FIG. 1a

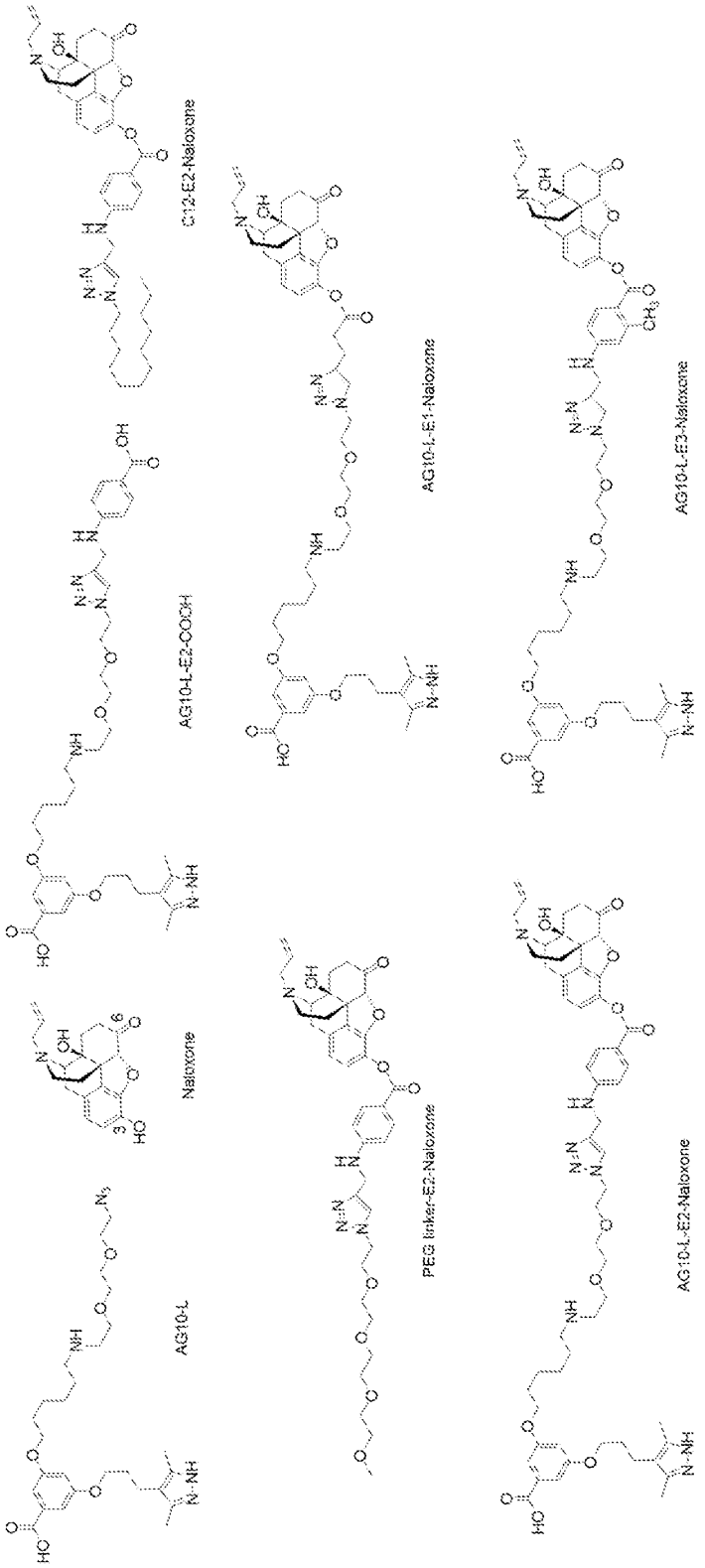

AG10-L
Naloxone
AG10-L-E2-COOH
C12-E2-Naloxone
PEG linker-E2-Naloxone
AG10-L-E1-Naloxone
AG10-L-E3-Naloxone
AG10-L-E2-Naloxone

FIG. 1a (Continued)

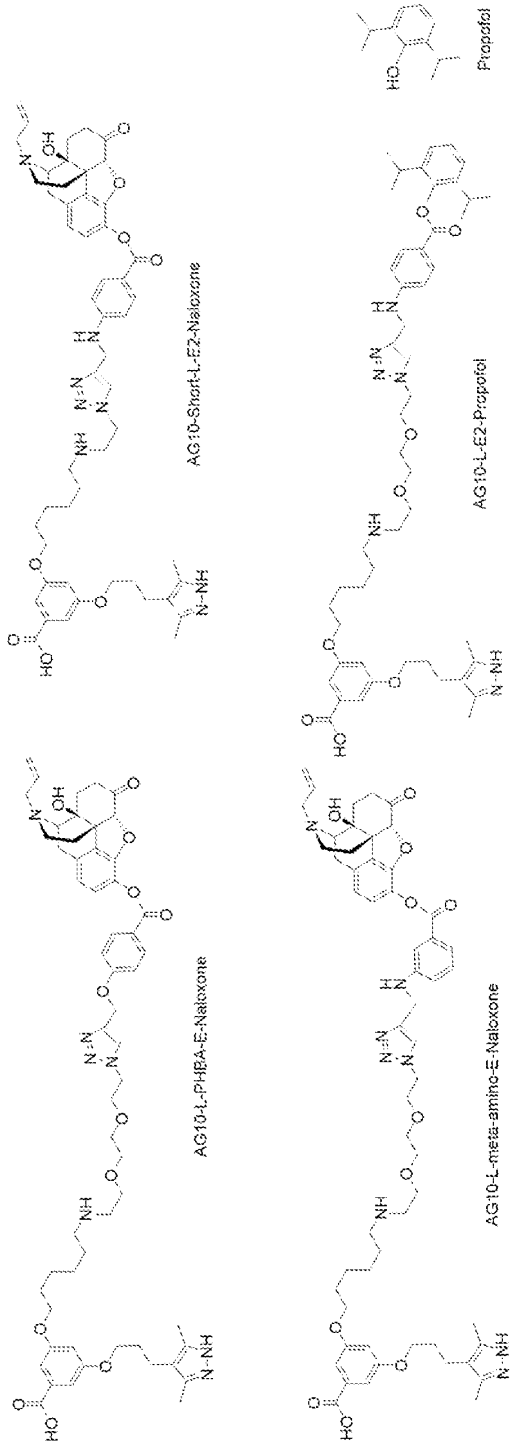
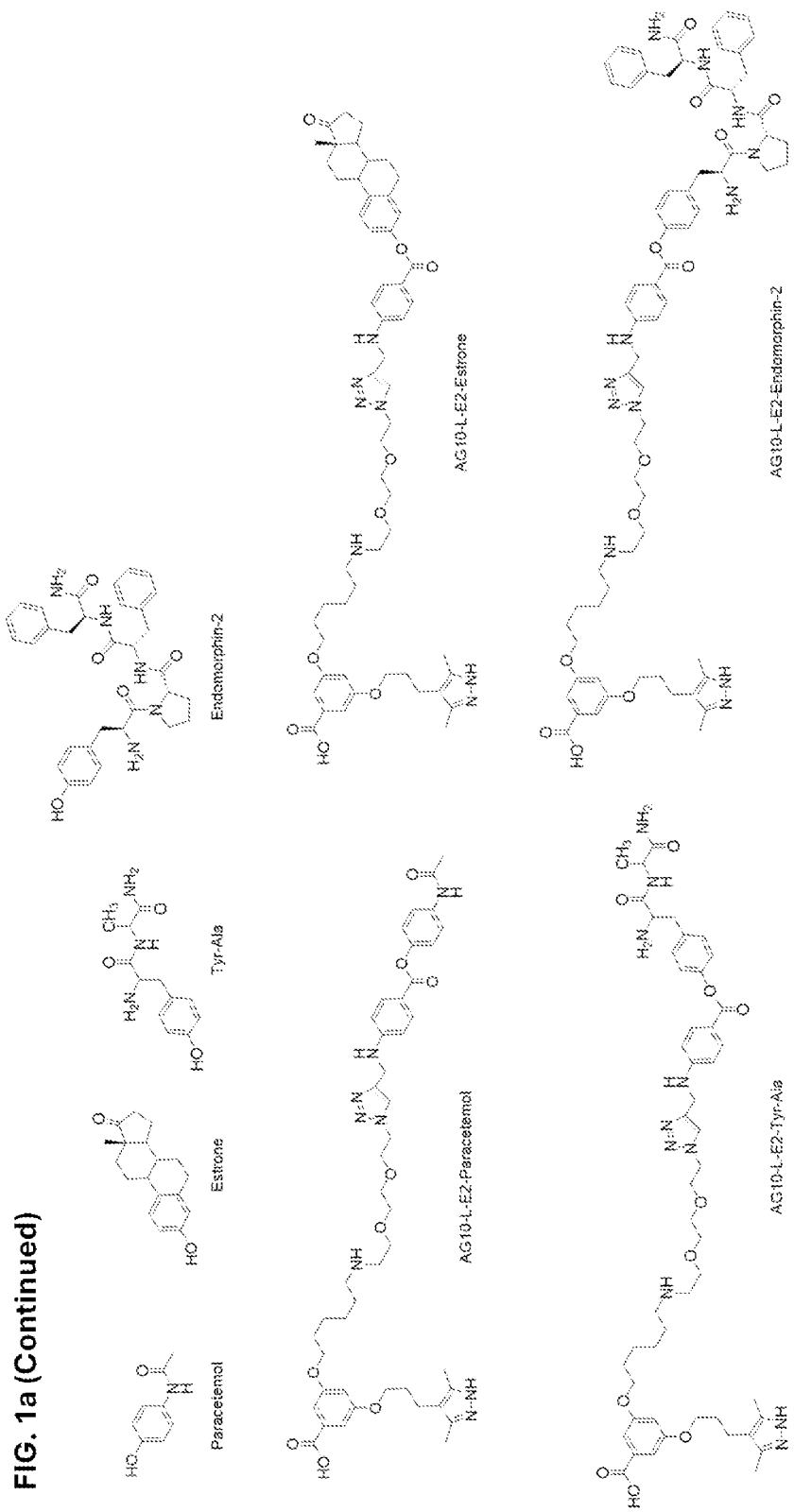


FIG. 1a (Continued)



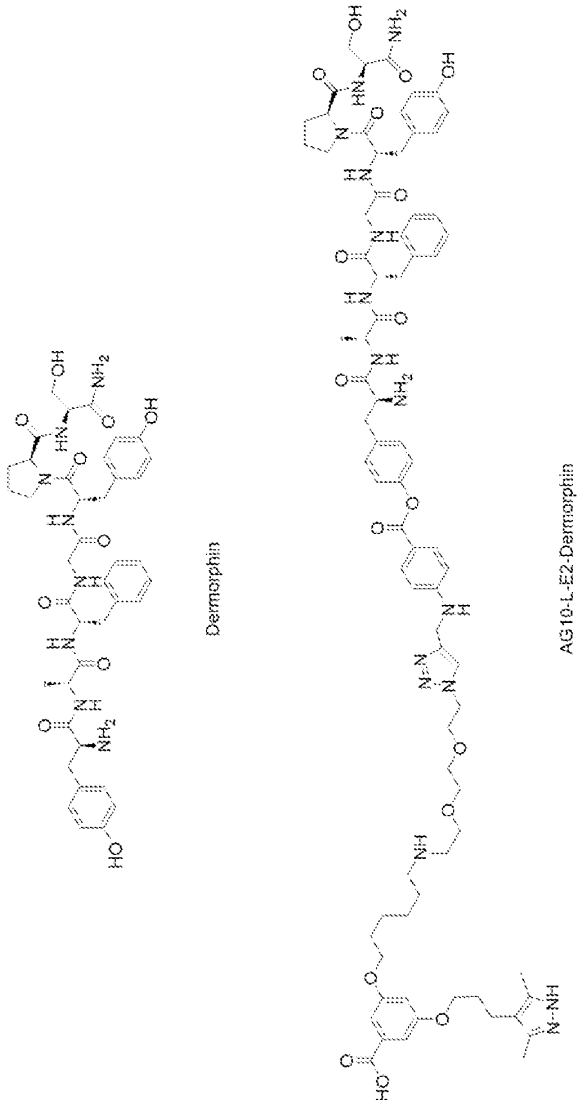


FIG. 1a (Continued)

FIG. 1b

Solubility and Log*D* pH 7.4
of test compounds

Compound	Solubility in water (pH 3.5)	Solubility in buffer (pH 7.4)	Log *D* (pH 7.4)
Naloxone	200 µM	170 µM	1.2
C12-E2-Naloxone	5 µM	0.3 µM	2.0
AG10-L-E2-Naloxone	200 µM	44 µM	1.5
AG10-L-E2-COOH	200 µM	200 µM	-1.6
AG10-L-E2-Ethyl	200 µM	97 µM	1.2

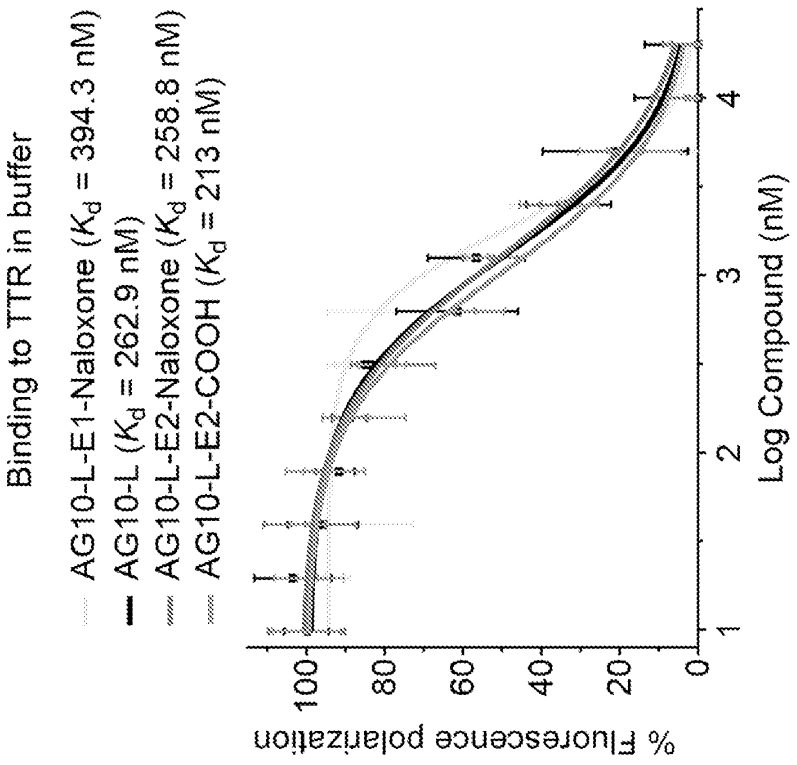

Binding to TTR in buffer
AG10-L-E1-Naloxone (K_d = 394.3 nM)
AG10-L (K_d = 262.9 nM)
AG10-L-E2-Naloxone (K_d = 258.8 nM)
AG10-L-E2-COOH (K_d = 213 nM)
% Fluorescence polarization
0
20
40
60
80
100
1
2
3
4
Log Compound (nM)

FIG. 1c

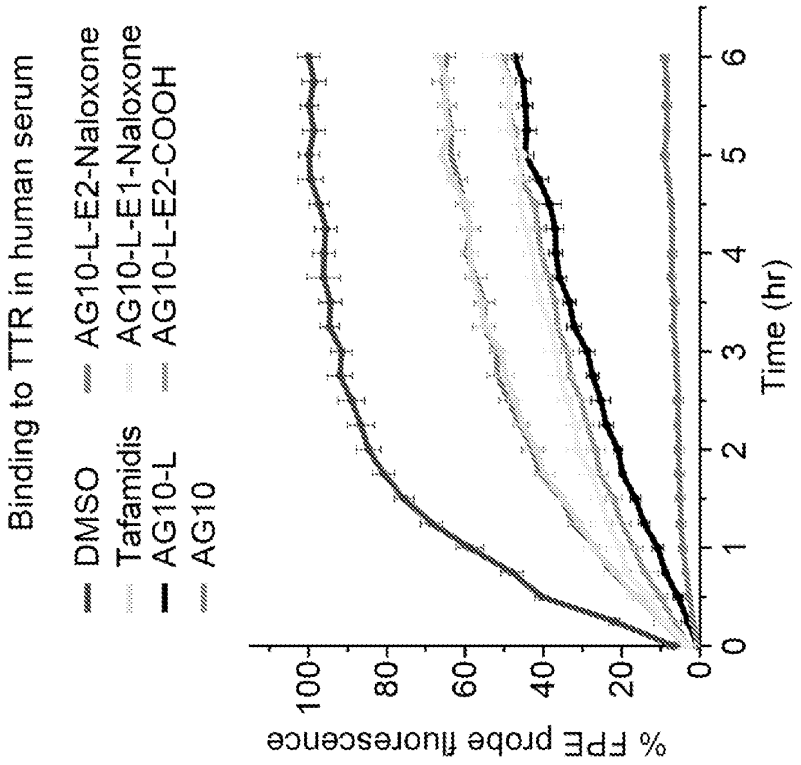

Binding to TTR in human serum
DMSO
Tafamidis
AG10-L
AG10
AG10-L-E2-Naloxone
AG10-L-E1-Naloxone
AG10-L-E2-COOH
% FPE probe fluorescence
100
80
60
40
20
0
0
1
2
3
4
5
6
Time (hr)

FIG. 1d

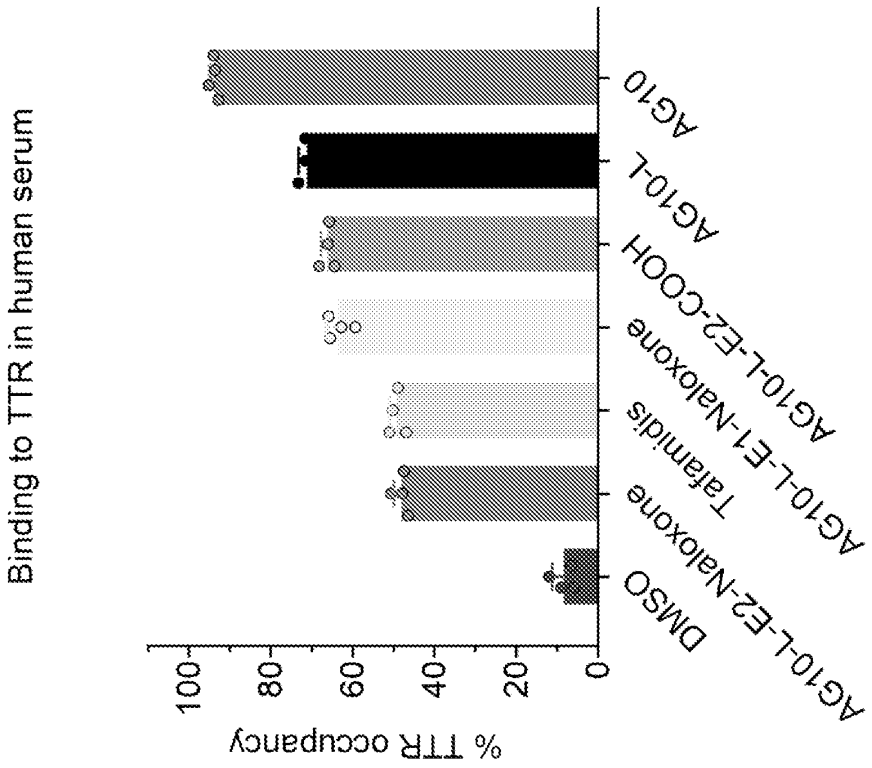

Binding to TTR in human serum
% TTR occupancy
0
20
40
60
80
100
DMSO
AG10-L-E2-Naloxone
Tafamidis
AG10-L-E1-Naloxone
AG10-L-E2-COOH
AG10-L
AG10

FIG. 1e

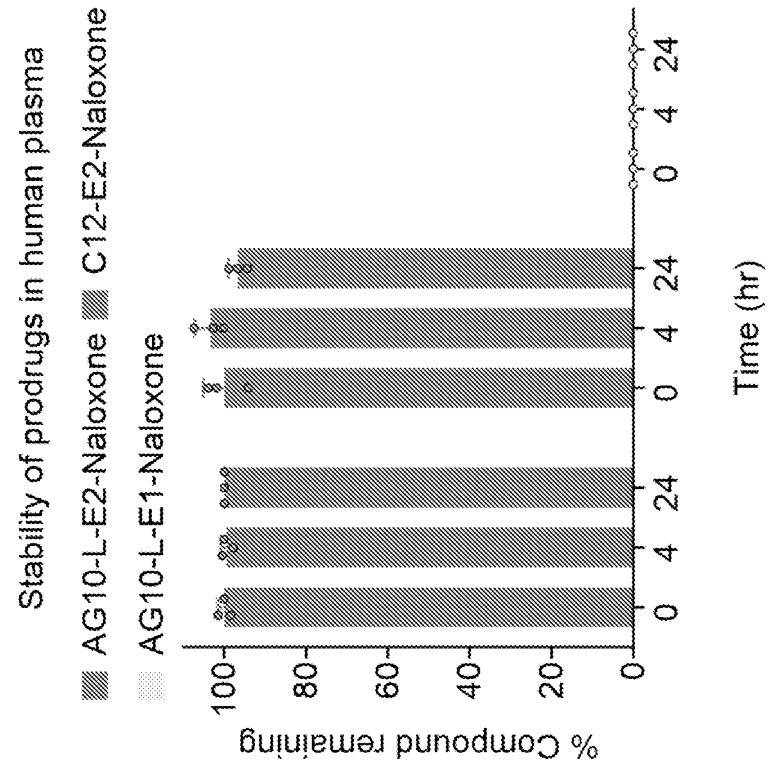

Stability of prodrugs in human plasma
AG10-L-E2-Naloxone
C12-E2-Naloxone
AG10-L-E1-Naloxone
% Compound remaining
100
80
60
40
20
0
0
4
24
0
4
24
0
4
24
Time (hr)

