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Bioinformatic and Molecular Interaction Studies Uncover That CCA-1.1 and PGV-1 Differentially Target Mitotic Regulatory Protein and Have a Synergistic Effect against Leukemia Cells

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ABSTRACT

CCA-1.1, a modified compound from PGV-1, inhibits tumor cell growth in breast and colorectal cancer. Here, we used bioinformatic and molecular interaction approaches to ascertain the potential activity of CCA-1.1 in targeting leukemia and applied the cytotoxic test in leukemia cells. Genomic data were collected from the Catalogue of Somatic Mutations in Cancer database by using the gene sets from K562 cells as a model for chronic myelogenous leukemia (CML). We identified CCA-1.1 and PGV-1 predicted targets through SwissTargetPrediction. The overlapping genes among CCA-1.1, PGV-1, and K562 cells were chosen for further analysis. We narrowed down the potential targets by using the list of genes involved in cell cycle and mitosis collected through GeneCards. Molecular docking study was performed to determine the molecular interaction of CCA-1.1 or PGV-1 with the predicted protein target. We carried out a cytotoxic test using trypan blue exclusion assay. We treated K562 cells with CCA-1.1 and PGV-1 alone or in combination to determine the half-maximal concentration growth-inhibitory concentration and combination index score. The two compounds shared similar predicted target genes in mitosis, and CCA-1.1 mainly targeted Aurora A kinase in K562 cells with relatively low docking scores against the inhibitor in molecular docking analysis. Both compounds exhibited a similar inhibitory influence, and their co-treatment resulted in a synergistic effect on K562 cells. In conclusion, we indicated that CCA-1.1 and PGV-1 possibly target mitosis in cell cycle progression and, along with their specific targets, exhibit their synergistic activity in CML. These findings should be validated through experimental studies to provide data on the pharmacological activities of CCA-1.1 for CML treatment.

Keywords: CCA-1.1, PGV-1, chronic myelogenous leukemia, bioinformatics, molecular docking, cytotoxic test

INTRODUCTION

Pentagamavunone-1 (PGV-1) showed anticancer activity against leukemia cells in preclinical studies; the proposed mechanism underlying its effective and irreversible inhibiting effect on chronic myeloid leukemia (CML) cells' proliferation (with half-maximal growth-inhibitory (GI_{50}) value less than 1 μM) includes prometaphase arrest and induced cell senescence through elevated intracellular reactive oxygen species

(ROS) levels (Lestari *et al*., 2019). PGV-1 also suppresses ROS-metabolizing enzymes, which eventually increase ROS levels and cause cell senescence. An *in vivo* study showed the importance of developing this compound due to its ability to efficiently impede tumor development with negligible side effects (Lestari *et al*., 2019; Meiyanto *et al*., 2019). A novel synthetic curcumin analog named CCA-1.1 was synthesized by modifying PGV-1 structure. CCA-1.1 exhibits similar cytotoxic effect to PGV-1 against several cancer cell lines and remains less toxic toward non-cancerous cells. In a molecular docking study, CCA-1.1 performed better than PGV-1 and showed a higher binding affinity toward several ROS scavengers that might have partly contributed to the increased intracellular ROS levels (Utomo *et al*., in press.). In experimental studies, CCA-1.1 halted cell cycle progression and apoptosis and enhanced ROS levels, and these effects are partly correlated with cellular senescence in breast cancer cells (Novitasari, *et al*., 2021; Novitasari, *et al*., 2021; Novitasari, *et al*., 2021; Wulandari *et al*., 2020). Similar activities were observed against colorectal tumor cells (Wulandari *et al*., 2021). Hence, CCA-1.1 must be further investigated to determine its activities toward other cancer cells.

This study focused on leukemia cells, particularly CML, an uncommon type of tumor that originates from the bone marrow (Eden & Coviello, 2021). This disease is associated with the translocation of the Philadelphia chromosome (BCR-ABL), a producer of tyrosine kinase, which leads to abnormal cell cycle progression (Faderl *et al*., 1999). Current existing drugs to cure CML, including imatinib, dasatinib, and the recently US-FDA-approved asciminib, are categorized as tyrosine kinase inhibitors (Hochhaus *et al*., 2020). In addition to targeting BCR-ABL, a new target for CML treatment must be identified by investigating other molecular mechanisms, such as cell cycle and cancer metabolism. Given that PGV-1 can target cell cycle arrest in prometaphase, show high selectivity against normal cells, and inhibit tumor growth in a xenograft model, the anticancer properties of CCA-1.1 against CML must also be explored.

