Iranian Journal of Pharmaceutical Sciences
2019: 15 (4): 73-82
www.ijps.ir

Original Article

Receptor Tyrosine Kinase Inhibitory Activities and Molecular Docking Studies of Some Pyrrolo[2, 3-*d*]pyrimidine Derivatives

Zühal Kilic-Kurt[a*], Emin Emre Kurt[b,c], Filiz Bakar-Ates[c]

[a]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey,*
[b]*Department of Medical Genetics, Faculty of Medicine, Yildirim Beyazit University, Ankara, Turkey,*
[c]*Department of Biochemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey,*

Abstract

In this study, we aimed to determine VEGFR-2, EGFR and PDGFR-β tyrosine kinase inhibitory activities of some pyrrolo[2,3-*d*]pyrimidine derivatives previously synthesized and showed potent cytotoxic and apoptotic effects against several cancer cell lines by our group and to evaluate the relationships between inhibitory activities and binding properties of the active compounds by molecular docking studies. VEGFR-2, EGFR ve PDGFR-β tyrosine kinases inhibitory activities of the tested compounds were determined using KDR Kinase Enzyme System Analysis Kit (Promega, #V2681), EGFR Kinase Enzyme System Analysis Kit (Promega, #V3831) and PDGFR-β Kinase Enzyme System Analysis Kits (Promega, #V3731) according to the manufacturer's instructions. The molecular docking studies were performed using Autodock vina program. Compounds 9a, 9b and 11b exhibited the weak inhibitory activities against VEGFR-2, EGFR and PDGFR-β, respectively. Molecular docking studies showed that one or two hydrogen bonding interactions were found between compounds 9a, 9b, 11b and VEGFR-2, EGFR, PDGFR-β tyrosine kinases. Biological activity and molecular docking results revealed that interactions of compounds with target protein active sites are not enough to obtain potent RTK inhibitory activity. It is necessary to design some compounds showing more interactions with the target proteins to obtain better activity results.

Keywords: Receptor tyrosine kinases inhibitors, pyrrolo[2,3-d]pyrimidines, molecular docking

Corresponding Authors: Zühal Kilic-Kurt, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey
Tel: +90(312) 203 30 73
E-Mail: zkurt@ankara.edu.tr
Cite this article as: Kilic-Kurt Z, Emre Kurt E, Bakar-Ates F , Receptor Tyrosine Kinase Inhibitory Activities and Molecular Docking Studies of Some Pyrrolo[2, 3-d]pyrimidine Derivatives, 15 (4): 73-82.

1. Introduction

Receptor tyosine kinases (RTK) play a major role in signal transduction pathway that regulates critical cellular processes such as cell growth, proliferation, differentiation, migration and metabolism. Under physiological conditions, the intrinsic activities of RTKs are strictly

controlled [1]. Overexpressed or increased activities of RTKs resulting from mutations, gene rearrangement or amplification have been correlated with tumor development and progression [2]. The epidermal growth factor receptor (EGFR), the first identified receptor of tyrosine kinases, is important for epithelial cell biology. It has been reported that EGFR is overexpressed in various solid tumor such as gastrointestinal tract, non-small cell lung, breast, prostate, bladder and ovarian carcinomas, head and neck cancers, glioblastoma [3,4]. Vascular endothelial growth factor receptors (VEGFRs) are known to stimulate angiogenesis and induce endothelial cell proliferation, migration, survival and permeability of blood vessel [5]. Aberrant activation of VEGFRs is reported to be linked with tumor growth and metastasis through promoting angiogenesis and enhancing vascular permability [6]. Platelet-derived growth factor (PDGF) signaling stimulates various cellular function such as survival, growth, proliferation and chemotaxis. Elevated levels of PDGF has been reported in many different human tumors [3,7].

