ANTİPLATELET ETKİDE YENİ HEDEF SİNYAL YOLAKLARI

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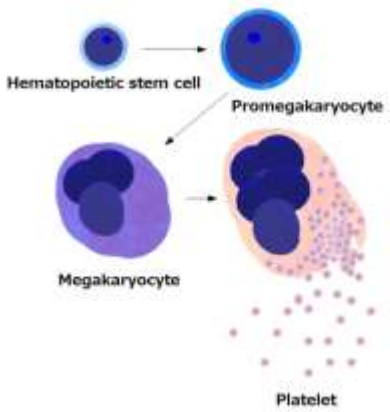
Biyokimya Anabilim Dalı

Platelet fonksiyonu ve agregasyonu

Güncel antiplatelet tedavide hedefler ve kısıtlamalar

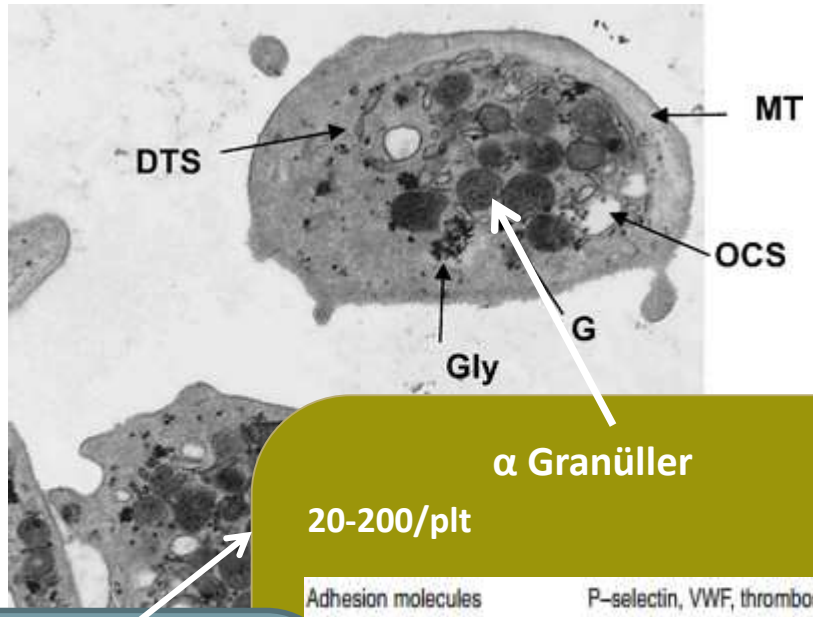
Yeni hedef olarak platelet GPVI reseptör sinyal yolu

Plateletler



1.5-3 µm ,çekirdeksiz hücreler
7-10 gün
150-400.000 plt/µl

Hemostaz
Tromboz
İnflamasyon
Anjiojenez
Yara iyileşmesi



α Granüller

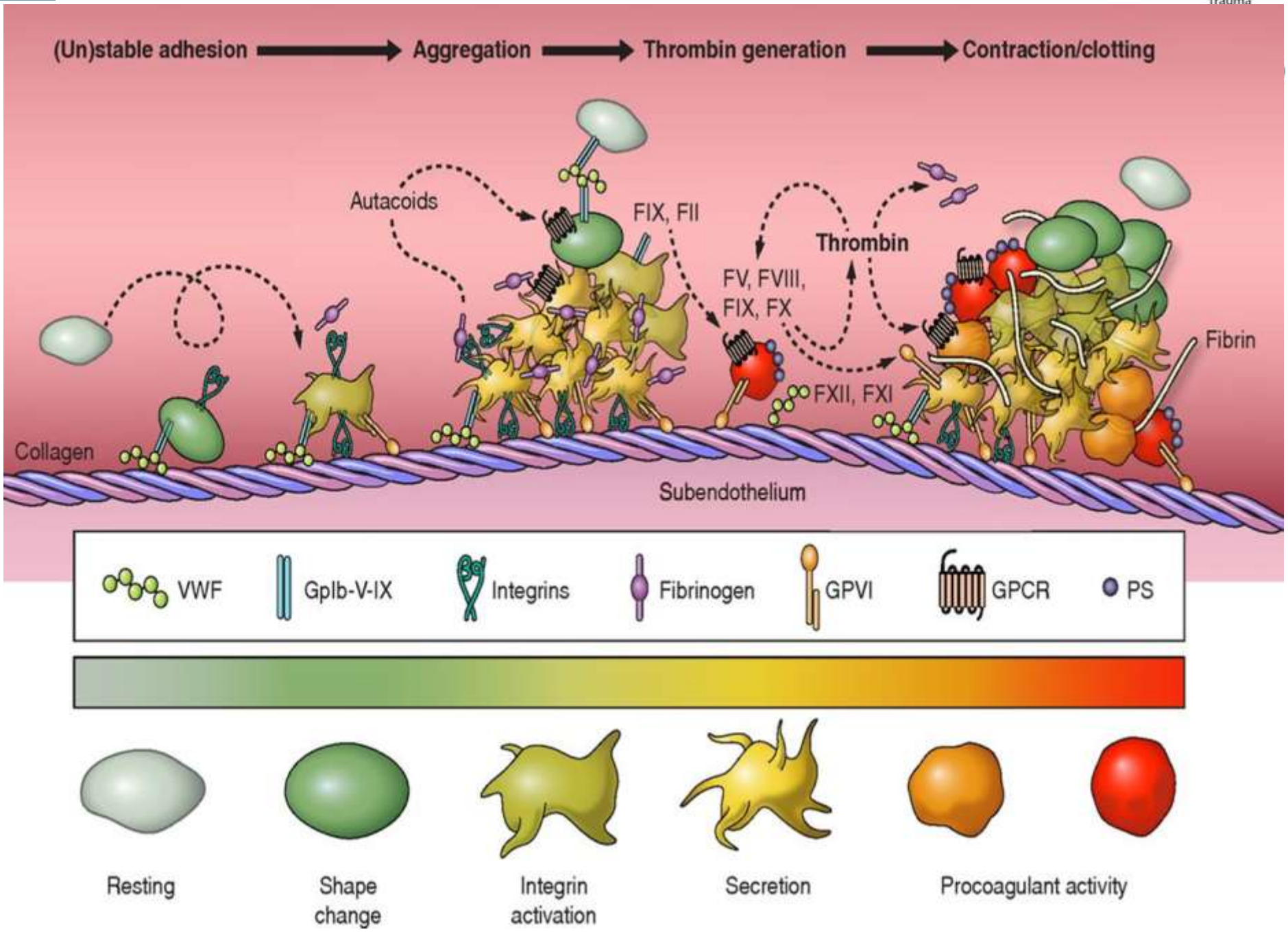
20-200/plt

Adhesion molecules	P–selectin, VWF, thrombospondin, fibrinogen, αIIbβ3, αvβ3, fibronectin
mokines	PF4, β–thromboglobulin, CCL3, 5, 7, 17
gulation factors	Fibrinogen, Factor V, Factor VIII
nolytic factors	A2 macroglobulin, plasminogen, PAI—1
vth and angiogenic	BFGF, EGF, HGF, IGF1, TGFβ,
actors	VEGF–A, –C, PDGF
unologic molecules	C1 inhibitor, IgG
r proteins	Albumin, α1–antitrypsin, Gas6, HMWK

