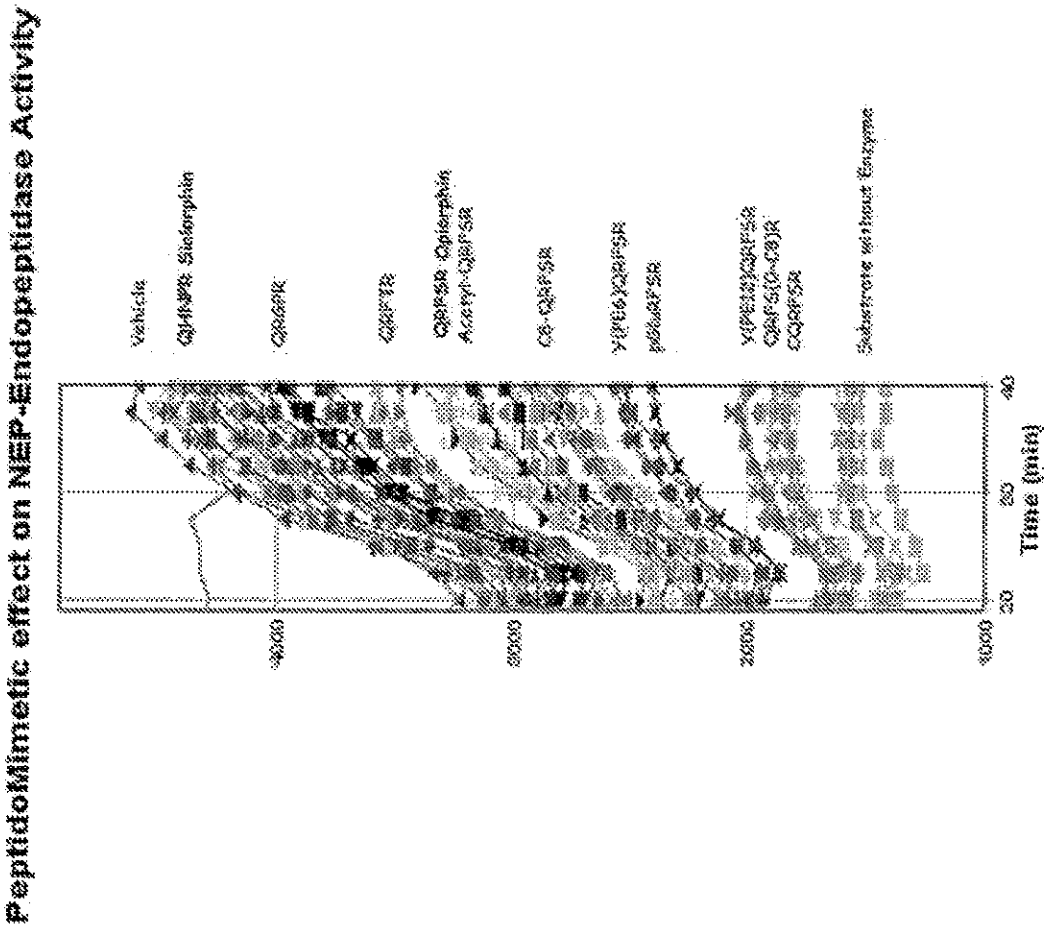

PeptidoMimetic effect on NEP-Endopeptidase Activity
Vehicle
QHNPR Sialorphin
QRSPR
QRFTR
QRFSR Opiorphin
Acetyl-QRFSR
C6-QRFSR
Substrate without Enzyme
Time (min)

FIG.2

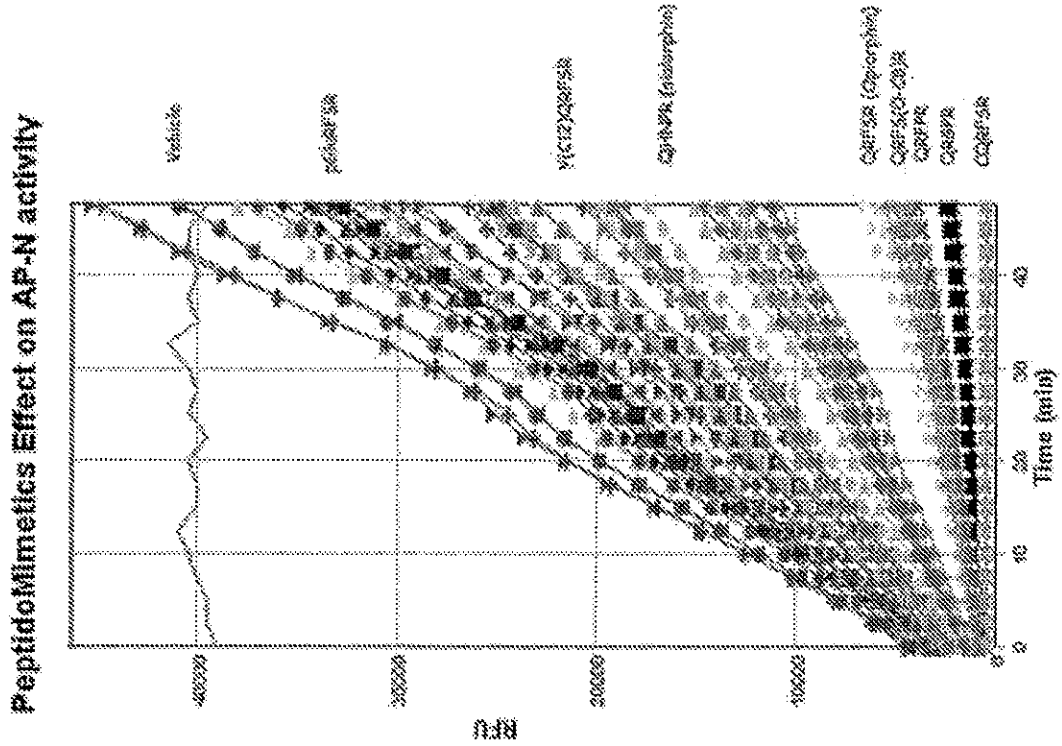

PeptidoMimetics Effect on AP-N activity
Vehicle
Y(C12)QRFSR
QHNPR (sialorphin)
QRFSR (Opiorphin)
QRFSR
Time (min)
RFU

FIG.1

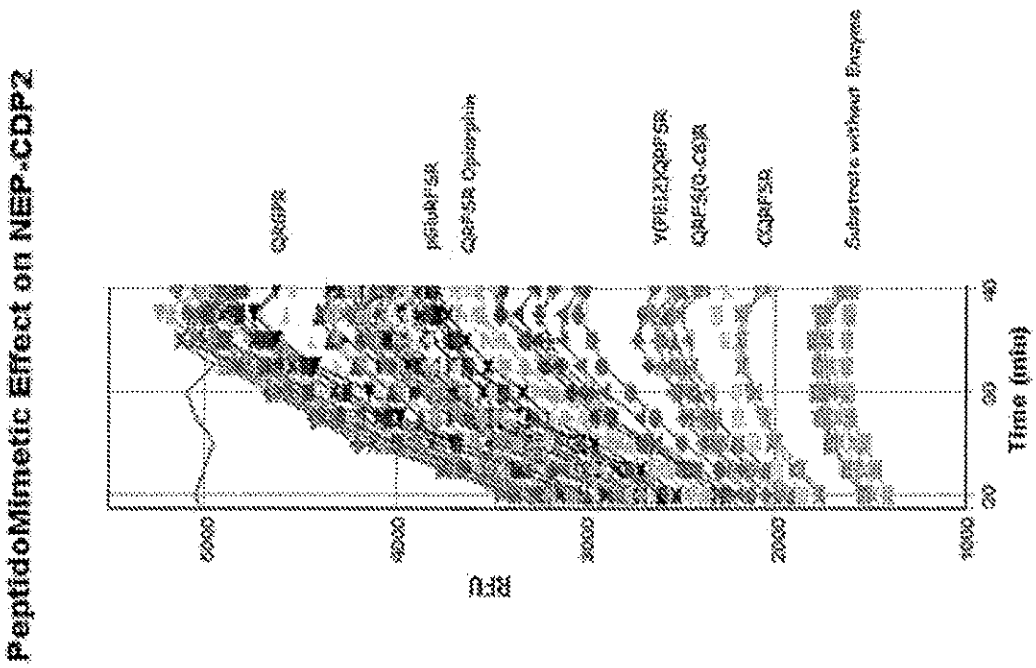

PeptidoMimetic Effect on NEP-CDP2
RFU
Time (min)
QAFSR
pSluAFSR
QAFSR Opiorphin
Y(PE1Z)QAFSR
QAFS(O-C8)R
CQAFSR
Substrate without Enzyme

