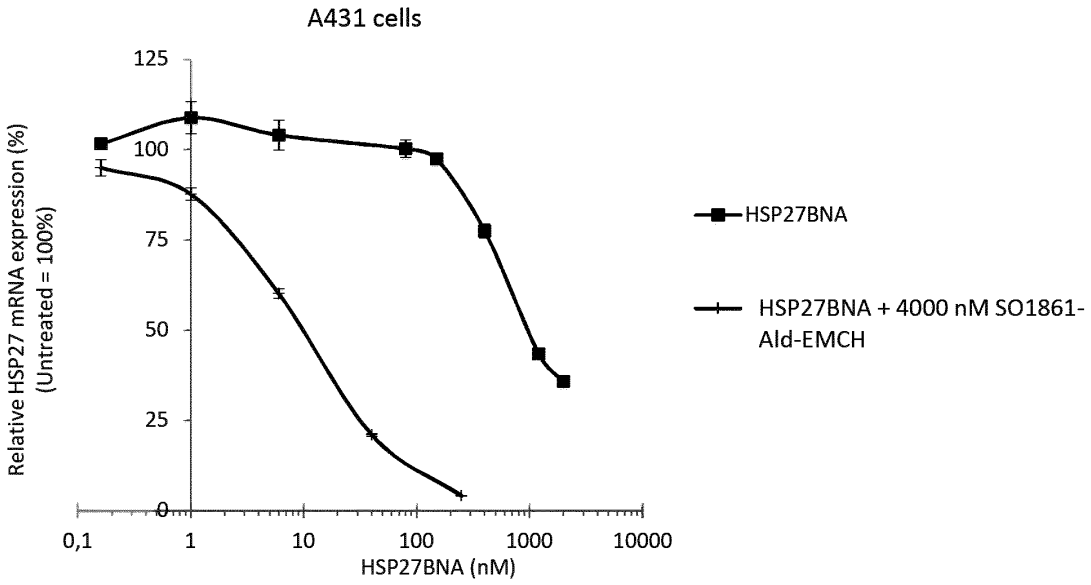


FIG. 57B

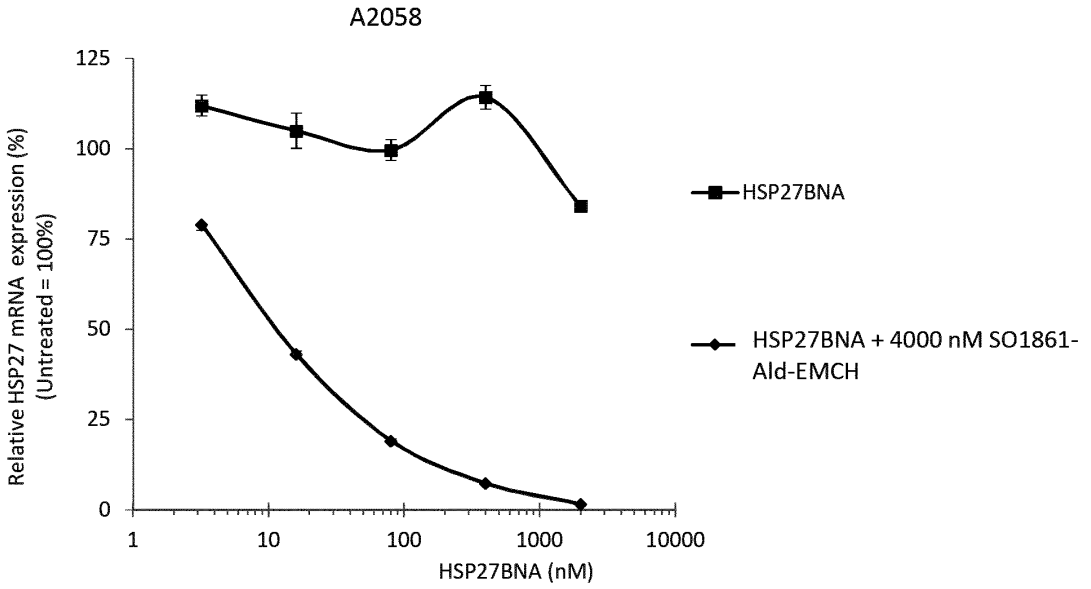


FIG. 58

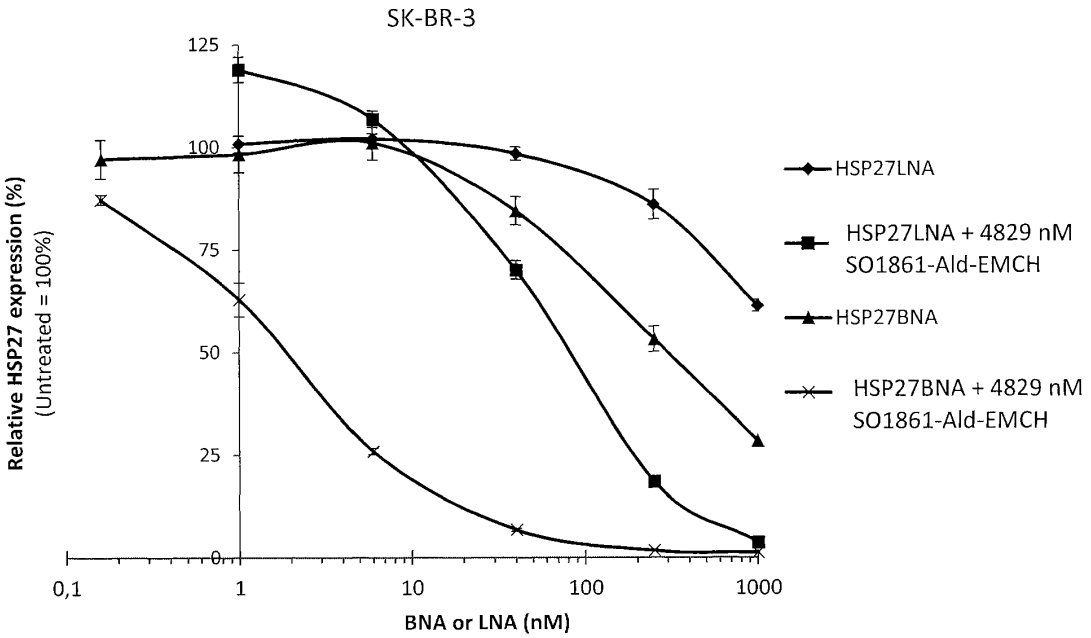


FIG. 59

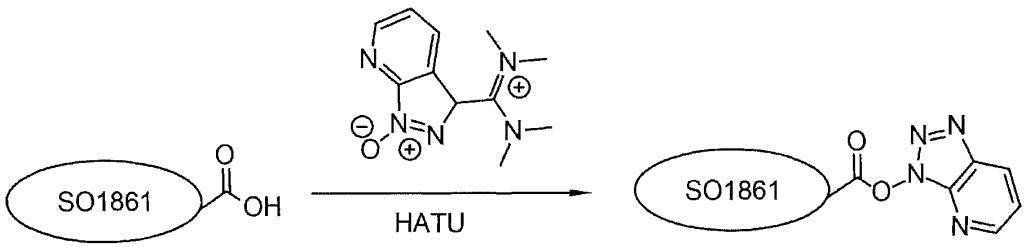


FIG. 60

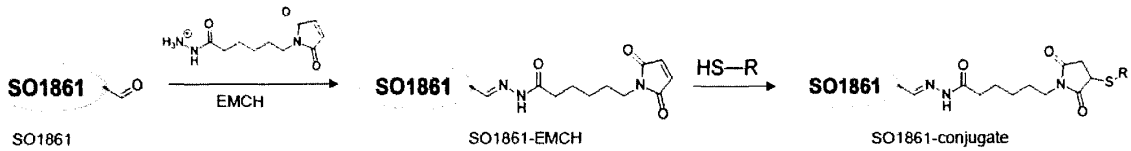