As curcumin analogs, PGV-1 still faces problems on bioavailability and stability. CCA-1.1 with the modified ketone to hydroxyl was purposely synthesized to achieve improved stability and good cytotoxic effects similar or even better than those of PGV-1. This study explores PGV-1 and CCA-1.1 (Figure 1A and 1B) as potential anticancer drugs for CML through bioinformatic analysis and subsequent in vitro screening with single and combination treatments. The results will provide insights and comprehensive scientific information on CCA-1.1 as a potential anticancer drug.

MATERIALS AND METHODS

Target prediction and data acquisition

The targets of CCA-1.1 and PGV-1 were estimated using the library database from Swiss Target Prediction (http://www.swisstargetprediction.ch/) (Daina *et al*., 2019) with default settings. We selected the expressed genes in CML K562 cells from the Catalogue of Somatic Mutations in Cancer (COSMIC) Cell Line Project (https://cancer.sanger.ac.uk/cosmic) (https://doi.org/10.1093/nar/gky1015) and gathered the list for the cell cycle genes in K562 from GeneCards (https://www.genecards.org/) by typing "cell cycle K562" on the search box. The top 500 genes generated in GeneCards were selected. A Venn diagram was drawn for the possible targets of each compound and the expressed genes in K562 cells, and the overlaps were generated as potential targets (PTs) in K562 cells.

Molecular docking analysis

We utilized the Molecular Operating Environment (MOE) version 2010.12 with default settings to generate the molecular interaction between ligand and receptor (unless otherwise mentioned). CCA-1.1 and PGV-1 structures were drawn using the compound builder in the program and energetically minimized in the MMFF94 forcefield. The conformational structure was then explored. Aurora kinase A (AURKA, PDB ID:4BYJ), cyclin-dependent kinase 1 (CDK1, PDB ID:6GU6), WEE1 (PDB ID:7N3U), and kinesin family member 11 (KIF11, PDB ID:3K5E) were chosen as the protein targets in this study. The docking protocol of Hasbiyani *et al*. (2021) was adopted. The ligand conformation with the lowest docking score was analyzed for the binding interaction.

Cytotoxic assay

The immortalized CML K562 cells were used as the model for *in vitro* screening and maintained in RPMI culture medium (Fujifilm Wako Chemical, Japan) and grown until they reached high confluence. In brief, 1×10^4 cells were plated in a 48-well plate. Concentration variances of CCA-1.1, PGV-1, or their combination at specific concentrations (i.e., fractions of IC_{50}) were diluted in the culture medium.

