Supplement Information

Molecular docking and MD simulations were carried out to elucidate the effect of vanillin on the stability of ligand-(P-gp） complexes, which is closely related to the absorption of actively transported drugs.

A molecular docking simulation was performed and analyzed using AutoDock 4.2.[1] The crystal structure of human P-gp was generated using homology modeling, which was carried out as previously reported and used for the docking study.[2] The structure of vanillin and other ligands (colchicine, quinidine, verapamil and berberine) was obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and optimized using Chimera1.12 (Figures 1 and 2).[3] The lowest docked free energy structure, which was in the P-gp hydrophobic pocket (MET35, LEU299, PHE270, PHE302, PHE303, PHE638, LEU634, LEU668, GLY691, VAL884, LEU885, PHE888, THR855), was chosen. Docking images were rendered by PyMOL Molecular Graphics System (Version 1.6.0.0; Schrödinger LLC, Cambridge, MA) and ProteinsPlus.[4]

To observe the effect of vanillin on P-gp, homology modeling of P-gp was taken as the initial structure for CG simulation in the central region of 512 DPPC molecules, and 709 vanillin molecules (30%) were randomly placed in the simulation box. The CG force field of P-gp used Martini2.2. The whole system was solvated in water and energy minimized for 2000 steps. Then, 50 ps NVT equilibration and 1ns NPT equilibration were performed with a time step of 10 fs. After equilibration, a 1ps production simulation was run with a time step of 20 fs for analysis. The system was constructed by CHARMM-GUI and Gromacs tools. The temperature was set at 323K using the V-rescale algorithm with a coupling time of 1.0 ps. The Berendsen barostat, semi-isotropic pressure coupling at a compressibility of 3×10-4/bar, and a time constant of 2.0ps were used to maintain pressure (1.0 bar).

We further used all-atom MD simulations to investigate the effect of vanillin on the stability of key residues in the P-gp binding pocket and transmembrane domains (TMDs). P-gp was placed in the appropriate position of 256 DPPC membrane by FlateGro script.[5] Next, 348 vanillin (20%) molecules were placed into the box randomly. The entire system was solvated in SPC water and neutralized by CL-1 ions. Lipids used the Berger force field, and P-gp used the GROMOSA97 force field.[6] The force field of vanillin was generated from the PRODRG server.[7]

After 5000 steps of energy minimization, we performed 50 ps NVT equilibration and 2.0 ns NPT equilibration with a time step of 2 fs and 10000 kJ/mol·nm^2 position restraint. The Berendsen method was selected for temperature and pressure coupling in the equilibration. The temperature was 323K, and semi-isotropic pressure coupling maintained pressure at 1.0 bar. Particle-Mesh Ewald was set to calculate long-range electrostatic interactions, and the Van der Waals cut-off was 1.0 nm. As long as the system was well-equilibrated, we immediately released the position restraints and ran a 50 ns production simulation with V-rescale temperature coupling and Parrinello-Rahman pressure coupling.

The docking energy and conformation of four natural substrates of P-gp, colchicine, quinidine, verapamil, and berberine were compared with vanillin at the binding site of P-gp. Murine P-gp (PDB:3G5U, 3.8Å) was used as a template to model human P-gp; validation of the structure of human P-gp is shown in Supplementary Figure 1-3. As presented in Supplementary Figure 6, vanillin remained at the binding site of P-gp with a docking energy of -4.78 kcal/mol, much higher than the docking energies of colchicine, quinidine, verapamil, and berberine (Supplementary Figure 6). These results indicated that P-gp is a low-affinity receptor for vanillin.

CG MD simulations were conducted to investigate the effect of vanillin on P-gp over a long time scale. We calculated the root mean square deviation (RMSD) of P-gp embedded in the membrane to determine the stability of the protein structure and then compared the RMSD with system-added vanillin molecules (Supplementary Figure 7). All-atom MD simulations were performed to calculate the RMSD of key residues in the binding pocket and TMDs of P-gp (Supplementary Figure 7). The graph reveals that the RMSDs of all systems fluctuated slightly compared with system-added vanillin molecules, suggesting that vanillin may not affect the stability of P-gp or the P-gp binding pocket. A possible explanation for this phenomenon is that the drug-binding pocket of P-gp is composed of extremely hydrophobic and aromatic residues and is located in the center of the membrane, which is highly hydrophobic. However, the log P value of vanillin (ChemIDplus RN: 121-33-5), 1.21, was lower than that of the hydrophobic substrates of P-gp, implying that vanillin could not take up the ligand binding site and inhibit the function of P-gp.