FIG. 61

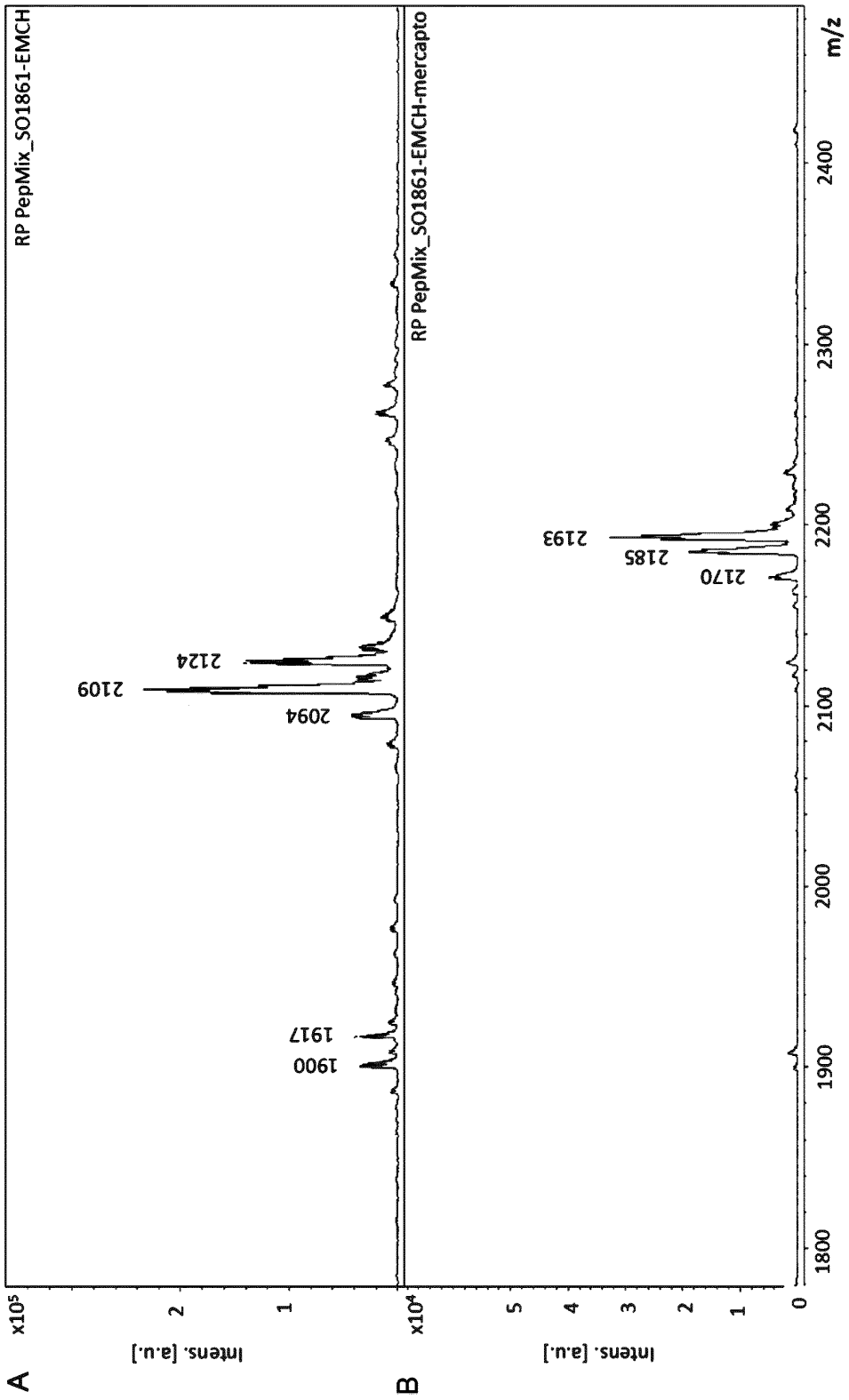

A
B
RP PepMix_SO1861-EMCH
RP PepMix_SO1861-EMCH-mercapto
Intens. [a.u.]
x10⁵
x10⁴
m/z
1800
1900
2000
2100
2200
2300
2400
1900
1917
2094
2109
2124
2170
2185
2193