In the last decades, since the understanding the key roles of RTKs in the tumor development and progression, inhibition of RTK to prevent cancer growth and metastasis has become an attractive approach for discovery of novel anticancer drug [8]. To date, a number of small-molecule RTK inhibitors structurally based on various heterocyclic scaffolds have been reported. Among them, kinazoline-based EGFR inhibitors such as gefitinib, erlotinib, lapatinib, vandetanib and afatinib and several VEGFR inhibitors such as indole-based sunitinib, nintedanib; indazole-based pazopanib and axitinib; urea derivative sorafenib and lenvatinib have reached widespread clinical use for cancer theraphy [9]. Urea moiety of sorafenib and lenvatinib (Figure 1) is known as an important pharmacophore in VEGFR inhibitory activity [10,11]. So, many urea compounds have been developed to inhibit RTKs. Several of these, such as PD173074 [12], ABT869 [13], CP-547632 [14], (Figure 1) have been investigated as potential anticancer agents in clinical trials.

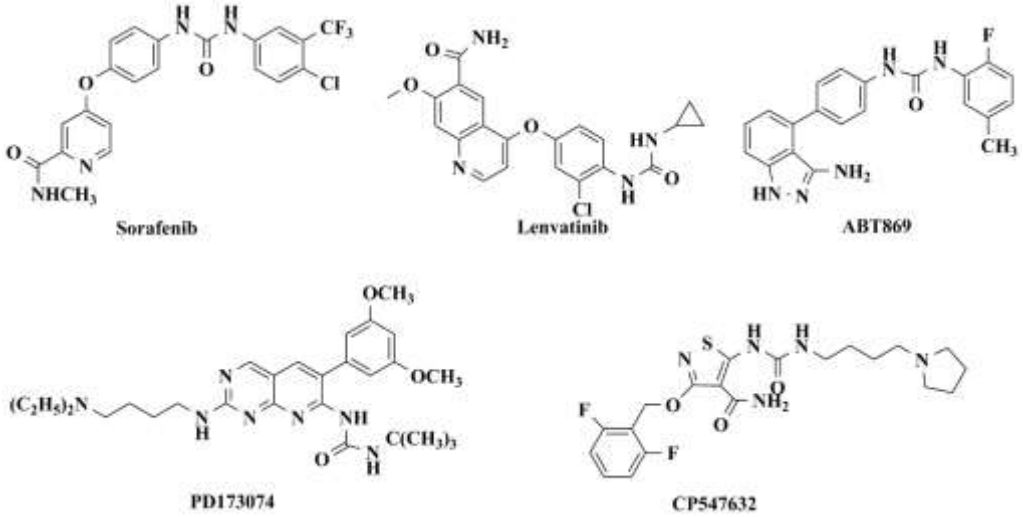


Figure 1. Receptor tyrosine kinase inhibitors in clinical trials.

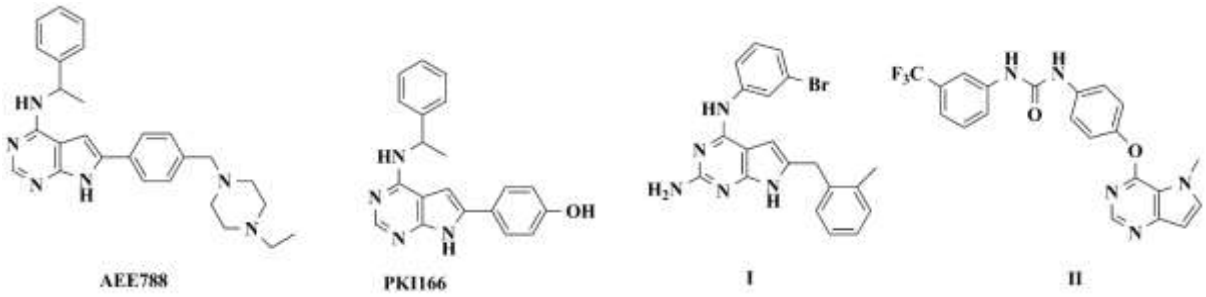


Figure 2. Pyrrolo[2,3-*d*]pyrimidine derivatives as RTK inhibitors.