FIG.4

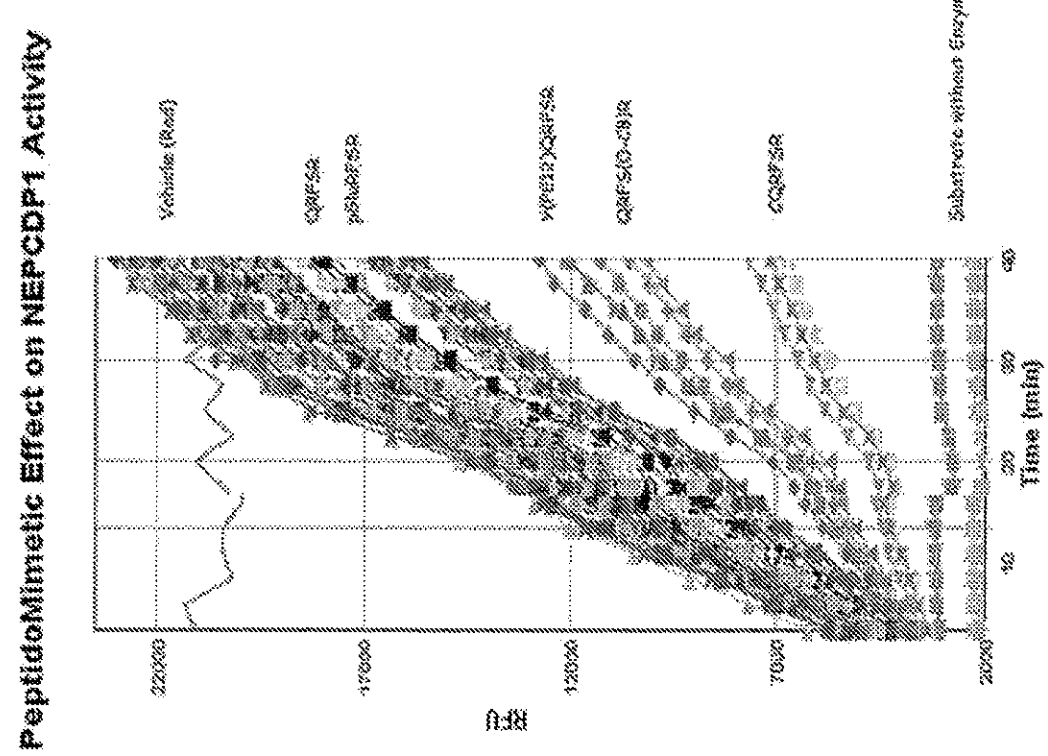

PeptidoMimetic Effect on NEPCDP1 Activity
RFU
Time (min)
Vehicle (Red)
QAFSR
pSluAFSR
Y(PE1Z)QAFSR
QAFS(O-C8)R
CQAFSR
Substrate without Enzyme

FIG.3

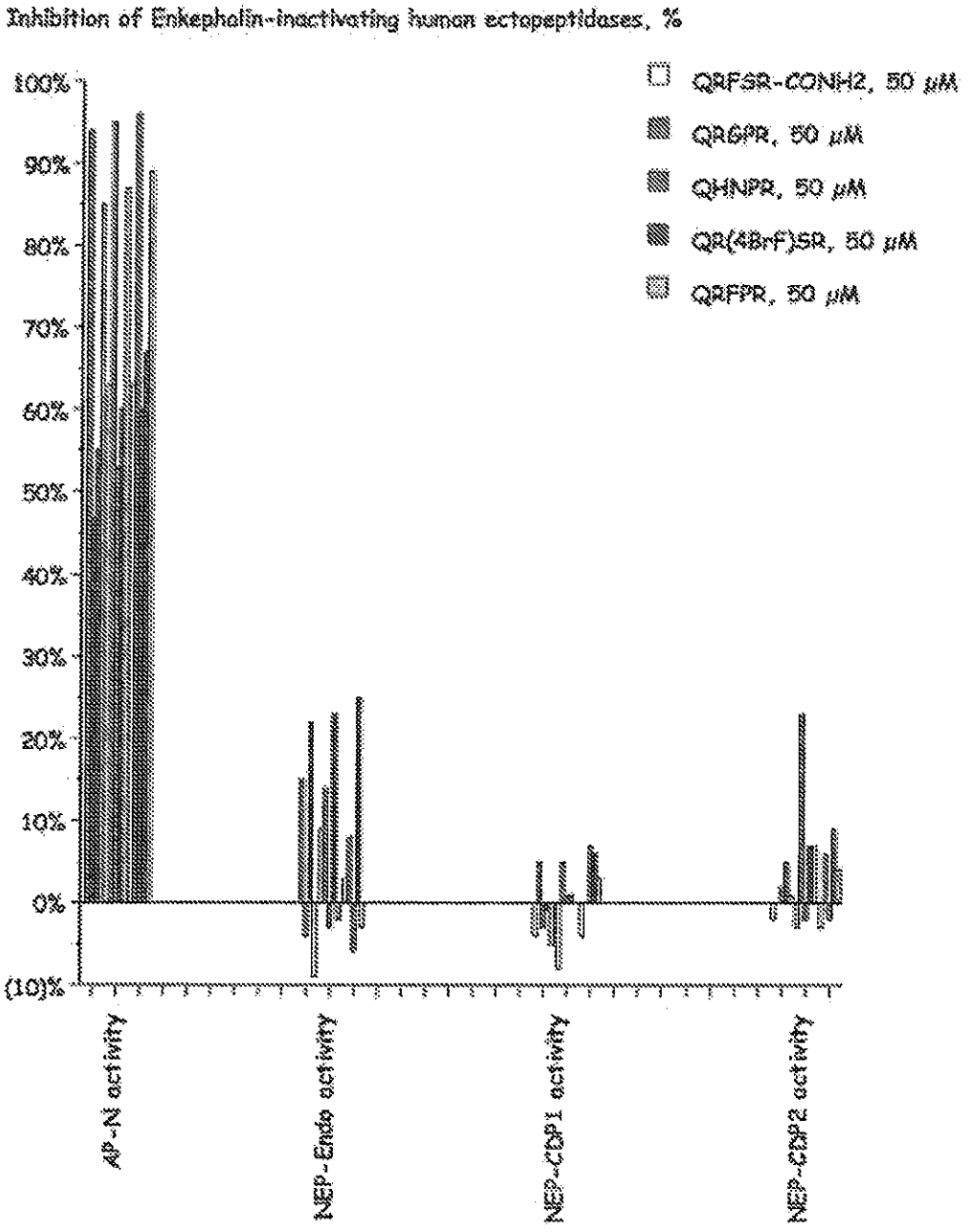

Inhibition of Enkephalin-inactivating human ectopeptidases, %
QRFSR-CONH2, 50 µM
QRGPR, 50 µM
QHNPR, 50 µM
QR(4BrF)SR, 50 µM
QRFPR, 50 µM
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
(10)%
Ap-N activity
NEP-Endo activity
NEP-CDP1 activity
NEP-CDP2 activity