Table.S1 physicochemical information of marker drugs

ID	Compound	miLogP	TPSA	natoms	MW	nON	nOHNH	nrotb	volume
1	acyclovir	-1.61	119.06	16	225.21	8	4	4	187.75
2	hydrochlorothiazide	-0.06	118.36	17	297.75	7	4	1	202.5
3	propranolol	2.97	41.49	19	259.35	3	2	6	257.82
4	carbamazepine	2.84	48.03	18	236.27	3	2	0	215.08

Table S2. The linear regression equation for maker drugs

Maker drug	Range(μM)	Equation (r2)
Acyclovir	0.5-100μM	y=1586.6x+1330.3 (0.9913)
Hydrochlorothiazide	0.5-100μM	y=2523.1x+759 (0.996)
Propranolol	0.5-100μM	y=657.04x-3.0026 (0.9958)
Carbamazepine	0.5-100μM	y=1759.7x-884.95 (0.9918)
Vinblastine	0.5-100μM	y=2169x-1080.2 (0.9907)

Table S3. Binding energy and interaction results of Ligand in the binding pocket of P-gp.

Compound	Binding energy(kcal/mol)	H-bonds	Hydrophobic residues
vanillin	-4.75	2	1
colchicine	-7.24	1	4
quinidine	-8.47	2	5
verapamil	-6.49	1	6
berberine	-8.59	0	6

1. Sequence alignment

Figure. S1 Sequence alignment of 3G5U and human P-gp obtained from ClustalW

2. 3D-structure of Human P-glycoprotein

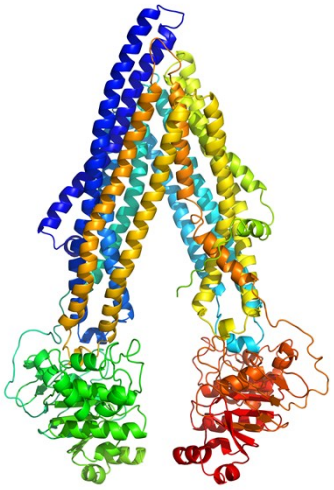

Figure. S2 The 3D structure of homology modeling of human P-gp.