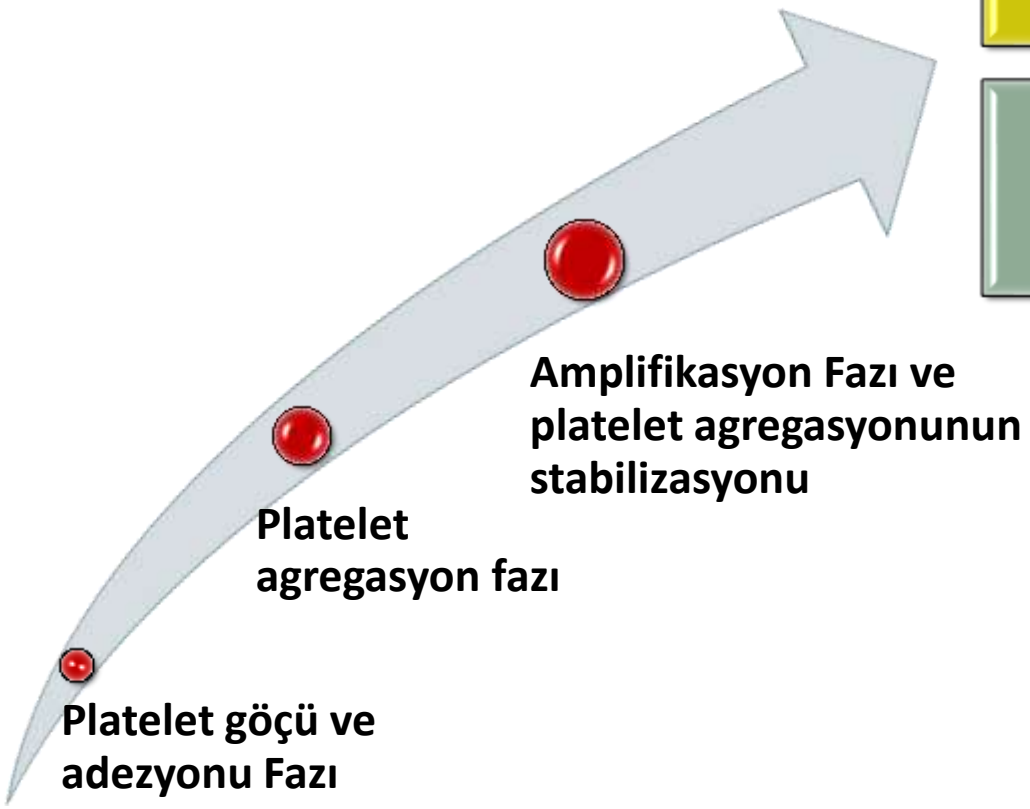
Dense Granüller

10-20/plt

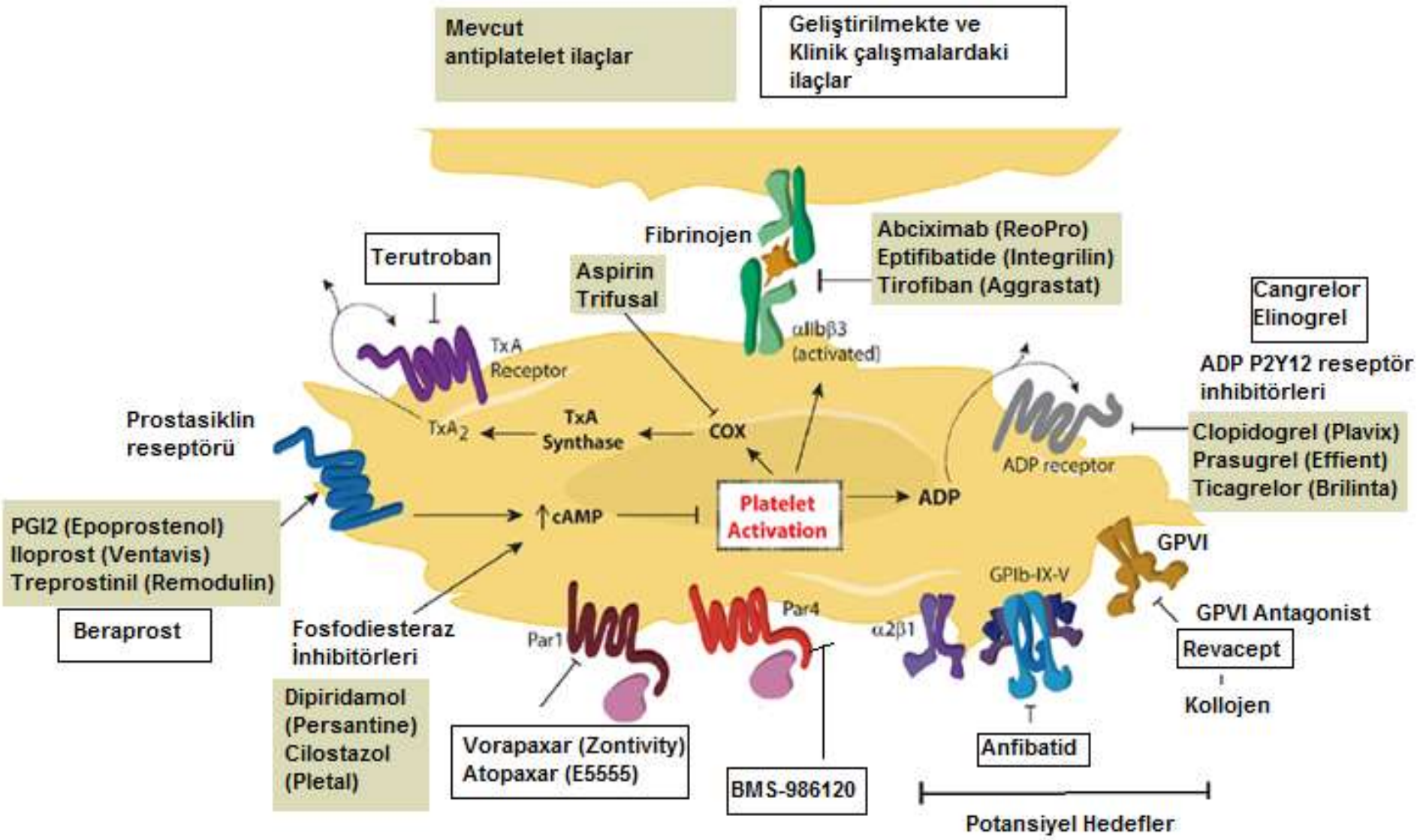
Ions	Calcium, magnesium, phosphate, pyrophosphate
Nucleotides	ATP, GTP, ADP, GDP
Membrane proteins	CD63, LAMP2
Transmitters	5-HT, epinephrine, histamine


(Un)stable adhesion
Aggregation
Thrombin generation
Contraction/clotting
Autacoids
FIX, FII
Thrombin
FV, FVIII,
FIX, FX
FXII, FXI
Fibrin
Collagen
Subendothelium
VWF
GpIb-V-IX
Integrins
Fibrinogen
GPVI
GPCR
PS
Resting
Shape change
Integrin activation
Secretion
Procoagulant activity

ANTİPLATELET TEDAVİDE HEDEFLER



- Agonist oluşumunun inhibisyonu
- Reseptör inhibisyonu
- Enzimatik kaskadın inhibisyonu
- G protein inhibisyonu



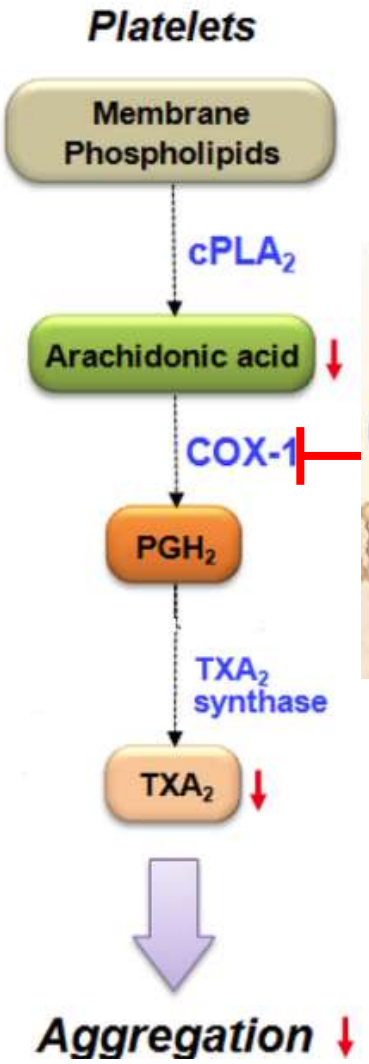
Arterioscler Thromb Vasc Biol June 2015

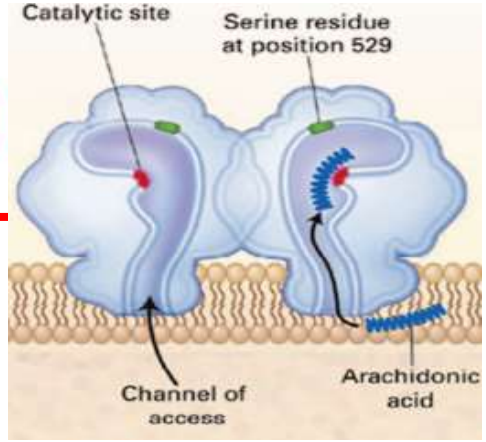
COX-1, TROMBOKSAN SENTAZ VE TROMBOKSAN-PROSTANOİD RESEPTÖR (TP)

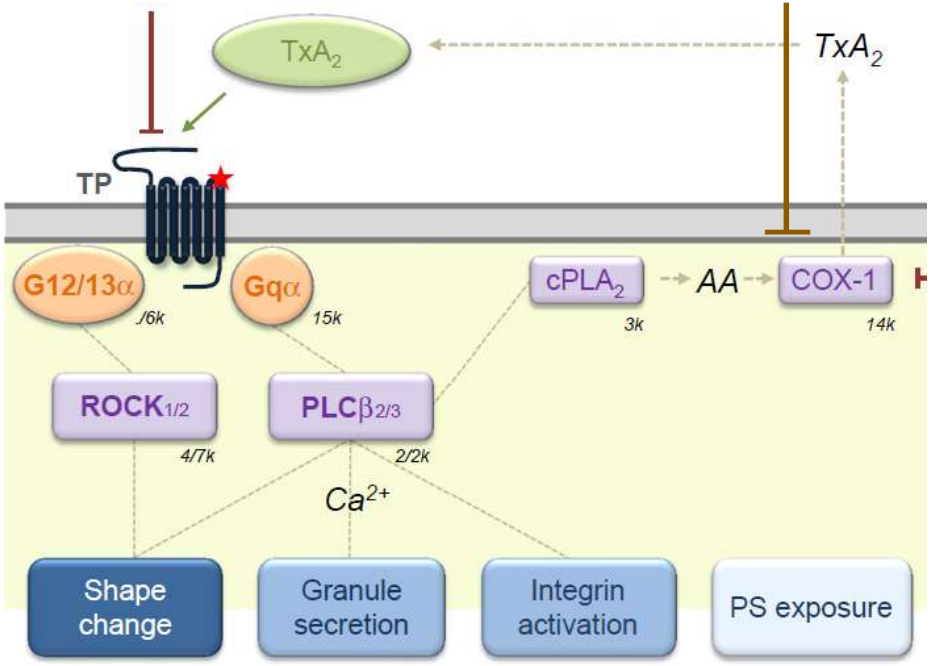
Terutroban
TP antagonist
TAIPAD
PERFORM Faz III