FIG. 1f

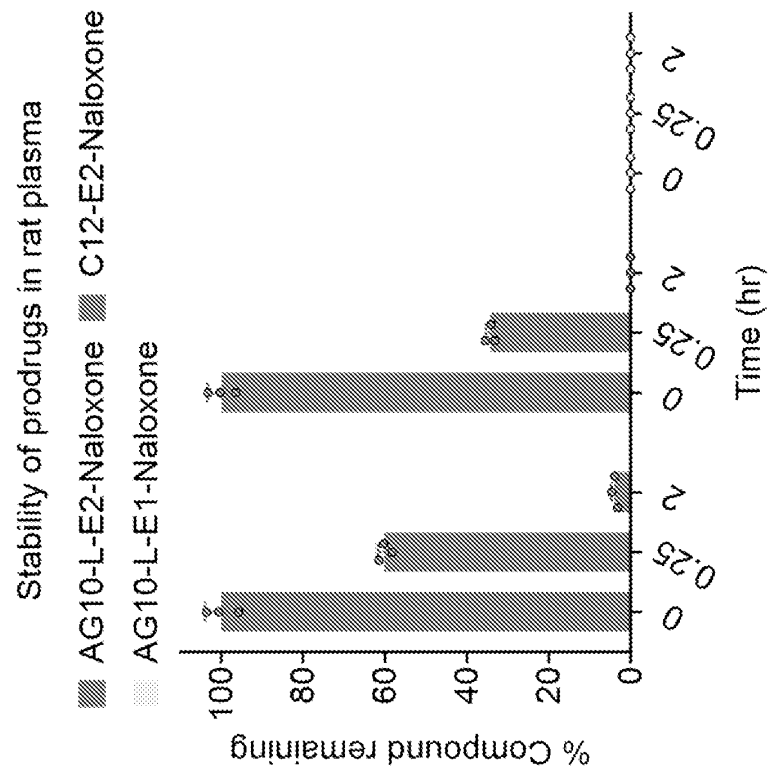

Stability of prodrugs in rat plasma
AG10-L-E2-Naloxone
C12-E2-Naloxone
AG10-L-E1-Naloxone
% Compound remaining
100
80
60
40
20
0
0
0.25
2
0
0.25
2
0
0.25
2
Time (hr)

FIG. 1g

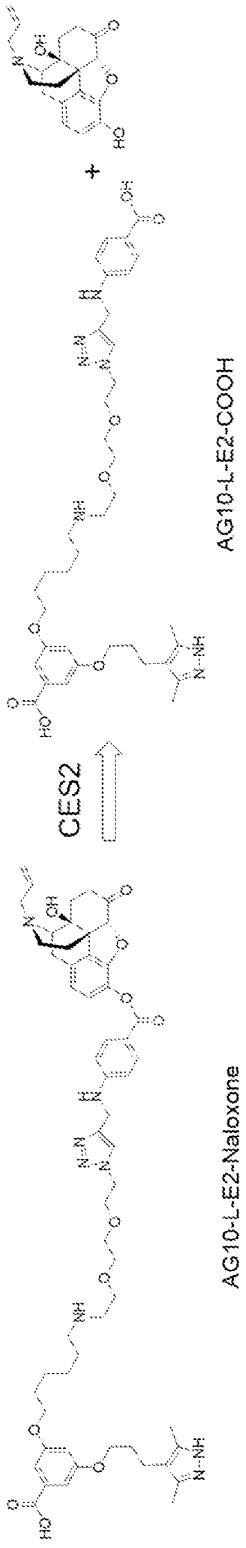


FIG. 2a

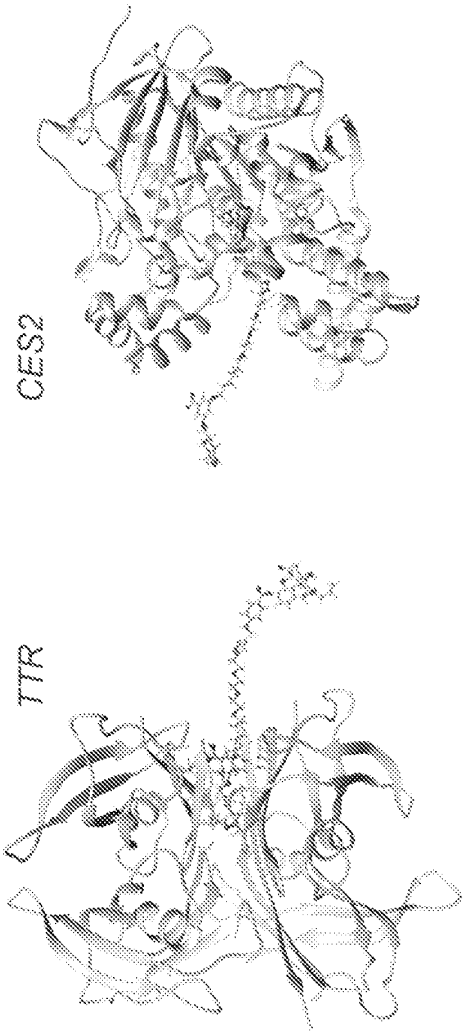


FIG. 2b

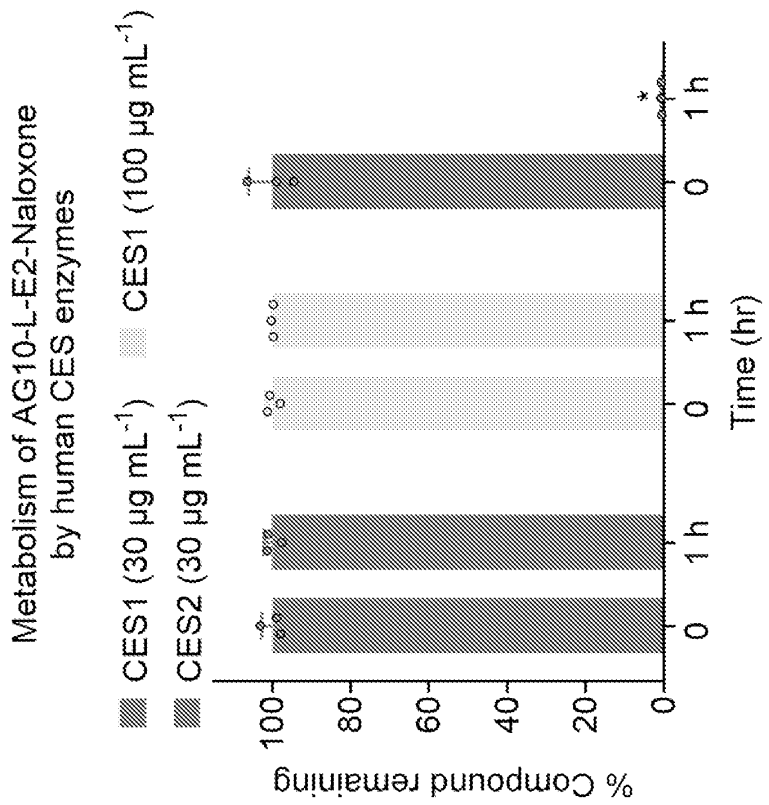

Metabolism of AG10-L-E2-Naloxone
by human CES enzymes
CES1 (30 µg mL-1)
CES2 (30 µg mL-1)
CES1 (100 µg mL-1)
% Compound remaining
0
20
40
60
80
100
0
1 h
0
1 h
0
1 h
Time (hr)

FIG. 2c

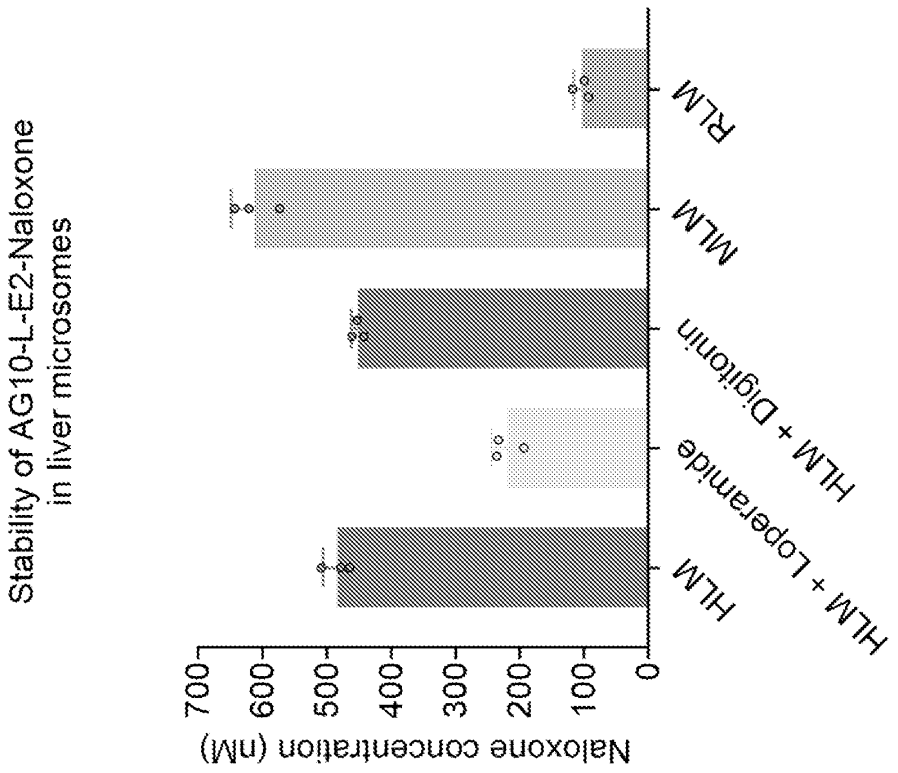

Stability of AG10-L-E2-Naloxone
in liver microsomes
Naloxone concentration (nM)
0
100
200
300
400
500
600
700
HLM
HLM + Loperamide
HLM + Digitonin
MLM
RLM

FIG. 2d

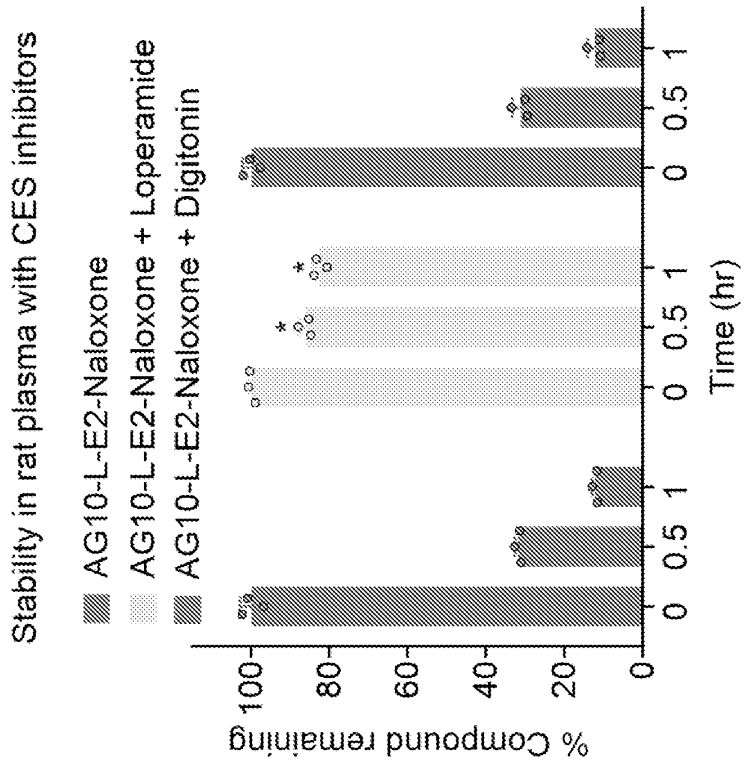

Stability in rat plasma with CES inhibitors
AG10-L-E2-Naloxone
AG10-L-E2-Naloxone + Loperamide
AG10-L-E2-Naloxone + Digitonin
% Compound remaining
0
20
40
60
80
100
0 0.5 1 0 0.5 1 0 0.5 1
Time (hr)

FIG. 2e

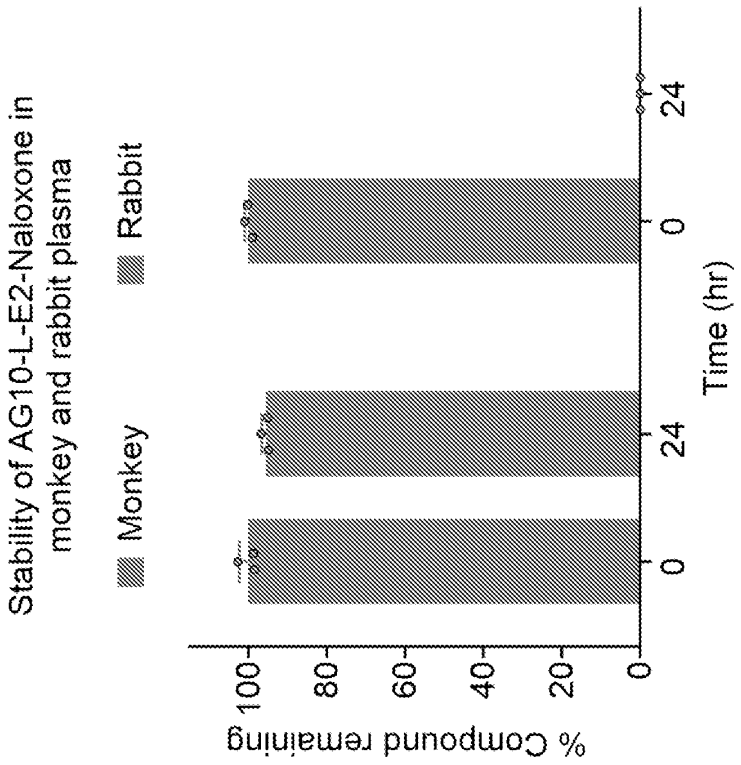

Stability of AG10-L-E2-Naloxone in monkey and rabbit plasma
Monkey
Rabbit
% Compound remaining
100
80
60
40
20
0
0
24
0
24
Time (hr)

FIG. 2f

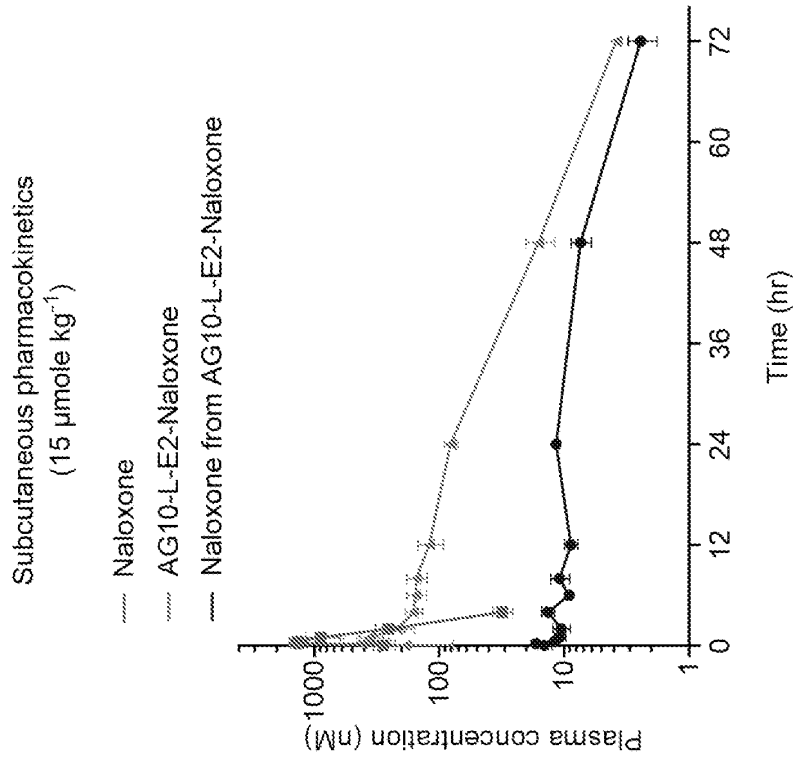

Subcutaneous pharmacokinetics
(15 µmole kg-1)
Naloxone
AG10-L-E2-Naloxone
Naloxone from AG10-L-E2-Naloxone
Plasma concentration (nM)
1000
100
10
1
0
12
24
36
48
60
72
Time (hr)

FIG. 3a

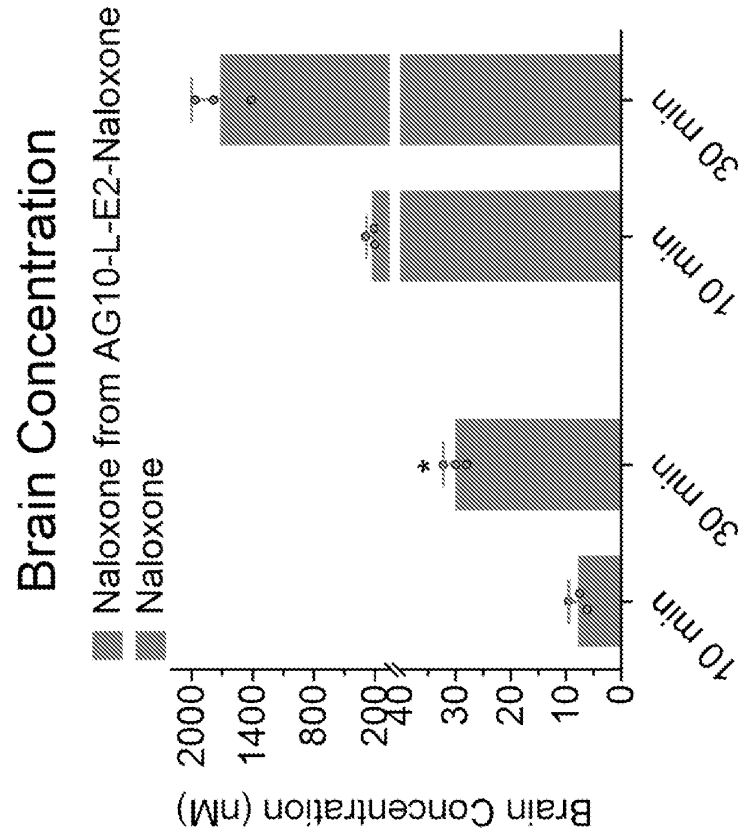

Brain Concentration
Naloxone from AG10-L-E2-Naloxone
Naloxone
Brain Concentration (nM)
2000
1400
800
200
40
30
20
10
0
*
10 min
30 min
10 min
30 min

FIG. 3b

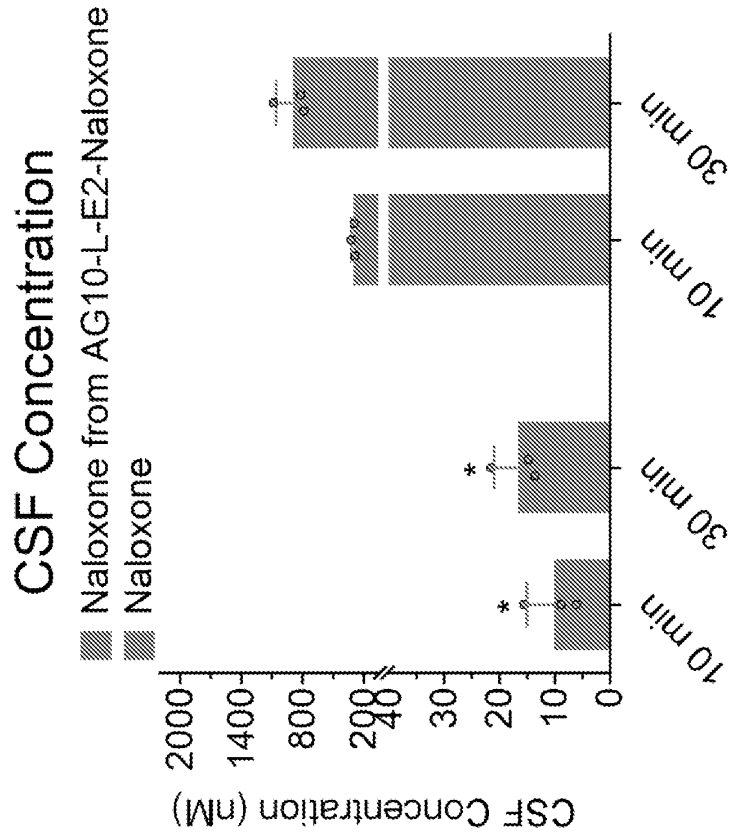

CSF Concentration
Naloxone from AG10-L-E2-Naloxone
Naloxone
CSF Concentration (nM)
2000
1400
800
200
40
30
20
10
0
*
*
10 min
30 min
10 min
30 min