FIG. 62

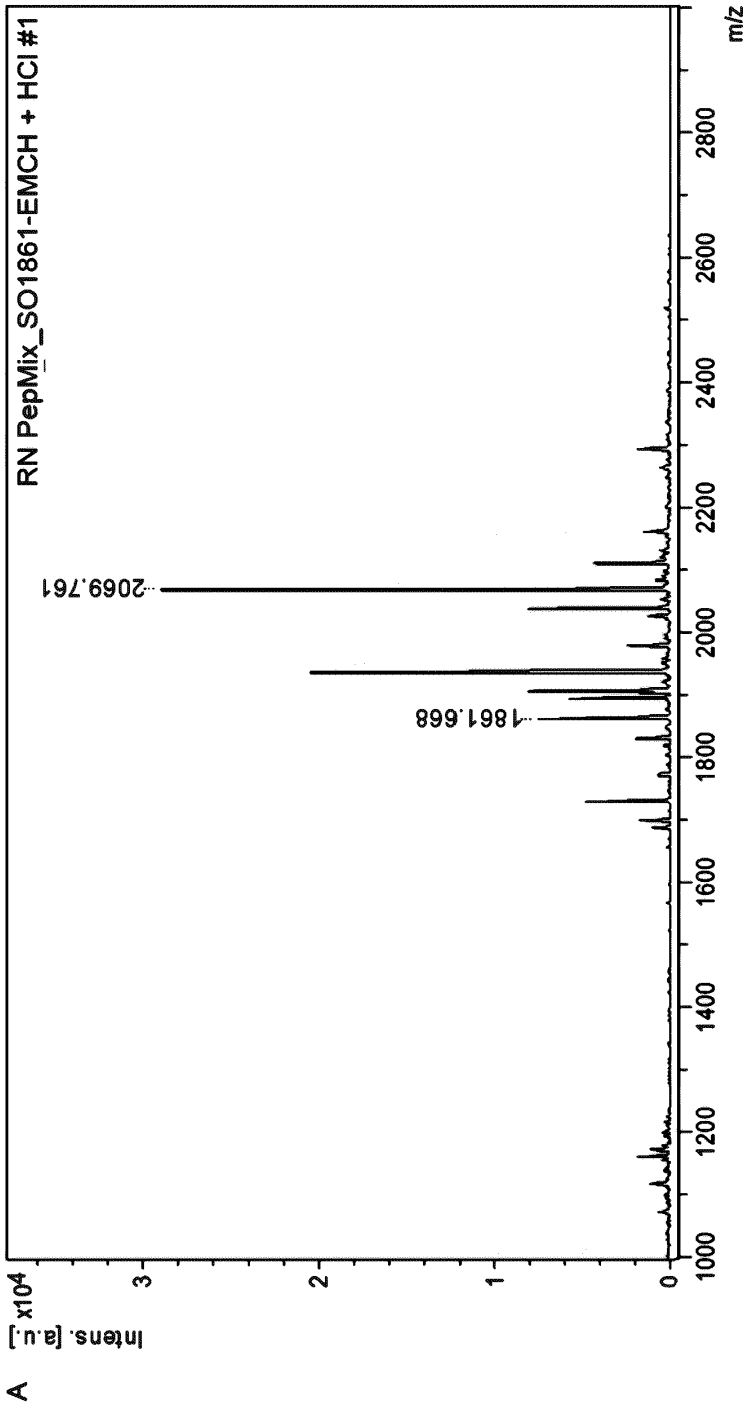

RN PepMix_SO1861-EMCH + HCl #1
2069.761
1861.668
m/z
Intens. [a.u.]
x10^4
A

FIG. 62

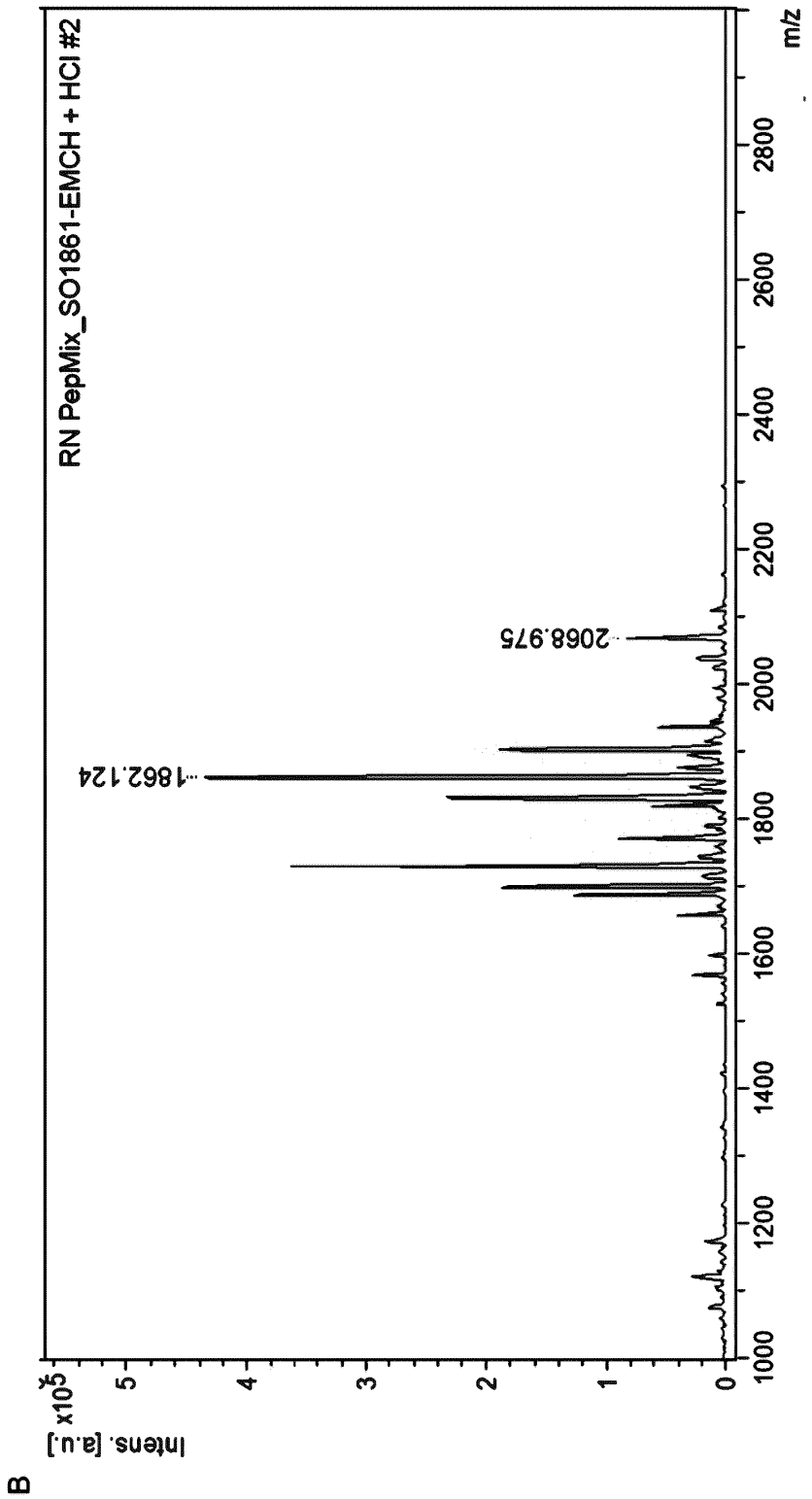

B
Intens. [a.u.]
x10^5
0
1
2
3
4
5
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
m/z
RN PepMix_SO1861-EMCH + HCl #2
1862.124
2068.975