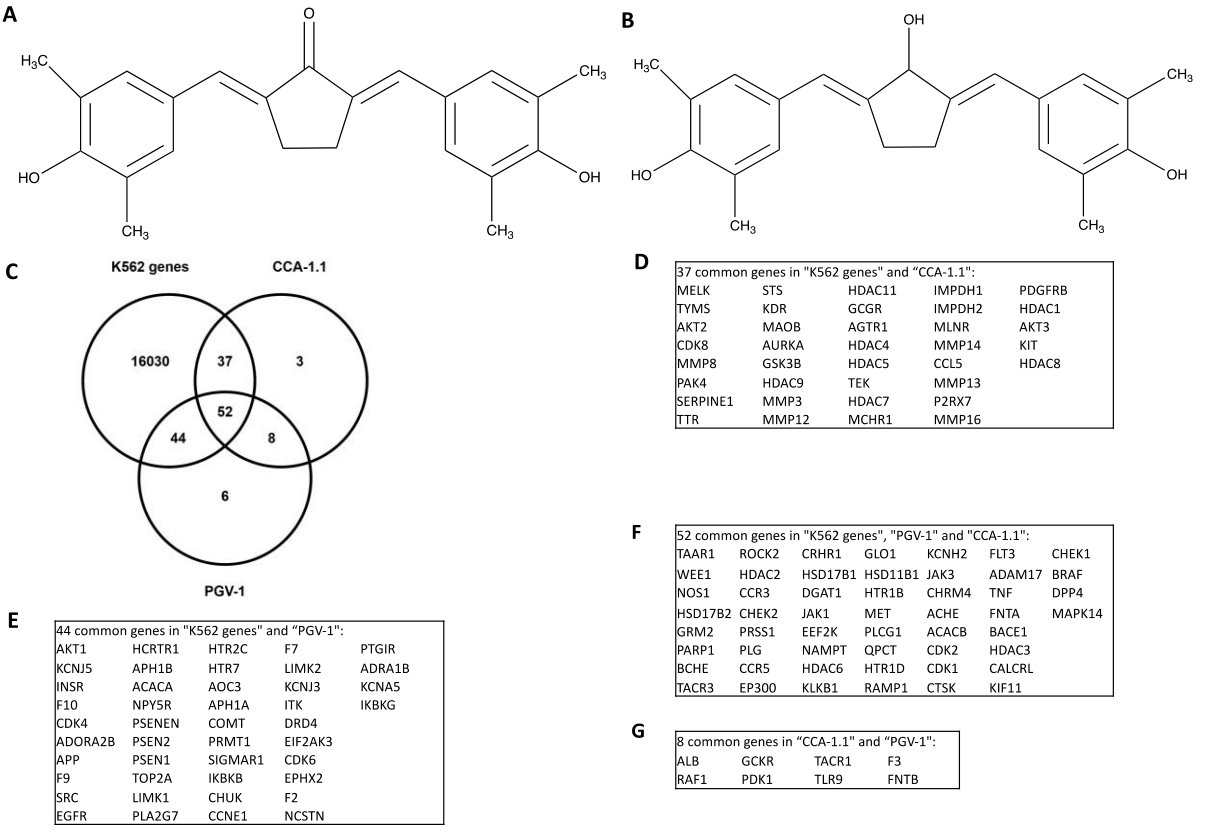


Figure 1. Chemical structure of (A) PGV-1 and (B) CCA-1.1. (C) A Venn diagram between the predicted target of CCA-1.1 and PGV-1 against regulated genes in K562 cells which collected from the COSMIC database. Overlapping genes from the common genes between (D) CCA-1.1 and K562 cells. (E) PGV-1 and K562 cells. (F) CCA-1.1, PGV-1, and K562 cells. (G) CCA-1.1 and PGV-1 only.

After 24 h, a certain amount of cell suspension was mixed with trypan blue (Fujifilm Wako Chemical, Japan), and the viable cells were counted under an inverted microscope. The quantified viable cells were plotted as linear regression and converted into half growth inhibitory (GI_{50}) value and combination index (CI) value using Excel MS Office.

RESULT AND DISCUSSION

Data acquisition for CCA-1.1 and PGV-1 potential target genes

The possible targets of CCA-1.1 and PGV-1 against leukemia were identified using bioinformatic approaches. From the experimental perspective, CCA-1.1 has anticancer activities against breast and colorectal cancer (Novitasari, *et al*., 2021; Novitasari, *et al*., 2021; Novitasari, Wulandari, *et al*., 2021; Wulandari *et al*., 2020, 2021) and exhibits a similar molecular phenomenon to PGV-1 (Lestari *et al*., 2019; Meiyanto *et al*., 2018, 2019). Given the preliminary *in vitro* results for these cancer cell lines, we explored the potential target genes of CCA-1.1 as a potential antitumor agent against CML. A total of 16,163 genes regulated within the K562 cells were extracted from the COSMIC database (Supplementary Table I). CCA-1.1 and PGV-1 target prediction using SwissTargetPrediction identified 100 genes for each compound (Supplementary Tables 2 and 3). Using a Venn diagram, we found that 37 and 44 genes overlapped specifically between CCA-1.1 and PGV-1 (Figures 1C, 1D, and 1E). Given that CCA-1.1 was synthesized on the basis of PGV-1 chemical structure, 52 genes were shared among CCA-1.1, PGV-1, and K562 cells (Figure 1F), and 8 genes intersected between CCA-1.1 and PGV-1, though not in K562 cells (Figure 1G). These data indicated that CCA-1.1 and PGV-1 exhibit distinct molecular mechanisms to inhibit tumor cell proliferation and

similar pharmacological activities. Considering that CCA-1.1 and PGV-1 induce cell cycle arrest in mitosis, we narrowed down the PTs of these curcumin analogs in cell cycle regulation, particularly in mitosis.