Picotamide
TPantagonist/
TxA2 sentaz
inihibitör

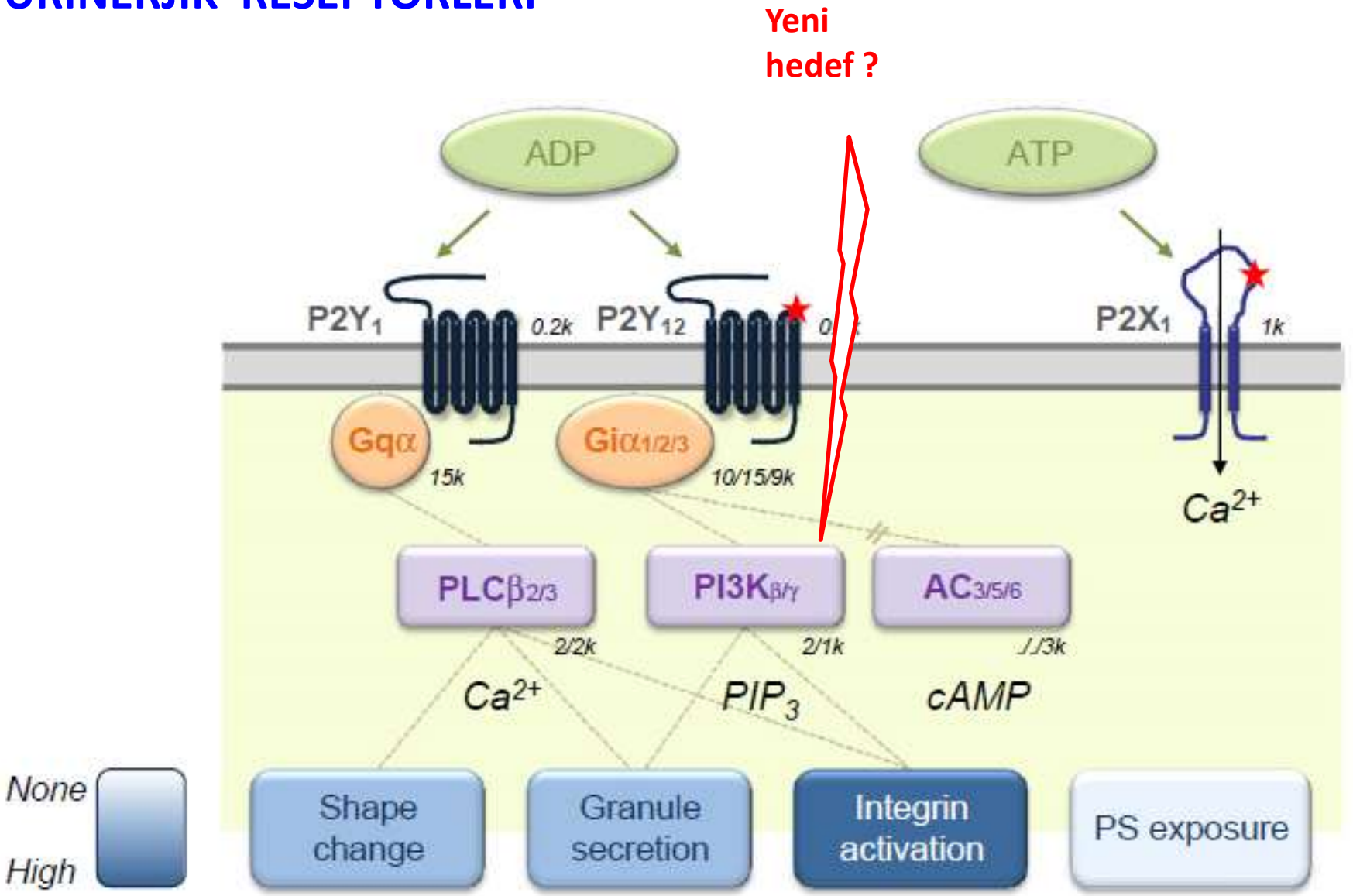
Triflusal
Avrupa, Asya,
Güney
Amerika

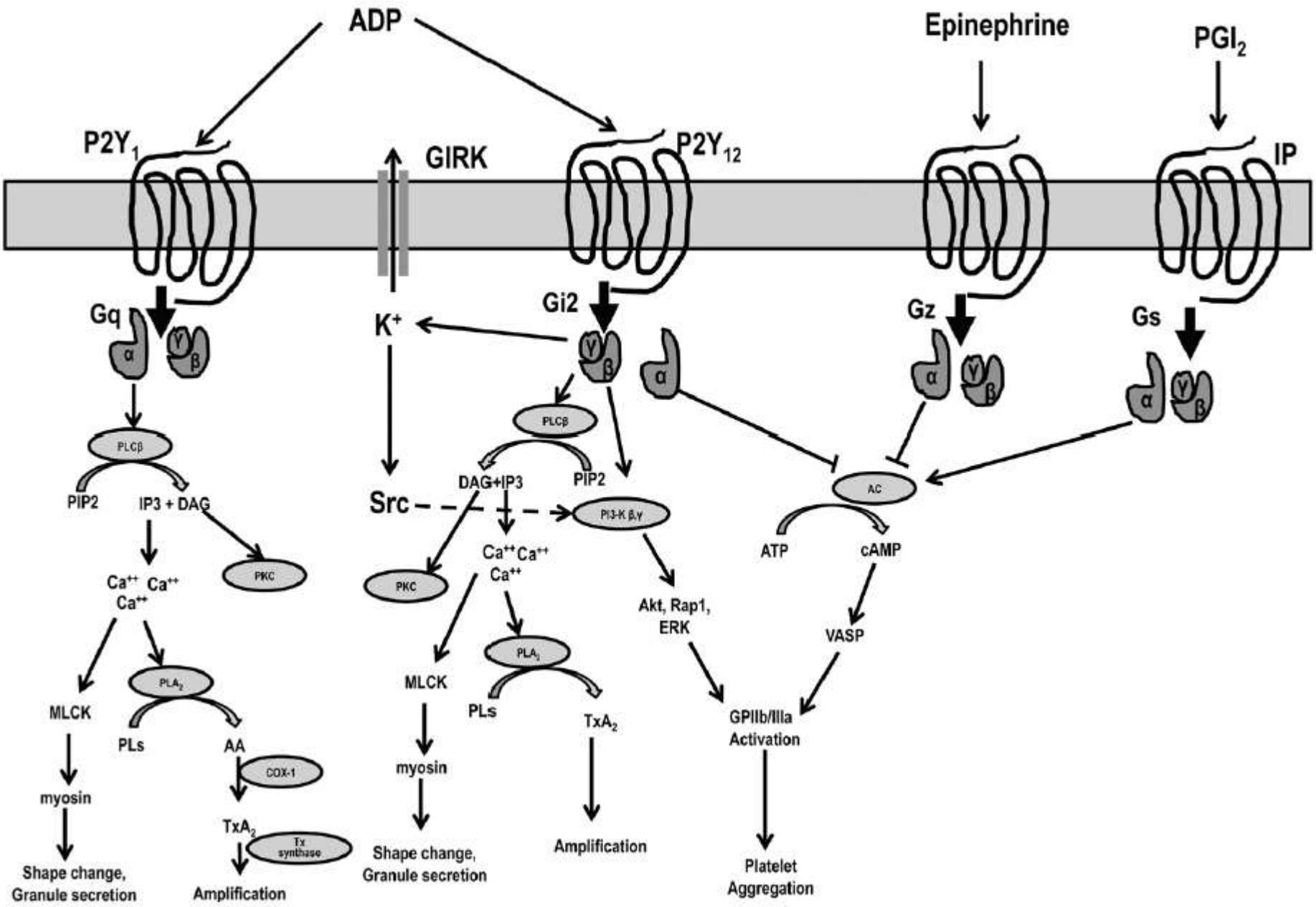






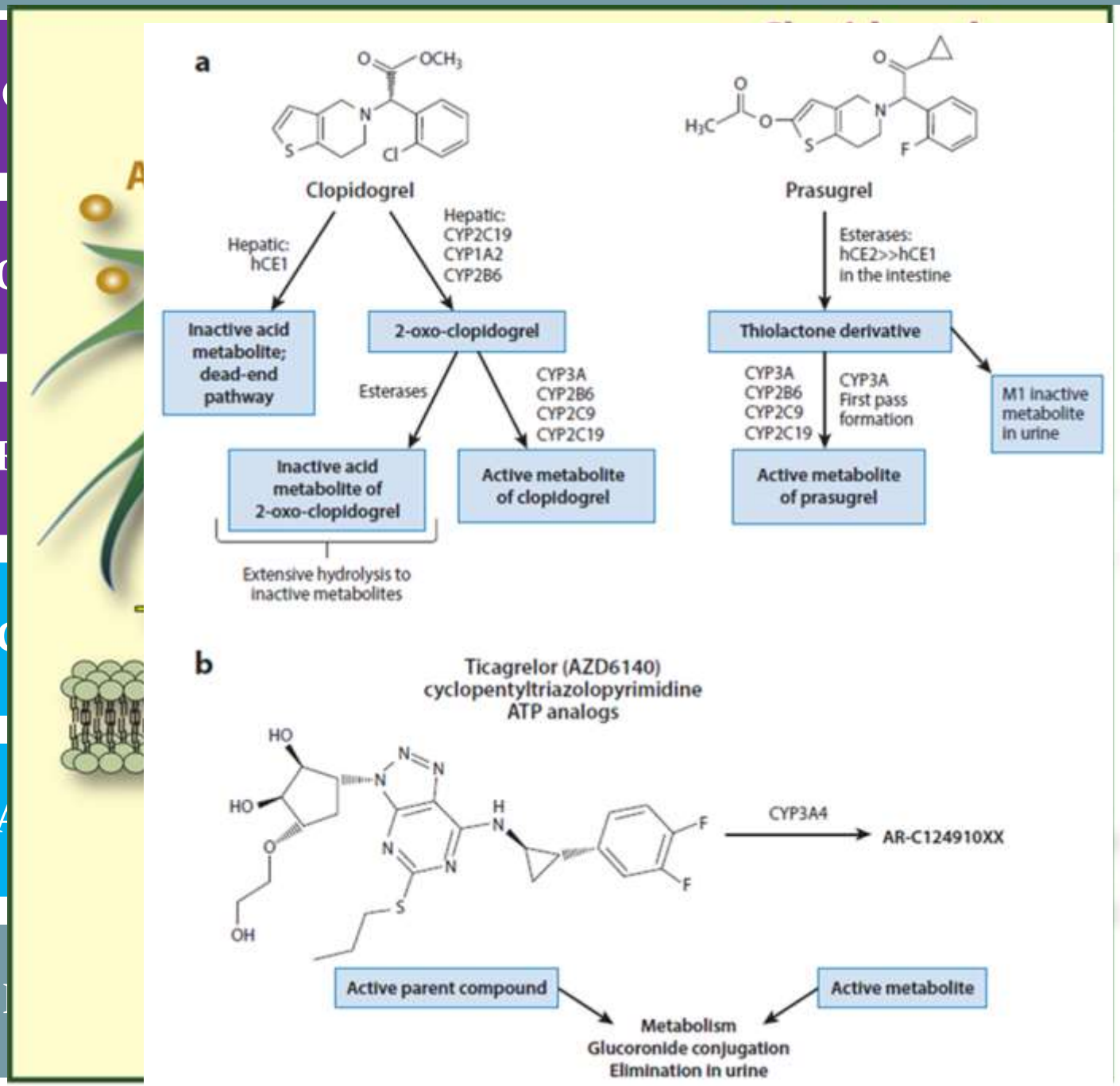
PURİNERJİK RESEPTÖRLERİ




ADP
Epinephrine
PGI2
P2Y1
GIRK
P2Y12
IP
Gq
K+
Gi2
Gz
Gs
PLCβ
PIP2
IP3 + DAG
DAG+IP3
PIP2
Src
PI3-K β,γ
AC
ATP
cAMP
PKC
Ca++ Ca++ Ca++
Ca++Ca++ Ca++
PKC
Akt, Rap1, ERK
VASP
PLA2
MLCK
PLA2
PLs
AA
COX-1
MLCK
PLA2
PLs
TxA2
GPIIb/IIIa Activation
myosin