Pyrrolo [2,3-*d*] pyrimidine are purine analogs that demonstrate a variety of biological activities such as antibacterial, antiviral, anticancer, anti-inflammatory, and antihyperglycemic activities [15]. There are several pyrrolo [2,3-*d*] pyrimidine derivatives of RTK inhibitors such as AE788, PKI166, compound I and II (Figure 2). AE788 is a potent inhibitor of EGFR and VEGFR-2 tyrosine kinase with IC_{50} value of 2 nM and 77 nM, respectively. Moreover, AE788 inhibited the proliferation of EGF- and VEGF-stimulated human umbilical vein endothelial cells and VEGF-induced angiogenesis in a murine implant model [16]. PKI is EGFR tyrosine kinase inhibitor in the low nanomolar range and shows potent antiproliferative effects in EGFR-overexpressing cell lines [17]. Compound I, previously reported by Gangjee et al., [18] was a potent VEGFR-2 inhibitor (IC_{50}= 0.25 μM) and displayed single-digit micromolar inhibition of EGFR in the cellular assays. Moreover, it demonstrated potent cytotoxic effect against A431 cell in culture and moderate antiangiogenic activity in the CAM assay. Compound II of urea-containing pyrrolo[3,2-*d*]pyrimidine derivatives was discovered as potent angiogenesis-related kinase inhibitors and showed strong inhibitory activity against both VEGF-stimulated human umbilical vein endothelial cells (HUVEC) proliferation with IC_{50} value of 4.4 nM [19].

Despite some of the pyrrolopyrimidine derivatives which were substituted urea motif at 4-position of pyrrolopyrimidine ring, were designed and synthesized against RTKs, pyrrolopyrimidines containing urea motif at 2-position have not been explored as multi-target VEGFR-2, EGFR and PDGFR-β inhibitors in published reports. In this paper, in vitro VEGFR-2, EGFR and PDGFR-β tyrosine kinase inhibitory activities of several urea derivatives of pyrrolo[2,3-*d*]pyrimidines, which were previously synthesized and determined anticancer activities by our research group [20] were evaluated. In addition, molecular docking studies of the active compounds with VEGFR-2, EGFR and PDGFR-β tyrosine kinases were carried out in an attempt to speculate the possible binding mode of these molecules in the active site of target enzymes.

2. Material and Methods

2.1. Molecular Docking

The crystal structure of the VEGFR-2-42Q1170 (PDB ID: 3VHE), EGFR-6309001 (5HG7) and PDGF-BB–PDGFR-β (3MJG) complex were obtained from the Protein Database (PDB, http://www.rcsb.org). The three-dimensional structures of the aforementioned compounds were constructed, then they were energetically minimized. All bound ligands and water molecules were removed from the proteins and the polar hydrogen was added to the proteins. Polar hydrogens and Gasteiger charges of co-crystalized ligands were assigned using AutoDockTools. The grid boxes were adjusted with a volumetric space of 30x30x30 and 40x40x40 for VEGFR-2 and EGFR tyrosine kinase proteins, respectively. Since no active site of PDGFR-β has been annotated in literature, a blind docking was performed with the whole protein as a target with volumetric space of 62x112x104. The docking study was performed using the Lamarckian genetic search algorithm implemented in AutoDock vina 1.1.2. The 3D compound-protein docking posses were analyzed manually using AutoDockTools.

2.2. Kinase Inhibitory Activity

At present study, we evaluated the effects of synthesized compounds on receptor tyrosine kinases like VEGFR-2, EGFR ve PDGFR-β. The assays were performed using KDR Kinase Enzyme System Analysis Kit (Promega, #V2681), EGFR Kinase Enzyme System Analysis Kit (Promega, #V3831) and PDGFR-β Kinase Enzyme System Analysis Kits (Promega, #V3731) according to the manufacturer's instructions. Kits include microwell plates that coated with tyrosine kinase receptor antibody. Briefly, the calibrator and synthesized compounds were added to the wells at 100, 10, 1 ve 0.1 µM concentrations with biotin-conjugated polyclonal antibody that was specific for enzyme receptor. 10 µl enzyme-substrate mix and 10 µl ATP-assay solution were then added and incubated for 15 minutes at 30 C. Following incubation step 25 µl ADP-Glo reagent were added to the microwells, mixed and incubated at room temperature 40 minutes. 50 µl kinase detection agent was added and incubated at room temperature for 30 minutes. Then luminescence levels were detected by Molecular Devices, SpectraMax M2 (United Kingdom) device.