CCA-1.1 and PGV-1 potentially target mitotic regulatory proteins in K562 cells

Experimental studies showed that PGV-1 and CCA-1.1 promote antineoplastic properties against CML K562 cells (Lestari *et al*., 2019; Utomo *et al*., in press.). The pharmacological effect of PGV-1 on K562 cells is expressed through several mechanisms: inhibit cell cycle progression, induce apoptosis and senescence, elevate intracellular ROS level, and suppress ROS-metabolizing enzymes. Therefore, we analyzed the potential proteins that affect CCA-1.1 and PGV-1 in cell cycle regulation as crucial points in cancer cell proliferation. Using the GeneCards database, we collected a total of 500 genes that regulate cell cycle in K562 cells (Supplementary Table IV). Venn diagram analysis revealed that 15 genes overlapped between CCA-1.1 and PGV-1 against cell cycle regulator protein in K562 cells (Figure 2A and 2B). Moreover, 12 genes were explicitly targeted by CCA-1.1 (Figure 2C), and 13 were presumed as target genes by PGV-1 (Figure 2D).

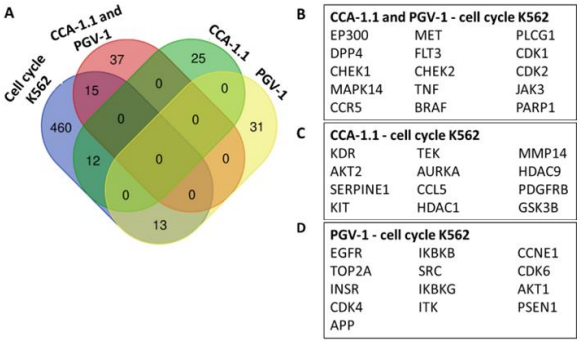


Figure 2. (A) A Venn diagram between the predicted target of CCA-1.1 and PGV-1 in K562 cells and the cell cycle regulator (CCR) genes in K562 cells collected from GeneCards website. The overlapping genes resulted in shared genes between (B) CCA-1.1, PGV-1, and CCR. (C) CCA-1.1 and CCR. (D) PGV-1 and CCR.

In vitro results displayed that PGV-1 inhibited mitosis during cell cycle, resulting in apoptosis and senescence. Using the list (Figure 2), we sorted the genes that might become the target of CCA-1.1 and PGV-1 against the mitotic genes regulated in K562 cells (Figure 3A). Only 15 genes (10 of them were shared with PGV-1) were sorted as PTs of CCA-1.1 in mitosis (Figures 3B and 3C), and 18 genes (8 of them were only specific to PGV-1) were listed as possible drivers of mitosis (Figures 3B and 3D). Some mitotic proteins including CDK1, KIF11, and WEE1 were targets of CCA-1.1 and PGV-1. CDK1 is involved during G2 transition to mitosis by binding to cyclin B and inducing chromosome condensation and microtubule dynamics (Enserink & Kolodner, 2010). Kinesin family member 11 (KIF11) functions in mitosis by interceding the centrosome segregation and conformation of the bipolar mitotic spindle (Jiang *et al*., 2017). WEE1 kinase protein ensures the inactivation of its downstream target CDK1 until the latter passes to enter mitosis (Ghelli Luserna di Rorà *et al*., 2020). A previous bioinformatic study using triple negative breast cancer also presented similar results and showed that CCA-1.1 targets several mitotic regulatory proteins (Novitasari, *et al*., 2021). Some known mitosis-driven proteins such as PLK1 and AURKB were not listed in the present study because each cancer cell has distinct mechanism and control in mitosis and some genes or protein levels differ across cancer types.

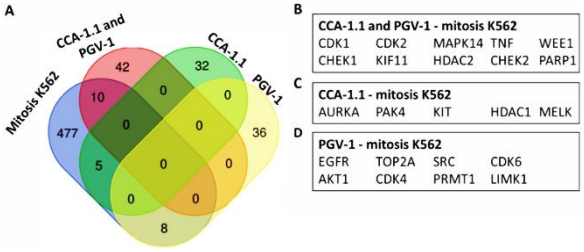


Figure 3. (A) A Venn diagram between the possible target of CCA-1.1 and PGV-1 in mitosis phase on K562 cells. The intersected genes resulted in shared genes between (B) CCA-1.1, PGV-1, and mitosis K562 cells. (C) CCA-1.1 and mitosis K562 cells. And (D) PGV-1 and mitosis K562 cells.