FIG. 3c

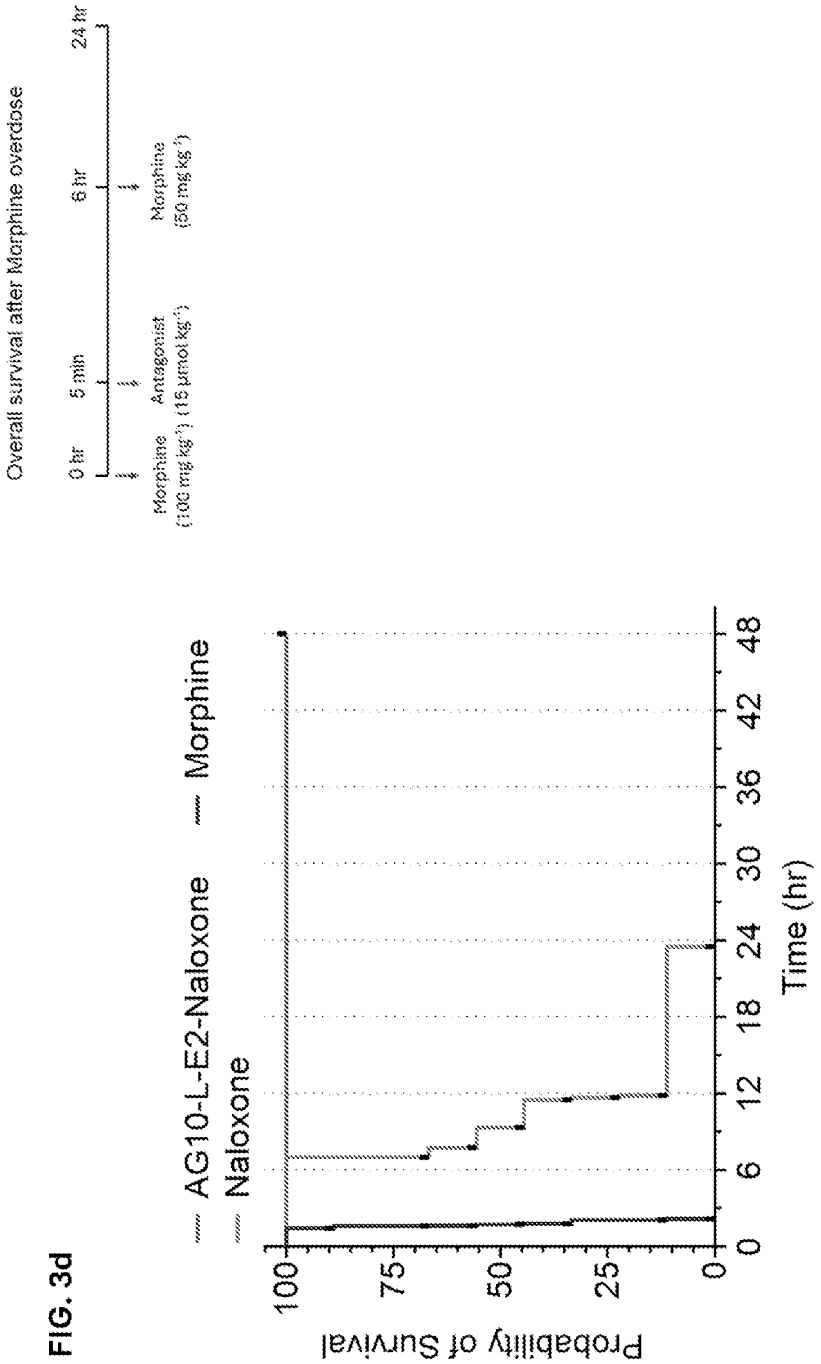

Overall survival after Morphine overdose
0 hr
5 min
6 hr
24 hr
Morphine (100 mg kg⁻¹)
Antagonist (15 µmol kg⁻¹)
Morphine (50 mg kg⁻¹)
AG10-L-E2-Naloxone
Naloxone
Morphine
Probability of Survival
100
75
50
25
0
0
6
12
18
24
30
36
42
48
Time (hr)

FIG. 3d

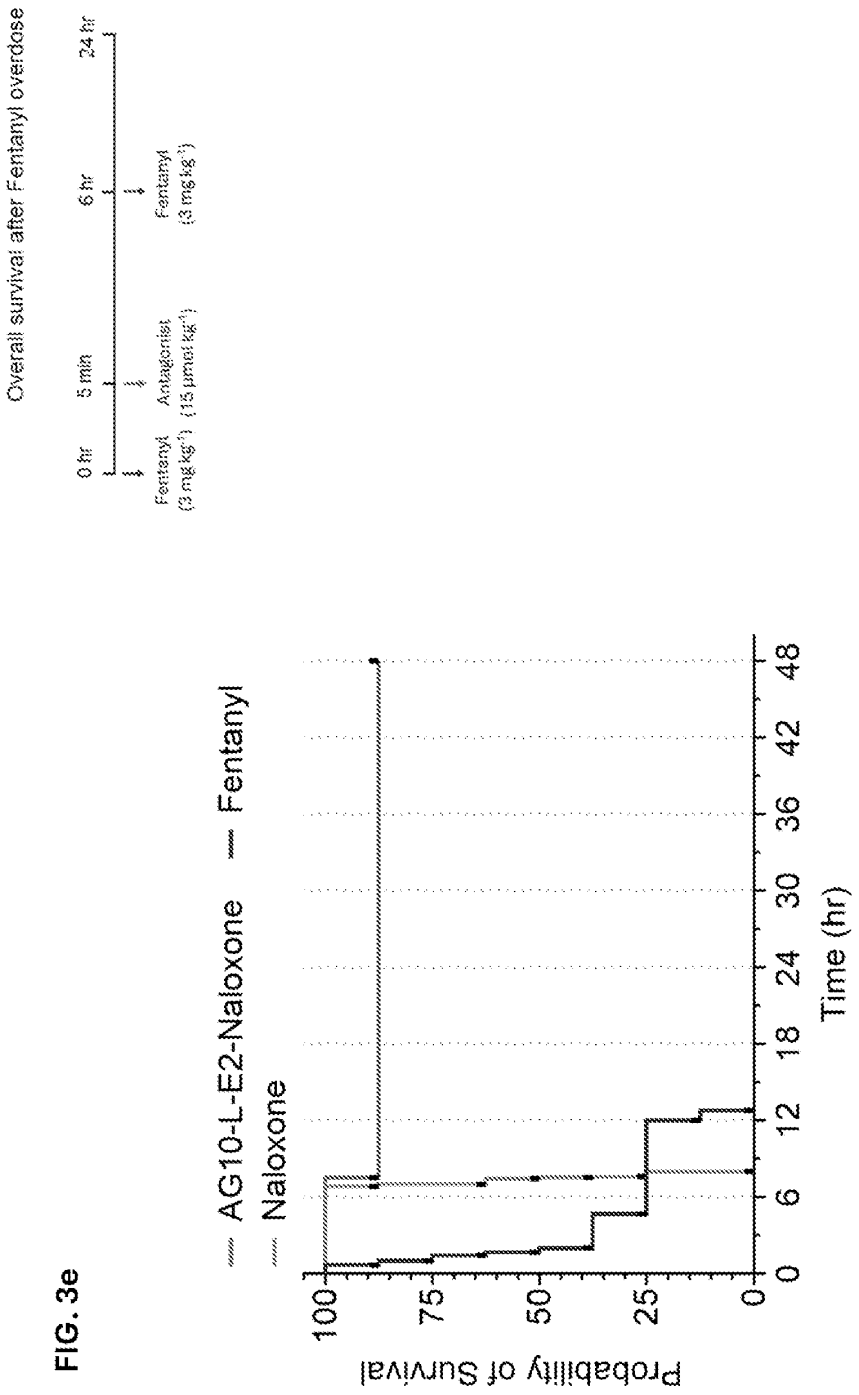
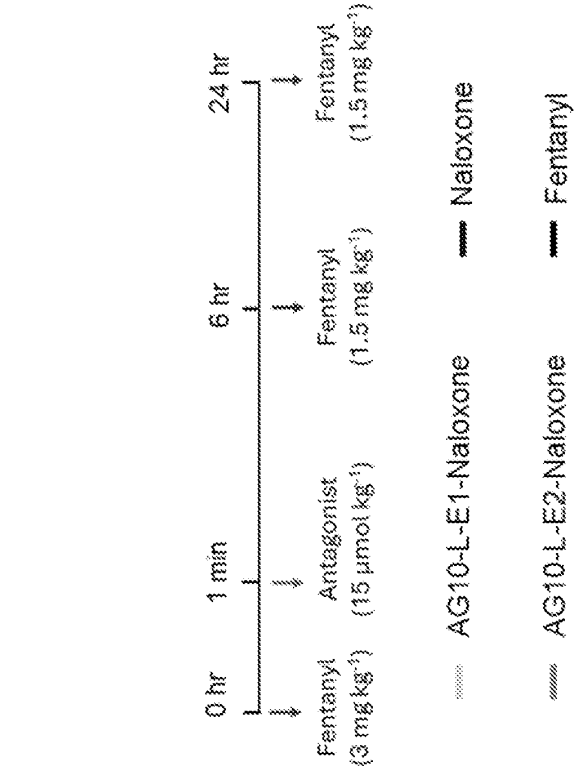

Overall survival after Fentanyl overdose
0 hr
5 min
6 hr
24 hr
Fentanyl (3 mg kg-1)
Antagonist (15 μmol kg-1)
Fentanyl (3 mg kg-1)
AG10-L-E2-Naloxone
Naloxone
Fentanyl
Probability of Survival
100
75
50
25
0
0
6
12
18
24
30
36
42
48
Time (hr)

FIG. 3e

FIG. 3f


0 hr
1 min
6 hr
24 hr
Fentanyl (3 mg kg-1)
Antagonist (15 µmol kg-1)
Fentanyl (1.5 mg kg-1)
Fentanyl (1.5 mg kg-1)
AG10-L-E1-Naloxone
Naloxone
AG10-L-E2-Naloxone
Fentanyl
C12-E2-Naloxone

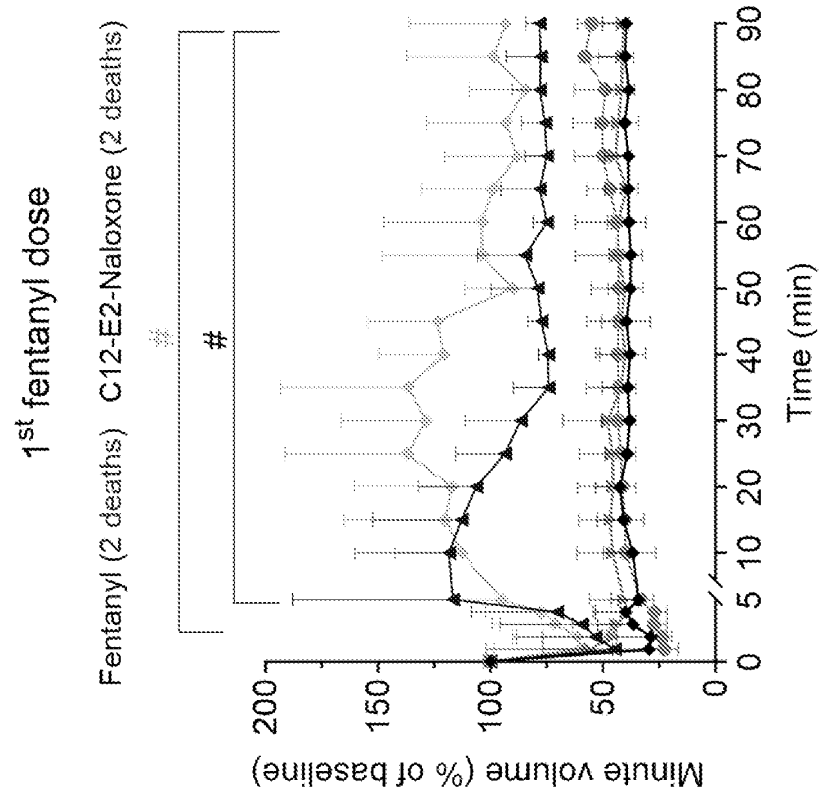

1st fentanyl dose
Fentanyl (2 deaths)
C12-E2-Naloxone (2 deaths)
*
#
Minute volume (% of baseline)
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 3g

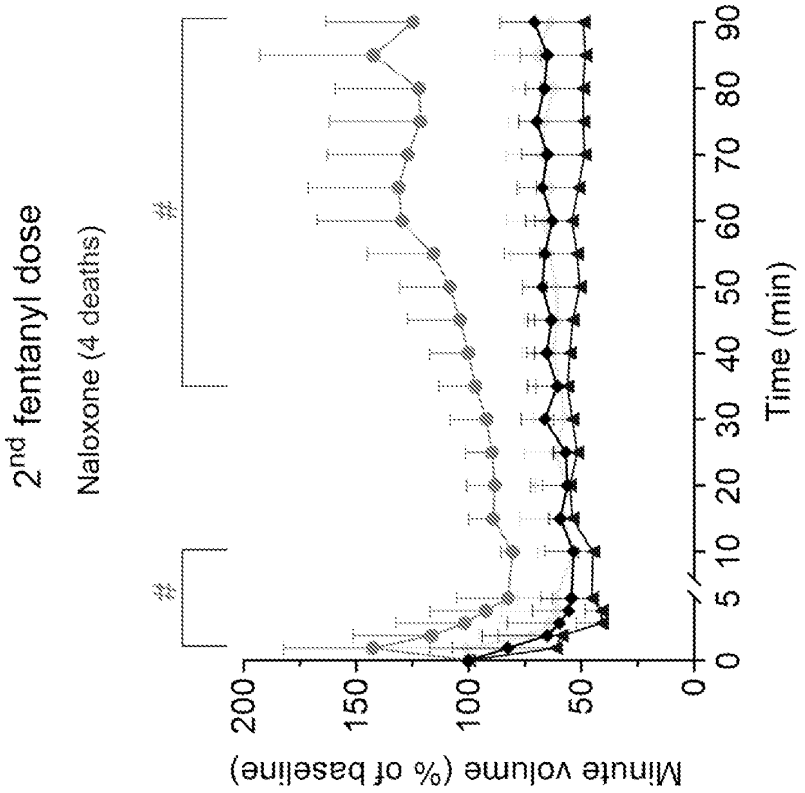

2nd fentanyl dose
Naloxone (4 deaths)
#
#
Minute volume (% of baseline)
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 3h

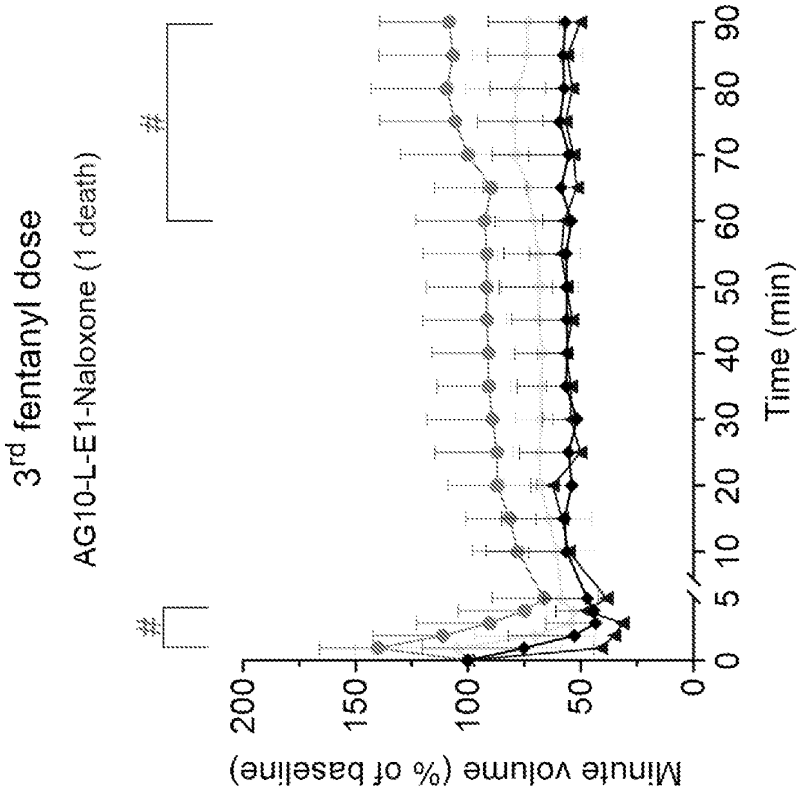

3rd fentanyl dose
AG10-L-E1-Naloxone (1 death)
##
##
Minute volume (% of baseline)
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 3i

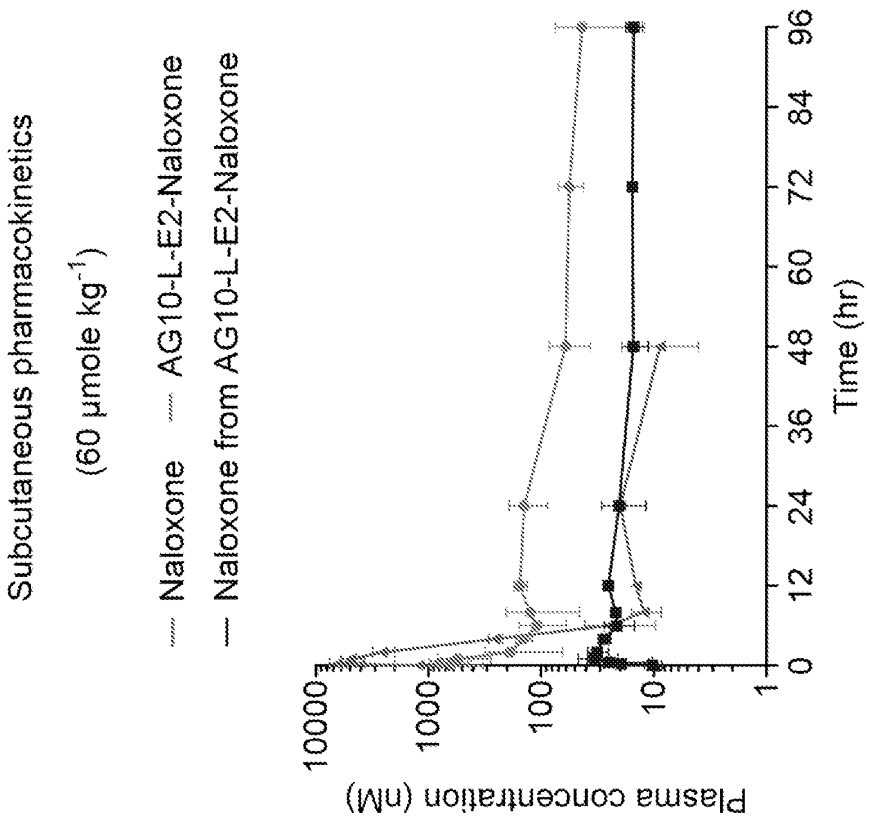
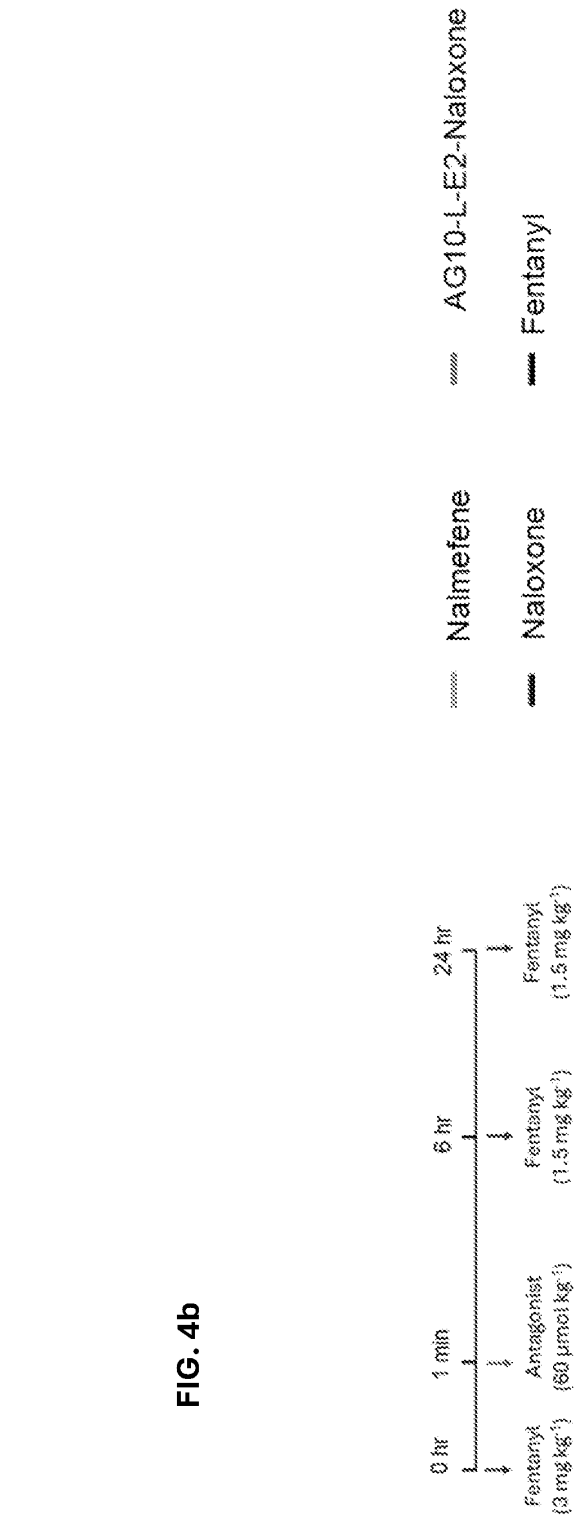