FIG.5

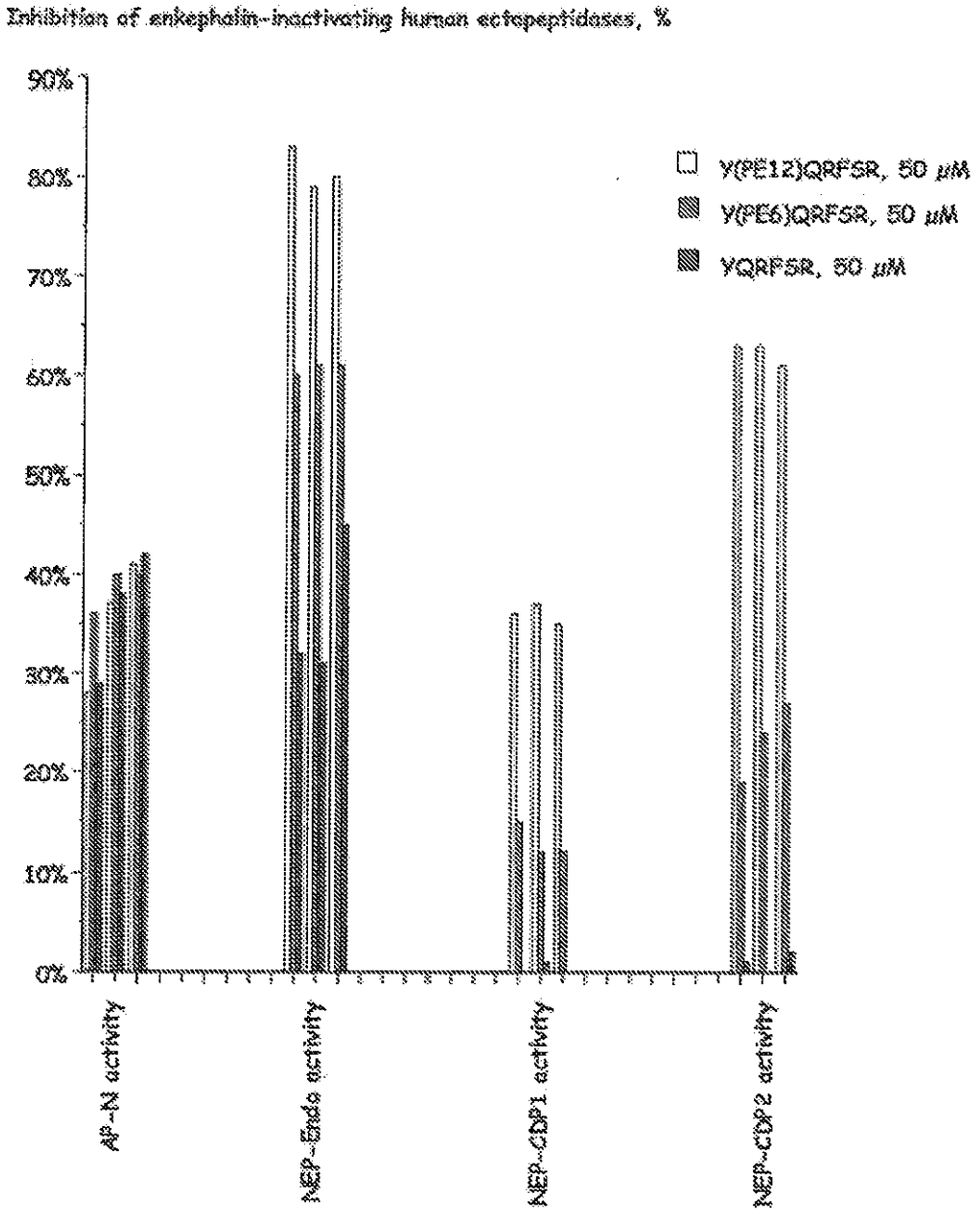

Inhibition of enkephalin-inactivating human ectopeptidases, %
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Y(PE12)QRFSR, 50 µM
Y(PE6)QRFSR, 50 µM
YQRFSR, 50 µM
AP-N activity
NEP-Endo activity
NEP-CDP1 activity
NEP-CDP2 activity

FIG.6

FIG.7

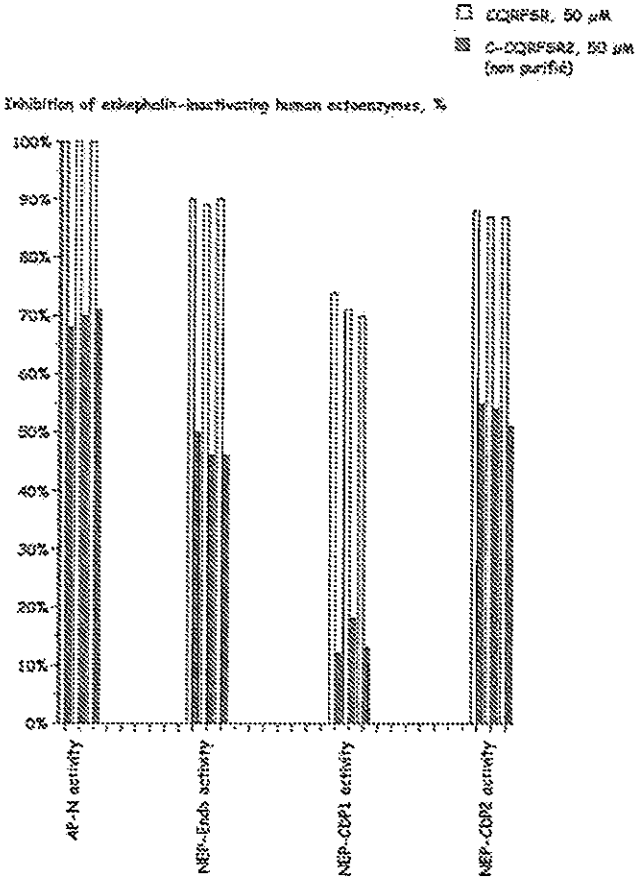
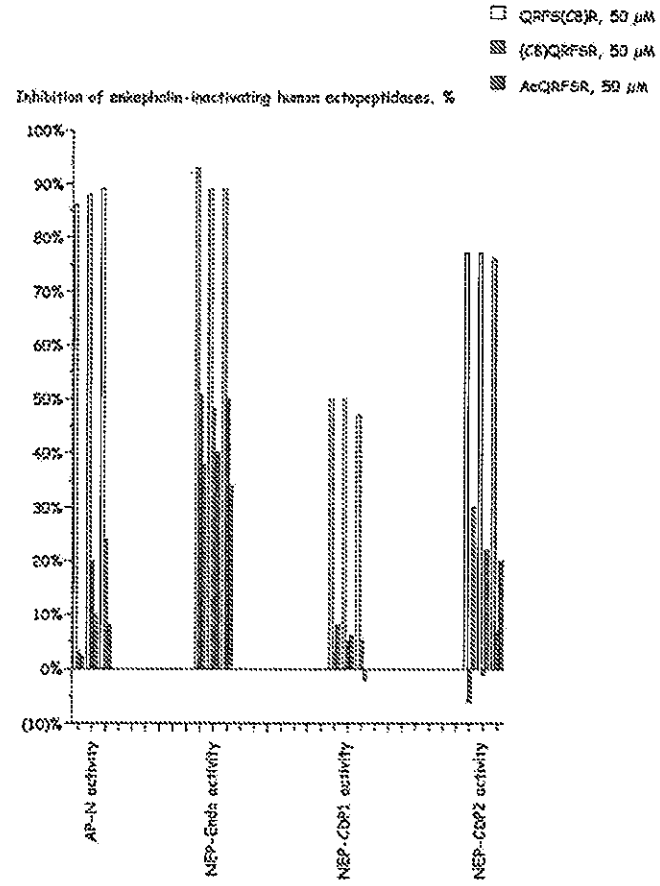

EQRFSR, 50 µM
C-EQRFSR2, 50 µM (non purified)
Inhibition of enkephalin-inactivating human ectoenzymes, %
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
AP-N activity
NEP-Endo activity
NEP-CDP1 activity
NEP-CDP2 activity

FIG.8


QRFS(CB)R, 50 µM
(CB)QRFSR, 50 µM
AcQRFSR, 50 µM
Inhibition of enkephalin-inactivating human ectopeptidases, %
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
(10)%
AP-N activity
NEP-Endo activity
NEP-CDP1 activity
NEP-CDP2 activity

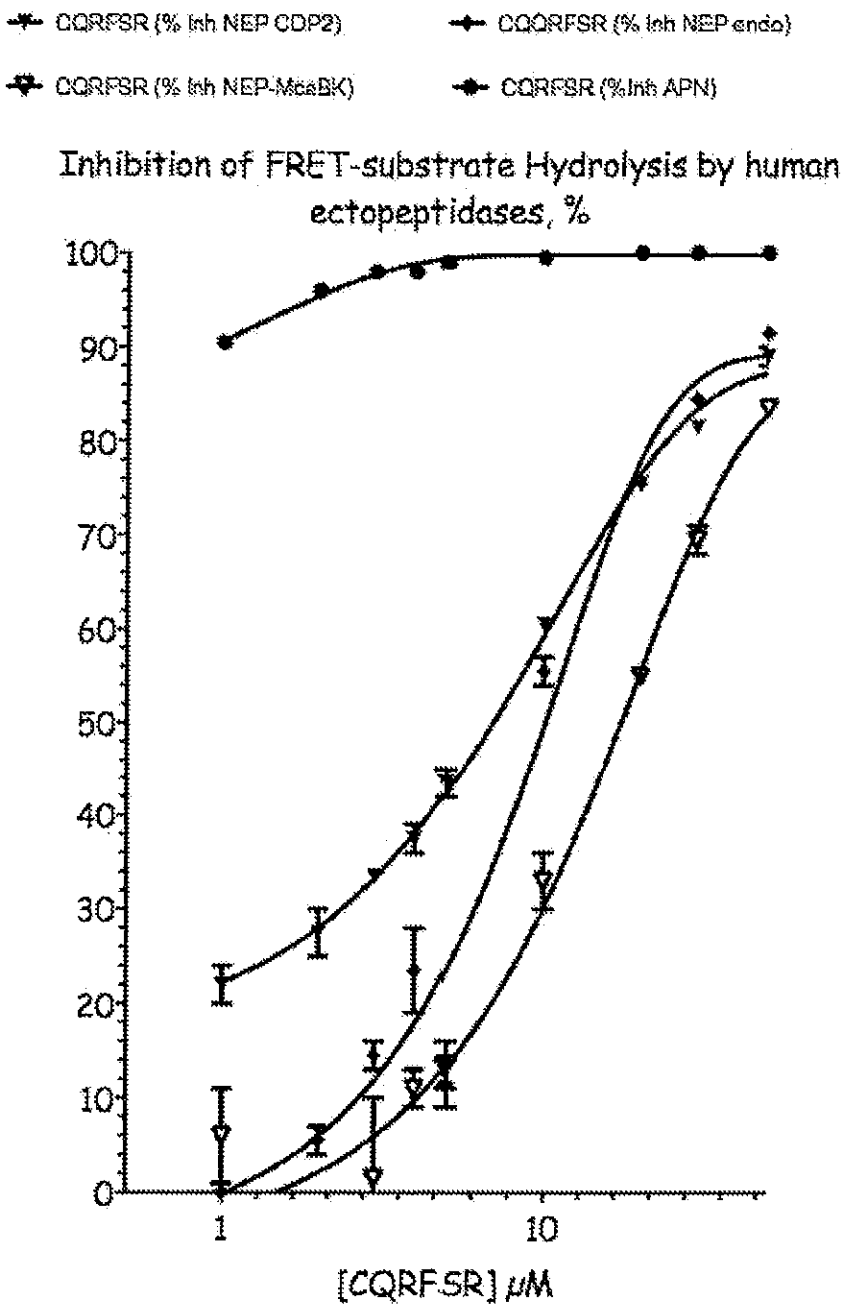

CQRFSR (% Inh NEP CDP2)
CQQRFSR (% Inh NEP endo)
CQRFSR (% Inh NEP-MceBK)
CQRFSR (%Inh APN)
Inhibition of FRET-substrate Hydrolysis by human ectopeptidases, %
100
90
80
70
60
50
40
30
20
10
0
1
10
[CQRFSR] µM