3. Result and Discussion

3.1. Biological Activity

Target compounds were evaluated for their inhibitory activities against RTK family members VEGFR-2, EGFR and PDGFR-β by screening at concentrations of 100, 10, 1 and 0.1 µM. Table1 present the percent inhibition of these RTK members at 100 µM concentration (Figure 3); the IC_{50} values of selected compounds are donated in parentheses. All compounds showed the inhibitory activities against all tested RTKs with less than 50% inhibition at 100 µM. Compound 9a bearing 3-trifluorophenylurea

moiety exhibited a dramatic increase in VEGFR-2 inhibitory activity with IC_{50} value of 140 µM compared to 4-fluorophenylurea derivative 11a (IC_{50}> 1000 µM). The VEGFR-2 inhibitory activity of compound 10a (IC_{50}= 198 µM), which has a chloro group at 4-position of 3-trifluorophenyl urea moiety, was well tolerated compared to 9a (IC_{50}= 140 µM). Similar VEGFR-2 inhibitory was observed in the case of the compound 9d (IC_{50}= 204 µM). Tested compounds had weak inhibitory activity against EGFR and PDGFR-β tyrosine kinases. EGFR tyrosine kinase inhibitory activities of 9b, 9c, 10b and 11b were all similar, with IC_{50} value of 249, 292, 280 and 259 µM, respectively. Compound 11b (IC_{50}= 129 µM) exhibited the best inhibitory activity against PDGF-β receptor kinase. Replacement of 4-fluorophenylurea moiety of 11b to 3-trifluoro- (9b) and 4-chloro-3-trifluorophenylurea (10b) resulted in approximately 2-fold reduction in PDGF-β RTK inhibitory activity.

3.2. Molecular Docking

In order to evaluate the binding modes and hydrogen bonding interactions of the compounds 9a, 9b and 11b which have the best inhibitory activity against tested RTKs, were docked in to the active sites of VEGFR-2, EGFR and PDGFR-β, respectively using Autodock vina. VEGFR-2, EGFR and PDGFR-β tyrosine kinase domain crystal structure with PDB code: 3VHE, 5HG7 and 3MJG were selected. The docking method was validated by re-docking of co-crystallized ligand 42Q1170 and 6309001 into the binding sites of VEGFR-2 and EGFR, respectively. The re-docked ligands were superimposed with co-crystallized ligand 42Q1170 and 6309001 with RMSD value below 2 Å. Also, re-docked ligand 42Q1170 showed hydrogen bond interactions with Cys919 and Asp1046 amino acids of VEGFR-2 active site residue, while co-crystallized ligand forms three hydrogen bonds with Cys919, Asp1046 and Glu885. For the other re-docked ligand 6309001 only one hydrogen bond was observed with Met793 in the active site of EGFR, despite co-crystallized 6309001 bound to the active site of EGFR via three hydrogen bonding interactions with Met793 and Gln791. Due to lack of annotated active site of PDGFR-β, a blind docking was performed for the compound 11b with both of the chains of the PDGF-BB–PDGFR-β complex and the complex as a whole [23].

The binding model of compound 9a, 9b and 11b and VEGFR-2, EGFR and PDGFR- β tyrosine kinases, respectively were depicted in figure 4A-C. Compound 9a located into the active site of VEGFR-2 by forming a hydrogen bond between urea moiety and the backbone of C=O Glu885. For the less potent compound 9b a similar molecular orientation as co-crystallized ligand 6309001 and hydrogen bond interaction of Met793 with the NH group of 4-chlorophenylamino moiety were observed. Compound 11b was anchored in PDGFR-β via two H-bonds between carbonyl of urea and 7-NH group with Cys99 and C=O group of Cys16.