Subcutaneous pharmacokinetics
(60 µmole kg-1)
Naloxone
AG10-L-E2-Naloxone
Naloxone from AG10-L-E2-Naloxone
Plasma concentration (nM)
10000
1000
100
10
1
0
12
24
36
48
60
72
84
96
Time (hr)

FIG. 4a

FIG. 4b


AG10-L-E2-Naloxone
Nalmefene
Fentanyl
Naloxone
0 hr
1 min
6 hr
24 hr
Fentanyl (3 mg kg-1)
Antagonist (60 µmol kg-1)
Fentanyl (1.5 mg kg-1)
Fentanyl (1.5 mg kg-1)

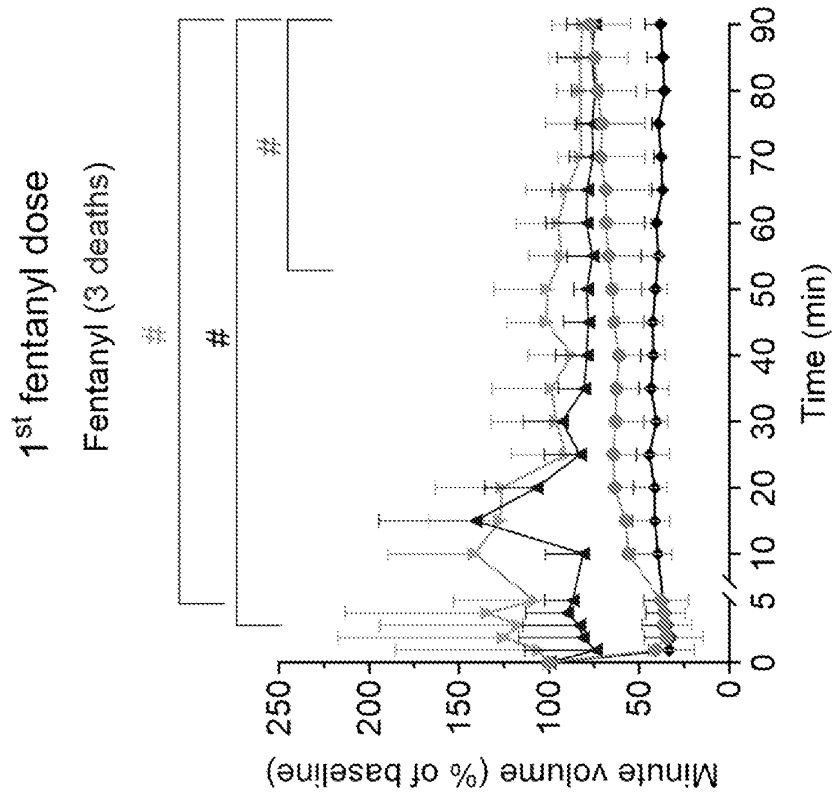

1st fentanyl dose
Fentanyl (3 deaths)
*
#
#
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 4c

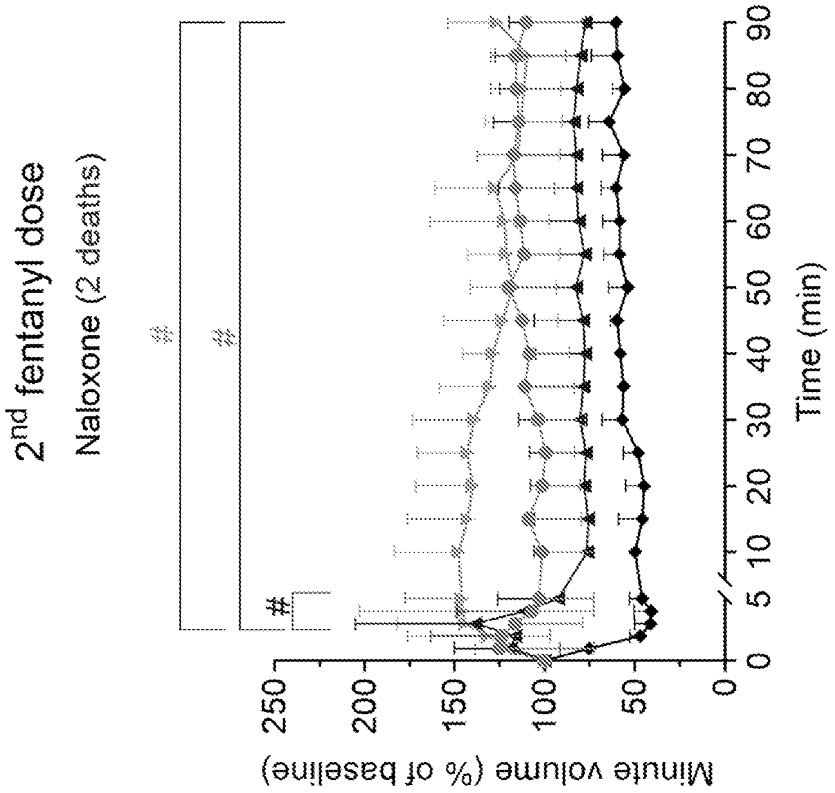

2nd fentanyl dose
Naloxone (2 deaths)
#
#
#
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 4d

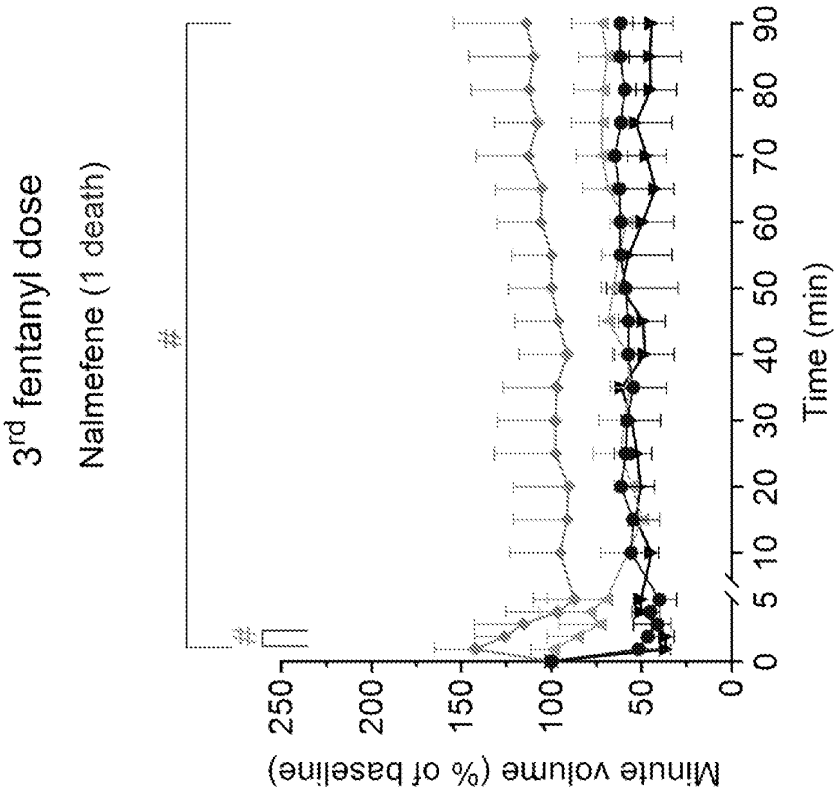

3rd fentanyl dose
Nalmefene (1 death)
#
#
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 4e

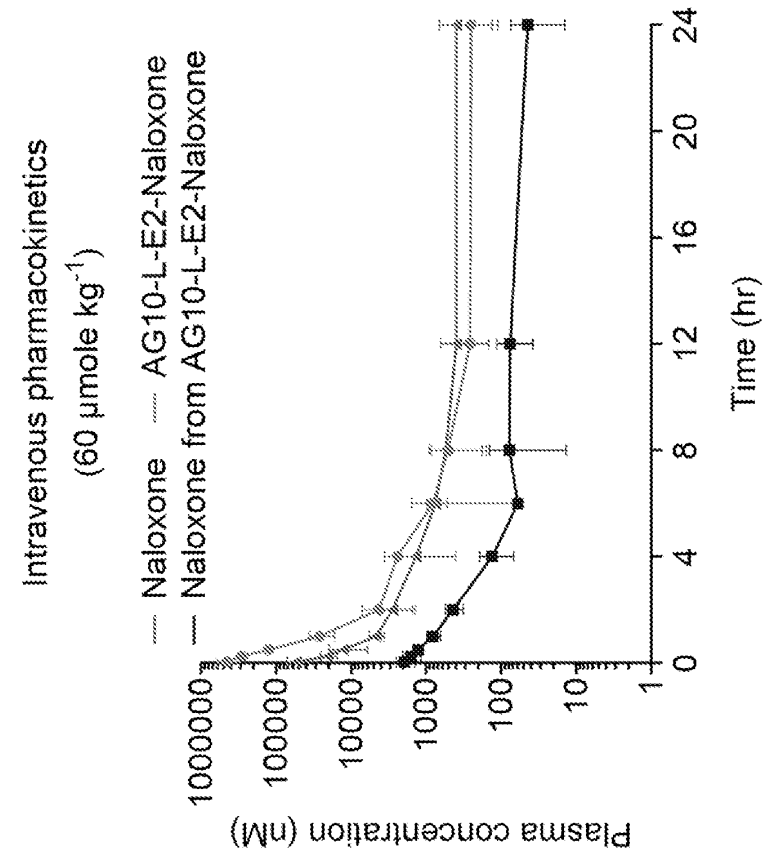

Intravenous pharmacokinetics
(60 µmole kg-1)
Naloxone
AG10-L-E2-Naloxone
Naloxone from AG10-L-E2-Naloxone
Plasma concentration (nM)
1000000
100000
10000
1000
100
10
1
0
4
8
12
16
20
24
Time (hr)

FIG. 4f

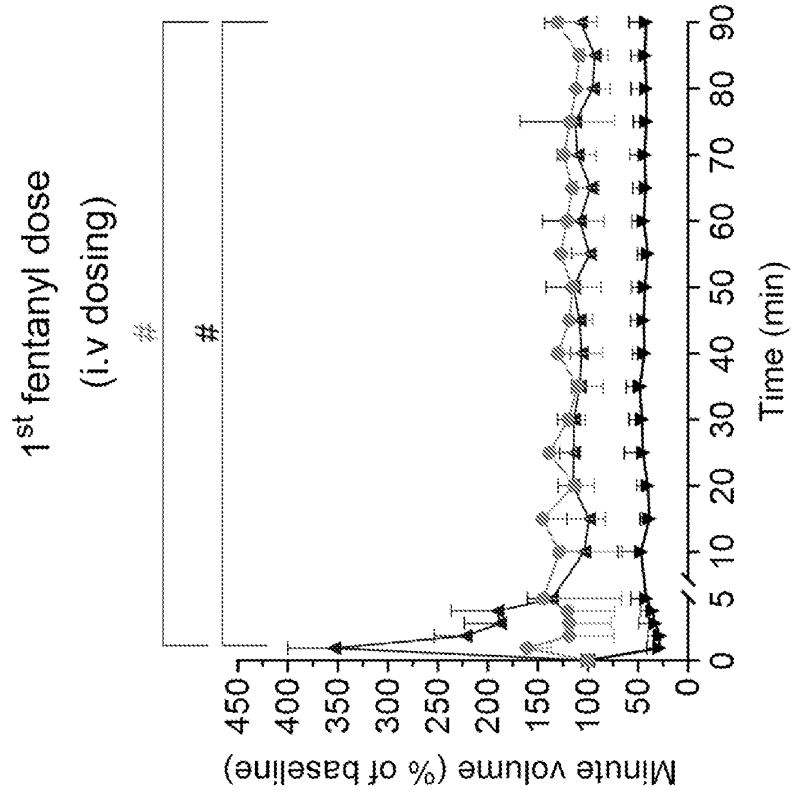

1st fentanyl dose
(i.v dosing)
##
#
Minute volume (% of baseline)
450
400
350
300
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 4g

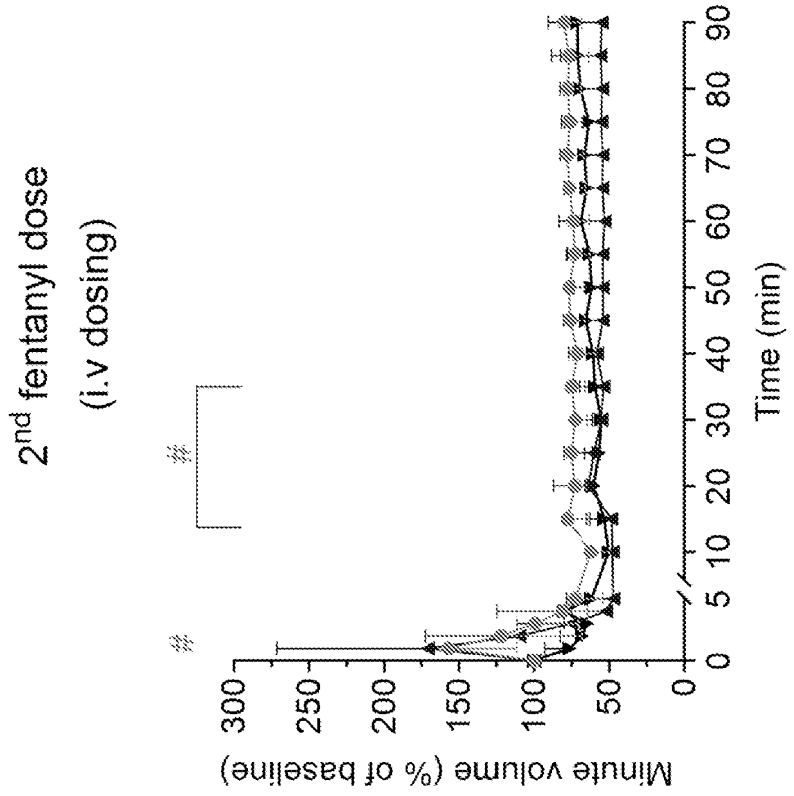

2nd fentanyl dose
(i.v dosing)
#
#
Minute volume (% of baseline)
300
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 4h

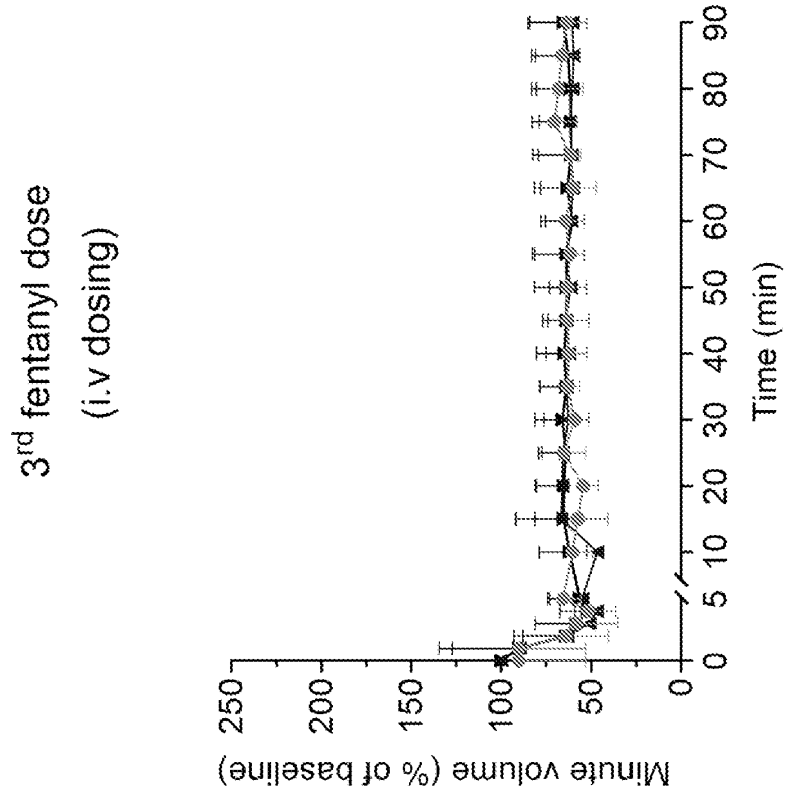
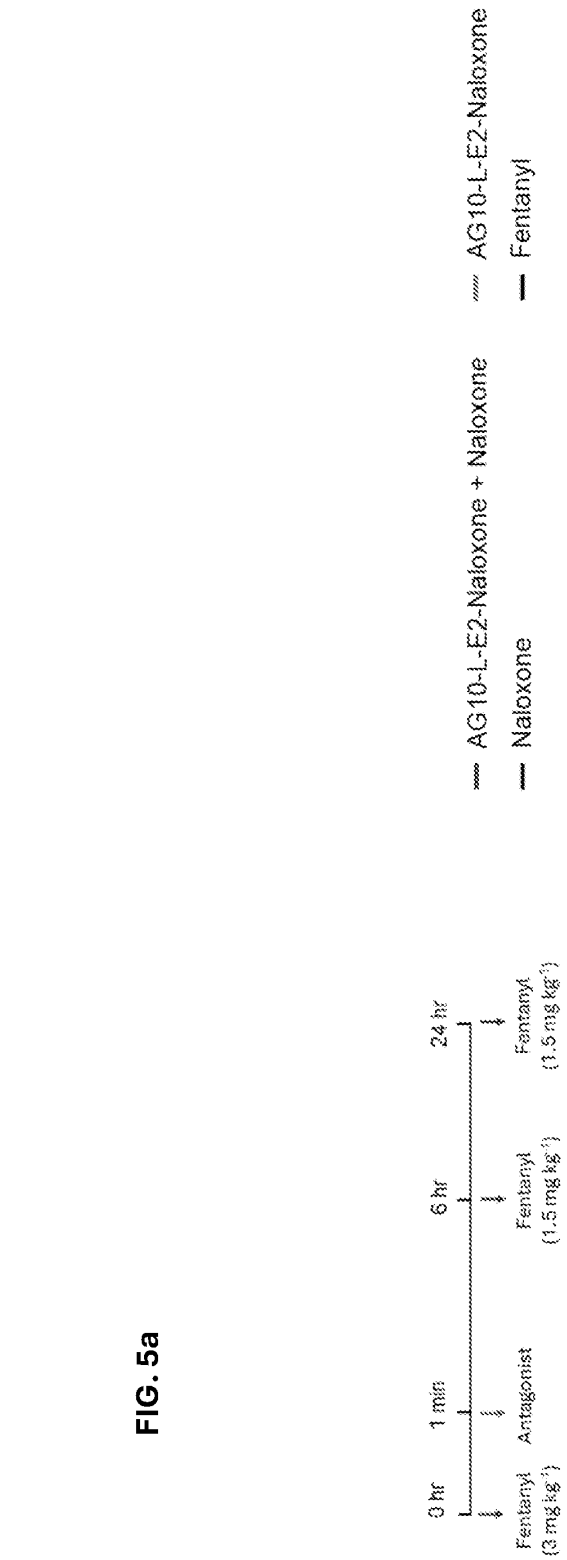

3rd fentanyl dose
(i.v dosing)
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 4i

FIG. 5a


AG10-L-E2-Naloxone + Naloxone
Naloxone
AG10-L-E2-Naloxone
Fentanyl
0 hr
1 min
6 hr
24 hr
Fentanyl (3 mg kg⁻¹)
Antagonist
Fentanyl (1.5 mg kg⁻¹)
Fentanyl (1.5 mg kg⁻¹)