FIG.9

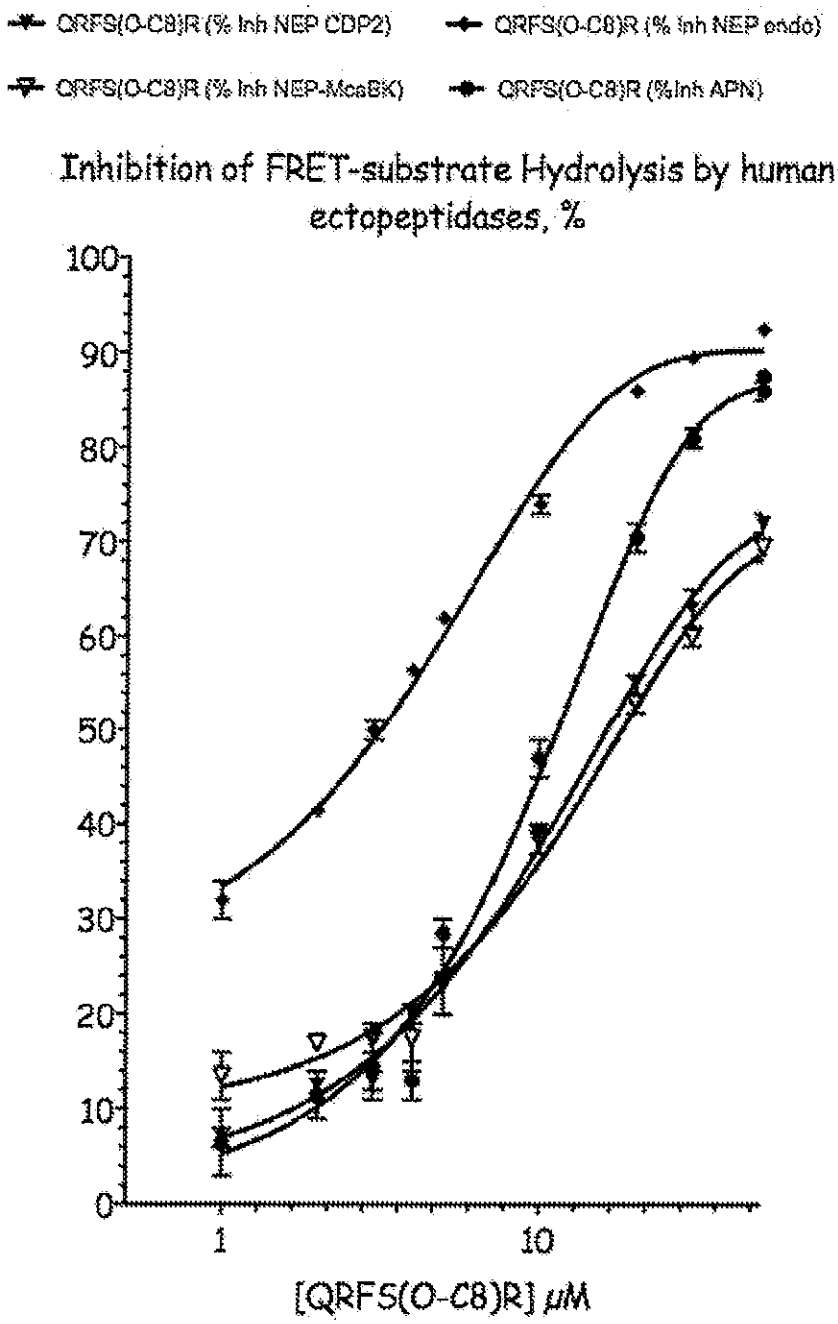

QRFS(O-C8)R (% Inh NEP CDP2)
QRFS(O-C8)R (% Inh NEP endo)
QRFS(O-C8)R (% Inh NEP-McaBK)
QRFS(O-C8)R (%Inh APN)
Inhibition of FRET-substrate Hydrolysis by human ectopeptidases, %
100
90
80
70
60
50
40
30
20
10
0
1
10
[QRFS(O-C8)R] µM

FIG.10

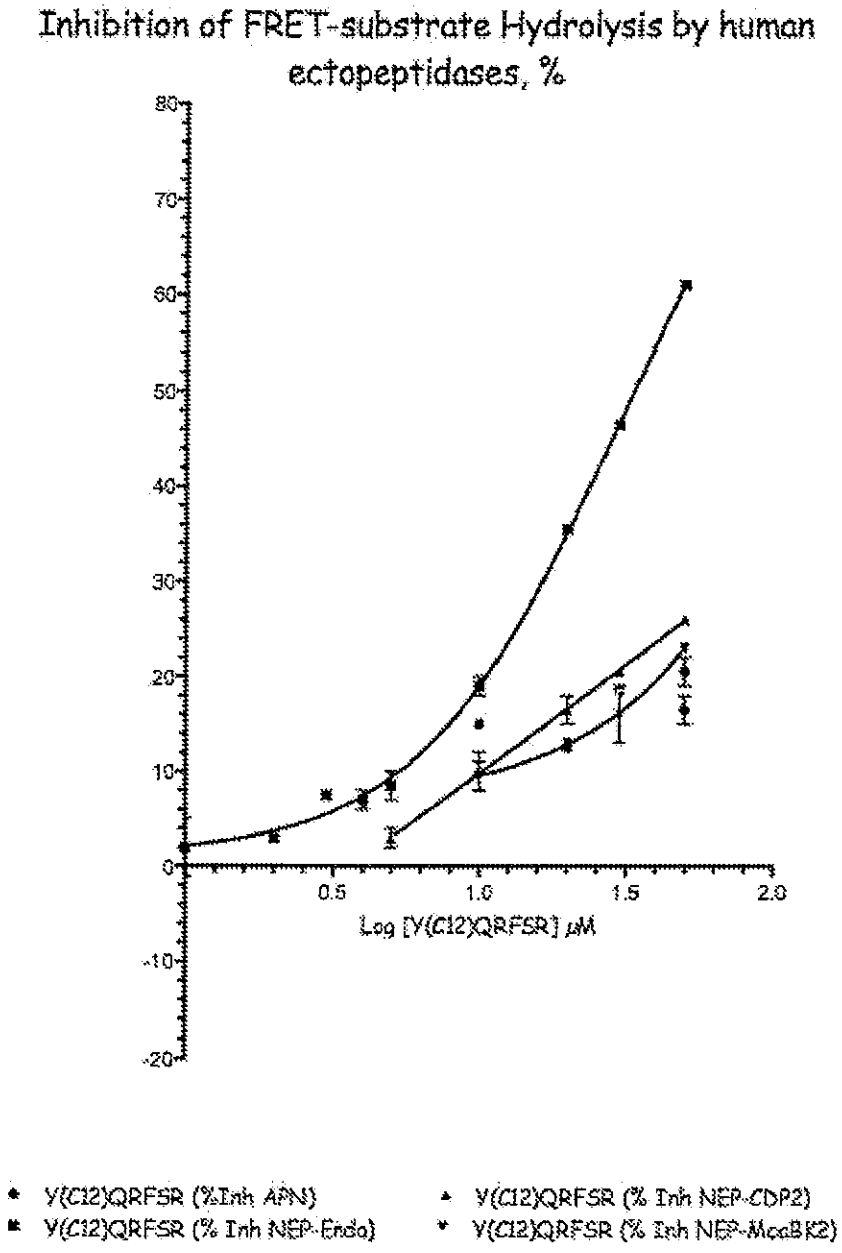

Inhibition of FRET-substrate Hydrolysis by human ectopeptidases, %
80
70
60
50
40
30
20
10
0
-10
-20
0.5
1.0
1.5
2.0
Log [Y(C12)QRFSR] µM
Y(C12)QRFSR (%Inh APN)
Y(C12)QRFSR (% Inh NEP-Endo)
Y(C12)QRFSR (% Inh NEP-CDP2)
Y(C12)QRFSR (% Inh NEP-MccBK2)