TxA2
Tx synthase
myosin
Shape change, Granule secretion
Amplification
Shape change, Granule secretion
Amplification
Platelet Aggregation

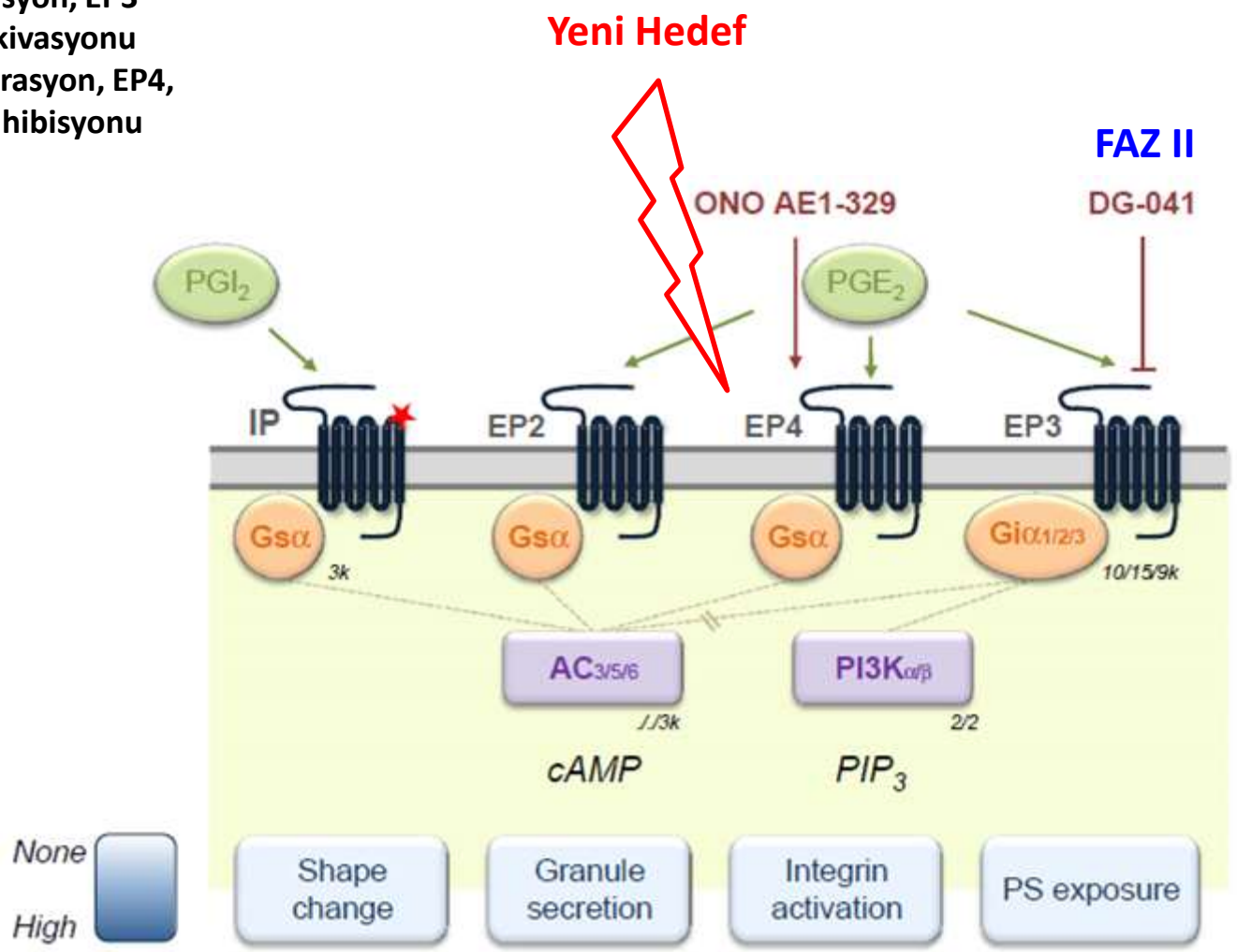
P_2Y_{12} ANTAGONİSTLERİ

- TİC
- CLO
- PR
- TİC
- CA
- EF

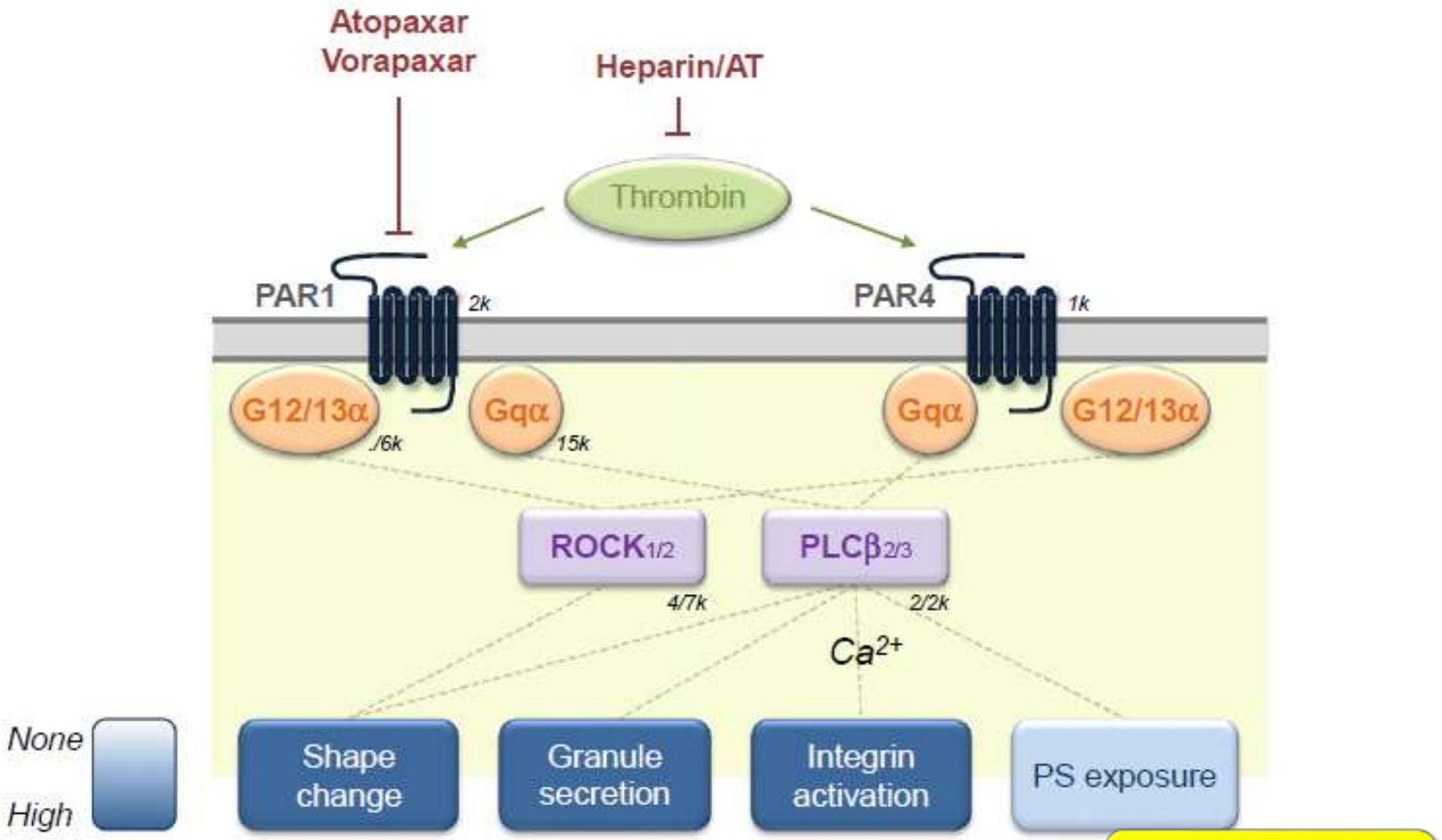


PROSTAGLANDİN RESEPTÖRLERİ: EP1-4

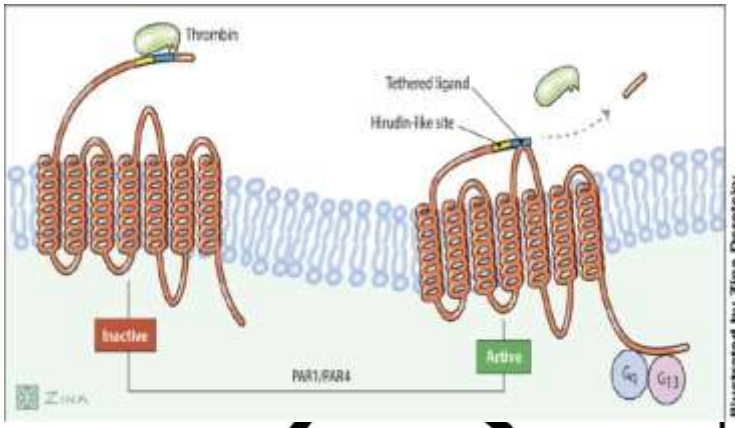
PGE_2 Düşük konsantrasyon, EP3 platelet agregasyon akivasyonu
PGE2, yüksek konsantrasyon, EP4, platelet agregasyon inhibisyonu