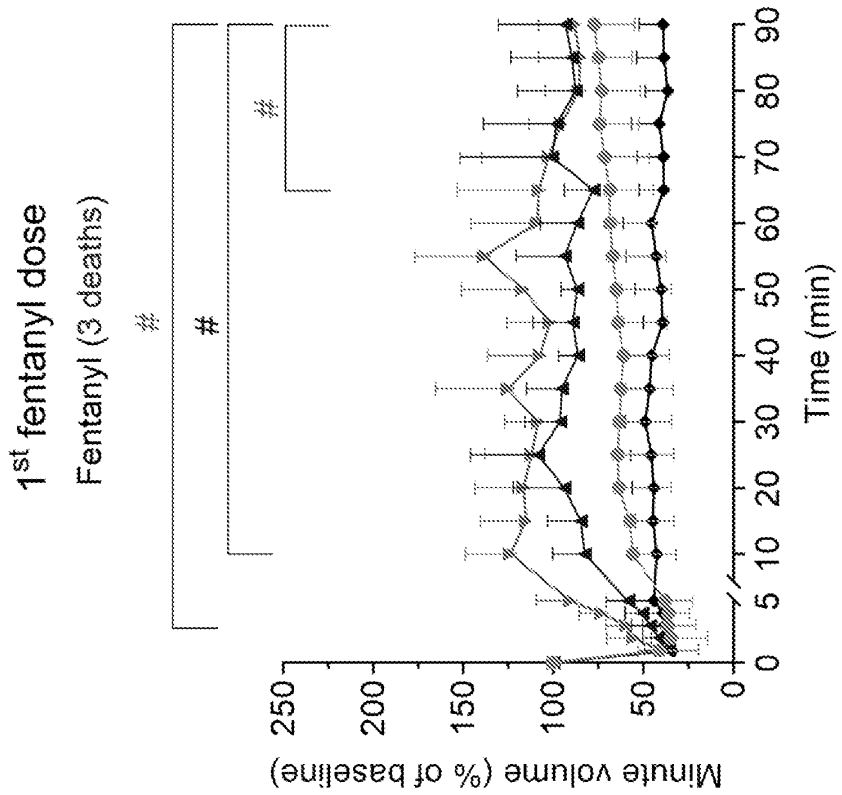

1st fentanyl dose
Fentanyl (3 deaths)
#
#
#
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 5b

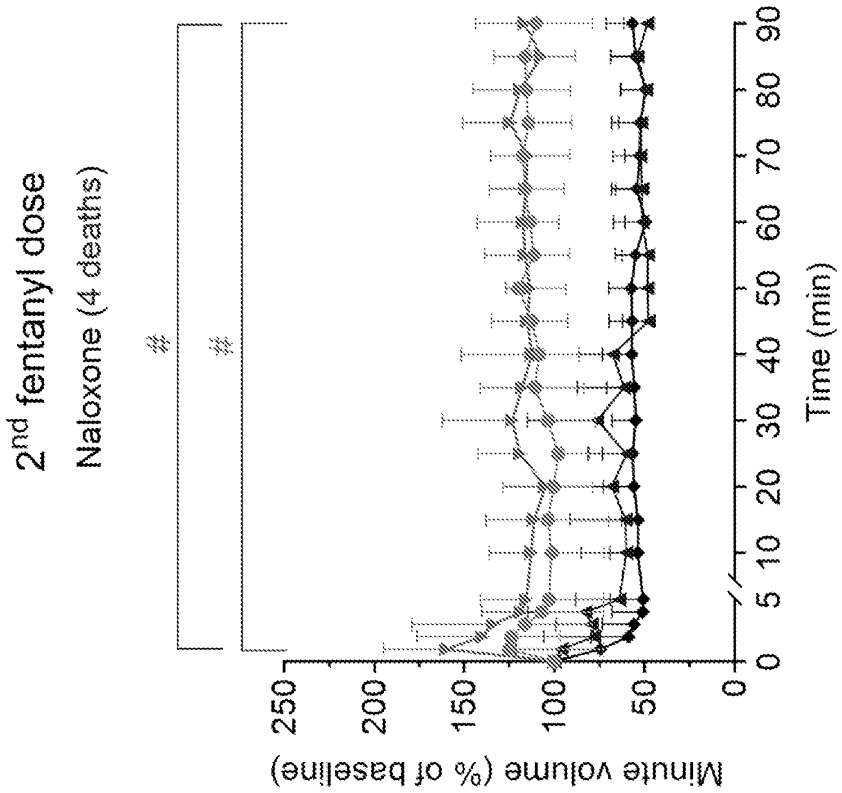

2nd fentanyl dose
Naloxone (4 deaths)
#
#
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 5c

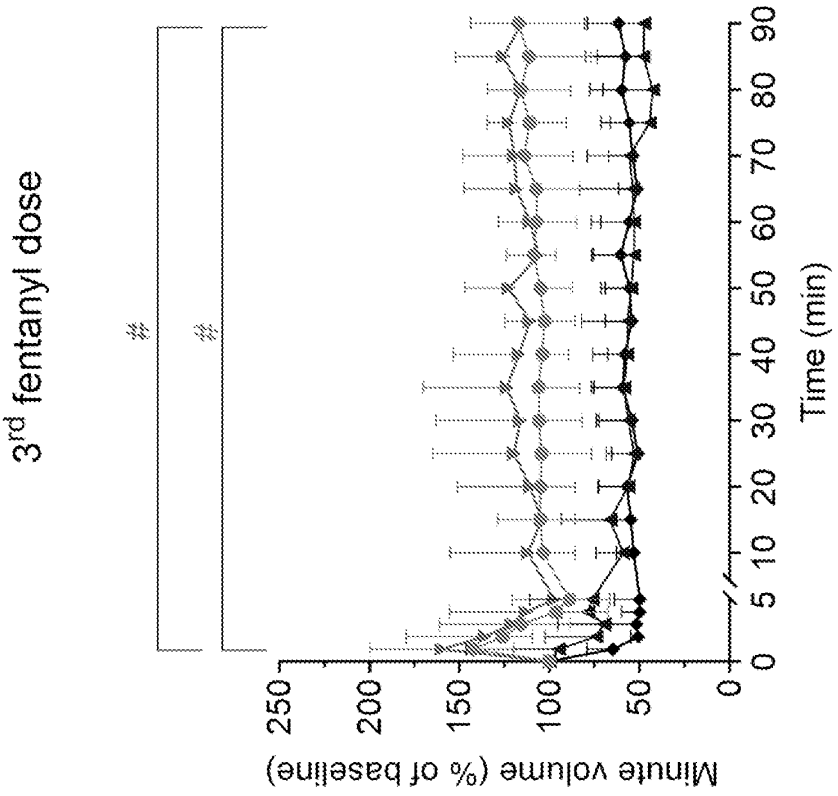

3rd fentanyl dose
##
##
Minute volume (% of baseline)
250
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 5d

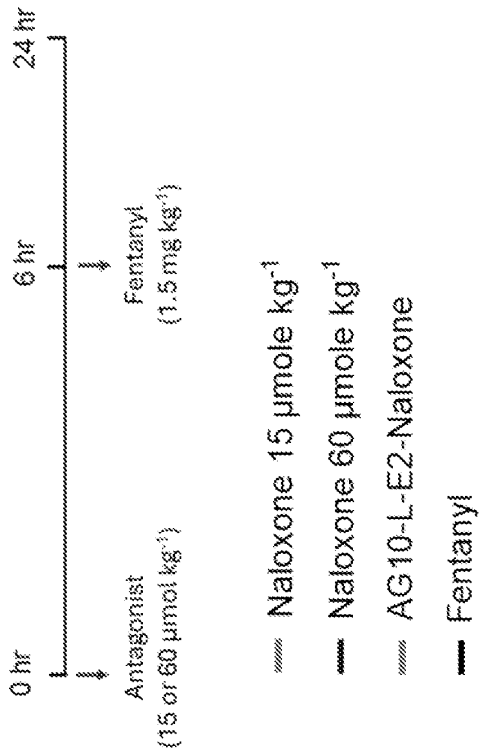

0 hr
6 hr
24 hr
Antagonist
(15 or 60 µmol kg-1)
Fentanyl
(1.5 mg kg-1)
Naloxone 15 µmole kg-1
Naloxone 60 µmole kg-1
AG10-L-E2-Naloxone
Fentanyl

FIG. 5e

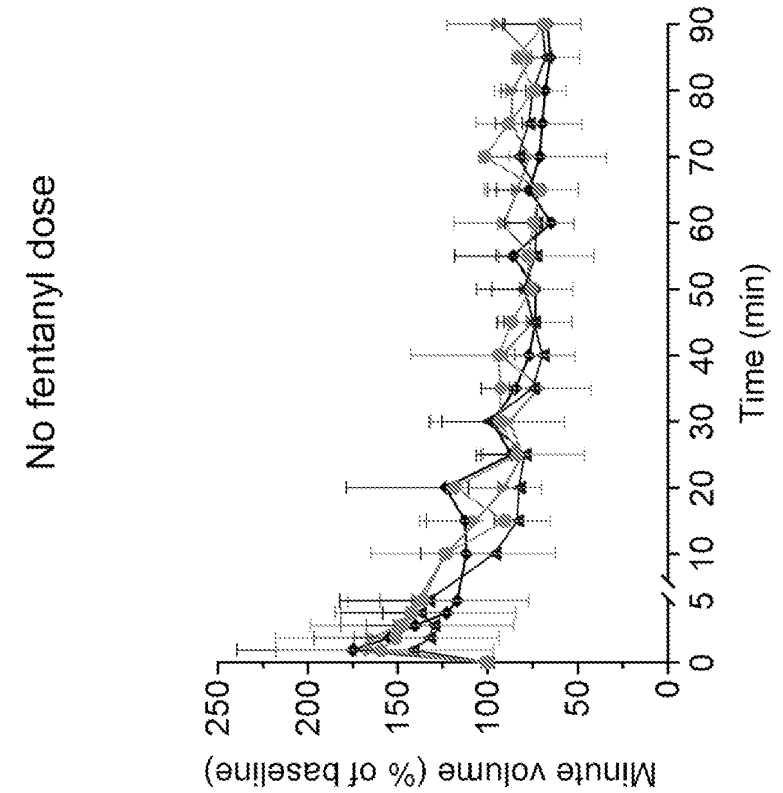

No fentanyl dose
Minute volume (% of baseline)
250
200
150
100
50
0
0 5 10 20 30 40 50 60 70 80 90
Time (min)

FIG. 5f

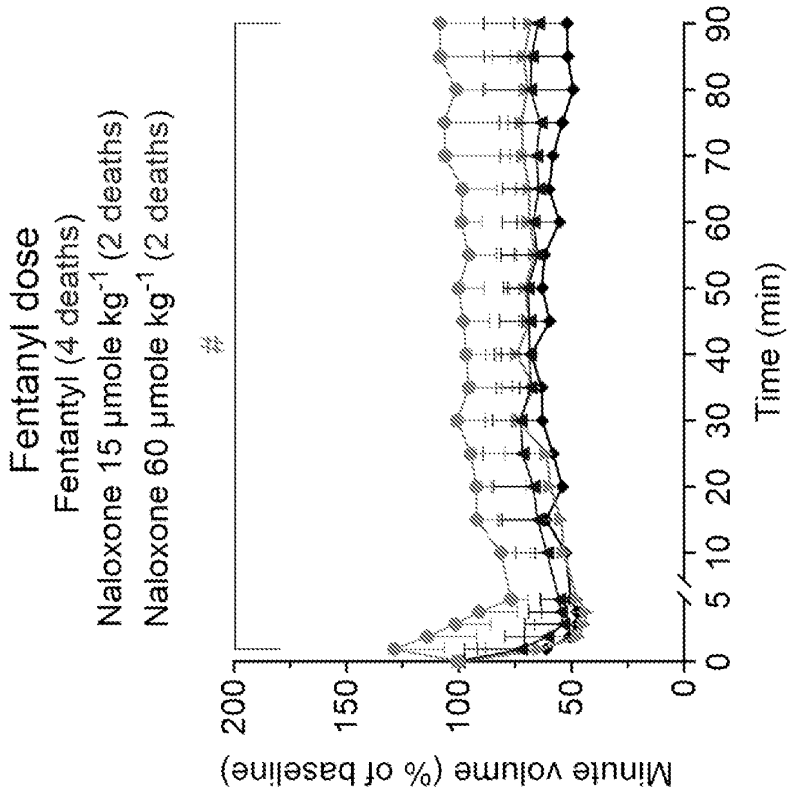

Fentanyl dose
Fentantyl (4 deaths)
Naloxone 15 µmole kg-1 (2 deaths)
Naloxone 60 µmole kg-1 (2 deaths)
#
Minute volume (% of baseline)
200
150
100
50
0
0
5
10
20
30
40
50
60
70
80
90
Time (min)

FIG. 5g

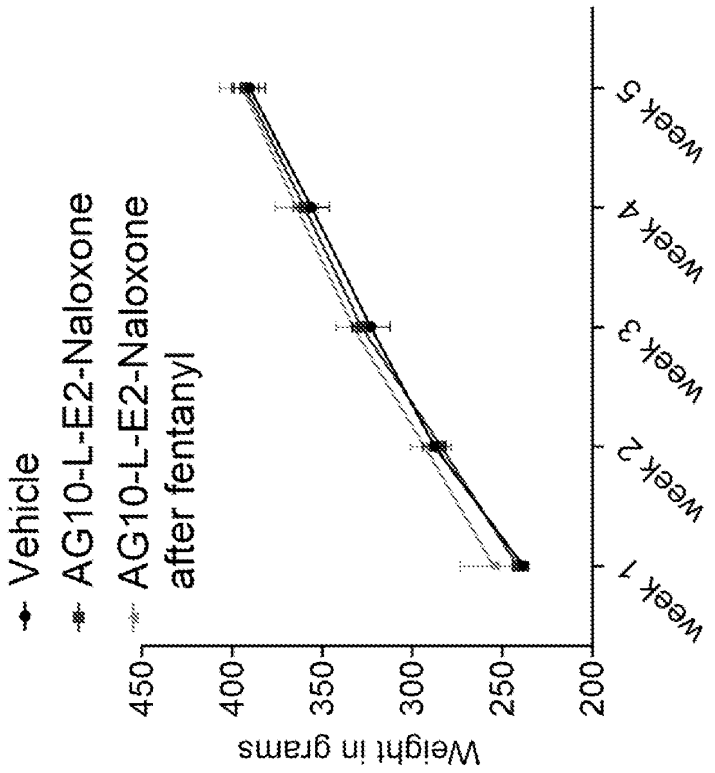

Vehicle
AG10-L-E2-Naloxone
AG10-L-E2-Naloxone after fentanyl
Weight in grams
450
400
350
300
250
200
week 1
week 2
week 3
week 4
week 5

FIG. 5h

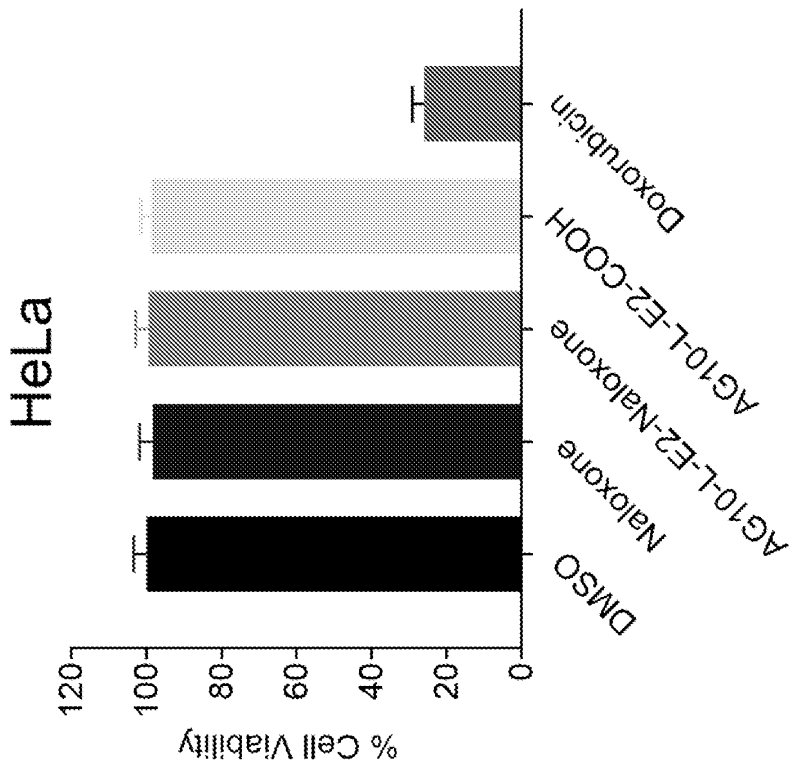

HeLa
% Cell Viability
120
100
80
60
40
20
0
DMSO
Naloxone
AG10-L-E2-Naloxone
AG10-L-E2-COOH
Doxorubicin

FIG. 5i

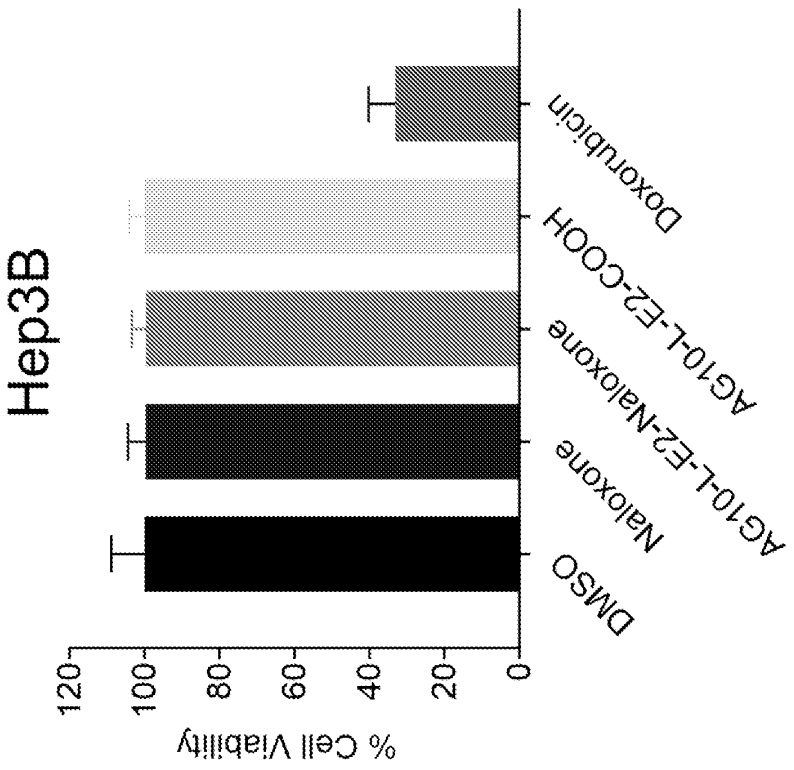

Hep3B
% Cell Viability
120
100
80
60
40
20
0
DMSO
Naloxone
AG10-L-E2-Naloxone
AG10-L-E2-COOH
Doxorubicin

FIG. 5j

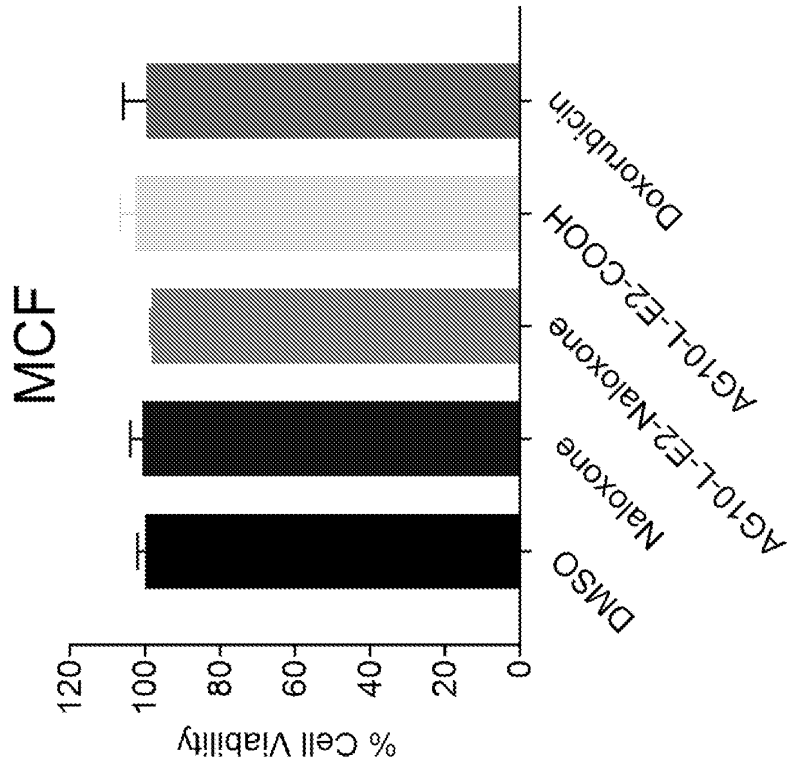

MCF
% Cell Viability
120
100
80
60
40
20
0
DMSO
Naloxone
AG10-L-E2-Naloxone
AG10-L-E2-COOH
Doxorubicin