3. Ramachandran plot for homology model

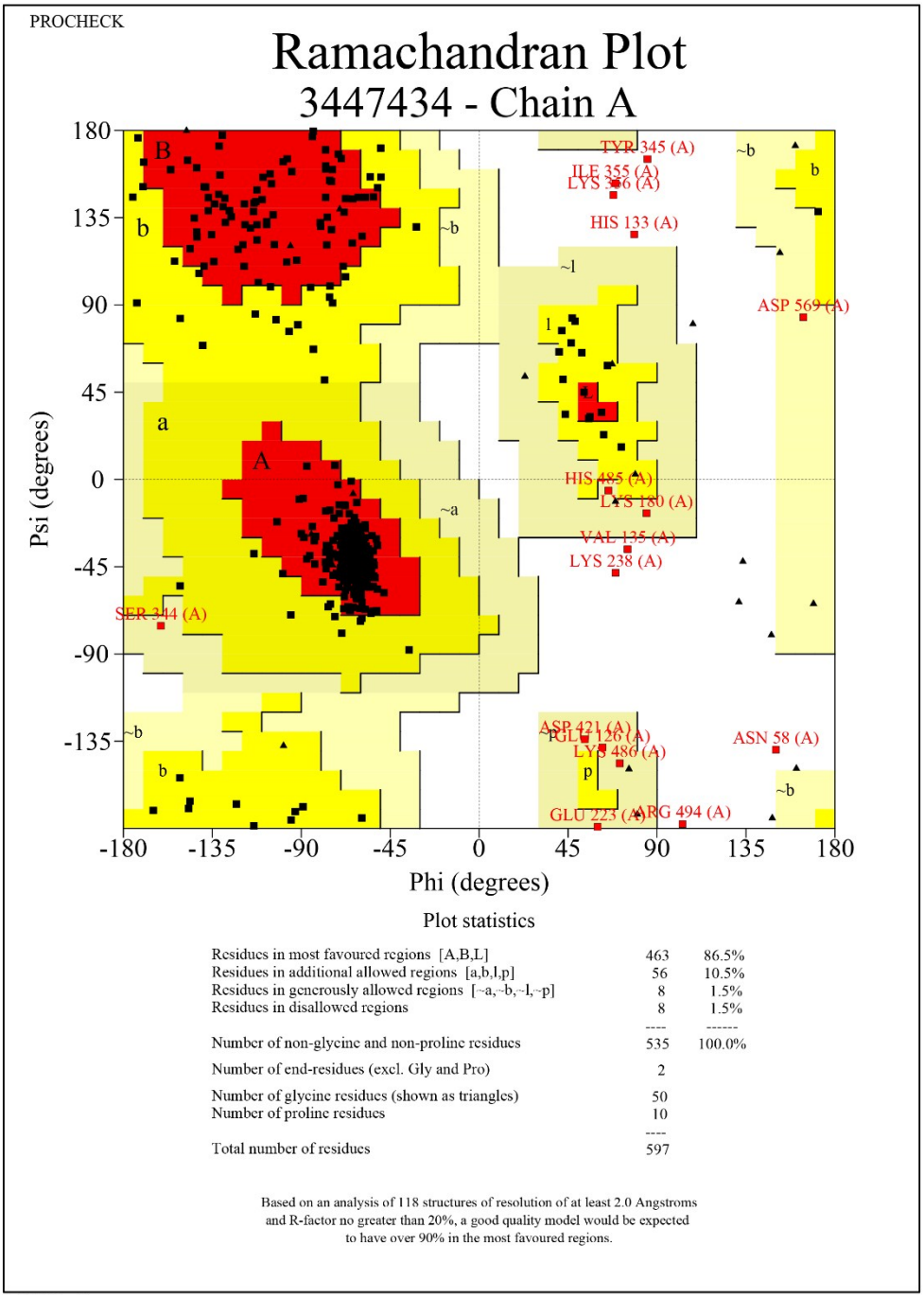

Figure. S3 Ramachandran plot for the Chain A of P-gp homology mode

4. Simulation snapshots of maker drugs located at various sites of membrane

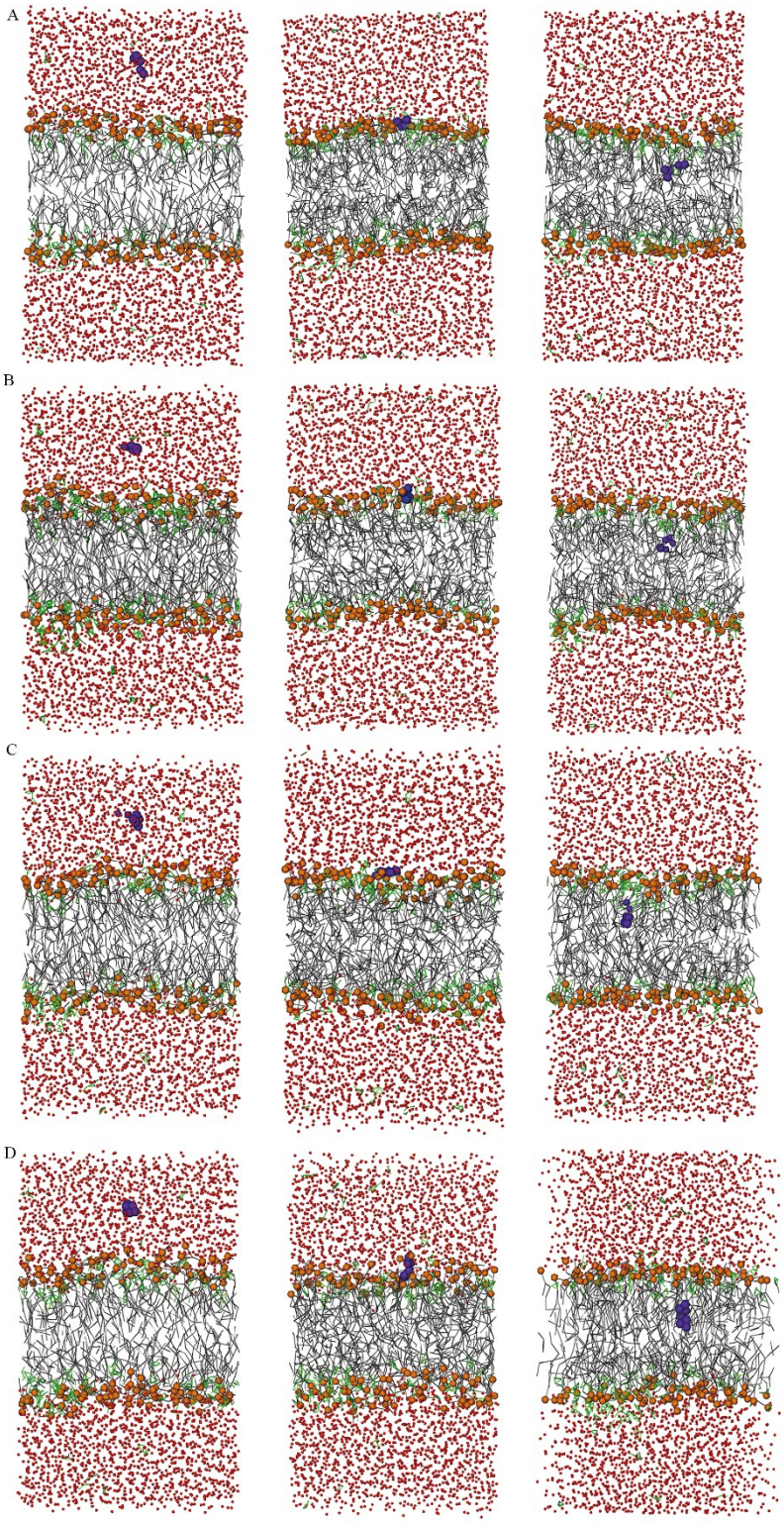

Figure. S4 Maker drugs with vanillin located at various sites of membrane: A.ACV B.HTZ C.PRO D.CBZ. In each snapshot, Maker drugs