FIG.11

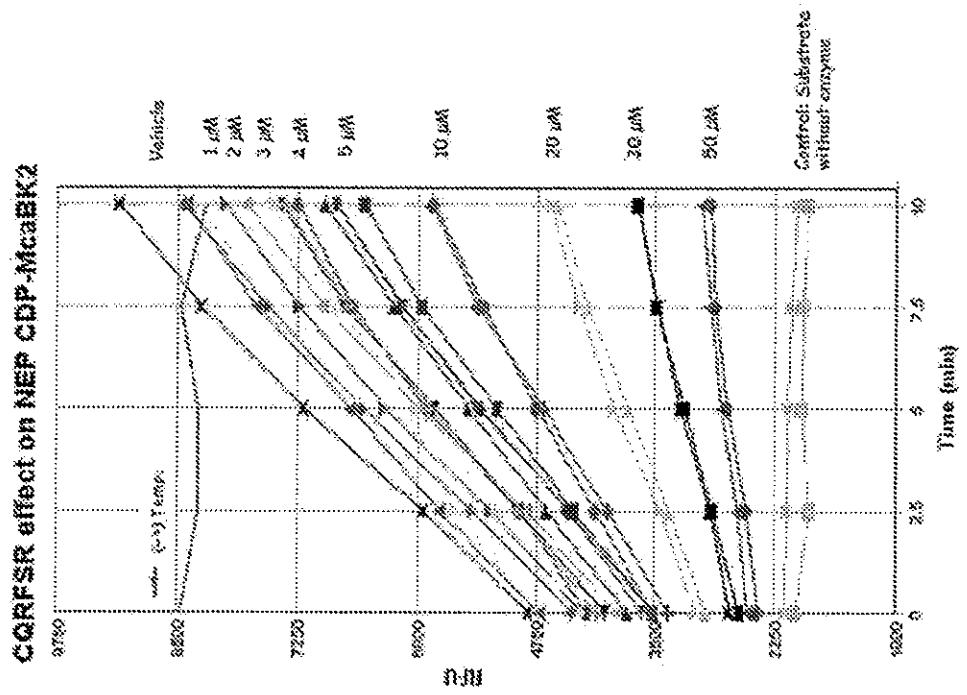

CQRFSR effect on NEP CDP-McaBK2
Vehicle
1 µM
2 µM
3 µM
4 µM
5 µM
10 µM
20 µM
30 µM
50 µM
Control: Substrate without enzyme
RFU
Time (min)

FIG.13

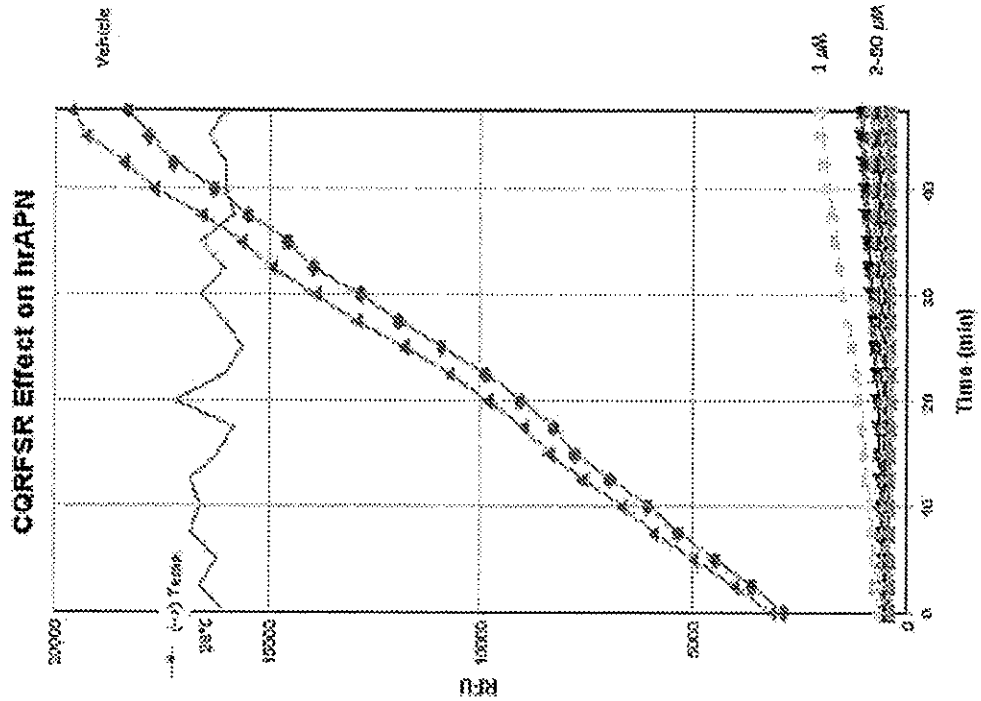

CQRFSR Effect on hrAPN
Vehicle
1 µM
2-50 µM
RFU
Time (min)

FIG.12

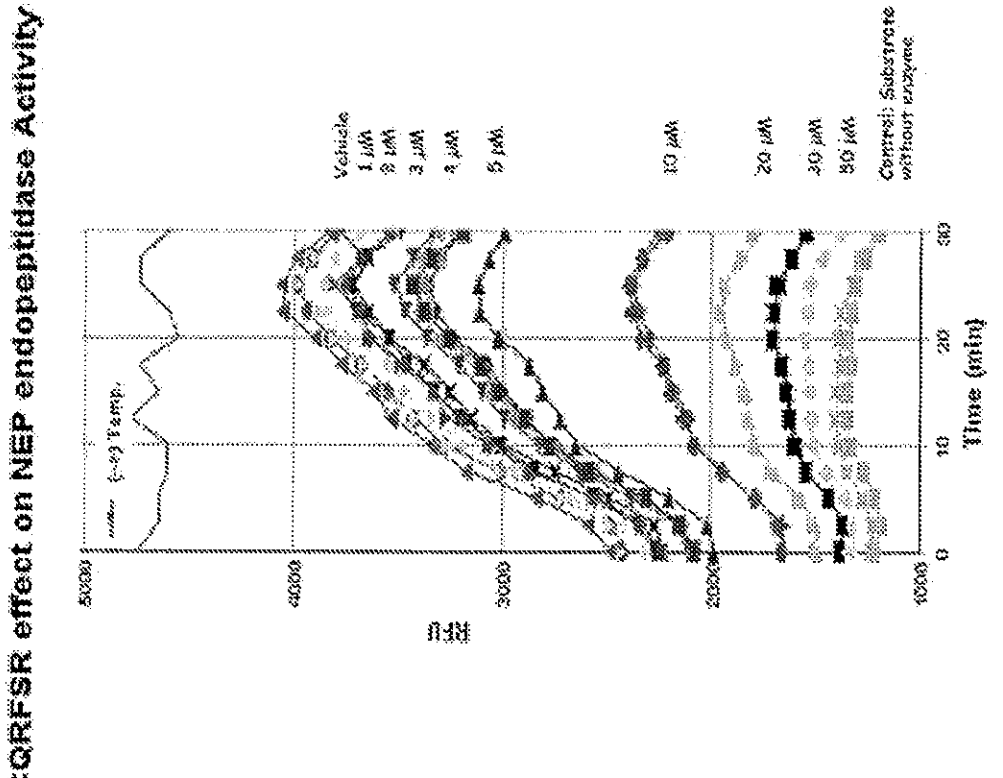

CQRFSR effect on NEP endopeptidase Activity
RFU
Time (min)
Vehicle
1 µM
2 µM
3 µM
4 µM
5 µM
10 µM
20 µM
30 µM
50 µM
Control: Substrate without enzyme