TROMBİN RESEPTÖRLERİ



F. Swieringa et al. / Blood Reviews 28 (2014) 9–21



Illustrated by Zina Deretsky

ADP

$P2Y_{12}$

Gi2

PI3-K β,γ

AC

ATP

cAMP

Akt, Rap1, ERK

VASP-P

GPIIb/IIIa Activation

Thrombin

GYPGOV

PAR-4

Gq

PLCβ

PIP2

IP3 + DAG

Ca^{++} Ca^{++} Ca^{++}

PKC

Shape change Granule secretion

Transient

G12/13

Rho-GEF

Shape change, Granule secretion

Shape change Granule secretion

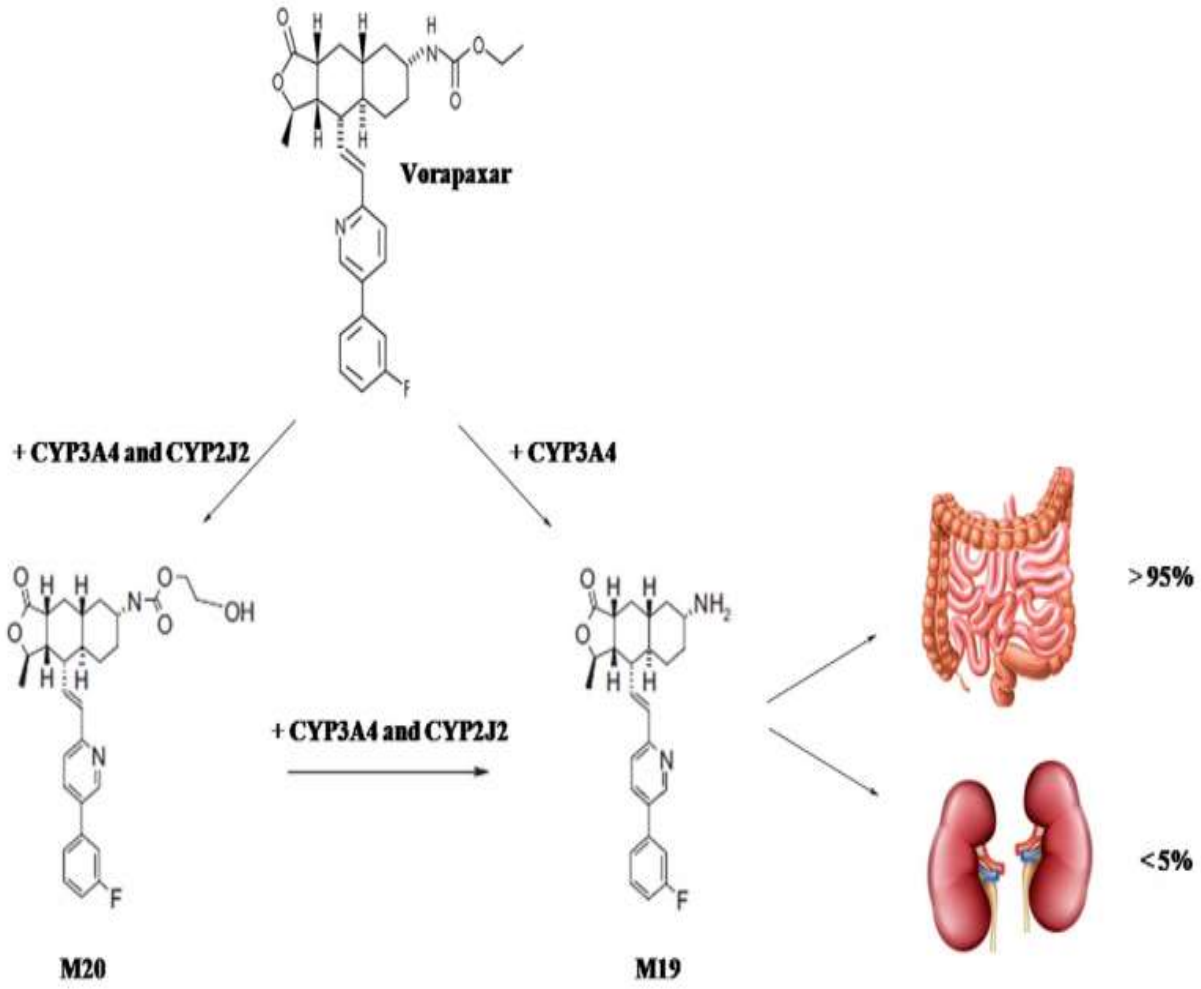
Prolonged

Reversible Aggregation

Irreversible Aggregation

PAR-1 ANTAGONİSTLERİ

- **Vorapaxar * (SCH 530348)**
- **Atopaxar (E 5555)**
- **Aday Bileşikler**



SCH 602539	Aday
PZ-128 (pepdusin)	(FAZ I)
Parmodulinler	Preklinik

Arterioscler Thromb Vasc Biol. 2015;35:500-512
Clinical pharmacology & Therapeutics 99(1):72-81, 2015

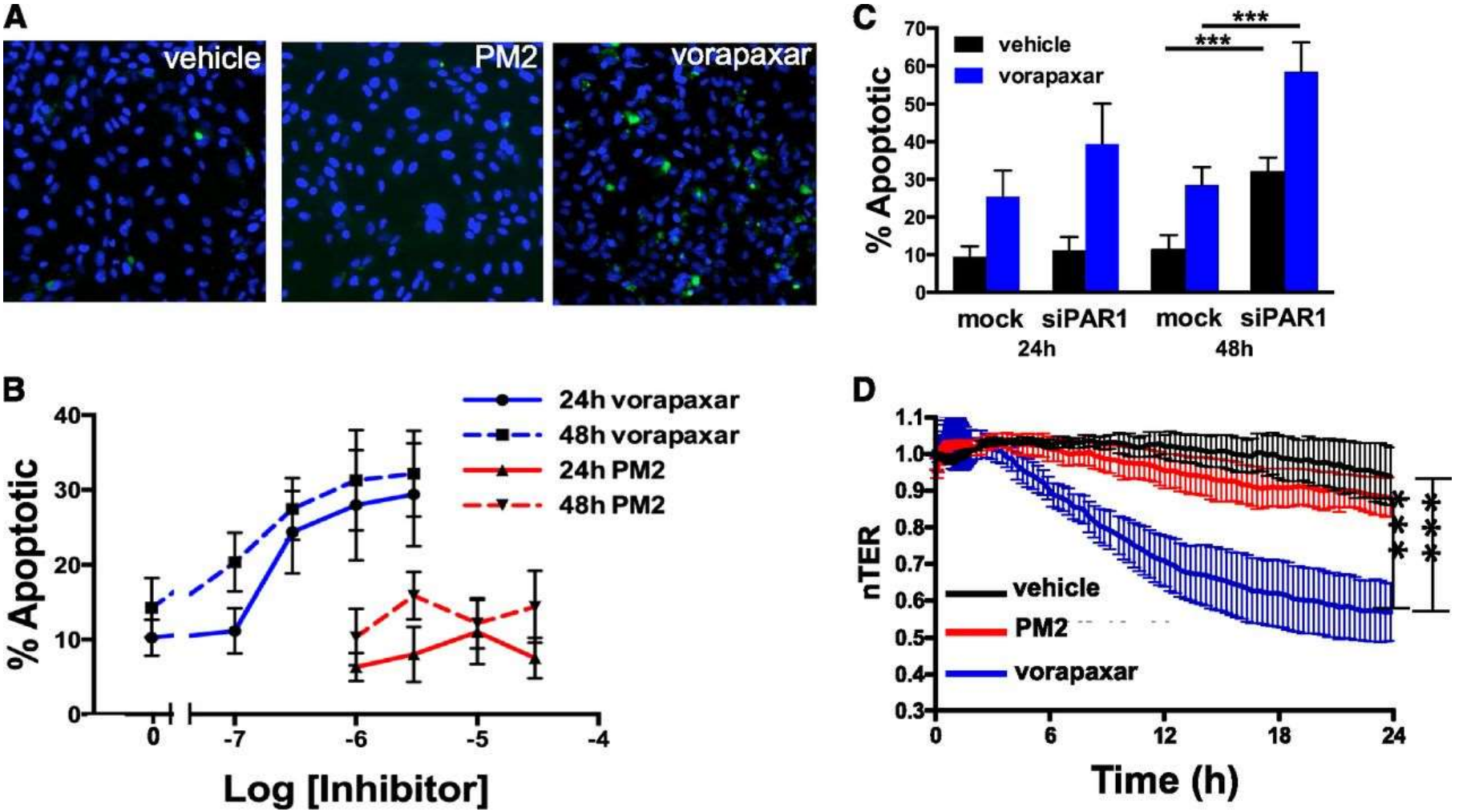
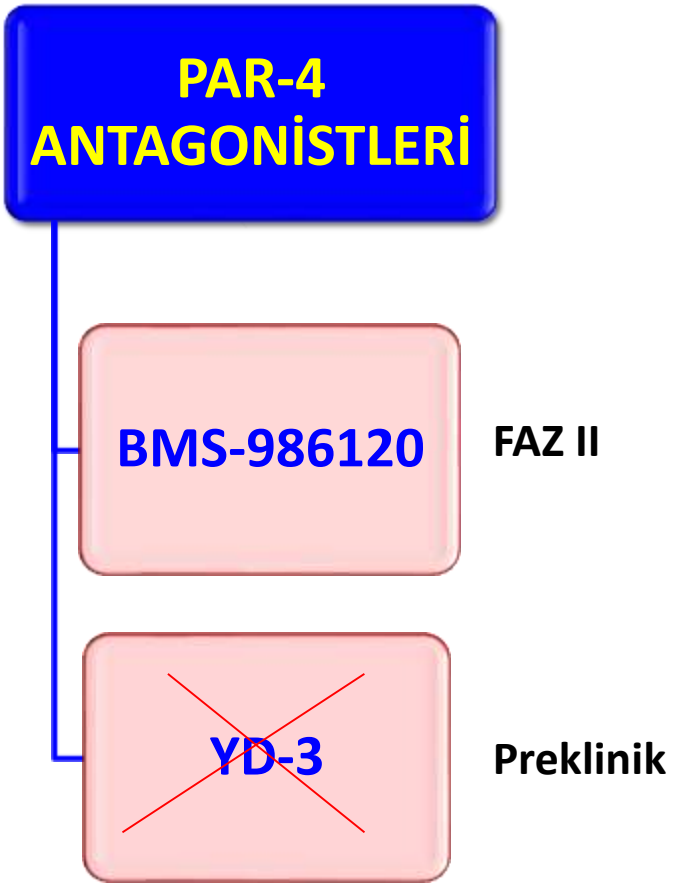