FIG. 5k

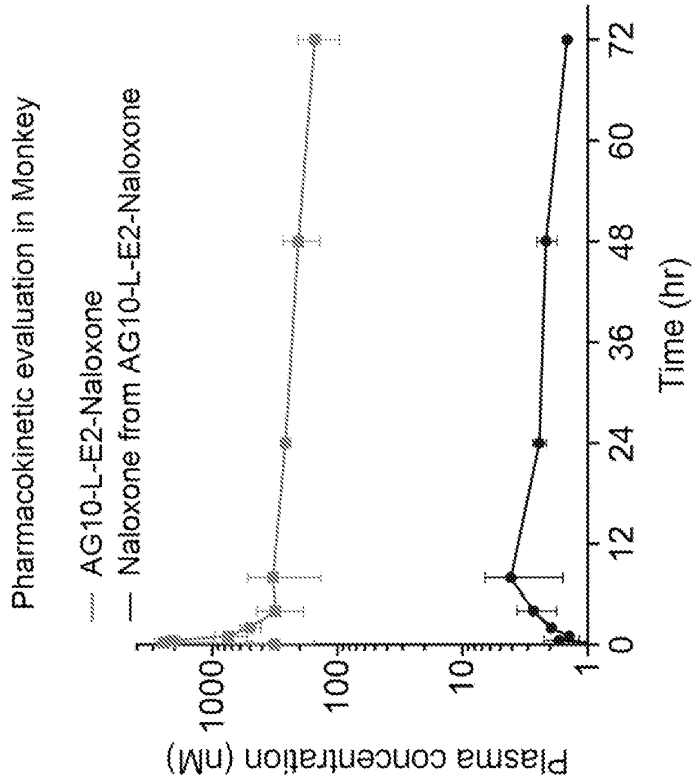

Pharmacokinetic evaluation in Monkey
AG10-L-E2-Naloxone
Naloxone from AG10-L-E2-Naloxone
Plasma concentration (nM)
1000
100
10
1
0
12
24
36
48
60
72
Time (hr)

FIG. 5I

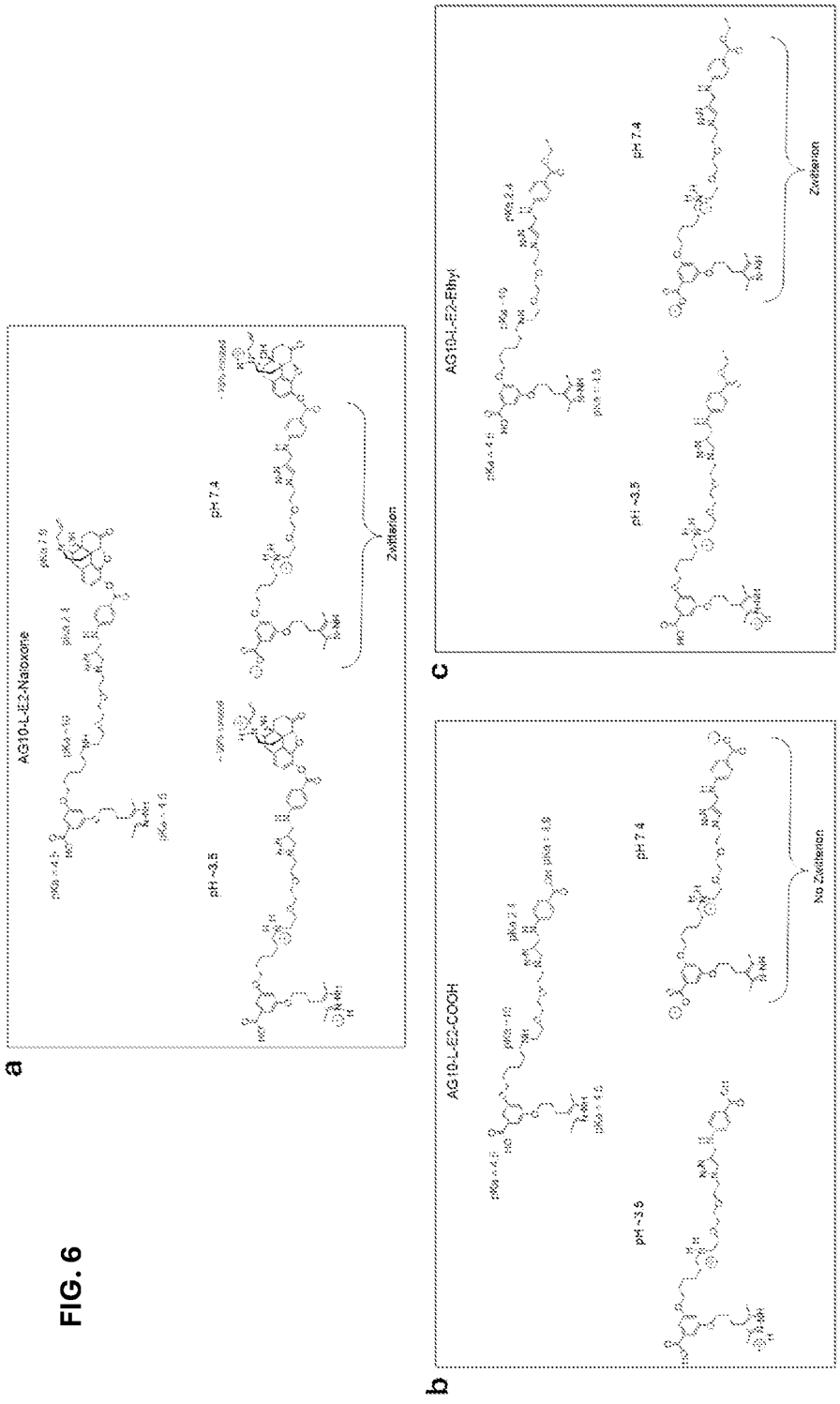

a
AG10-L-E2-Naloxone
pH ~3.5
pH 7.4
Zwitterion
b
AG10-L-E2-COOH
pH ~3.5
pH 7.4
No Zwitterion
c
AG10-L-E2-Ethyl
pH ~3.5
pH 7.4
Zwitterion

FIG. 6

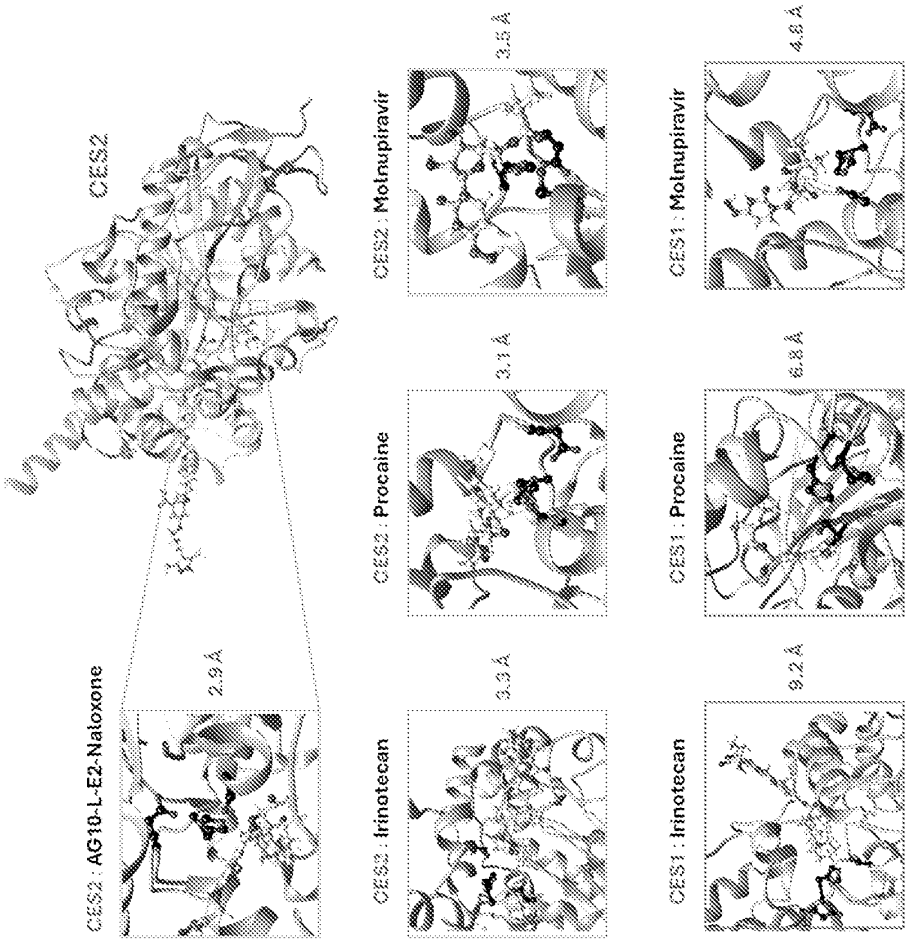

CES2
CES2 : AG10-L-E2-Naloxone
2.9 Å
CES2 : Irinotecan
3.3 Å
CES2 : Procaine
3.1 Å
CES2 : Molnupiravir
3.5 Å
CES1 : Irinotecan
9.2 Å
CES1 : Procaine
6.8 Å
CES1 : Molnupiravir
4.6 Å

FIG. 7

FIG. 8a

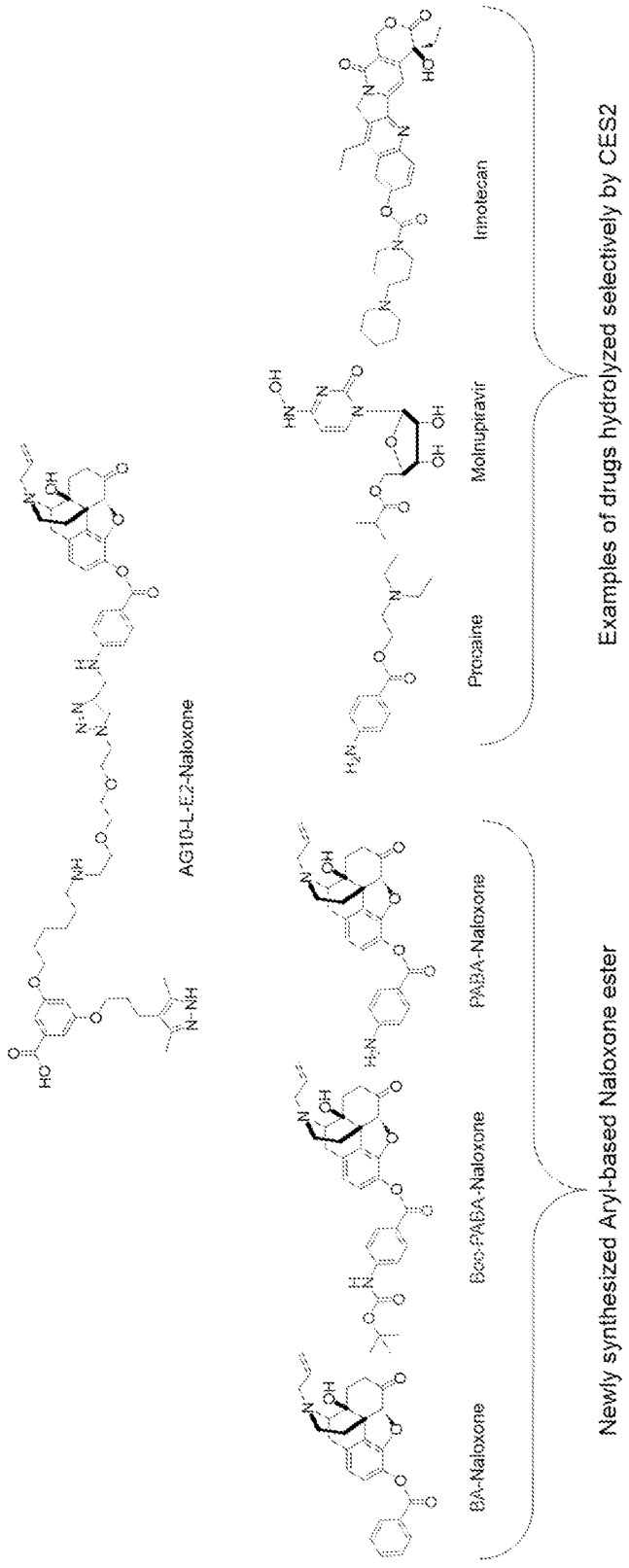

AG10-L-E2-Naloxone
Examples of drugs hydrolyzed selectively by CES2
Irinotecan
Molnupiravir
Procaine
Newly synthesized Aryl-based Naloxone ester
PABA-Naloxone
Boc-PABA-Naloxone
BA-Naloxone

FIG. 8c

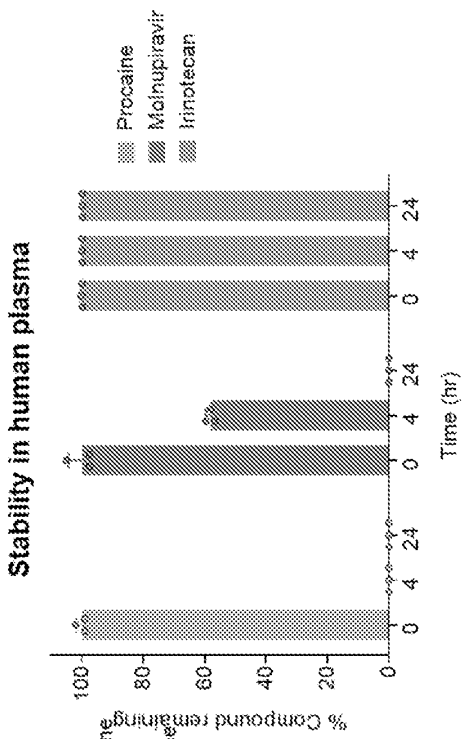

Stability in human plasma
% Compound remaining
Time (hr)
Procaine
Molnupiravir
Irinotecan

FIG. 8b

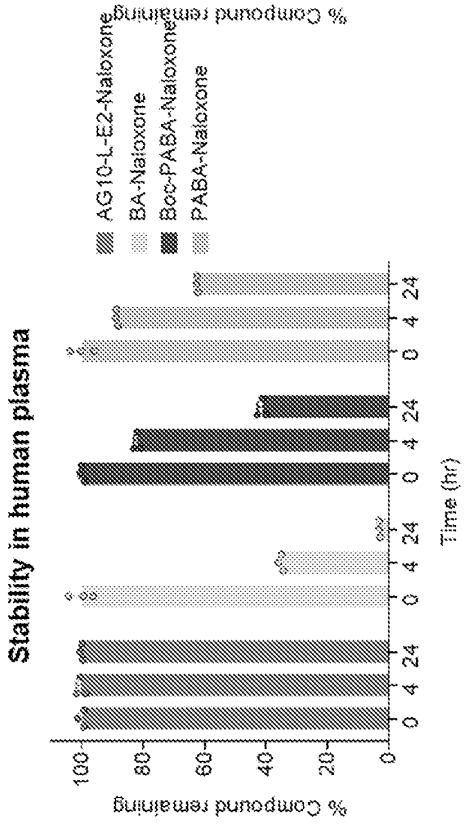

Stability in human plasma
% Compound remaining
Time (hr)
AG10-L-E2-Naloxone
BA-Naloxone
Boc-PABA-Naloxone
PABA-Naloxone

FIG. 8d

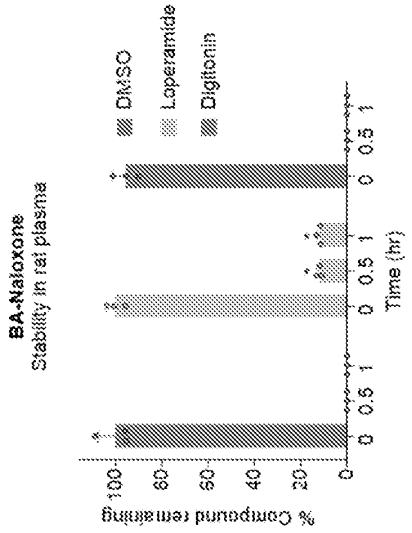


FIG. 8e

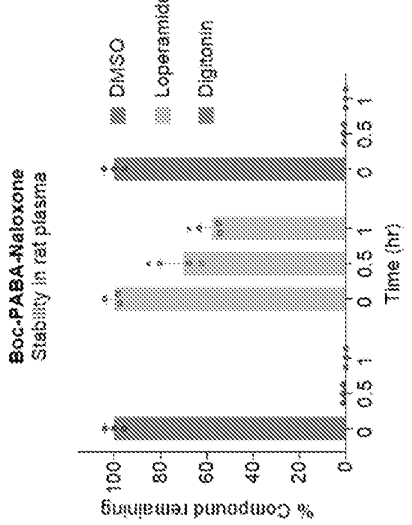


FIG. 8f

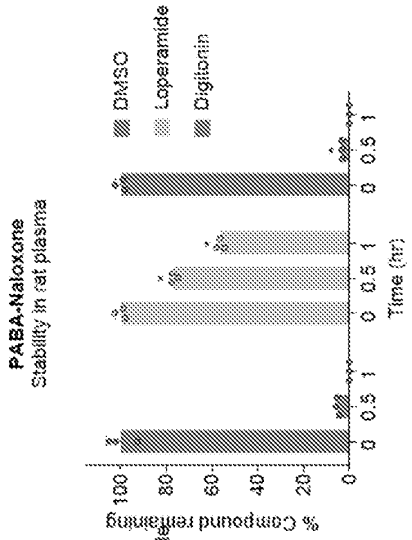


FIG. 8g

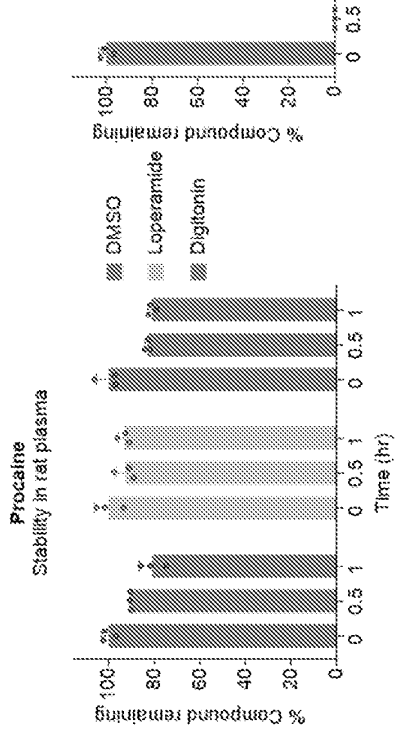


FIG. 8h

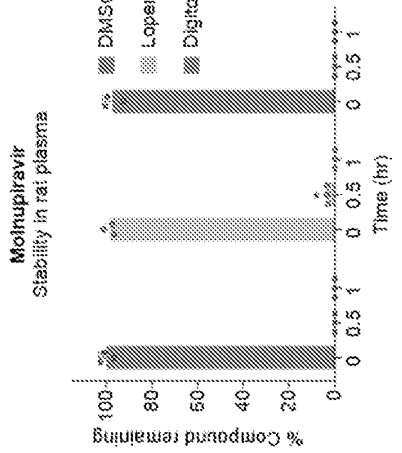


FIG. 8i

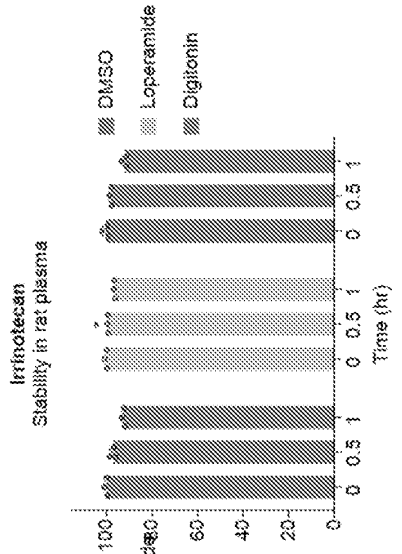
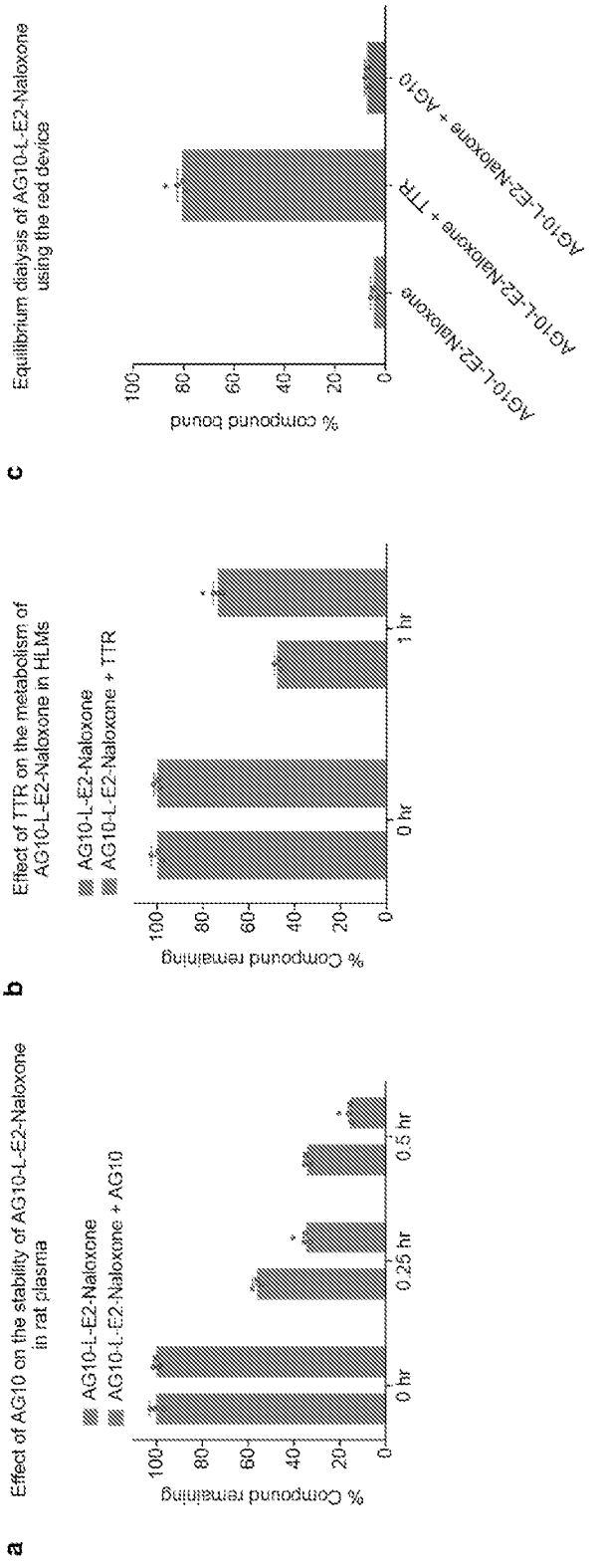