FIG. 63A

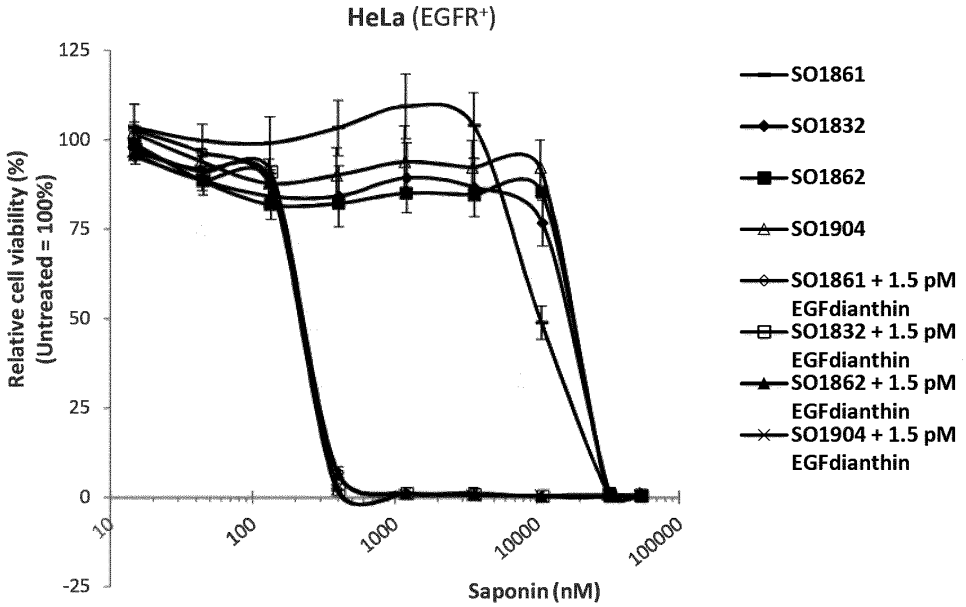


FIG. 63B

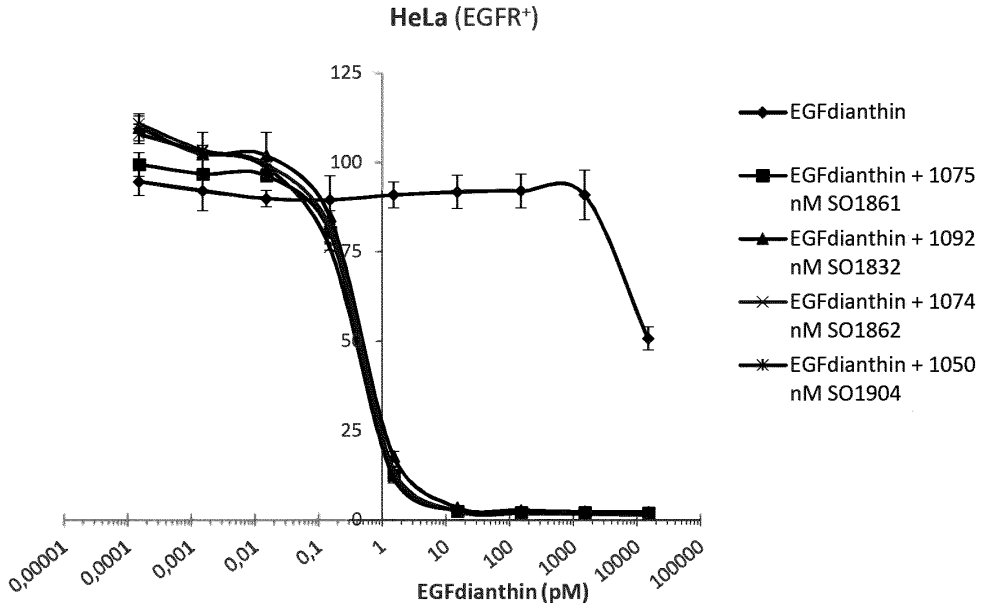


FIG. 63C

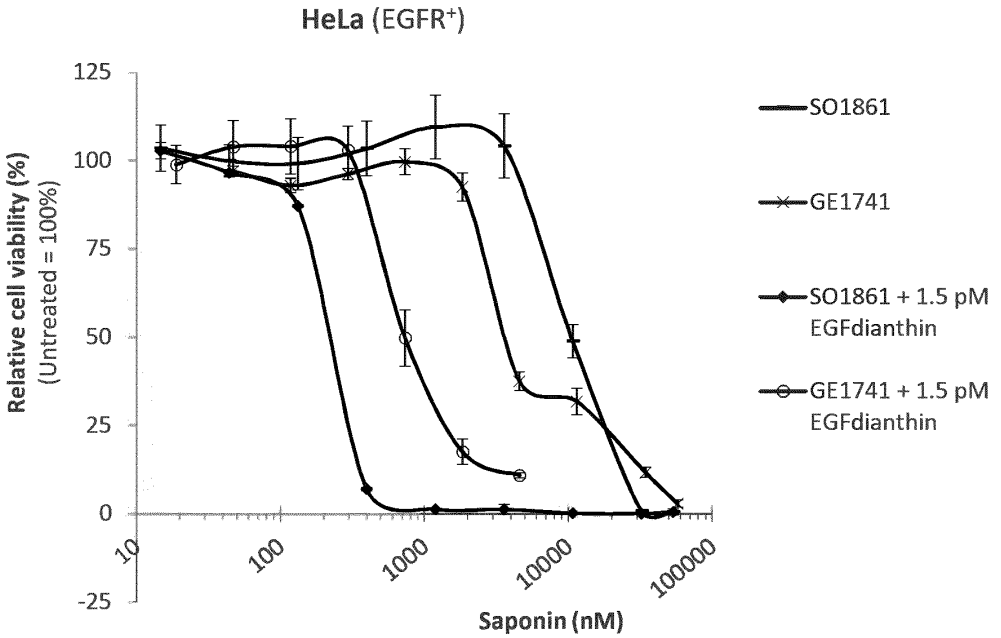


FIG. 63D

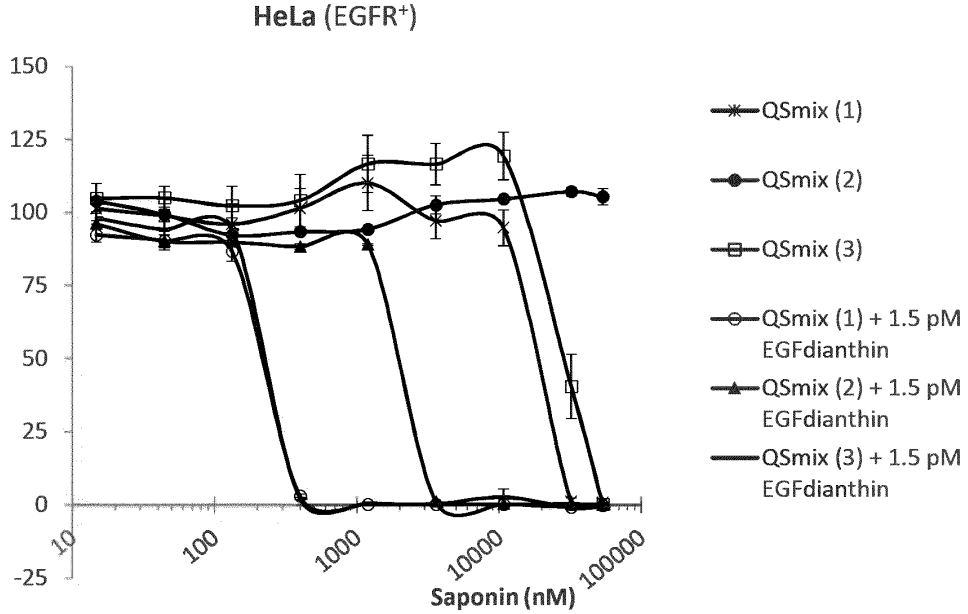


FIG. 64A

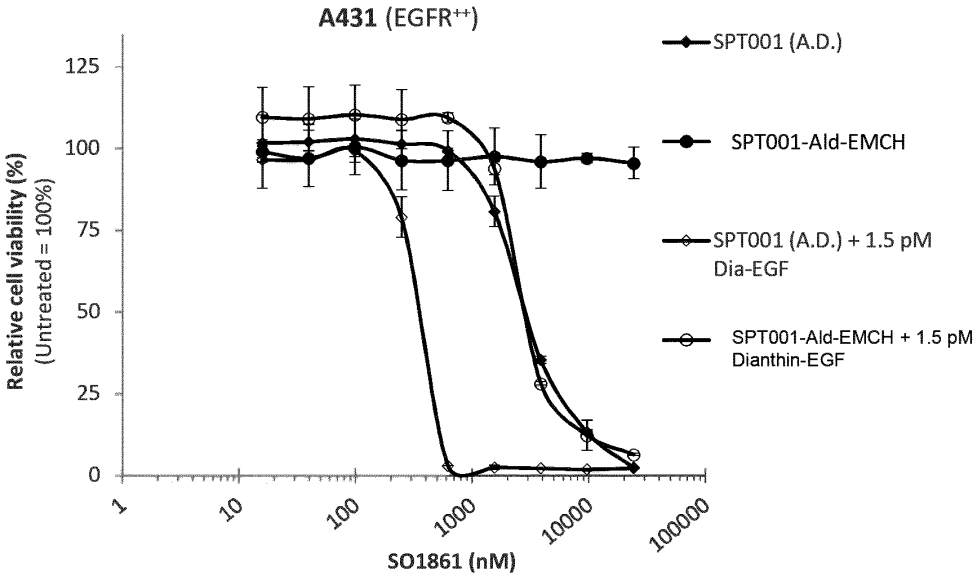


FIG. 64B