Regardless of the minor modification in CCA-1.1 from PGV-1, at least five proteins involved in mitosis were specifically targeted by CCA-1.1, but not by PGV-1. We highlighted that CCA-1.1 was predicted to target *AURKA,* which encodes Aurora A kinase protein synthesis. Aurora A is a serine/threonine kinase required in the cell cycle, and its activity peaks during mitosis. Aurora A controls the CDK1/cyclin B complex to enter mitosis and localizes differently according to the cell cycle phase (in centrosome during late G2 and in spindle along with mitosis) (Wysong *et al*., 2009).

Table I**.** Docking score of ligands against AURKA, CDK1, KIF11, and WEE1 through MOE program.

Protein	Ligand	ΔGibbs (kcal/mol)	RMSD (Å)	Residue(s)
AURKA (PDB ID : 4BYJ)	FH5	−9.5224	1.3518	Arg 137; Leu 139; Ala 213; Lys 162
	CCA-1.1	−11.1132	0.7971	Leu 139; Ala 213
	PGV-1	−11.1229	0.9811	Leu 139; Ala 213
CDK1	Dinaciclib	−14.8272	1.4671	Val A18; Phe A82
(PDB ID : 6GU6)	CCA-1.1	−15.4429	0.8507	Asp A146
	PGV-1	−12.5535	1.4564	Lys A89
WEE1	ZN-c3	−15.5438	0.9672	Phe 411; Asp 463
(PDB ID : 7N3U)	CCA-1.1	−11.7940	1.3534	Ile 305; Lys 328
	PGV-1	−12.1074	1.2483	Phe 433
KIF11	Enastrol	−10.4167	1.2695	Gly 117
(PDB ID: 3K5E)	CCA-1.1	−10.1758	1.4507	Pro 27; Phe 113
	PGV-1	−11.4045	1.9923	Thr 112; Glu 116