FIG.15

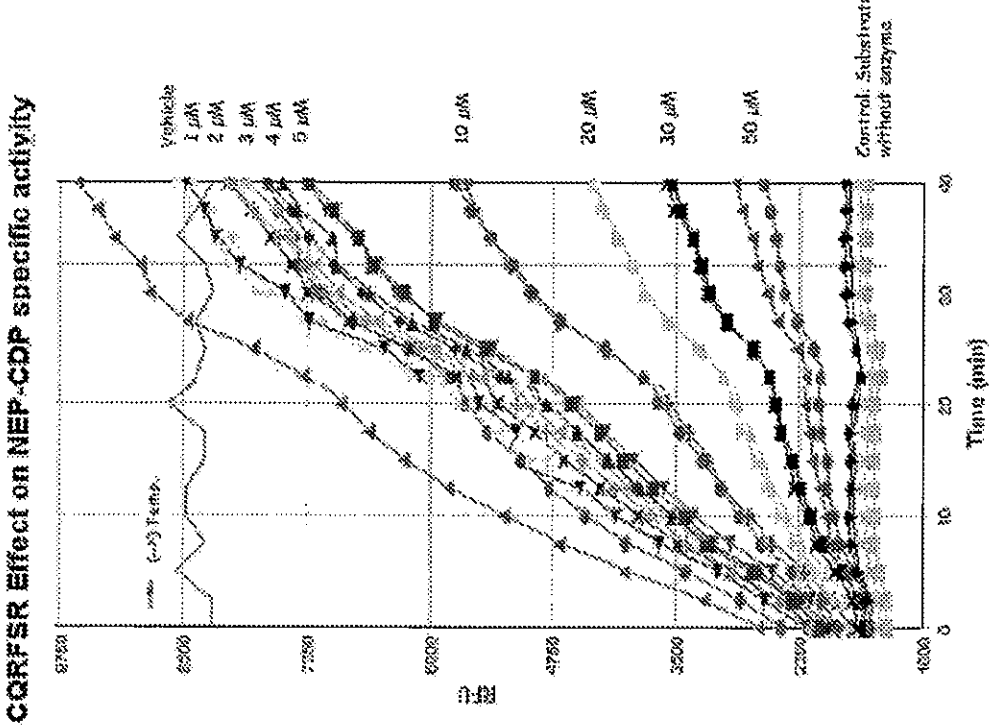

CQRFSR Effect on NEP-COP specific activity
RFU
Time (min)
Vehicle
1 µM
2 µM
3 µM
4 µM
5 µM
10 µM
20 µM
30 µM
50 µM
Control: Substrate without enzyme

FIG.14

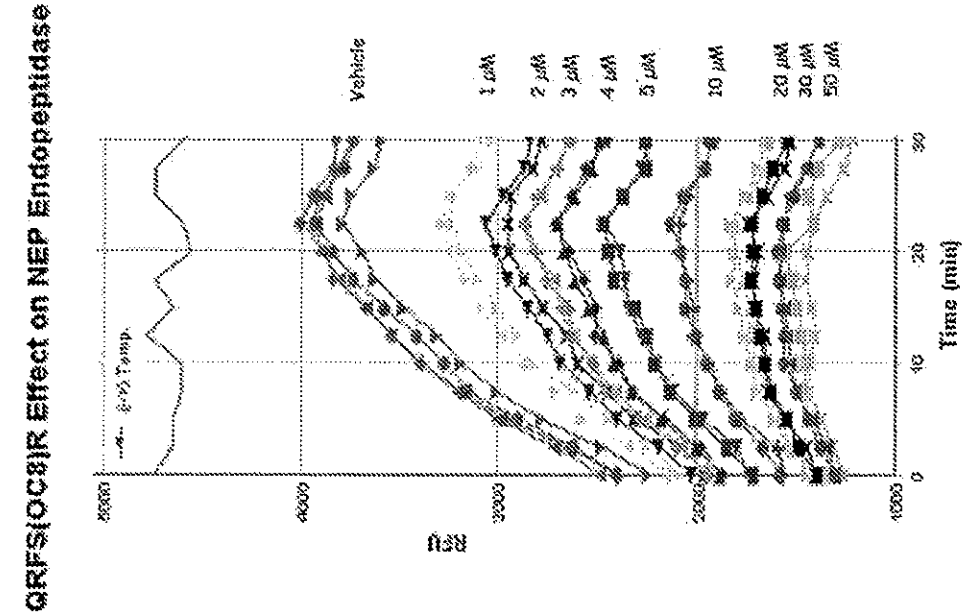

QRFS(QC8)R Effect on NEP Endopeptidase
RFU
Time (min)
Vehicle
1 µM
2 µM
3 µM
4 µM
5 µM
10 µM
20 µM
30 µM
50 µM

FIG.17

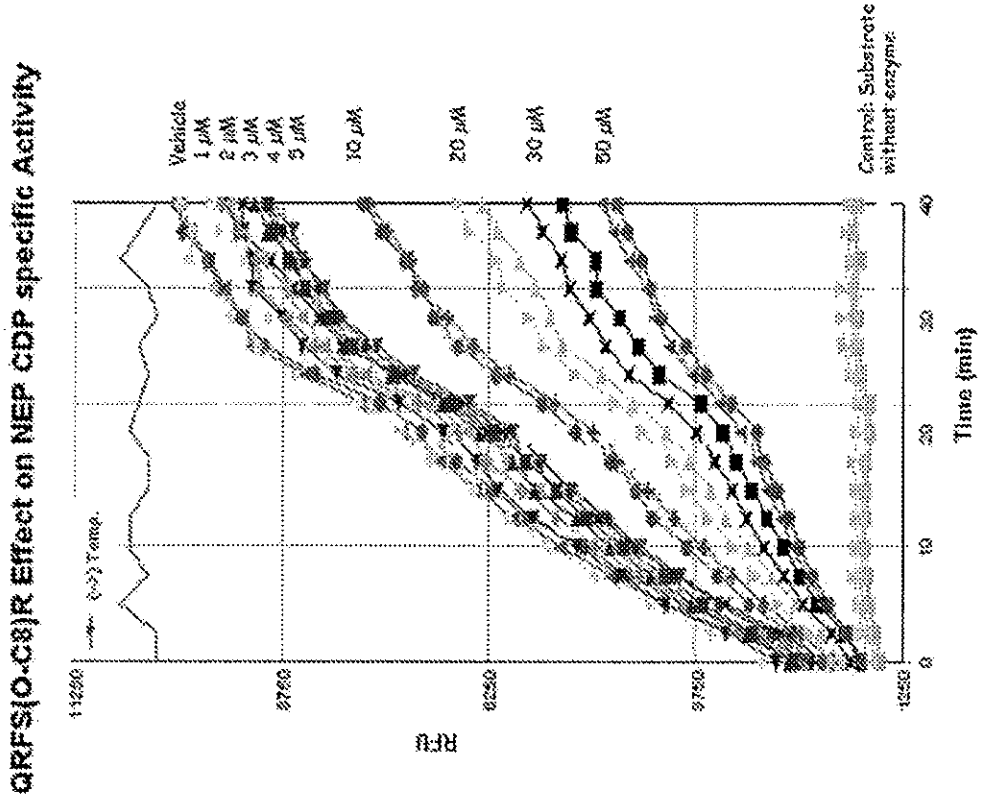

QRFS(Q-C8)R Effect on NEP CDP specific Activity
RFU
Time (min)
Vehicle
1 µM
2 µM
3 µM
4 µM
5 µM
10 µM
20 µM
30 µM
50 µM
Control: Substrate without enzyme

FIG.16

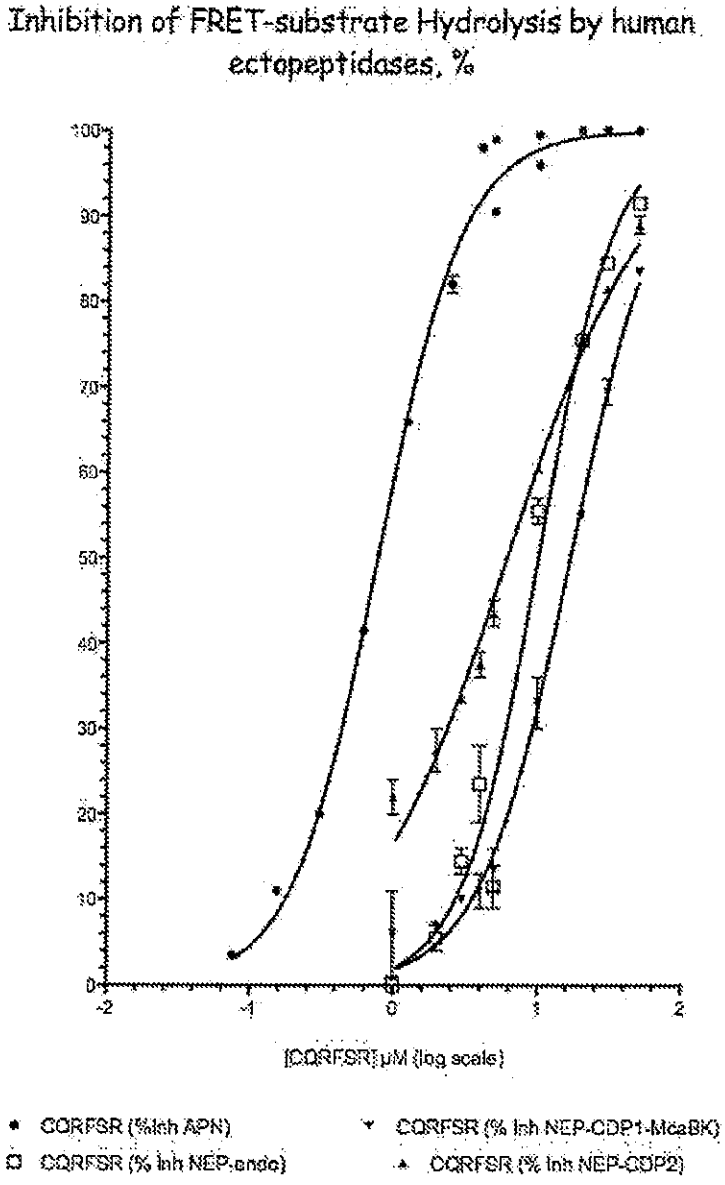

Inhibition of FRET-substrate Hydrolysis by human ectopeptidases, %
100
90
80
70
60
50
40
30
20
10
0
-2
-1
0
1
2
[CQRFSR] µM (log scale)
CQRFSR (%Inh APN)
CQRFSR (% Inh NEP-endo)
CQRFSR (% Inh NEP-CDP1-McaBK)
CQRFSR (% Inh NEP-CDP2)