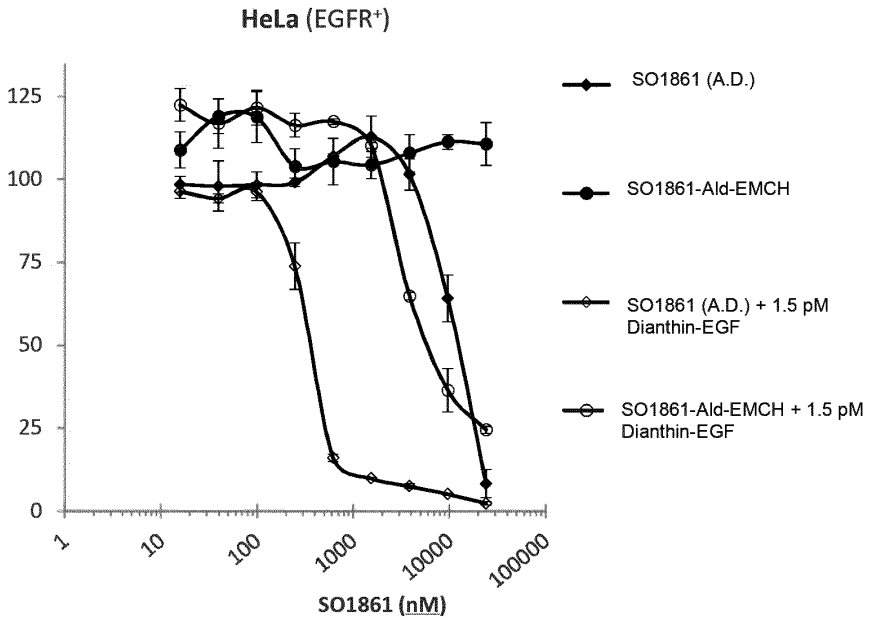


FIG. 64C

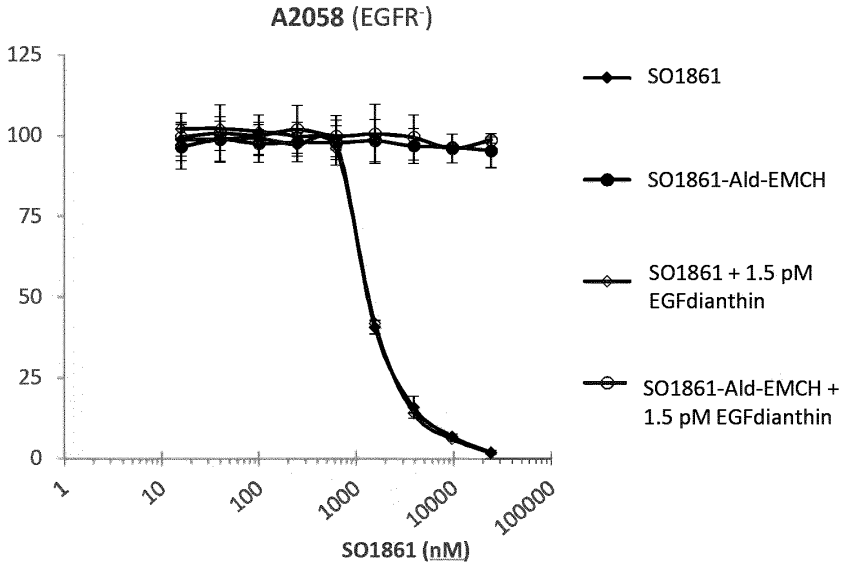


FIG. 64D

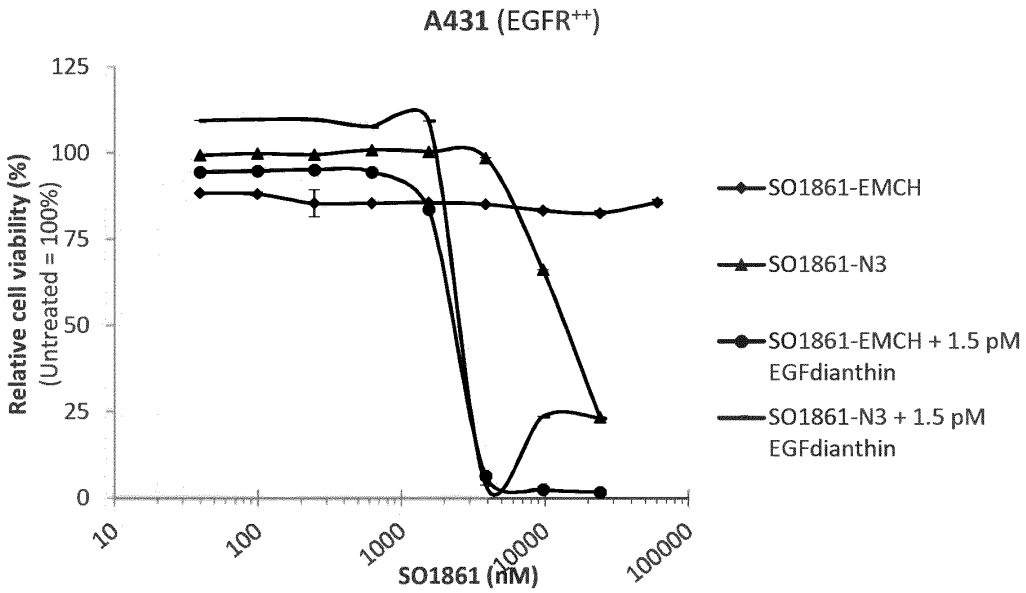


FIG. 64E

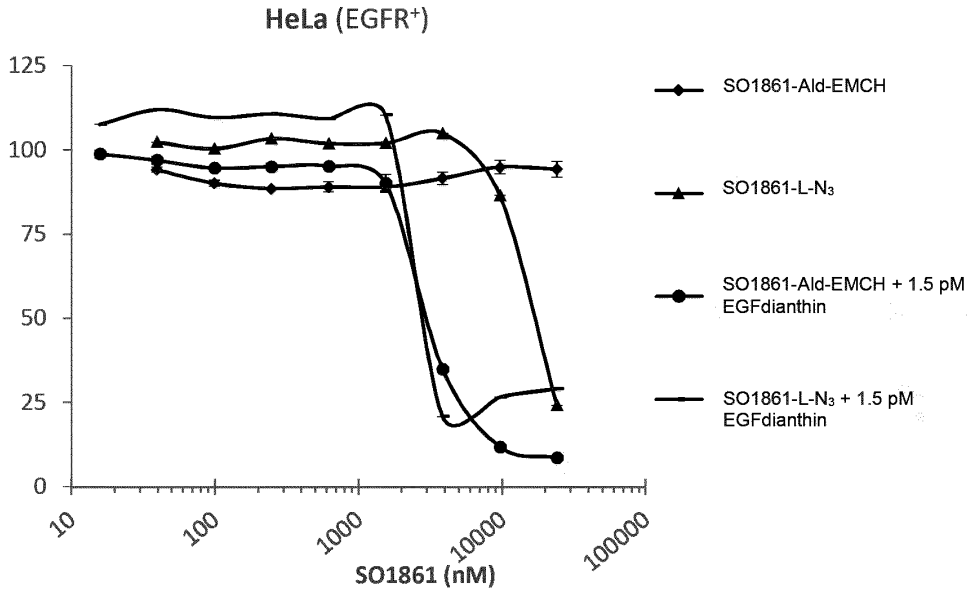


FIG. 65

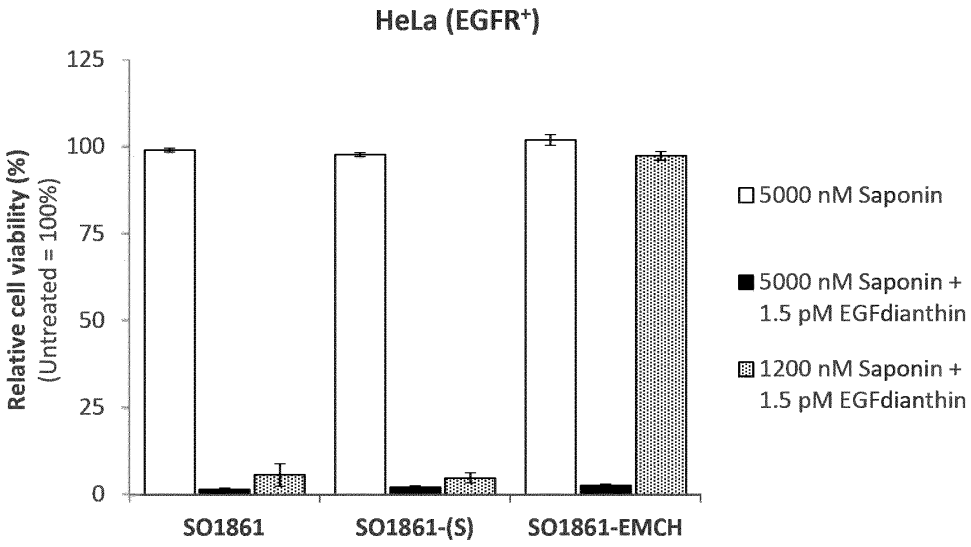


FIG. 66 A

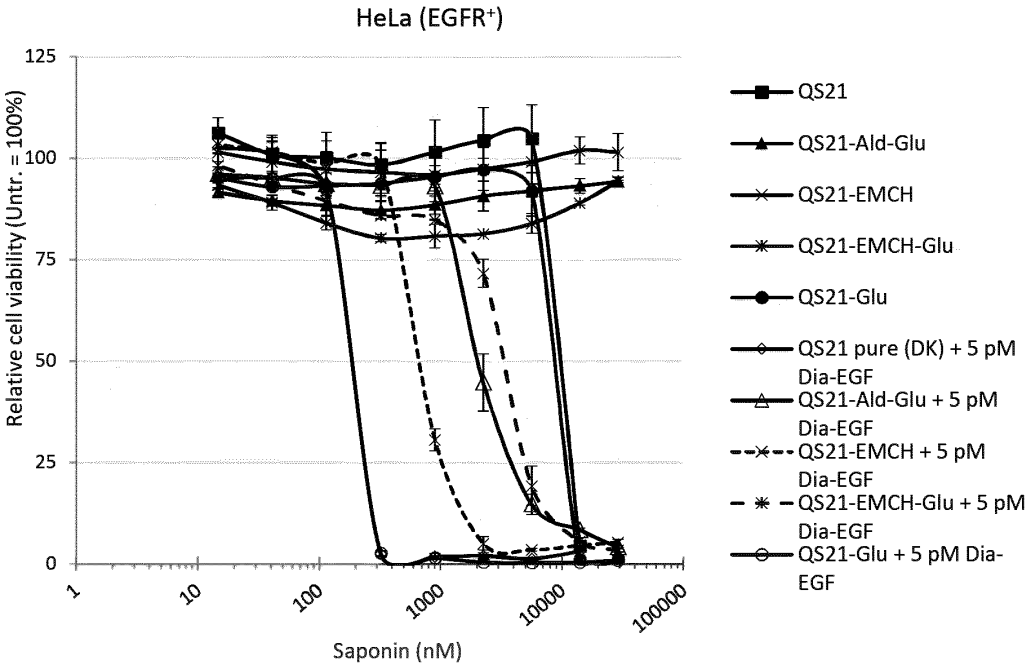