FIG. 9


a
Effect of AG10 on the stability of AG10-L-E2-Naloxone in rat plasma
AG10-L-E2-Naloxone
AG10-L-E2-Naloxone + AG10
% Compound remaining
0 hr
0.25 hr
0.5 hr
b
Effect of TTR on the metabolism of AG10-L-E2-Naloxone in HLMs
AG10-L-E2-Naloxone
AG10-L-E2-Naloxone + TTR
% Compound remaining
0 hr
1 hr
c
Equilibrium dialysis of AG10-L-E2-Naloxone using the red device
% compound bound
AG10-L-E2-Naloxone
AG10-L-E2-Naloxone + TTR
AG10-L-E2-Naloxone + AG10

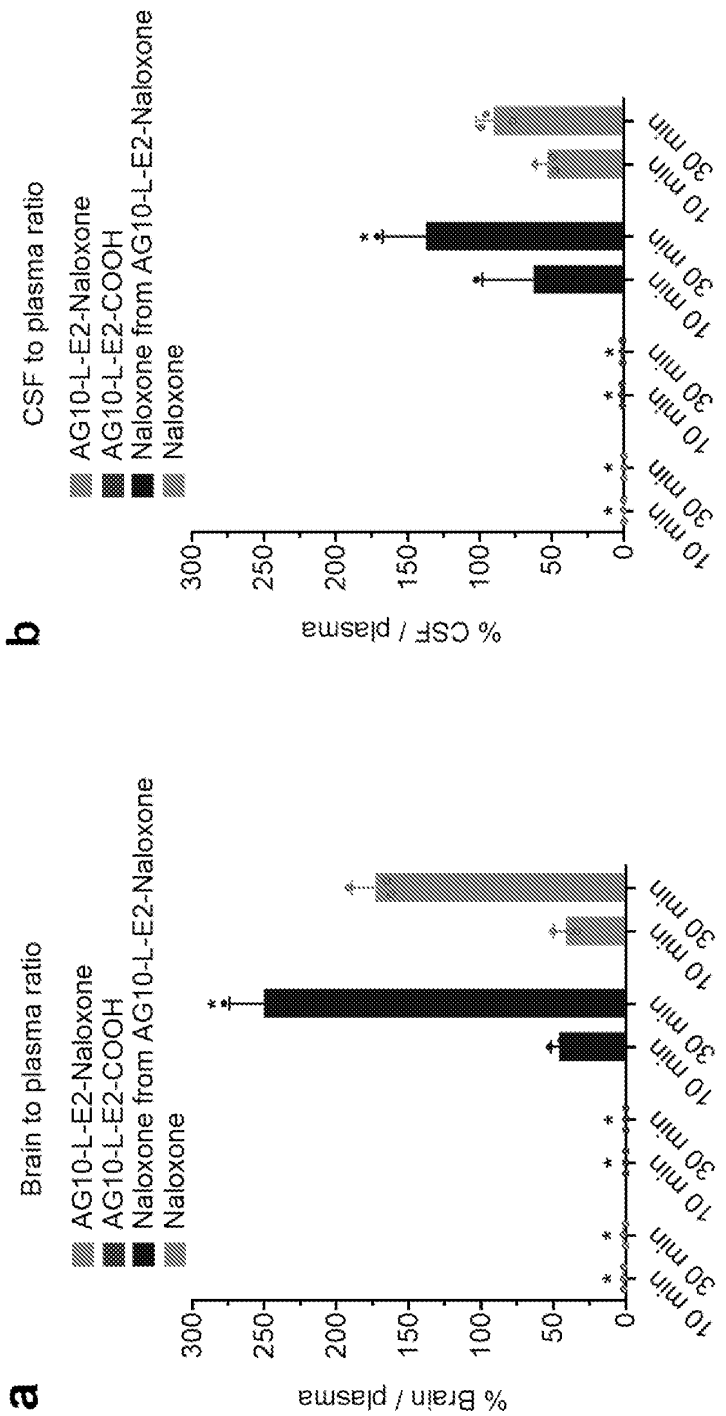

a
Brain to plasma ratio
AG10-L-E2-Naloxone
AG10-L-E2-COOH
Naloxone from AG10-L-E2-Naloxone
Naloxone
% Brain / plasma
0
50
100
150
200
250
300
10 min
30 min
b
CSF to plasma ratio
AG10-L-E2-Naloxone
AG10-L-E2-COOH
Naloxone from AG10-L-E2-Naloxone
Naloxone
% CSF / plasma

FIG. 10

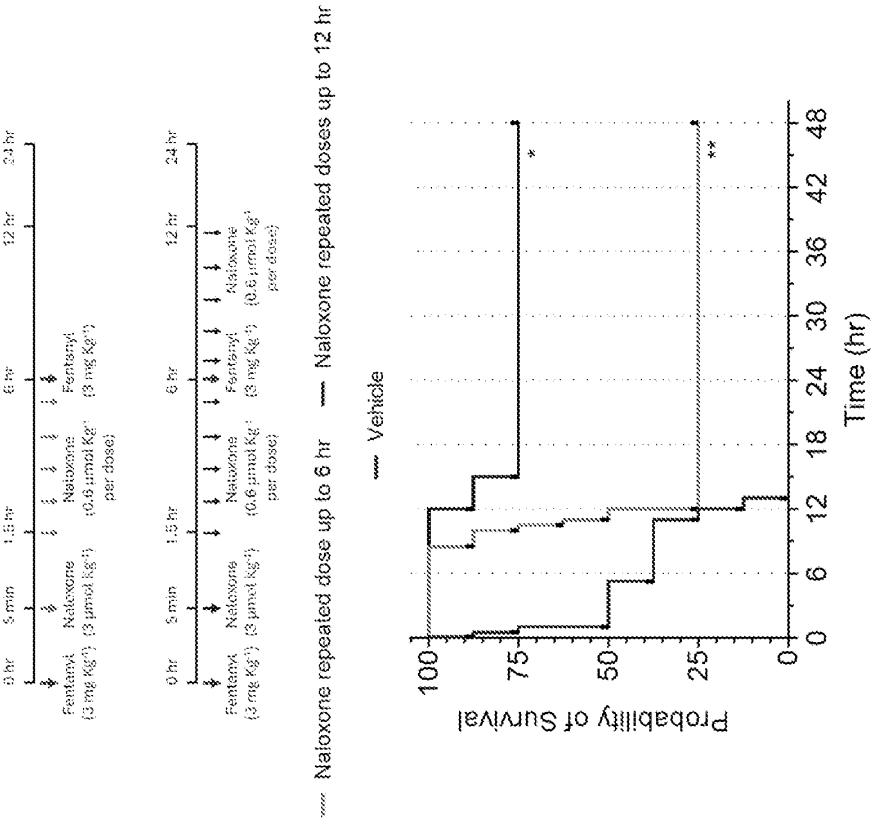

0 hr
5 min
1.5 hr
6 hr
12 hr
24 hr
Fentanyl (3 mg Kg-1)
Naloxone (3 µmol Kg-1)
Naloxone (0.6 µmol Kg-1 per dose)
Fentanyl (3 mg Kg-1)
Naloxone repeated dose up to 6 hr
Naloxone repeated doses up to 12 hr
Vehicle
Probability of Survival
100
75
50
25
0
0
6
12
18
24
30
36
42
48
Time (hr)
*
**

FIG. 11

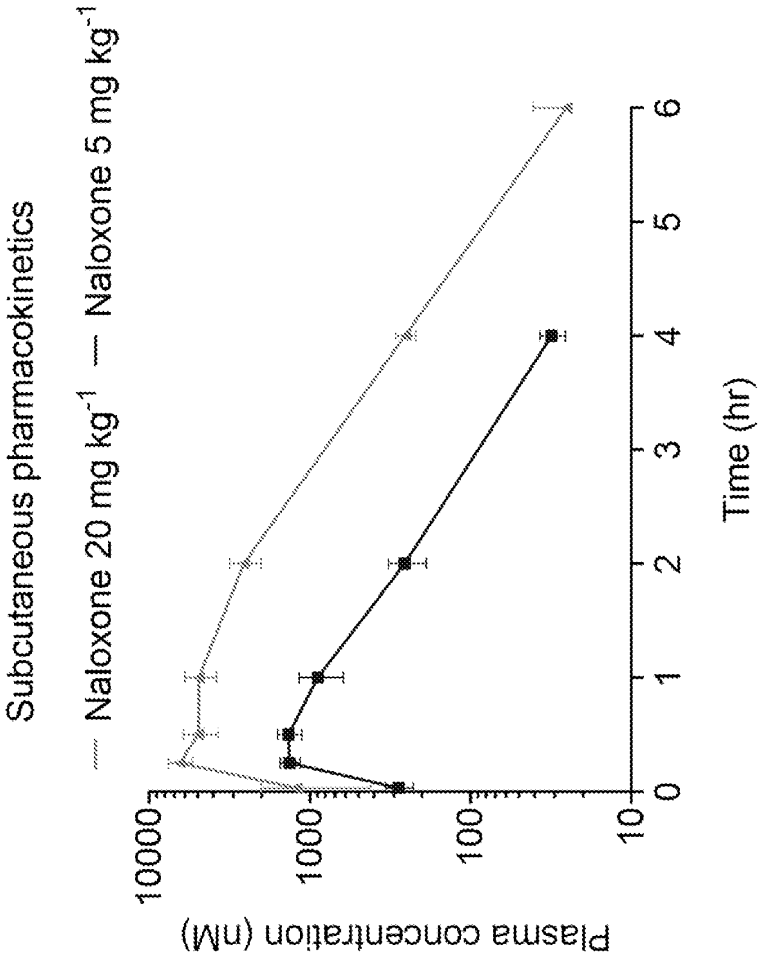
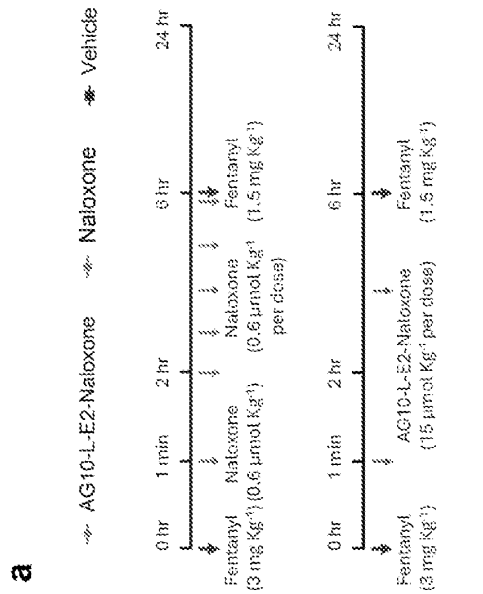

Subcutaneous pharmacokinetics
Naloxone 20 mg kg-1
Naloxone 5 mg kg-1
Plasma concentration (nM)
10000
1000
100
10
0
1
2
3
4
5
6
Time (hr)

FIG. 12

FIG. 13


a
AG10-L-E2-Naloxone
Naloxone
Vehicle
0 hr
1 min
2 hr
6 hr
24 hr
Fentanyl (3 mg Kg-1)
Naloxone (0.6 µmol Kg-1)
Fentanyl (1.5 mg Kg-1)
(0.6 µmol Kg-1 per dose)
AG10-L-E2-Naloxone (15 µmol Kg-1 per dose)
b
1st fentanyl dose
Vehicle (3 deaths)
Minute volume (% of baseline)
Time (min)
c
2nd fentanyl dose
Minute volume (% of baseline)
Time (min)

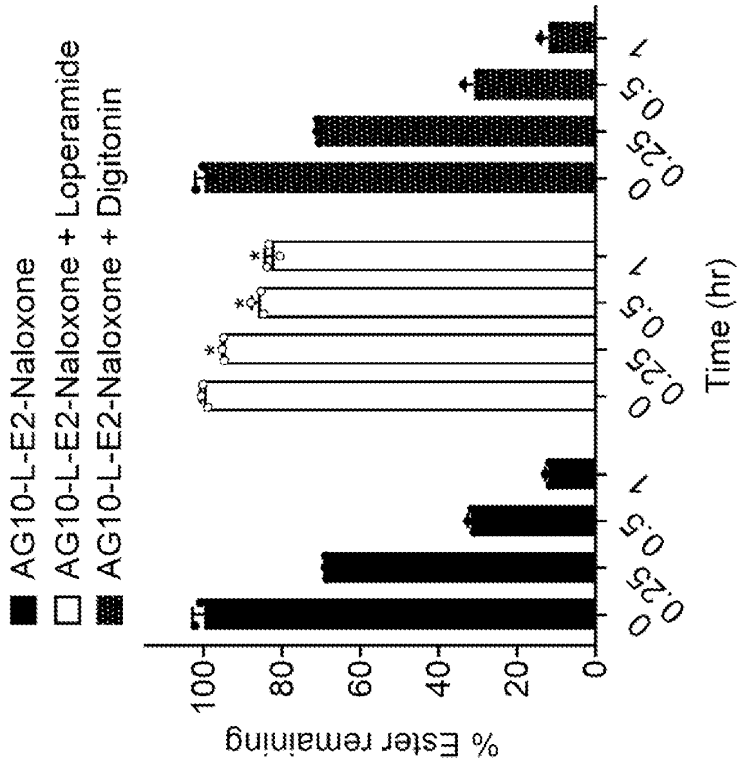

AG10-L-E2-Naloxone
AG10-L-E2-Naloxone + Loperamide
AG10-L-E2-Naloxone + Digitonin
% Ester remaining
100
80
60
40
20
0
0
0.25
0.5
1
Time (hr)

FIG. 14a

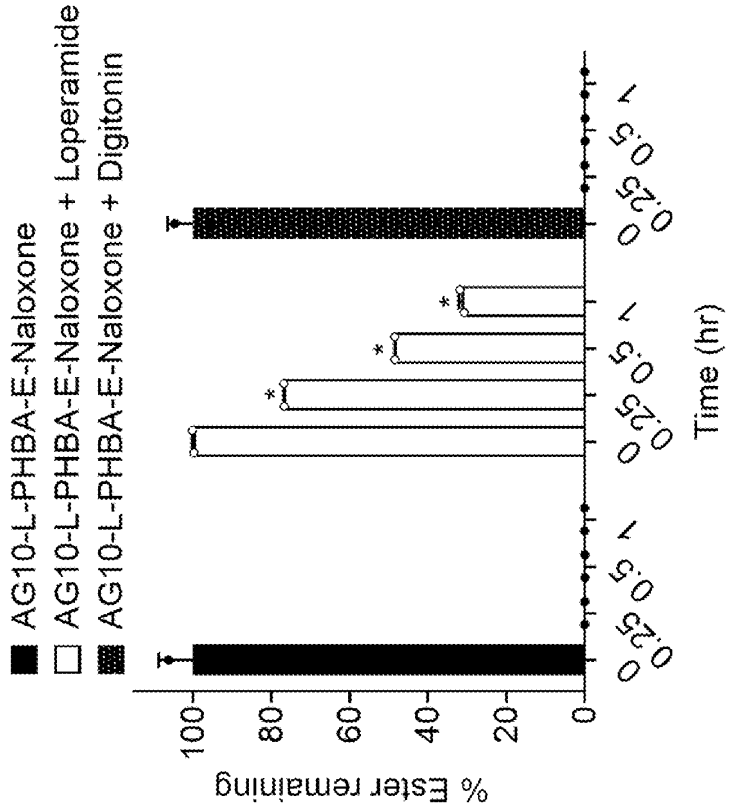

AG10-L-PHBA-E-Naloxone
AG10-L-PHBA-E-Naloxone + Loperamide
AG10-L-PHBA-E-Naloxone + Digitonin
% Ester remaining
100
80
60
40
20
0
0
0.25
0.5
1
Time (hr)
*

FIG. 14b

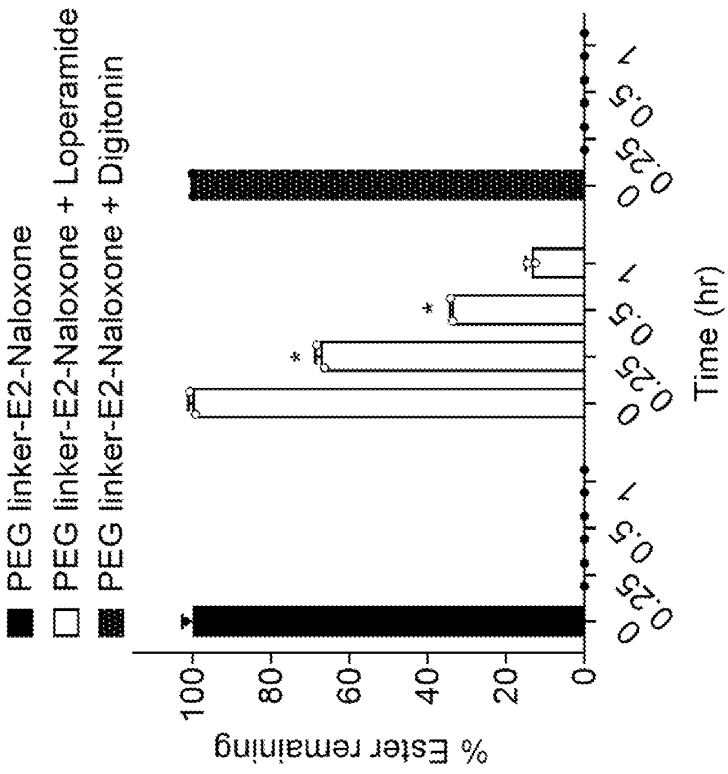

PEG linker-E2-Naloxone
PEG linker-E2-Naloxone + Loperamide
PEG linker-E2-Naloxone + Digitonin
% Ester remaining
100
80
60
40
20
0
0
0.25
0.5
1
Time (hr)
*
*
*