were shown as purple, the vanillin in green and the phosphorus atoms in brown. The remaining membrane atoms are shown as grey lines and water as red balls.

5. Simulation snapshots of protein-ligand system

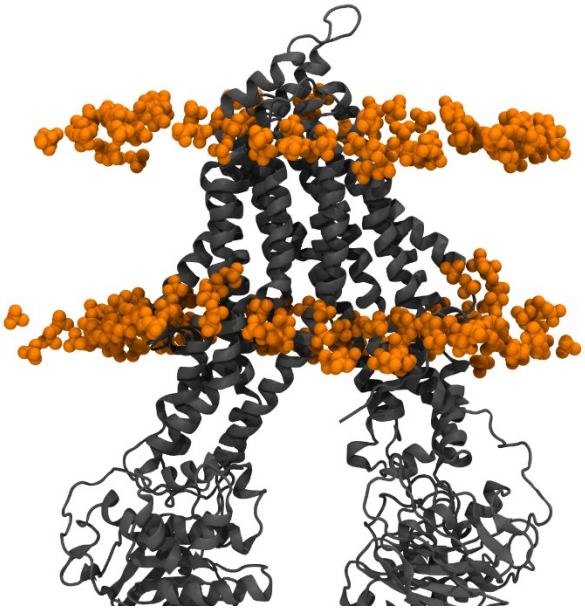

Figure. S5 Protein-lipid system for 10ns equilibration. The protein is shown as gray and the head of lipid as brown. Water have been removed for clarity.