Figure 6 Vorapaxar, but not parmodulin 2, induces endothelial dysfunction upon prolonged exposures. (A) HUVECs were exposed to vehicle alone, 10 μM parmodulin 2, or 0.3 μM vorapaxar, as indicated, for 48 hours and subsequently stained for apoptosis using YO-PRO-1. (B) HUVECs were incubated with the indicated concentrations of either vorapaxar (blue) or parmodulin 2 (red) for either 24 hours (solid lines) or 48 hours (dashed lines) and assayed for apoptosis. Data are presented as means ± SEM (n = 5). (C) Mock-transfected (mock) and PAR1 siRNA–transfected (siPAR1) HUVECs were incubated with either vehicle (black) or 0.3 μM vorapaxar (blue) for either 24 or 48 hours. Samples were subsequently assayed for apoptosis. In each condition, addition of vorapaxar led to a significant increase in apoptosis compared with the unexposed sample ($P < .001$). Knockdown of PAR1 also increased apoptosis. ***$P < .001$. Data are presented as means ± SEM (n = 5). (D) HMVEC barrier function was continuously monitored by transendothelial resistance for 24 hours after exposure to either 10 μM parmodulin 2 (red) or 0.3 μM vorapaxar (blue). ***$P < .001$.

Blood 2015 125:1976-1985; doi:10.1182/blood-2014-09-599910

YENİ HEDEF

Arteryal trombozun önlenmesinde potansiyel hedef



4-[4-[[6-methoxy-2-(2-methoxyimidazo[2,1b][1,3,4]thiadiazol-6-yl)-1-benzofuran-4-yl]oxymethyl]-5-methyl-1,3-thiazol-2-yl]morpholine

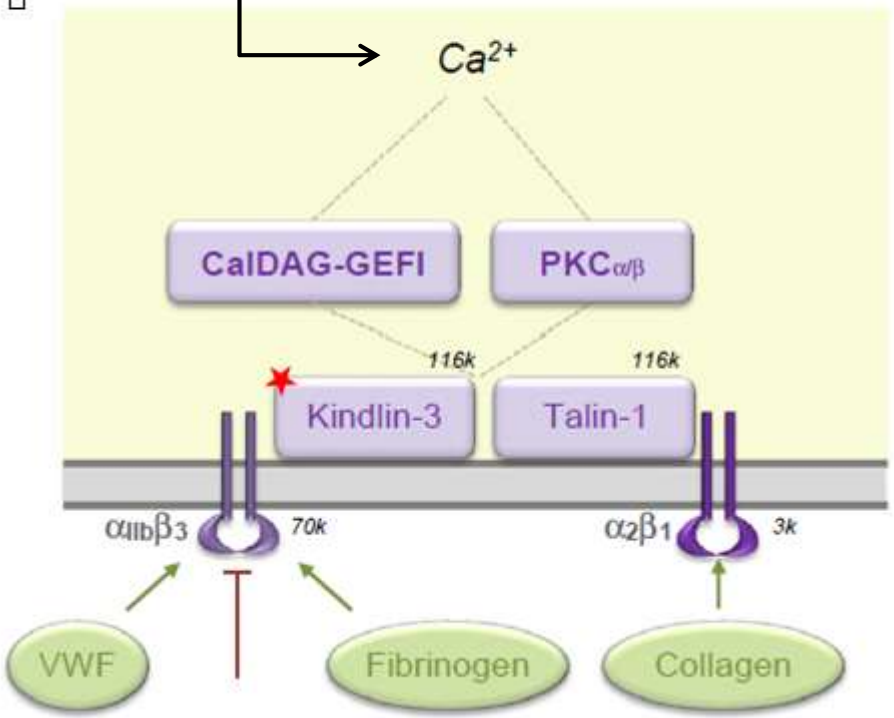
1-benzyl-3-(ethoxycarbonylphenyl)-indazole)

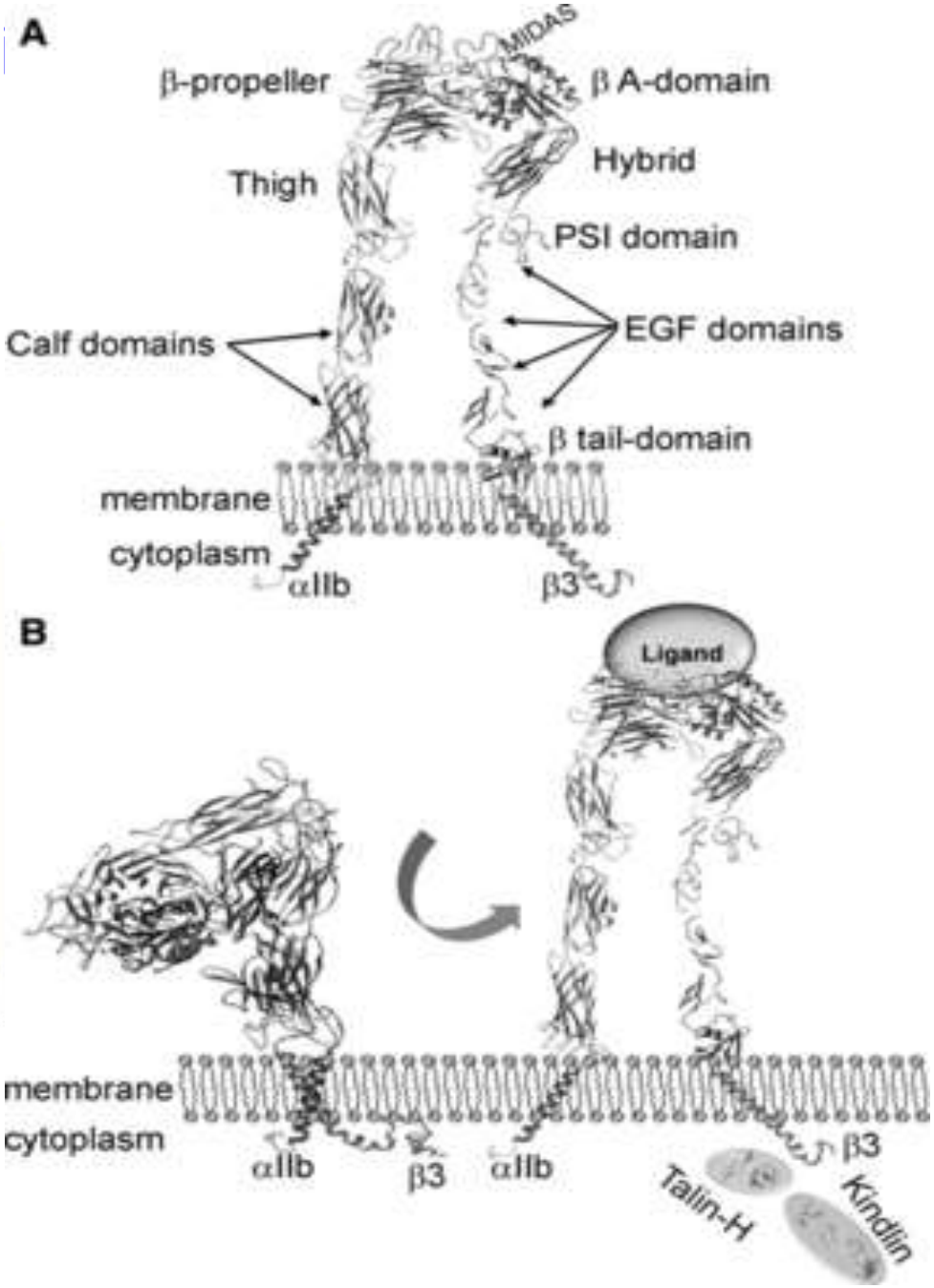
GPIIb/IIIa ($\alpha IIb\beta_3$) RESEPTÖRLER