FIG. 66 B

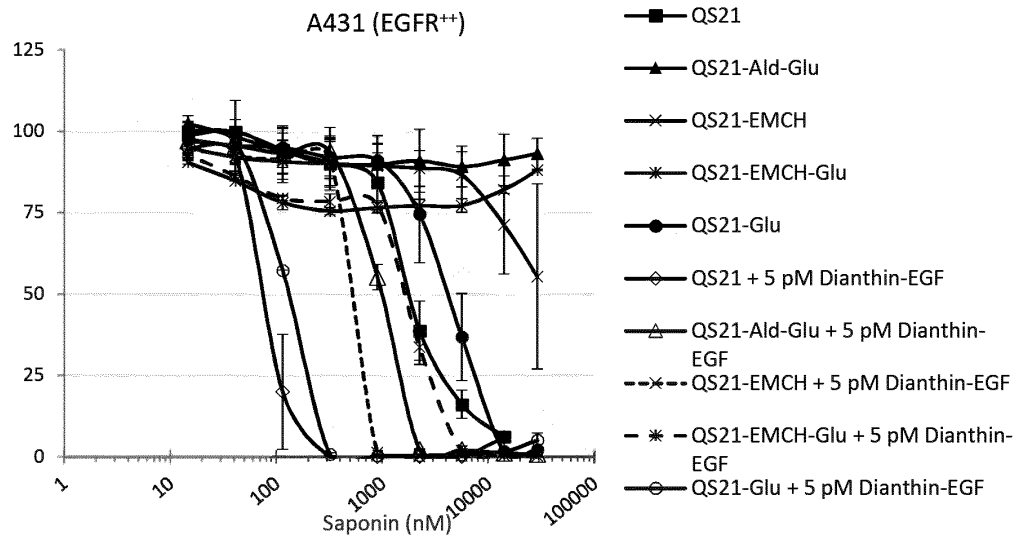


FIG. 67

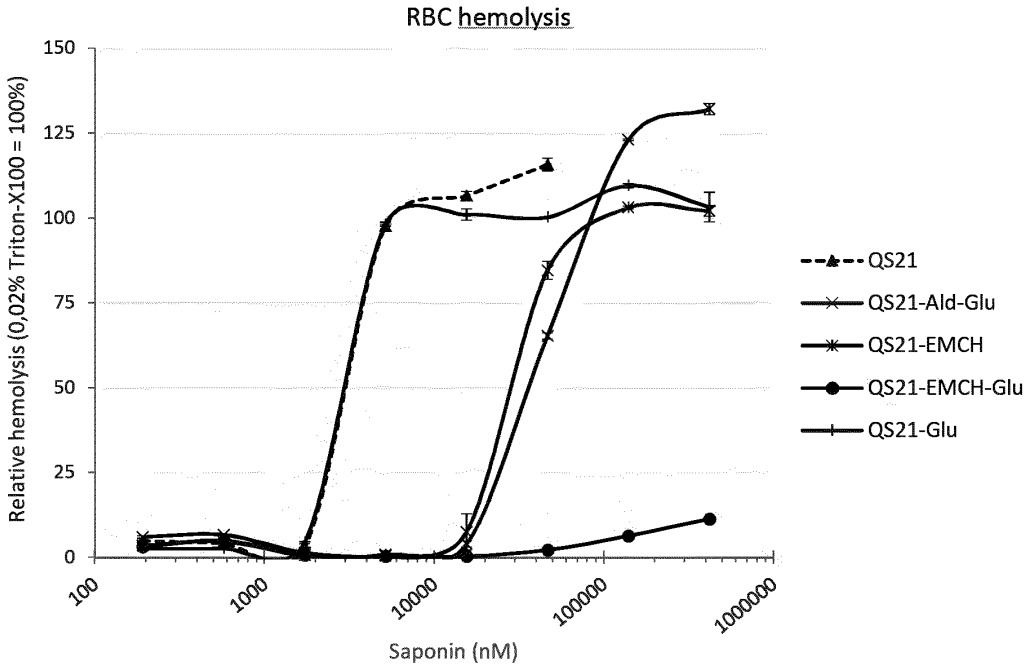

RBC hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
150
125
100
75
50
25
0
100
1000
10000
100000
1000000
Saponin (nM)
QS21
QS21-Ald-Glu
QS21-EMCH
QS21-EMCH-Glu
QS21-Glu