FIG.18

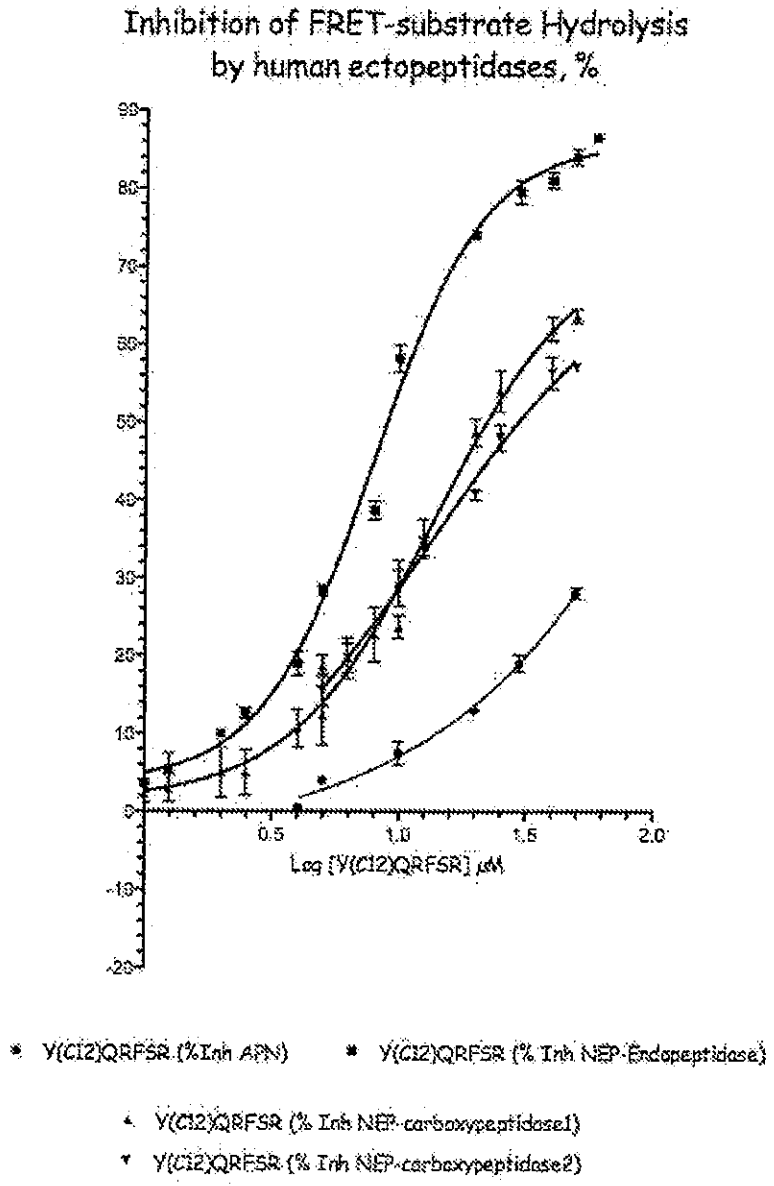

Inhibition of FRET-substrate Hydrolysis
by human ectopeptidases, %
90
80
70
60
50
40
30
20
10
0
-10
-20
0.5
1.0
1.5
2.0
Log [Y(Cl2)QRFSR] µM
Y(Cl2)QRFSR (%Inh APN)
Y(Cl2)QRFSR (% Inh NEP-Endopeptidase)
Y(Cl2)QRFSR (% Inh NEP-carboxypeptidase1)
Y(Cl2)QRFSR (% Inh NEP-carboxypeptidase2)

FIG.19

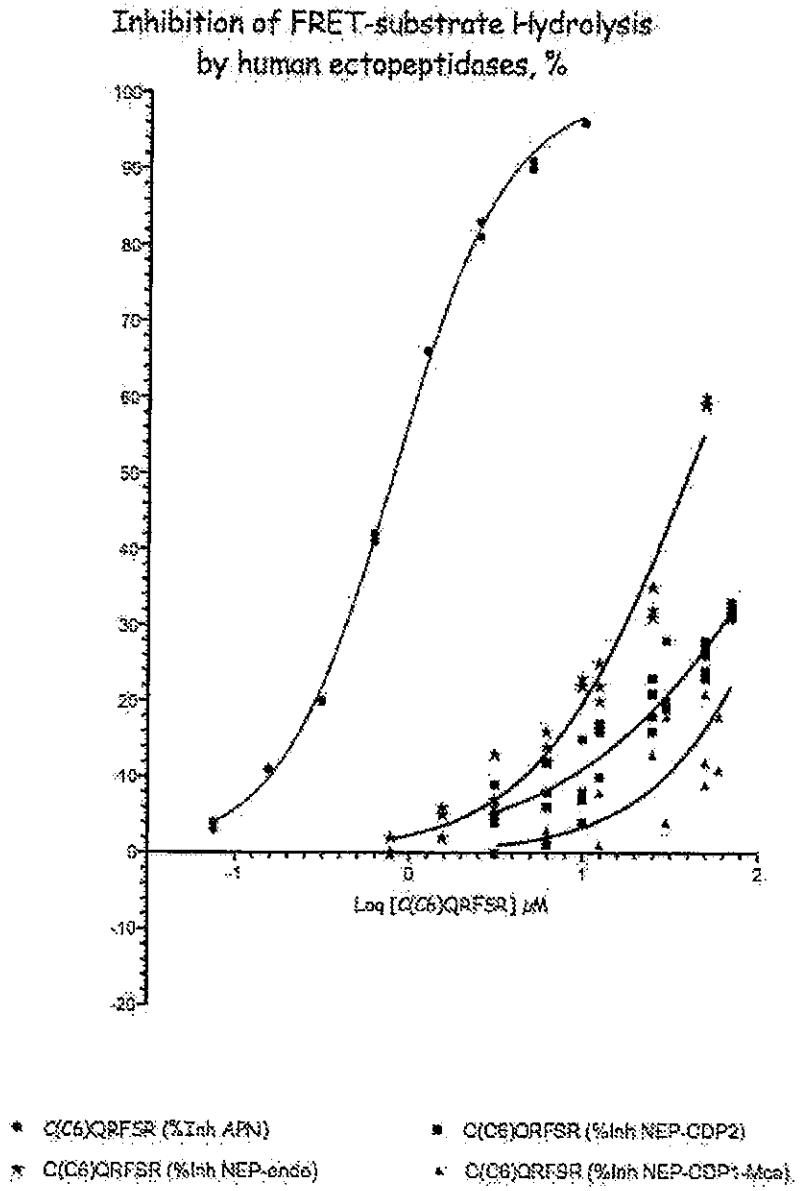

Inhibition of FRET-substrate Hydrolysis
by human ectopeptidases, %
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-1
0
1
2
Log [Q(C6)QRFSR] µM
C(C6)QRFSR (%Inh APN)
C(C6)QRFSR (%Inh NEP-CDP2)
C(C6)QRFSR (%Inh NEP-endo)
C(C6)QRFSR (%Inh NEP-CDP1-Mca)