İntegrin reseptörleri, 80.000 / platelet

GPIbα-VWF, GPVI/α2β1-collagen, P2Y12-ADP, PARs-thrombin

Inside-out signaling





GPIIb/IIIa ($\alpha IIb\beta 3$) ANTAGONİSTLERİ

Oral GPIIb/IIIa inhibitör gelişimi başarısız

- **Abciximab*** ReoPro, Clotinab
  - Kimerik antibadi Fab fragmanı, iv.,
- **Eptifibatide** Integrilin
  - Siklik KGD heptapeptid, iv.,
- **Tirofiban*** Aggrastat
  - L-tirozin türevi, iv.,
    - arginin-glisin-aspartik asit(RGD) sekansı

Güncel Antiplatelet İlaçlardaki Problemeler

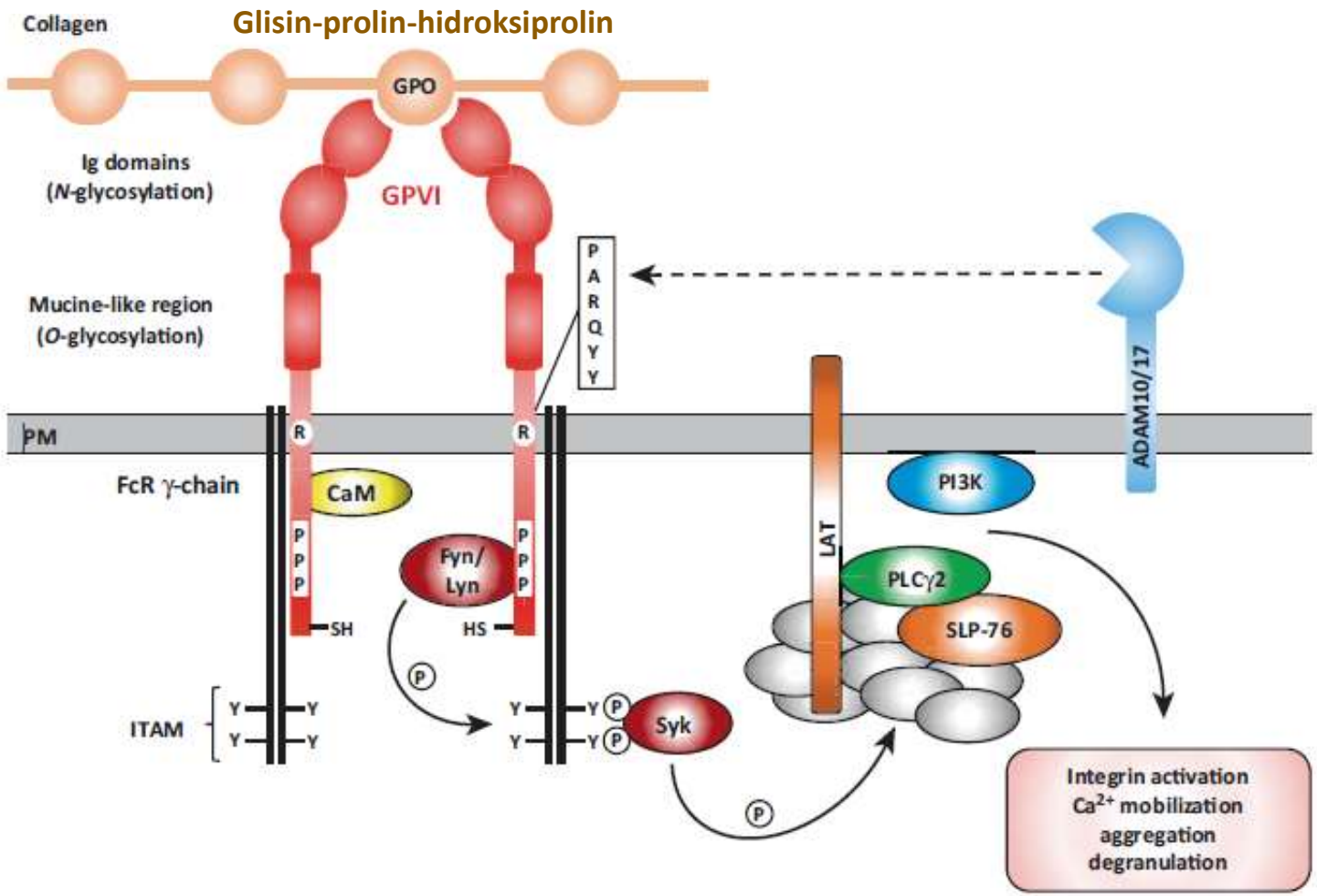
- ✓ Kanama ve kanama riski
- ✓ İlaç direnci
  Stokrom P450 enzim sistemi, polimorfizm
  ilaç etkileşimleri
  (omeprazol, statinler, kalsiyum kanal blokörleri)
- ✓ İv. uygulamaya bağlı doz ayarlanması gerekliliği
- ✓ İmmünojenite
- ✓ Uzun süreli ve irreversibl platelet inhibisyonu
  acil kardiyopulmoner bypass uygulanacak ACS hastalarında

İyileştirme
Yeni Hedefler

GPVI KOLLOJEN RESEPTÖRÜ

9600/plt

Fizyolojik ligand
(kollojen Tip I, Tip III, fibrin)
Sentetik spesifik ligand CRP



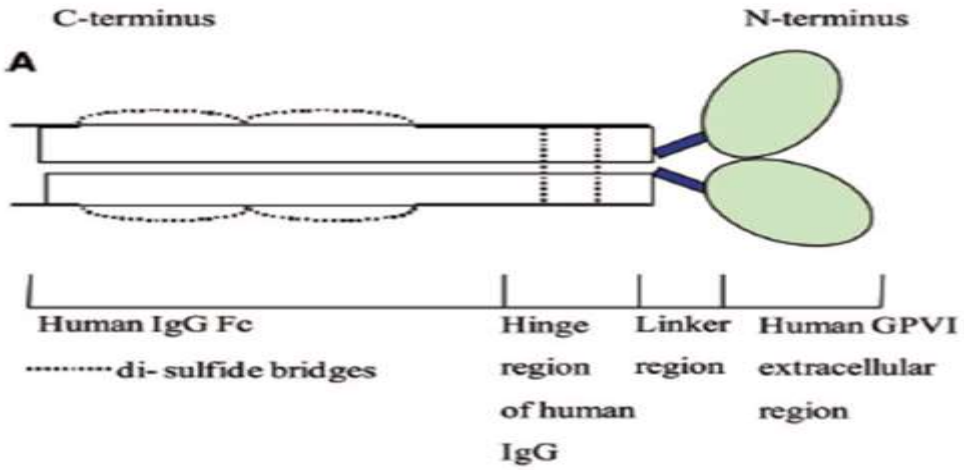
Nieswandt B.et.al.Trends in Pharmacological Sciences November 2012, Vol. 33, No. 11