FIG. 68 A

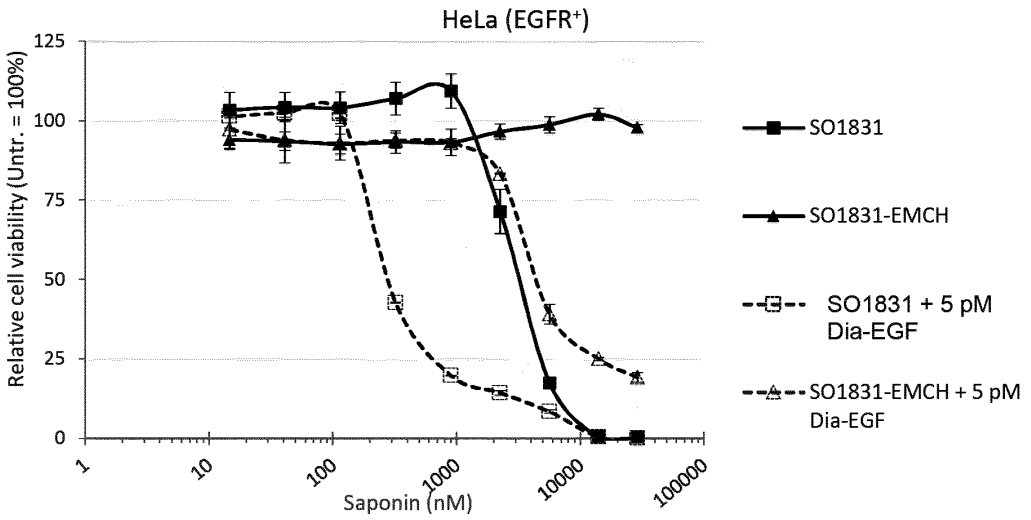


FIG. 68 B

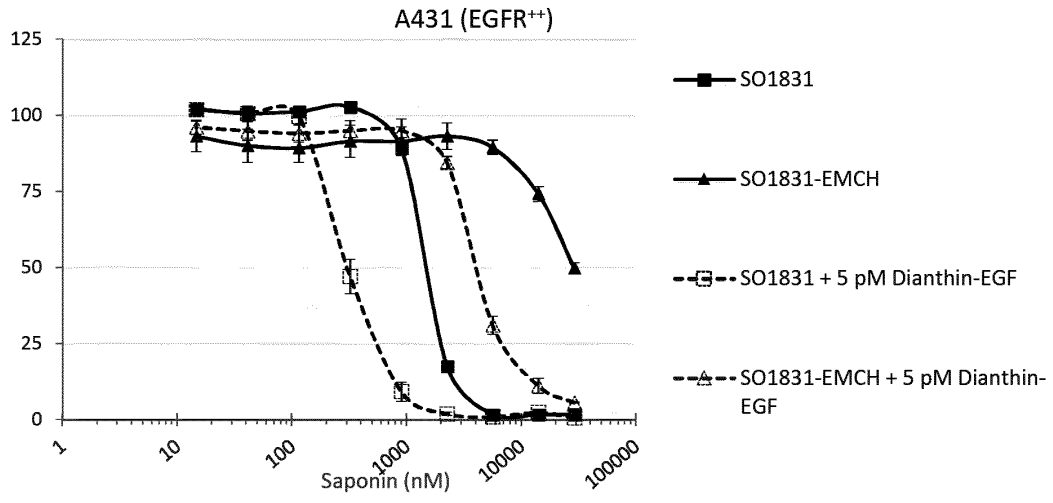


FIG. 69 A

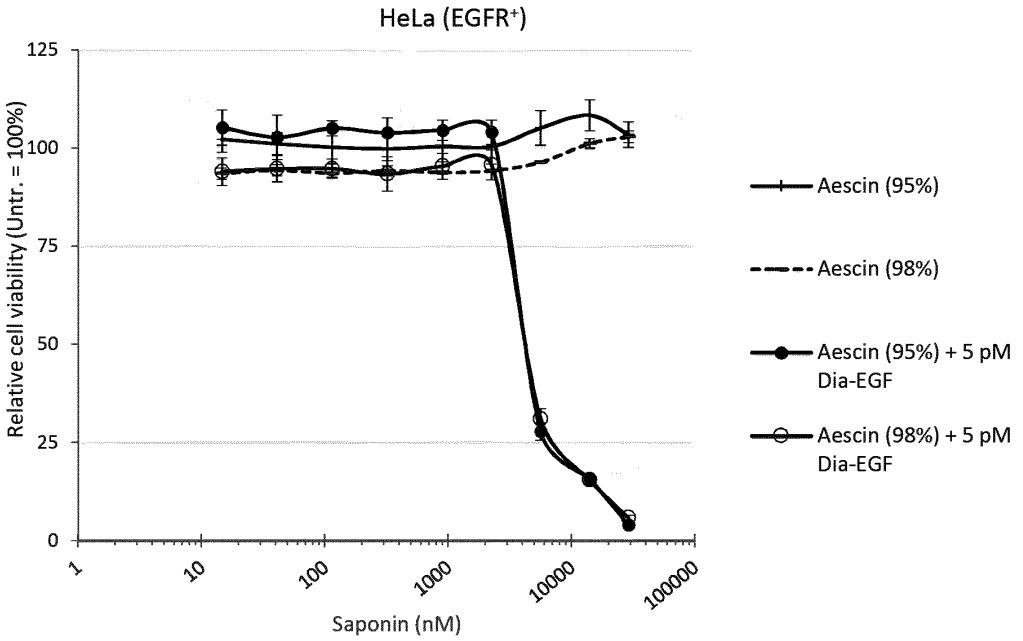


FIG. 69 B

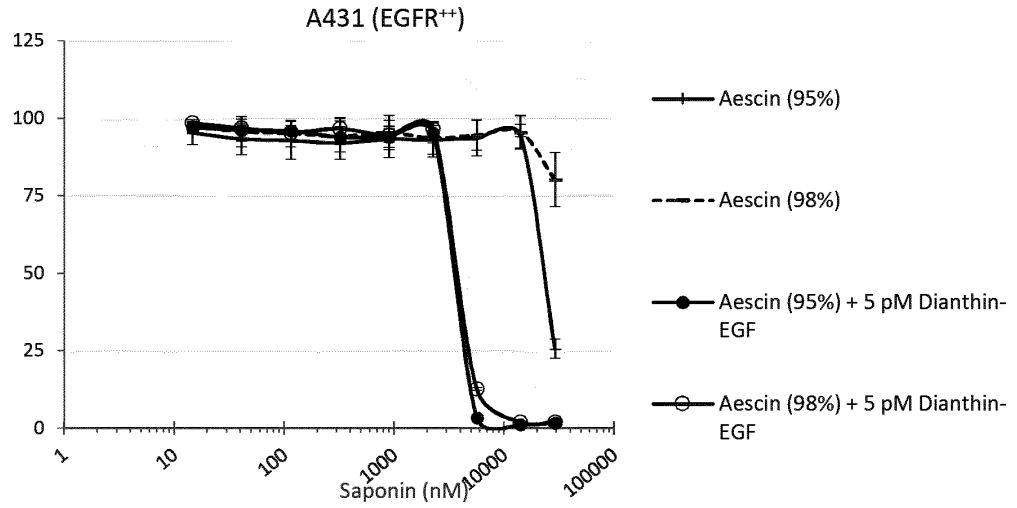


FIG. 70

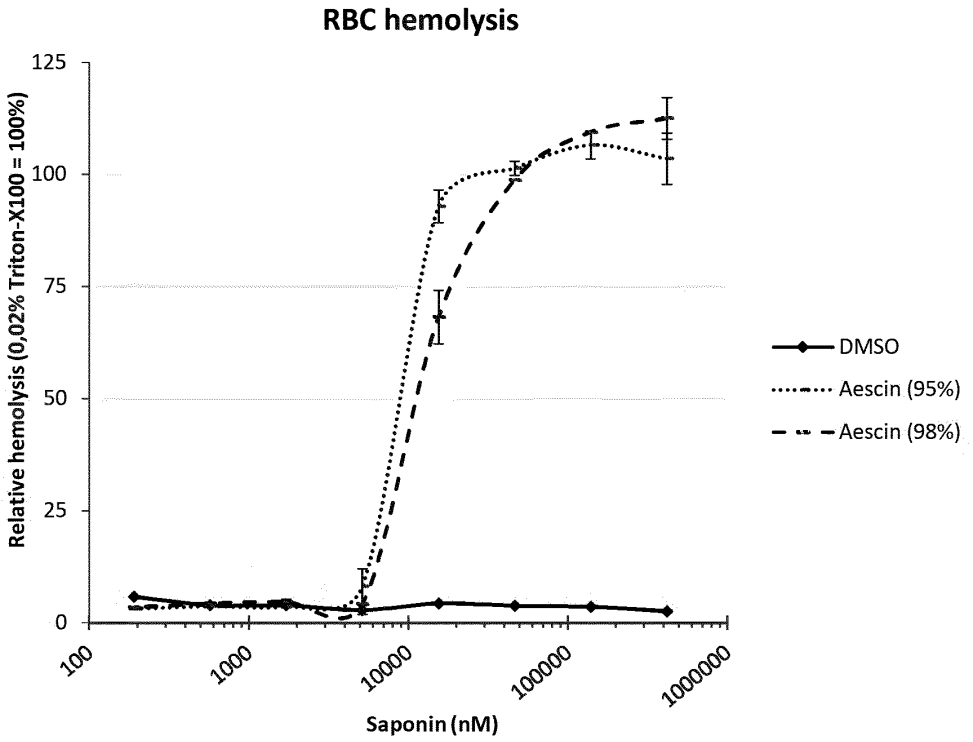

RBC hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
125
100
75
50
25
0
100
1000
10000
100000
1000000
Saponin (nM)
DMSO
Aescin (95%)
Aescin (98%)