6. Drug docking models in the human P-gp binding pocket.

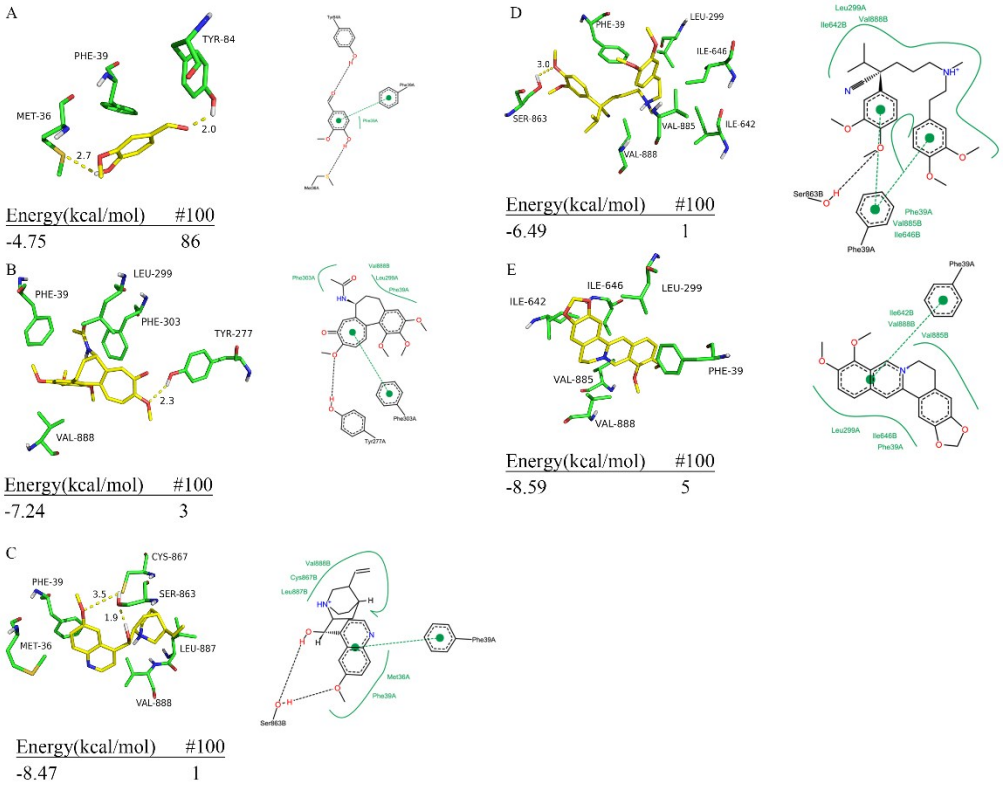

Figure. S6 Drug docking models in the human P-gp binding pocket. A. vanillin; B. colchicine; C. quinidine; D. verapamil; E. berberine. The energy of the binding pose and the number of runs were shown below. The information of H-bonds and hydrophibic interactions between drugs and P-gp are highlighted in the right. Color code: drug=yellow; residues=green; O=red; S= orange; N= blue; H=white.