FIG.20

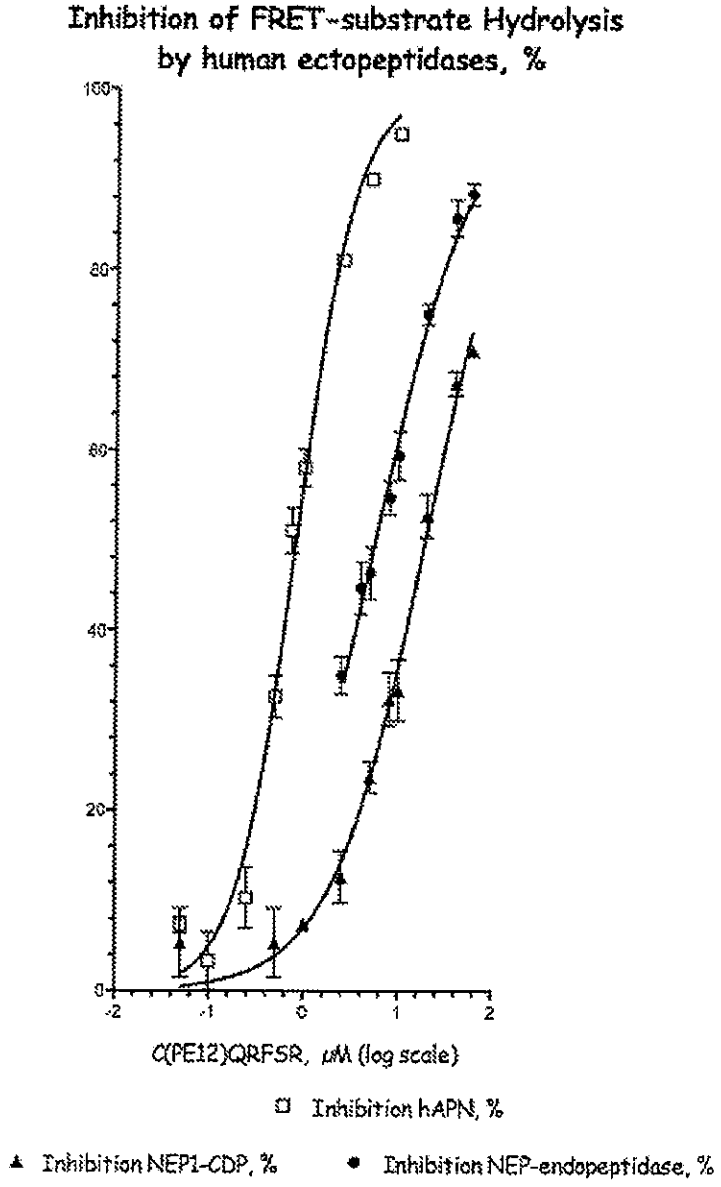

Inhibition of FRET-substrate Hydrolysis
by human ectopeptidases, %
100
80
60
40
20
0
-2
-1
0
1
2
C(PE12)QRFSR, µM (log scale)
Inhibition hAPN, %
Inhibition NEP1-CDP, %
Inhibition NEP-endopeptidase, %

FIG.21

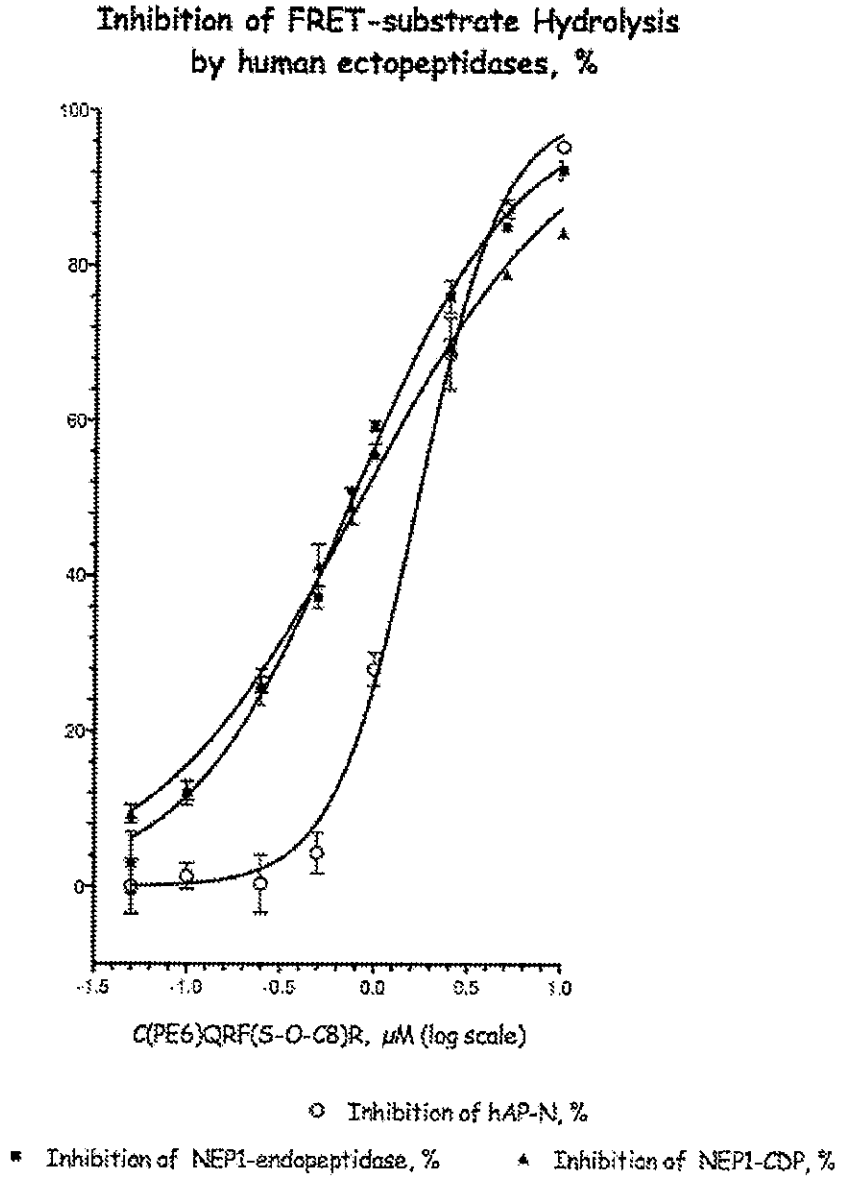

Inhibition of FRET-substrate Hydrolysis
by human ectopeptidases, %
100
80
60
40
20
0
-1.5
-1.0
-0.5
0.0
0.5
1.0
C(PE6)QRF(S-O-C8)R, μM (log scale)
Inhibition of hAP-N, %
Inhibition of NEP1-endopeptidase, %
Inhibition of NEP1-CDP, %

FIG.22

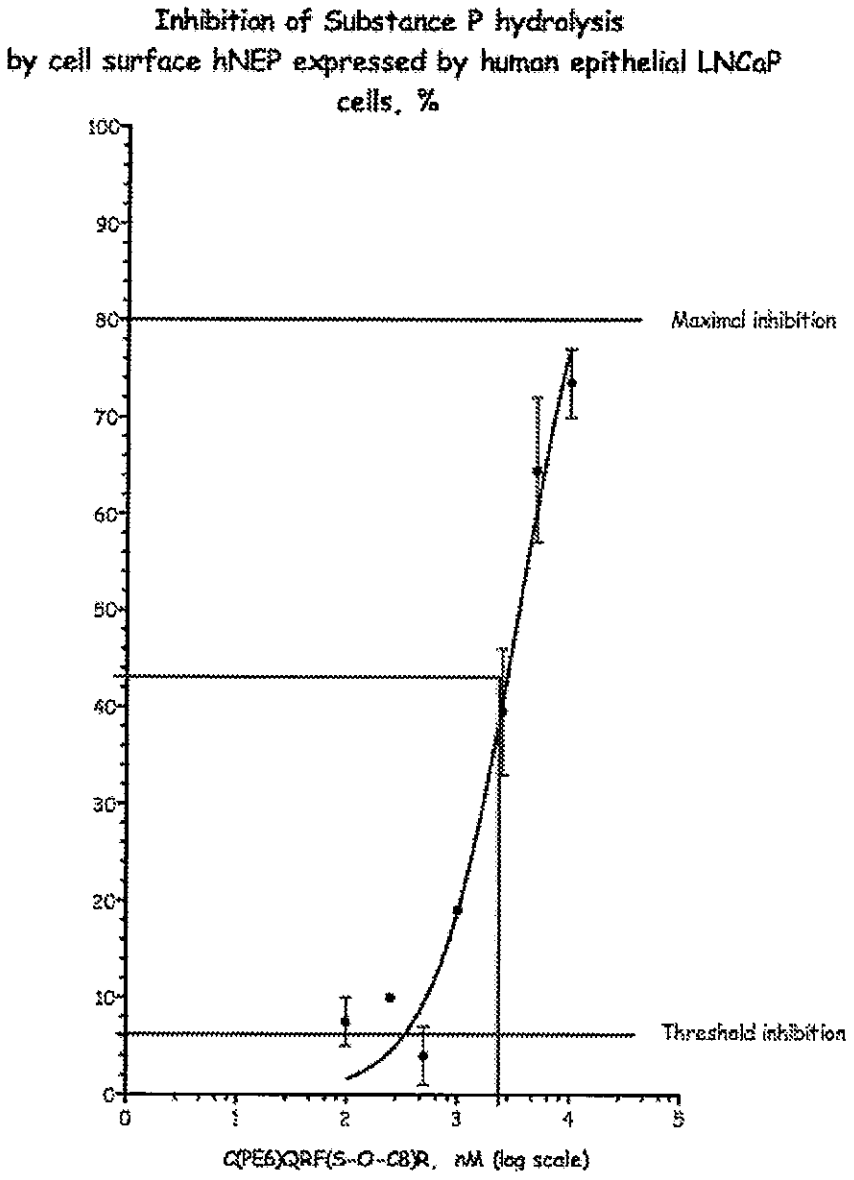

Inhibition of Substance P hydrolysis
by cell surface hNEP expressed by human epithelial LNCaP
cells, %
100
90
80
70
60
50
40
30
20
10
0
Maximal inhibition
Threshold inhibition
0
1
2
3
4
5
C(PEG)QRF(S-O-C8)R, nM (log scale)

FIG.23