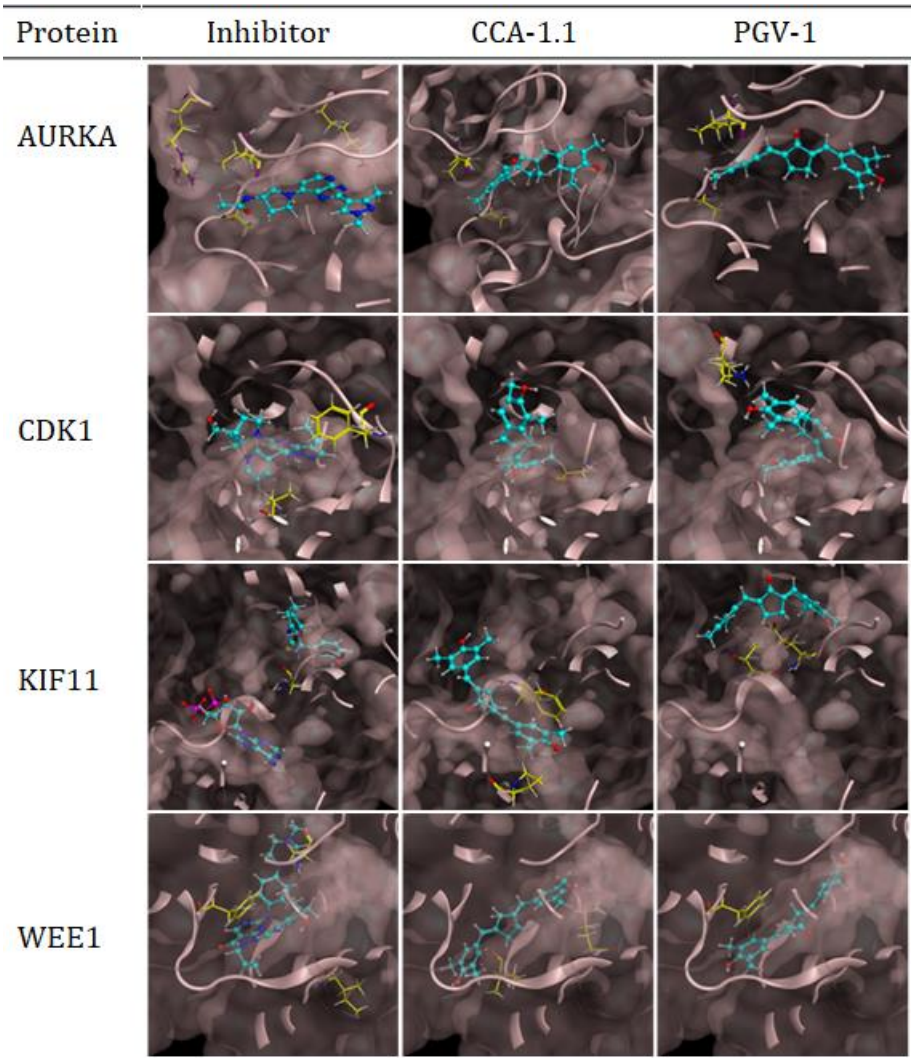


Figure 4. Binding interaction between native ligand, CCA-1.1, and PGV-1 in the pocket site of AURKA, CDK1, KIF11, and WEE1.

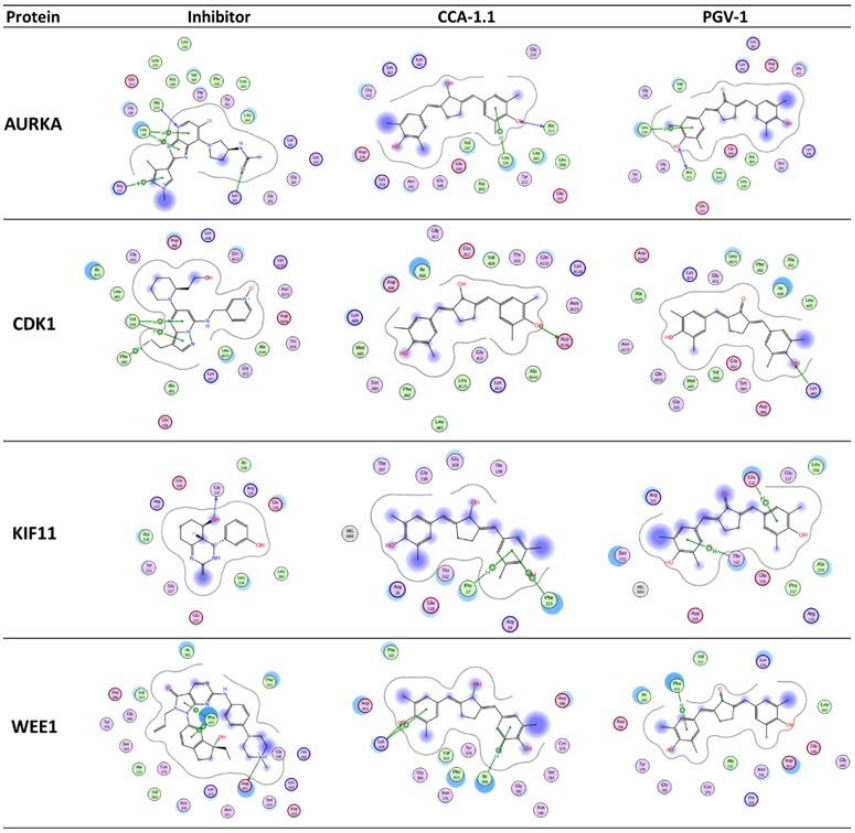
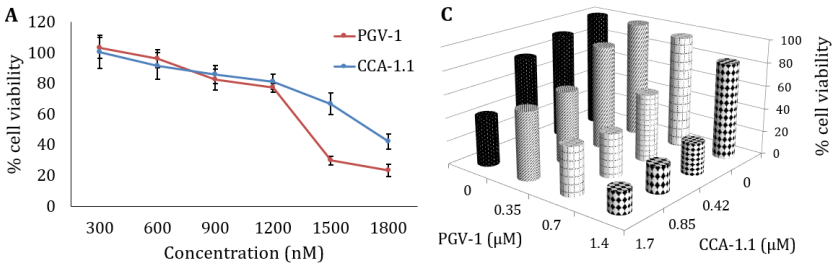


Figure 5. Binding interaction between native ligand, CCA-1.1, and PGV-1 with amino acid residue in AURKA, CDK1, KIF11, and WEE1 protein.