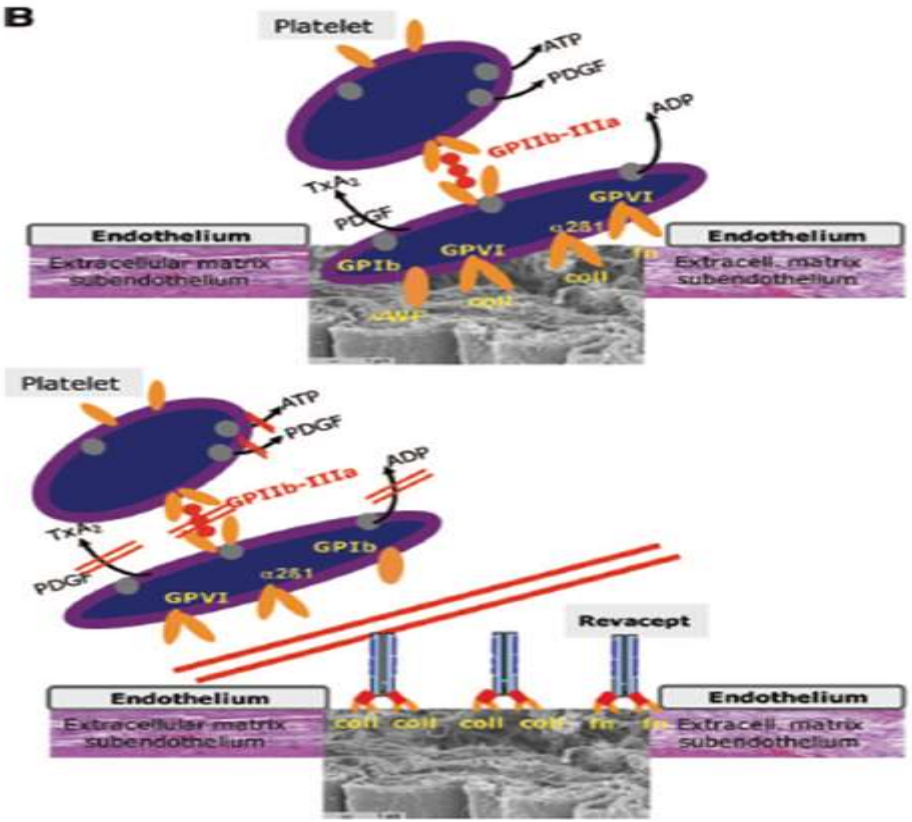
GP VI Hedefli moleküller

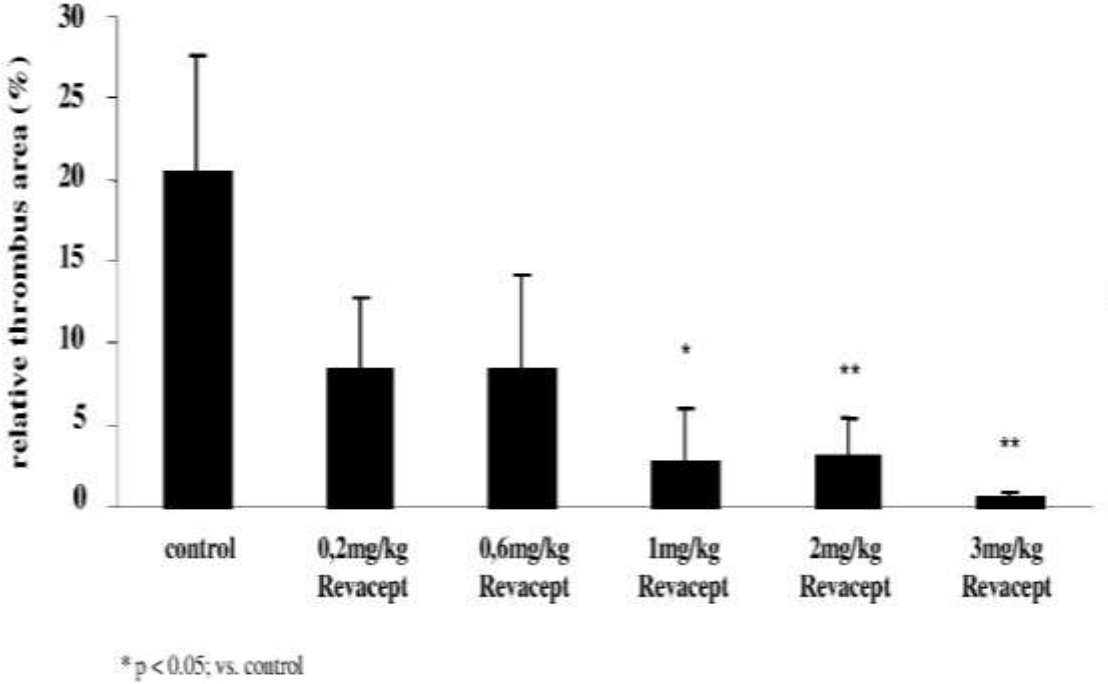
- Revacept
- Antibadiler mAb 9B12
- Losartan

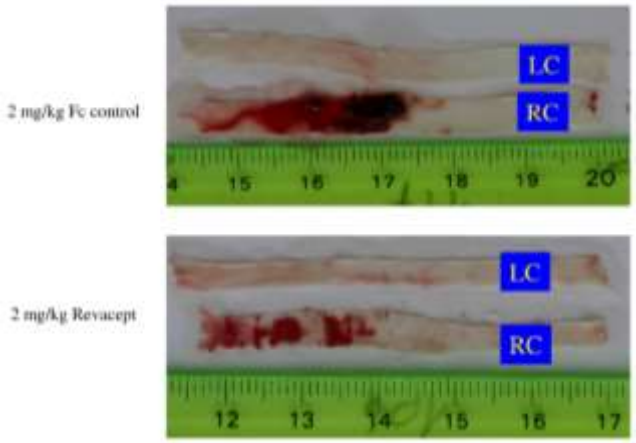
S
FAZ

PL









PLoS ONE, 2013, DOI: 10.1371/journal.pone.0071193

Structure and activity of compounds **7–24**: Percent reduction of platelet aggregation induced by agonists AA (700 μM), collagen (2 μg/ml) and ADP (10 μM).

Cmpd no	R	AA		Collagen	ADP
		1 μM	10 μM	10 μM	10 μM
7	Piperidine	92.89	94.77	71.13 (76.78)[a]	NI[b]
8	Morpholine	92.74	94.81	70.87 (74.21)[a]	11.34
9	4-Aminopiperidine	84.87	80.53	73.56 (77.59)[a]	8.14
10	4-Methoxyaniline	94.23	79.93	72.14 (43.56)[a]	3.92
11	4-Chloroaniline	80.93	74.31	70.48 (25.71)[a]	7.93
12	4-F-phenylpiperazine	NI	79.74	32.25	NI
13	4-CH_3-phenylpiperazine	3.96	97.59	20.12	NI
14	4-CF_3-piridin-2-ylpiperazine	10.86	65.46	16.02	6.8
15	2-Pyrimidinylpiperazine	NI	NI	22.86	2.7
16	4-Heptyloxyphenol	3.87	93.03	61.89 (32.24)[a]	NI
17	4-Methoxyphenol	6.05	84.25	24.16	NI
18	4-Methylphenol	23.55	95.00	48.51	NI
19	4-Cyclopentylphenol	NI	86.70	19.87	NI
20	4-(Imidazol-1-yl)phenol	5.25	90.05	2.11	NI
21	3-*i*-Propylphenol	8.97	84.00	42.27	NI
22	2-Cl-3-pyridinol	NI	86.52	8.49	NI
23	2-Naphthol	2.36	86.31	22.44	3.34
24	*i*-Pentanol	19.00	91.16	66.7 (34.16)[a]	6.77

[a] Percent inhibition at 1 μM.
[b] No detectable inhibition.

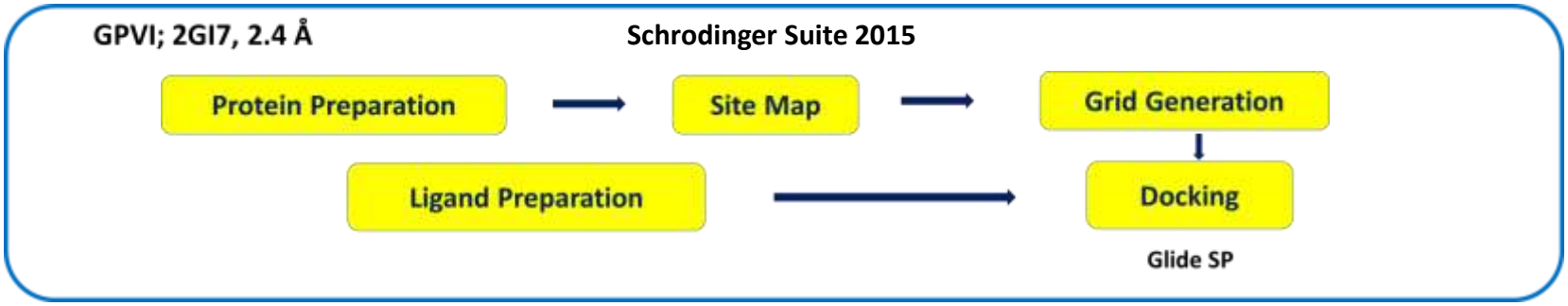
1,5-Diarilpirazol-3-karboksamid

IC_{50} values for antiplatelet activity of selected compounds against AA-induced platelet aggregation.

Cmpd no	IC_{50} (μM)	Cmpd no	IC_{50} (μM)
7	0.041	**35**	>10
8	0.083	**36**	>10
9	0.11	**41**	>10
10	0.40	**42**	>10
11	0.61	**45**	0.92
13	4.67	**46**	1.08
14	5.49	**50**	0.0079
24	1.33	**51**	0.0073
26	19.6	**59**	5.03
27	>20	**60**	18.4
29	2.09	**61**	0.015
30	12.6	**62**	0.0057
33	>10	**Aspirin**	7.76
34	>10	**Indomethacin**	1.62

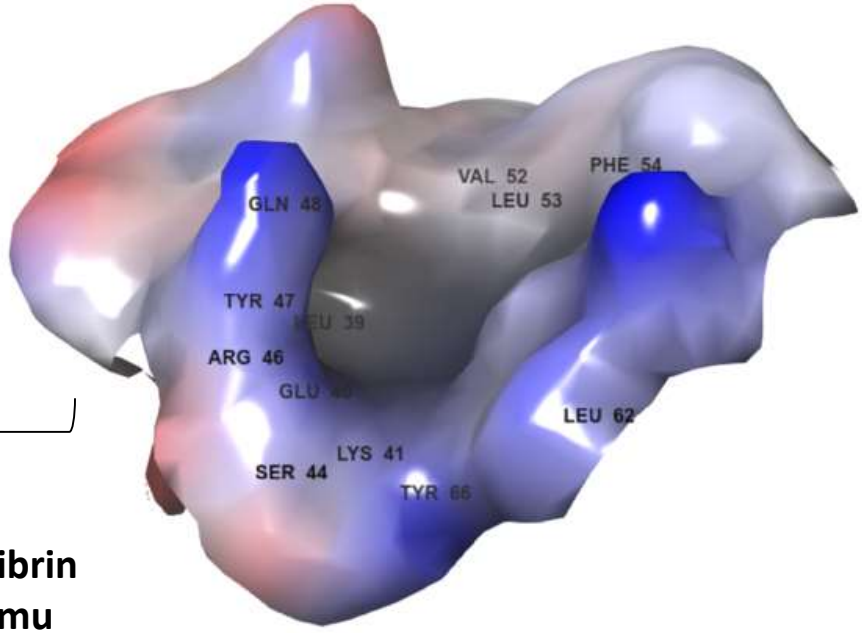
European Journal of Medicinal Chemistry 64 (2013) 42-53

FDA onaylı ilaç databank



İnsan Platelet GPVI kristal Yapısı

GPVI hidrofobik Bağlanma Bölgesi



Agregasyon çalışması (platelet)
recombinant GPVI bağlanma , kollojen ve fibrin
GPVI transfekte hücrelerde aktivasyon oluşumu

Araştırma Grubumuz

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http://evias.com.tr/tr/

Birmingham Platelet Group

Group leaders: Dr Alexander Brill, Dr Paul Harrison, Dr Neil Morgan, Prof Yotis Senis, Dr Steve Thomas and Prof Steve Watson