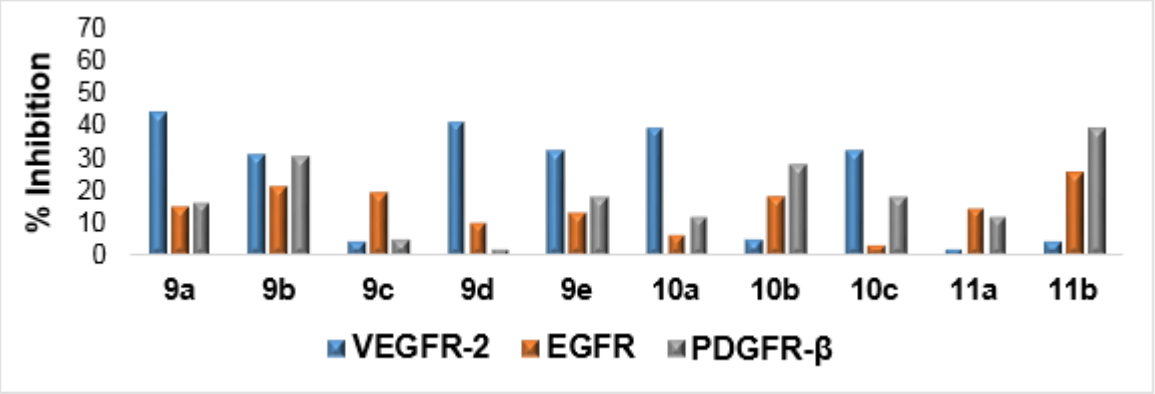
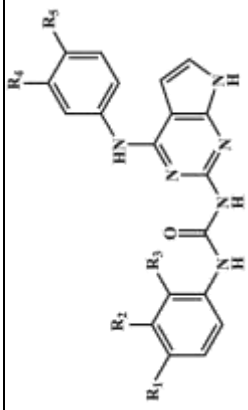


Figure 3. The effects of test compounds on VEGFR-2, EGFR and PDGFR-β inhibition at 100 µM concentration.

Table 1. Inhibitory activities of some pyrrolo[2,3-*d*]pyrimidine derivatives against VEGFR-2, EGFR and PDGFR-β tyrosine kinases.



Cmpd	R_1	R_2	R_3	R_4	R_5	VEGFR-2[a]	EGFR[a]	PDGFR-β[a]
9a	-H	$-CF_3$	-H	-Br	-H	44.38±0.017 (140)	15.15±0.189	15.69±0.220
9b	-H	$-CF_3$	-H	-Cl	-H	31.41±0.009	21.03±0.174 (249)	30.05±0.182 (227)
9c	-H	$-CF_3$	-H	-Cl	-F	3.83±0.016	19.13±2.051 (292)	5.23±0.221
9d	-H	$-CF_3$	-H	-H	-F	40.94±0.201 (204)	9.99±0.183	1.73±0.250
9e	-H	$-CF_3$	-H	$-CF_3$	-H	31.65±0.009	12.97±0.174	17.68±0.247
10a	-Cl	$-CF_3$	-H	-Br	-H	38.55±0.009 (198)	5.84±0.172	12.48±0.142
10b	-Cl	$-CF_3$	-H	-H	-Cl	5.43±0.054	17.96±0.171 (280)	27.57±0.196 (268)
10c	-Cl	$-CF_3$	-H	-Cl	-F	32.12±0.008	2.59±0.158	17.98±0.172
11a	-F	-H	-H	-Br	-H	1.59±0.011	13.77±0.130	11.68±0.247
11b	-F	-H	-H	-H	-Cl	3.99±0.017	24.71±0.173 (259)	39.02±0.223 (129)
Erlotinib						10 nM[c]	2 nM[b]	10 nM[c]
Sunitinib								

[a]The percent inhibition of each RTK family member at 100 µM concentration of the compounds. Representative derivative of potent inhibitor with the IC_{50} value denoted in micromolar units in parentheses. [b]ref. 21, [c]ref. 22.