7. Plots of RMSD of P-gp versus time(ns) obtained after 50ns and 1ps of production run.

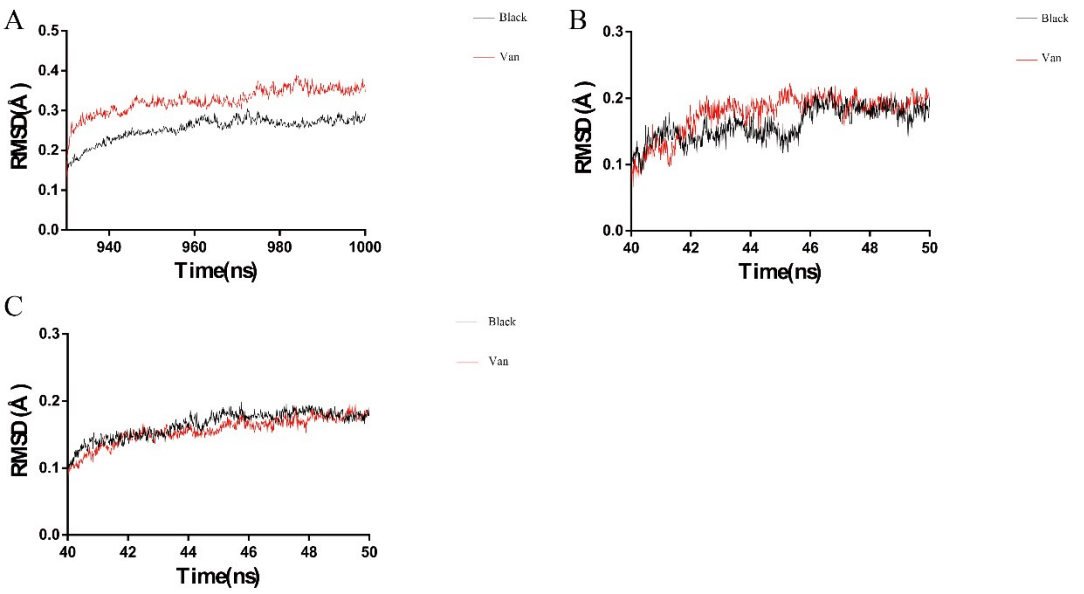

Figure. S7 Plots of RMSD of P-gp versus time(ns) obtained after 50ns and 1ps of production run. A. Coarse-grained MD simulations. B-C: All-atom MD simulations. A. Protein; B. Drug binding residues; C. TMDs of P-gp. The red plots are the system with 20% vanillin molecules and the black plots are the control group.

8. Typical chromatogram

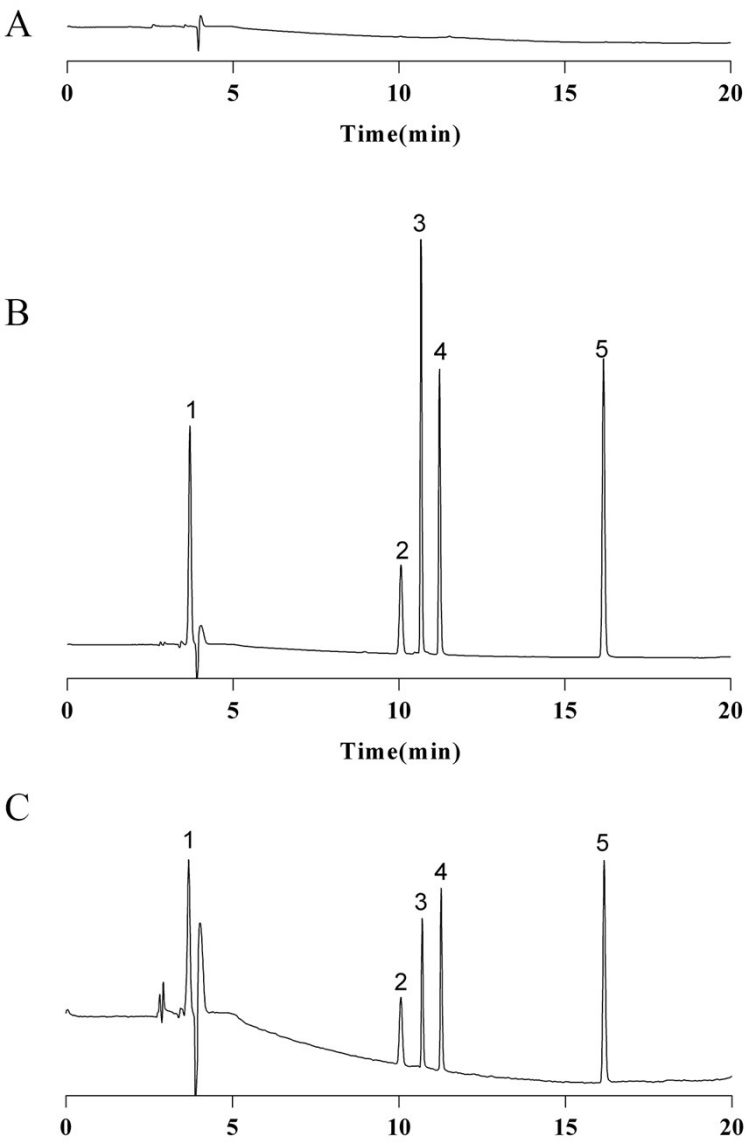

Figure. S8 Typical chromatogram of (A) HBSS buffer, (B) HBSS buffer spiked with ACV, HTZ, PRO, VIN, CBZ (50μM), (C) HBSS buffer obtained from Caco-2 cell bi-directional transport experiment. (peak 1: ACV; peak 2: HTZ; peak 3: PRO; peak 4: VIN; peak5: CBZ)

References

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