FIG. 14c

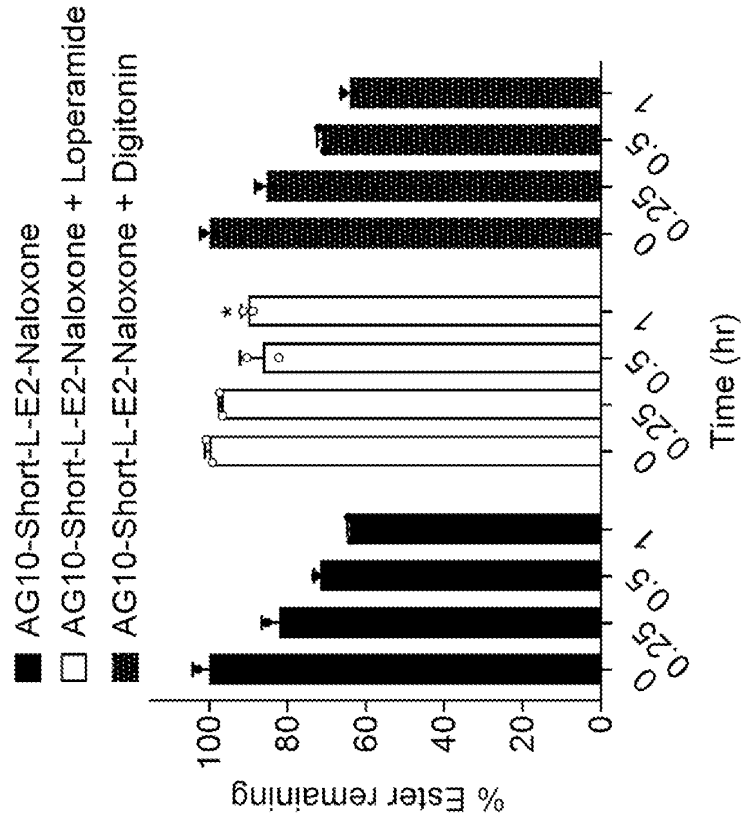

AG10-Short-L-E2-Naloxone
AG10-Short-L-E2-Naloxone + Loperamide
AG10-Short-L-E2-Naloxone + Digitonin
% Ester remaining
100
80
60
40
20
0
0.25
0.5
1
Time (hr)

FIG. 14d

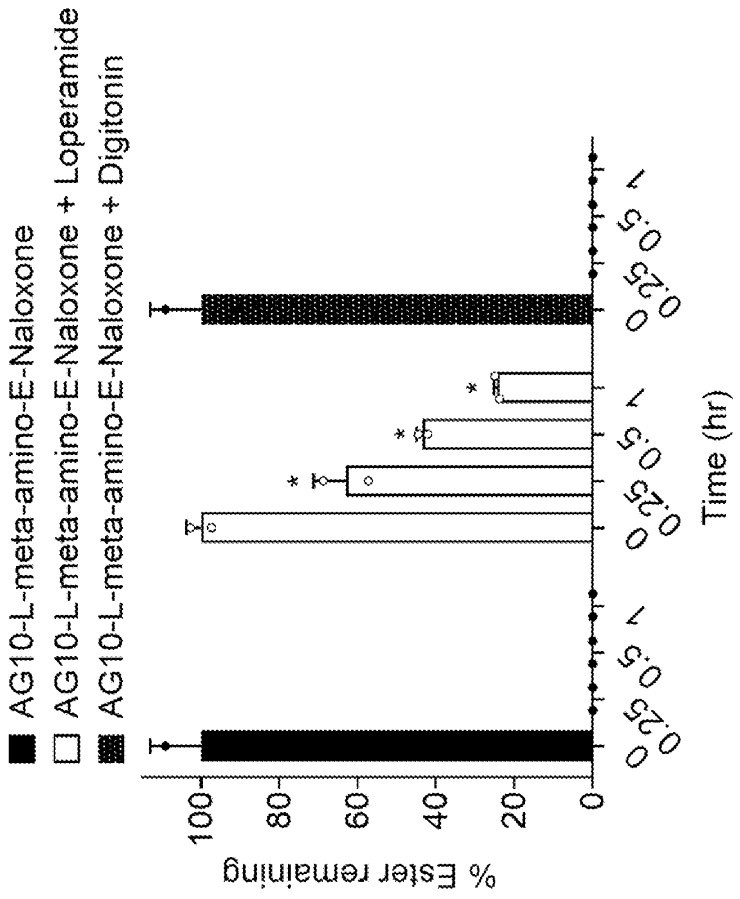

AG10-L-meta-amino-E-Naloxone
AG10-L-meta-amino-E-Naloxone + Loperamide
AG10-L-meta-amino-E-Naloxone + Digitonin
% Ester remaining
100
80
60
40
20
0
*
*
*
*
0
0.25
0.5
1
Time (hr)

FIG. 14e

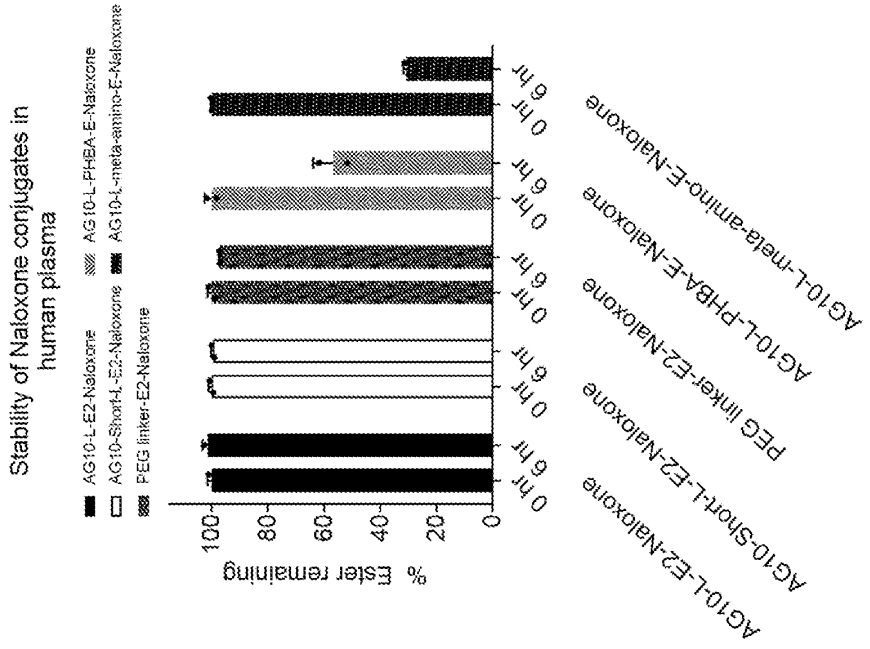

Stability of Naloxone conjugates in human plasma
AG10-L-E2-Naloxone
AG10-Short-L-E2-Naloxone
PEG linker-E2-Naloxone
AG10-L-PHBA-E-Naloxone
AG10-L-meta-amino-E-Naloxone
% Ester remaining
100
80
60
40
20
0
0 hr
6 hr
AG10-L-E2-Naloxone
AG10-Short-L-E2-Naloxone
PEG linker-E2-Naloxone
AG10-L-PHBA-E-Naloxone
AG10-L-meta-amino-E-Naloxone

FIG. 15

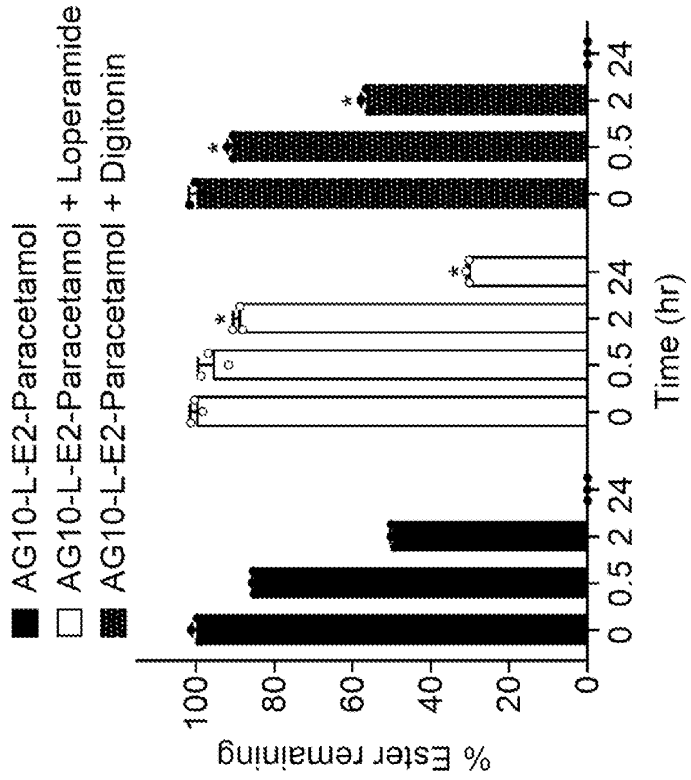

AG10-L-E2-Paracetamol
AG10-L-E2-Paracetamol + Loperamide
AG10-L-E2-Paracetamol + Digitonin
% Ester remaining
100
80
60
40
20
0
0 0.5 2 24
0 0.5 2 24
0 0.5 2 24
Time (hr)

FIG. 16a

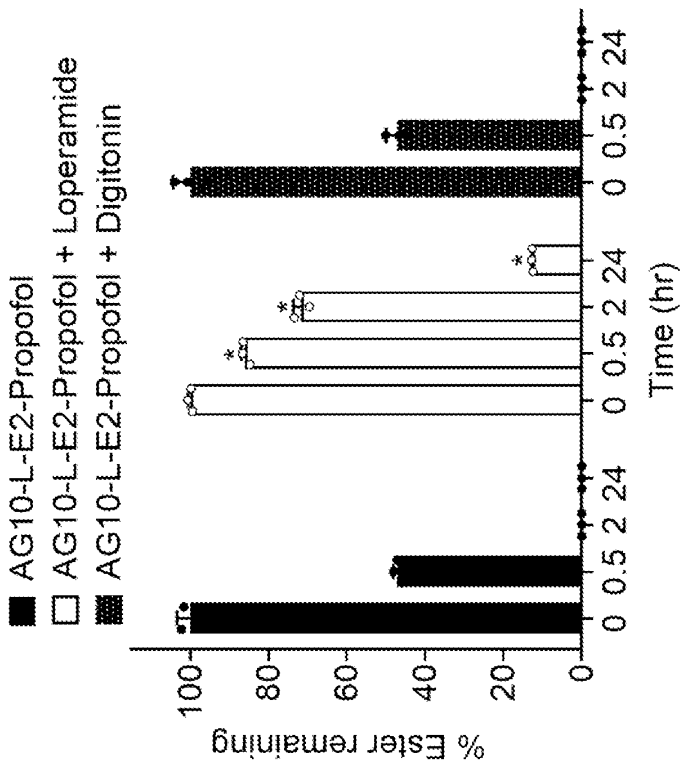

AG10-L-E2-Propofol
AG10-L-E2-Propofol + Loperamide
AG10-L-E2-Propofol + Digitonin
% Ester remaining
100
80
60
40
20
0
0 0.5 2 24
0 0.5 2 24
0 0.5 2 24
Time (hr)

FIG. 16b

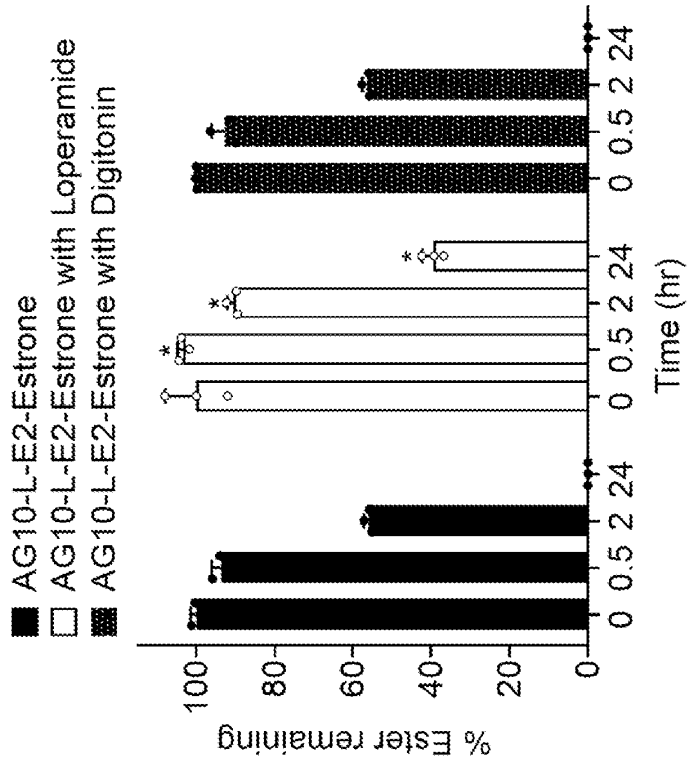

AG10-L-E2-Estrone
AG10-L-E2-Estrone with Loperamide
AG10-L-E2-Estrone with Digitonin
% Ester remaining
0
20
40
60
80
100
0 0.5 2 24
0 0.5 2 24
0 0.5 2 24
Time (hr)

FIG. 16c

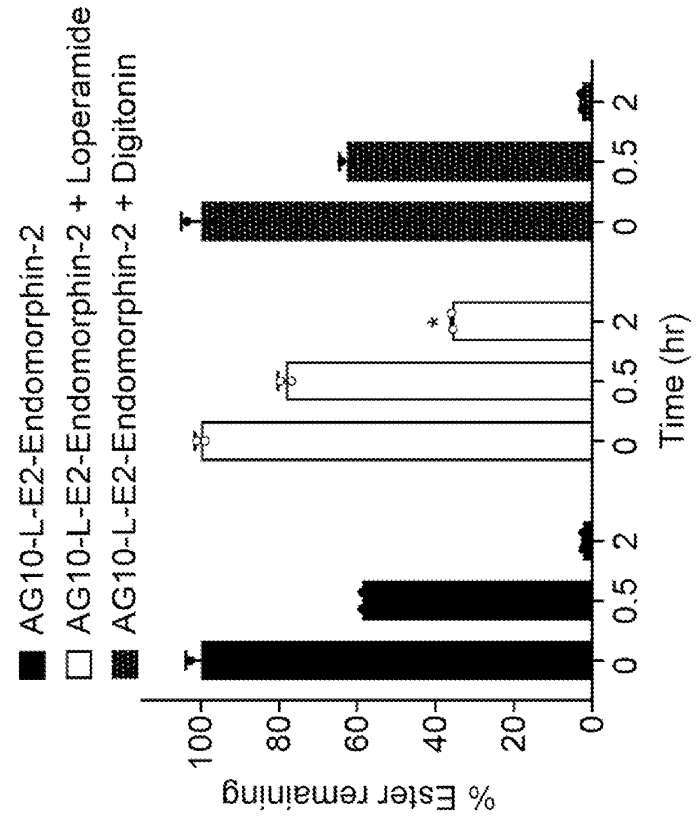

AG10-L-E2-Endomorphin-2
AG10-L-E2-Endomorphin-2 + Loperamide
AG10-L-E2-Endomorphin-2 + Digitonin
% Ester remaining
100
80
60
40
20
0
0 0.5 2 0 0.5 2 0 0.5 2
Time (hr)
*

FIG. 16d

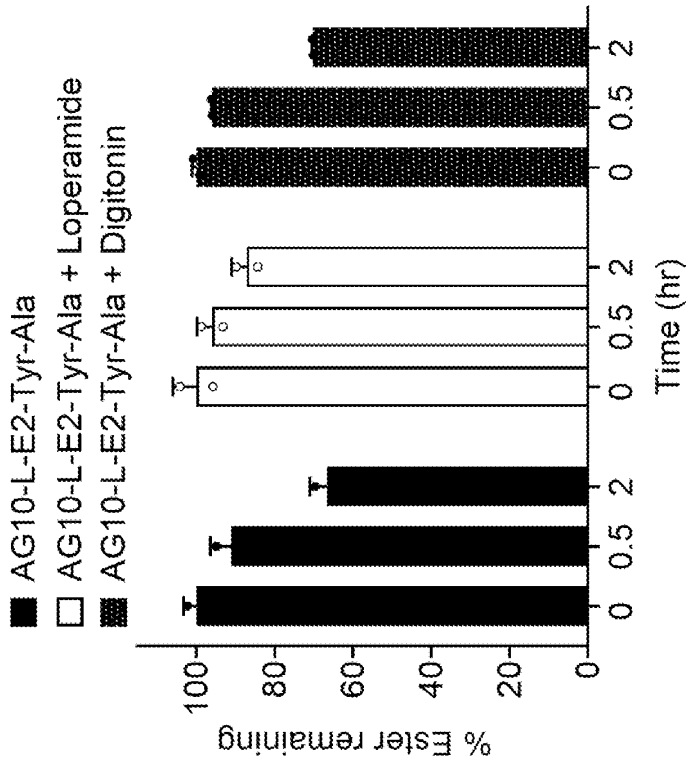

AG10-L-E2-Tyr-Ala
AG10-L-E2-Tyr-Ala + Loperamide
AG10-L-E2-Tyr-Ala + Digitonin
% Ester remaining
100
80
60
40
20
0
0
0.5
2
0
0.5
2
0
0.5
2
Time (hr)

FIG. 16e

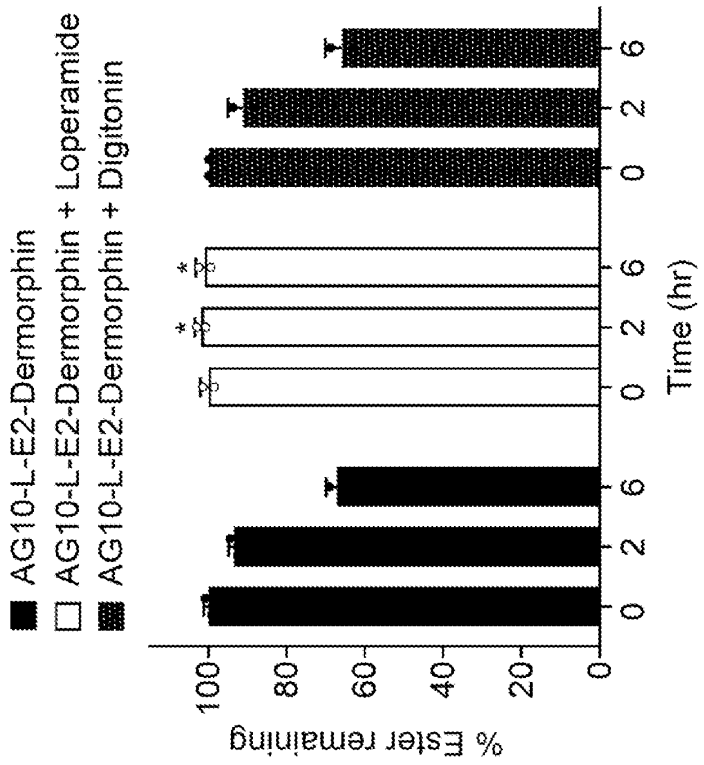

AG10-L-E2-Dermorphin
AG10-L-E2-Dermorphin + Loperamide
AG10-L-E2-Dermorphin + Digitonin
% Ester remaining
100
80
60
40
20
0
0
2
6
0
2
6
Time (hr)
*
*

FIG. 16f

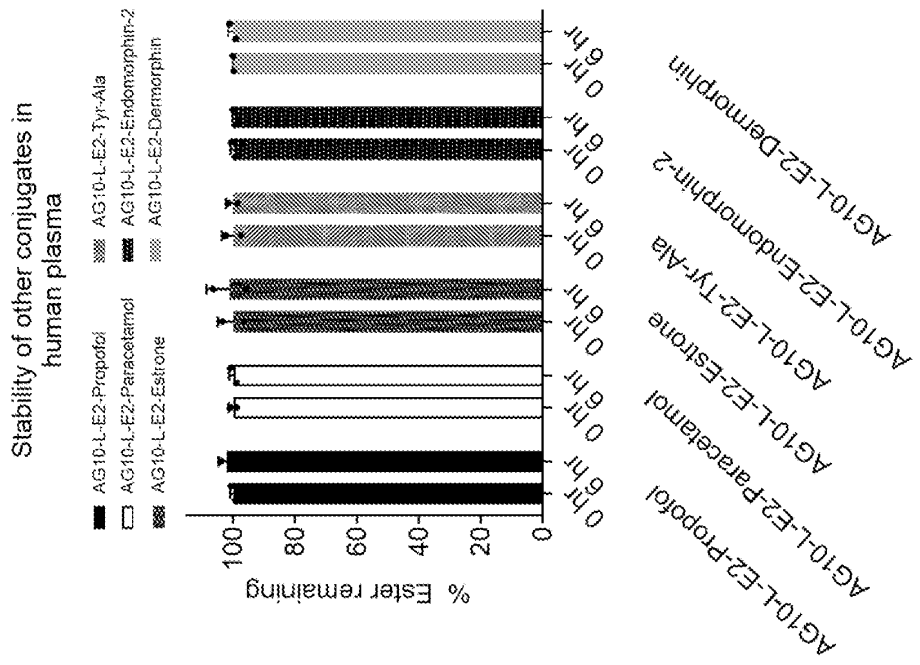

Stability of other conjugates in human plasma
AG10-L-E2-Propofol
AG10-L-E2-Paracetamol
AG10-L-E2-Estrone
AG10-L-E2-Tyr-Ala
AG10-L-E2-Endomorphin-2
AG10-L-E2-Dermorphin
% Ester remaining
100
80
60
40
20
0
0 hr
6 hr
AG10-L-E2-Propofol
AG10-L-E2-Paracetamol
AG10-L-E2-Estrone
AG10-L-E2-Tyr-Ala
AG10-L-E2-Endomorphin-2
AG10-L-E2-Dermorphin

FIG. 17