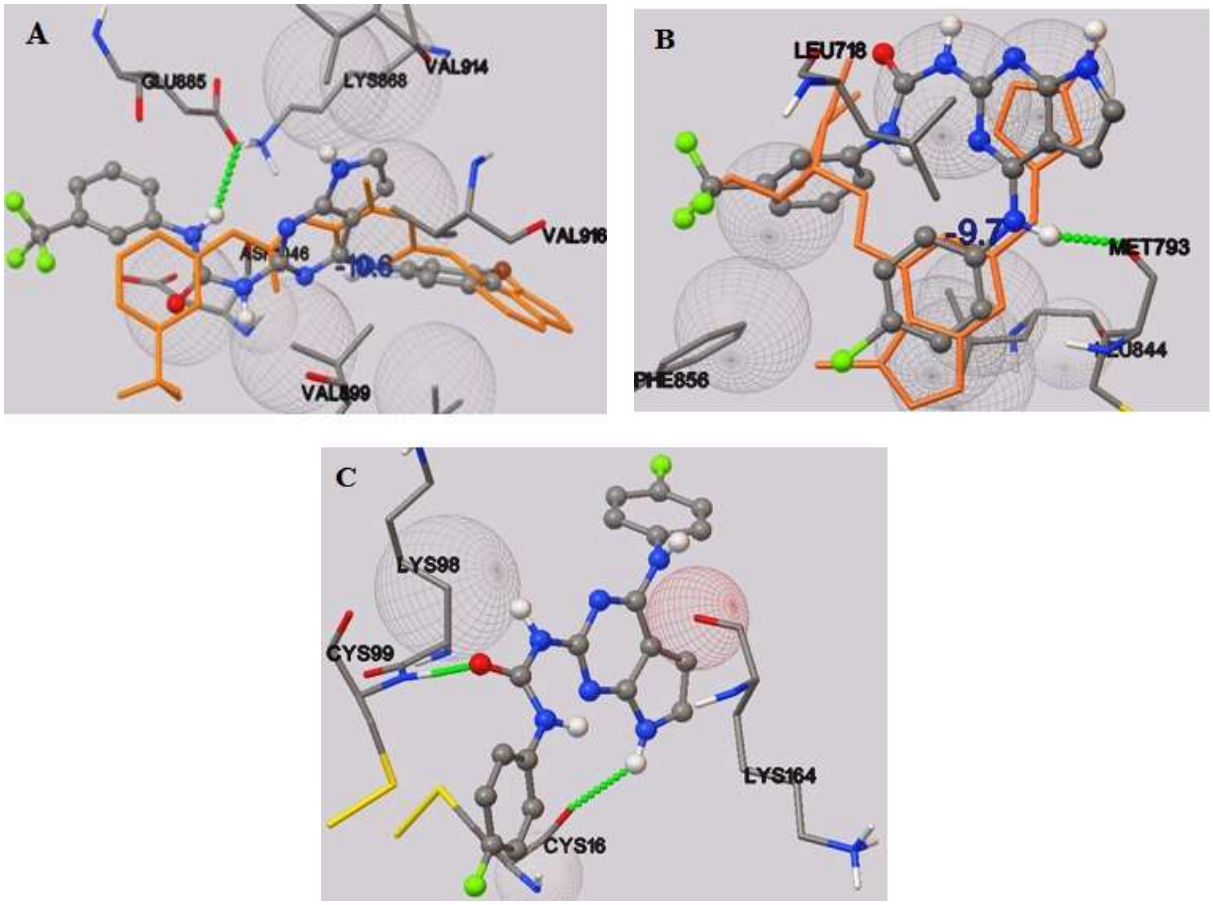


Figure 4. The predicted binding properties of the compounds **9a** (A), **9b** (B) and **11b** (C) in the catalytic site of VEGFR-2, EGFR and PDGFR- β tyrosine kinases, respectively. Co-crystallized ligands **42Q1170** (A) and **6309001** (B) were shown in orange coloured stick. The predicted pose of compounds are shown by element coloured stick and ball. H-bonds are presented as green dashed lines.

4. Conclusion

In this study, inhibitory activities of some pyrrolo[2,3-*d*] pyrimidine derivatives against VEGFR-2, EGFR and PDGFR-β tyrosine kinases and relationships between biological activity and binding properties of the compounds were evaluated. All of the compounds exhibited poor inhibition on the tested RTKs. The best inhibitory activity was obtained from the compounds 9a, 9b and 11b against VEGFR-2, EGFR and PDGFR-β tyrosine kinases with IC_{50} value of 140, 249 and 129 µM. While the co-crystallized ligands 42Q1170 and 6309001 bind VEGFR-2 and EGFR tyrosine kinases active sites with three hydrogen bonds, compounds 9a and 9b showed only one hydrogen bonding interactions with the proteins, which might explain the weak inhibitory activities of 9a and 9b. Compound 11b interacted with PDGFR-β tyrosine kinases by forming two hydrogen bond with Cys99 and Cys16 amino acides. The better inhibitory activity of the compound 11b than 9a and 9b could be resulted from the number of hydrogen bonds. In conclusion, it may be necessary to design some compounds showing more interaction with the target proteins to obtain better activity results.

Acknowledgement

This work was supported by grant from The Scientific and Technological Research Council of Turkey (TUBITAK, 214S573).

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