FIG. 71

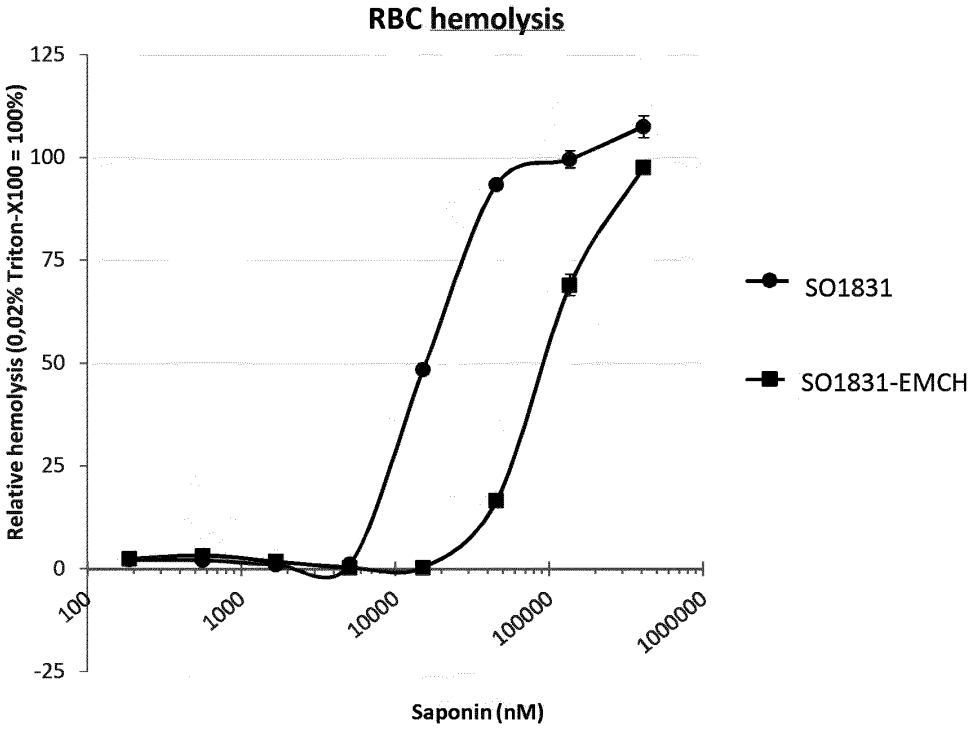

RBC hemolysis
Relative hemolysis (0,02% Triton-X100 = 100%)
125
100
75
50
25
0
-25
100
1000
10000
100000
1000000
Saponin (nM)
SO1831
SO1831-EMCH

FIG. 72

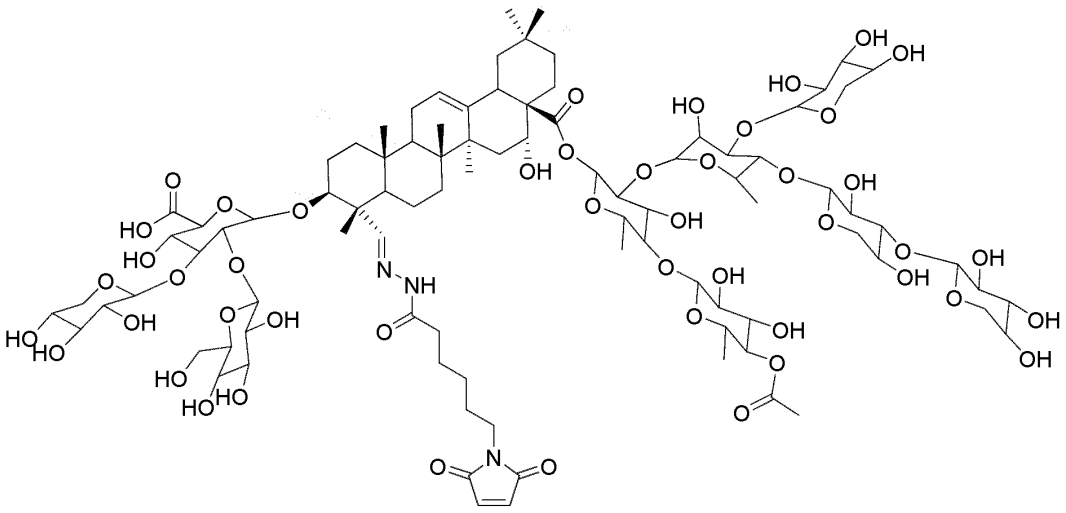
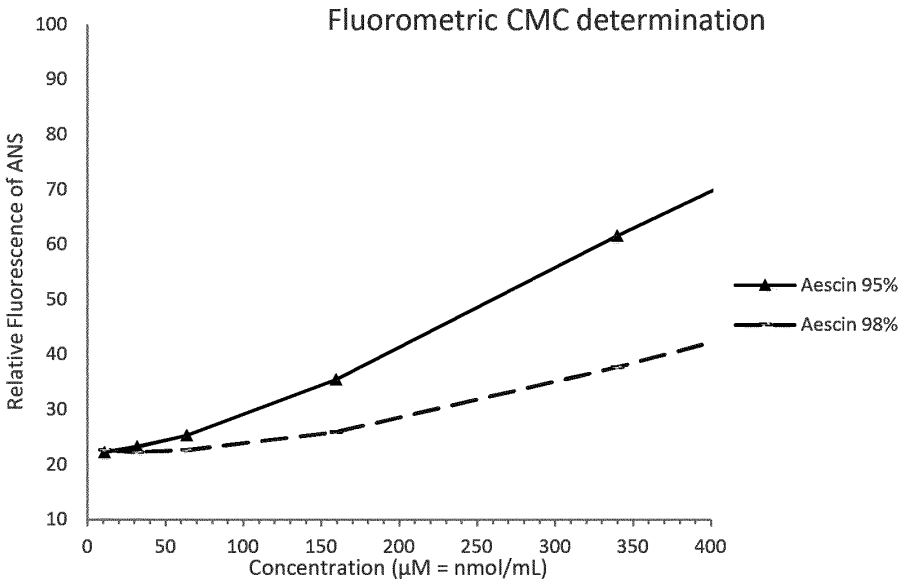
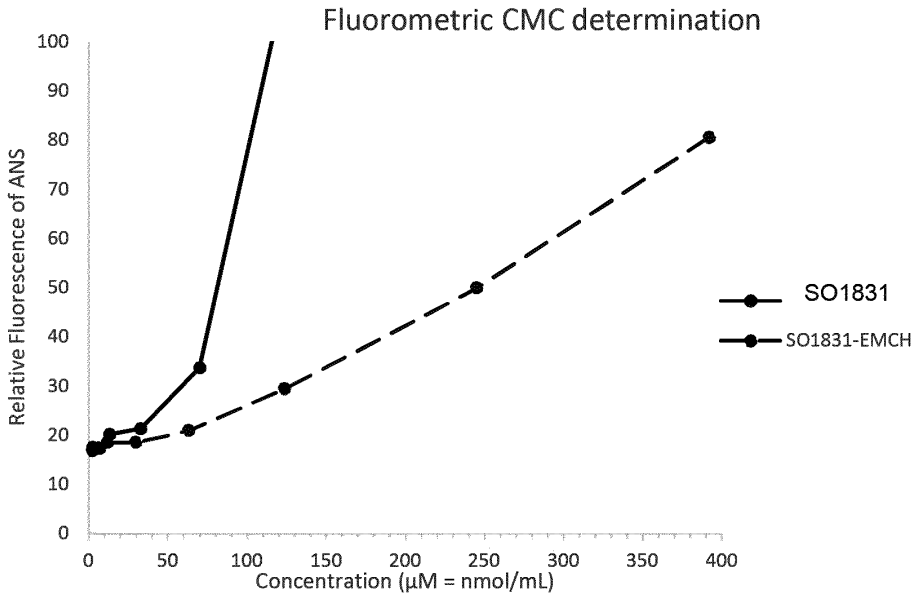


FIG. 73

A.


Fluorometric CMC determination
Relative Fluorescence of ANS
100
90
80
70
60
50
40
30
20
10
0
50
100
150
200
250
300
350
400
Concentration (µM = nmol/mL)
Aescin 95%
Aescin 98%

B.


Fluorometric CMC determination
Relative Fluorescence of ANS
100
90
80
70
60
50
40
30
20
10
0
50
100
150
200
250
300
350
400
Concentration (µM = nmol/mL)
SO1831
SO1831-EMCH