B

GI_{50} value (µM)	
PGV-1	1.4
CCA-1.1	1.7

D

PGV-1 (µM)	CCA-1.1 (µM)		
	0.42	**0.85**	**1.70**
0.35	0.34	0.30	0.50
0.70	0.24	0.27	0.46
1.40	0.25	0.32	0.45

Figure 6. Cytotoxic effects of (A) single treatment of CCA-1.1 and PGV-1 in K562 cells. The half growth-inhibitory value of each tested compound after treatment for 24 h (B) The percentage of cells viability upon the combination of CCA-1.1 and PGV-1 in K562 cells after 24 h of administration. The combinatory index (CI) value of the co-treatment of CCA-1.1 with PGV-1. The cytotoxic assay was experimented with according to the method. The data resulted as the average of 3 independent data ± SD.

The malfunction of Aurora A control in leukemia is uniquely found in acute megakaryocytic leukemia; as a result, the cells fail to exit mitosis and form as polyploids (Goldenson & Crispino, 2015). Owing to its involvement in the cell cycle, Aurora A becomes a hotspot target for antimitotic drugs. One preclinical study found that MLN8237 (the selective inhibitor of Aurora A) effectively kills CML with T315I mutation, which is responsible for the disease' resistance to therapy, and exhibits BCR-ABL independent mechanism (Kelly *et al*., 2011). We then investigated the binding interaction of CCA-1.1 and PGV-1 with the target protein through *in silico* studies.

Molecular docking analysis revealed that CCA-1.1 and PGV-1 interact with specific properties

Overcoming checkpoints that control cell cycle progression is essential for oncological disease treatments. By using the bioinformatics data, we evaluated the possible interaction of CCA-1.1 and PGV-1 with some target protein markers in the mitosis phase including AURKA, CDK1, KIF11, and WEE1 protein based on molecular docking. We used the inhibitor of each protein as a native ligand, that is, FH5 molecule in AURKA, dinaciclib in CDK1, enastrol in KIF11, and ZN-c3 in WEE1.

CCA-1.1 and PGV-1 interacted in the pocket side of AURKA with lower energy compared with the native ligand, though they shared a similar binding site with the inhibitor (Table I). Henceforth, the involvement of CCA-1.1 in Aurora A regulation is promising and warrants further studies. CCA-1.1 had a lower binding affinity than dinaciclib against CDK1, and PGV-1 exhibited a slightly higher docking score against dinaciclib. KIF11 and WEE1 showed similar docking scores with their native ligand, and each compound bound to different residues in the pocket site. Therefore, we suggested that CCA-1.1 and PGV-1 bind to several protein targets in mitosis with particular features (Figures 4 and 5).

CCA-1.1 and PGV-1 synergistically inhibit K562 cell proliferation

We tested the cytotoxic activity of CCA-1.1 and PGV-1 against CML K562 cells through trypan blue exclusion assay. Both curcumin analogs repressed cell growth depending on their tested concentration and had similar GI_{50} values (1.7 and 1.4 μM for CCA-1.1 and PGV-1, respectively) (Figure 6A). Using each fraction of their GI_{50} value (1; 1/2 and 1/4), we compared the ability to suppress cell viability between combination and single treatments. The combinatory index scores were less than 1 and ranged between 0.2 and 0.5 (Figure 6B), indicating that the combination treatment synergistically inhibited the cell proliferation.

Although CCA-1.1 and PGV-1 vary by only one functional group, their different predicted targets indicate that their combination treatment has a synergistic effect on K562 cells. This argument is also supported by the molecular docking results, which showed that CCA-1.1 and PGV-1 bound to the different residues of amino acid upon interaction with the receptor. Considering that both analogs are less toxic against non-cancerous cells, we suggested that their combination could be explored further to examine their synergistic activities. Biological experiment studies should verify these hypotheses. Nevertheless, this study presents valuable information and narrows down the possible mechanism of CCA-1.1 (also PGV-1) antitumor effect against leukemia cells.

CONCLUSION

Bioinformatic studies predicted that CCA-1.1 distinctly targets Aurora A in K562 cells and shares similar biomarkers with PGV-1 in mitotic regulatory proteins (CDK1, KIF11, and WEE1) as revealed by molecular docking analysis. We suggested that their different targets lead to the synergistic effect of their co-treatment on inhibiting K562 cell growth.

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