In silico modification of Zn$^{2+}$ binding group of suberoylanilide hydroxamic acid (SAHA) by organoselenium compounds as Homo sapiens class II HDAC inhibitor of cervical cancer

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Abstract. Cervical cancer is the most common cancer in women, and ranks seventh of all cancers worldwide, with 529000 cases in 2008 and more than 85% cases occur in developing countries. One way to treat this cancer is through the inhibition of HDAC enzymes which play a strategic role in the regulation of gene expression. Suberoyl Anilide Hydroxamic Acid (SAHA) or Vorinostat is a drug which commercially available to treat the cancer, but still has some side effects. This research present in silico SAHA modification in Zinc Binding Group (ZBG) by organoselenium compound to get ligands which less side effect. From molecular docking simulation, and interaction analysis, there are five best ligands, namely CC27, HA27, HB28, IB25, and KA7. These five ligands have better binding affinity than the standards, and also have interaction with Zn$^{2+}$ cofactor of inhibited HDAC enzymes. This research is expected to produce more potent HDAC inhibitor as novel drug for cervical cancer treatment.

1. Introduction

Cervical cancer occurs in the hollow area between the vagina and uterus, precisely in the cervical region [1]. This cancer is the second most common in women [2], as well as ranked seventh of all cancers in the world with as many as 529000 cases in 2008 and more than 85% of cases occur in developing countries. High-risk areas are found in East and West Africa, with a cumulative risk (0-74) of about 3.8%, South Africa (2.9%), South-Central Asia (2.6%), and Central Africa and the South Americas (2.5%) [3].

Cervical cancer is caused by human papilomavirus (HPV). HPV is from the family Papillomaviridae with the core material of double-stranded DNA and does not have a sheath (envelope) [4]. HPV enters the body through mucous membranes, does not circulate in the blood, but localized in the infected place, and cannot be grown in vitro [5].

A drug that used to suppress the growth of cervical cancer cells is an inhibitor of the enzyme histone deacetylase (HDAC). HDAC (EC.3.5.1) is an enzyme that acts as a catalyst for histone deacetylase [6]. It is a medium that can bind with oncogene transcription of genes with the aim of transforming the processes of cells into the media of the viral proliferation [7].
The most often used Inhibitors for class II HDAC activity is Vorinostat or suberoylanilide hydroxamic acid (SAHA). SAHA compound is having carbonyl and hydroxyl amine group. It binds zinc ion, Zn\(^{2+}\), with aliphatic chains as a linker, a hydrophobic group at the other tail. SAHA is a drug that has been sold in the market, but has the side effect as inhibitor of osteoblast maturation [8]. It also raises a variety of symptoms, such as dizziness, diarrhea, vomiting, hyperglycemia, increased levels of protein in urine, to a shortage of platelets (trombocytopenia) [9]. With the side effects of vorinostat or SAHA, it is necessary to modify the group of these compounds to obtain drugs that have toxicity and lower side effects [10].

In a recent study, it was found that the element selenium has an important role in the body, it also acts as an anti-cancer. Organoselenium in the form of compounds of selenometionin and metilselenosistein are proven to be therapeutic compounds on colon cancer cases [11]. In other studies, the organoselenium metabolites can inhibit HDAC in the cases of prostate cancer [11].

An inhibitor of HDAC has at least three sides/regions, namely the attachment of the Zn\(^{2+}\) cofactor/the enzyme active site (Zn\(^{2+}\) chlating/binding region) [12], close to the hydrophobic site (hydrophobic cap) [13,14], and liaison (linker) [15] containing the connecting unit/CU with electronegative group.

This study will construct various ligand models derived from SAHA compounds with organoselenium modifications based on Zinc Binding Group (ZBG).

2. Material and Methods

2.1 Preparation of the 3D structure of class II HDAC Homo sapiens.
The sequence data of *Homo sapiens* Class II HDACs (4, 5, 6, 7, 9, 10) was prepared by downloading them from the protein database at NCBI site (http://www.ncbi.nlm.nih.gov). Furthermore, the sequence was copied and stored with notepad in FASTA format. 3D structure of the *Homo sapiens* Class II HDACs was obtained from the Protein Data Bank (PDB) and stored in .pdb format. If there were proteins that were not available in the PDB, it was modeled by SWISS model and the data was obtained in .pdb format.

2.2 Sequence conservation of Homo sapiens Class II HDAC.
This alignment was done on the NCBI BLAST (Basic Local Alignment Search Tool) server and Clustal W at EMBL-EBI. Sequences of class II HDAC *Homo sapiens* enzymes were uploaded to the server, then performed the BLASTP (BLAST Protein).

2.3 Preparation of Homo Sapiens Class II HDAC enzyme.
HDACs sequence data were obtained and stored with .pdb format in the previous procedure, opened with the MOE (Molecular Operating Environment) 2008.10 software. Furthermore, the removal of amino acids chains, ligands/inhibitors, and unwanted solvents (usually H\(_2\)O) was conducted. Thus, 3D protonate menu was used on the compute section to add the polar hydrogen and led the charge of Zn\(^{2+}\). The addition of polar hydrogen provides partial charge in the enzyme. The determination of the partial load was carried on the menu of compute mode that has been parameterized with AMBER99 forcefield. Then, the menu was utilized to minimize enzyme’s energy to reach the lowest level. Enzymes are stored in .moe or .mdb format and used for molecular docking simulations.

2.3 Design and preparation of Homo sapiens Class II HDAC Ligand inhibitor.
Ligands were drawn in two dimensions using offline software program of ACDLabs ChemSketch 12.0. They are SAHA and its derivatives. Various SAHA modifications were utilized. The ligands were saved in MDL Molfile format. Ligands storage format was converted to MDL Mol using Vegazz software. All ligand then forwarded into MOE database. Ligand molecule was opened within .mol
format using MOE software. Furthermore, the ligand was computed with "wash" menu. Ligand was adjusted with "partial charge" and optimized using MMFF94 forcefield.

### 2.4 Docking simulation.
Docking process was done by using the menu Compute-Simulation-Dock in MOE 2008.10 software. Docking results were stored in .mdb format. The selected area was the docking residues. The placement method was using a triangle matcher with repetition of energy readings every 1000000 position and other corresponding parameters contained in MOE 2008.10. The next stage of scoring function was using London dG, refinement by using forcefield. The first repetition was done in 30 times, while the latter only just showed one of the best from 30 repetitions.

### 2.5 Analysis of Molecular Docking.
Results were analyzed using molecular docking of MOE software. Results data is opened in the .mdb format. To make it easier to sort the results based on the $\Delta G_{\text{binding}}$, the data file was converted to .xls format by using the export menu. Ligand conformation that has the lowest binding energy was chosen to determine protein-ligand conformation from docking result. The selected enzyme-ligand complex was having a low binding energy value and the highest inhibition constants for further analysis.

### 2.6 Visualization of Molecular Interactions.
Visualization of 3D complex interactions with ligands enzyme was done through the rendering process. After going through the process, 3D structure of the enzyme can be illustrated through maps on the navigation of surface and compute menu. Then, the interacting ligand, cofactor, and the enzyme amino acid residues could be displayed. Interaction of amino acid residues in enzymes with ligands can be viewed by using the features of LigX on MOE. Initially, ligand was selected using the browser menu. Enzyme-ligand complex energy was minimized with the LigX Minimize menu, then clicked the Interaction menu. After that, an analysis of the interactions that occurred between amino acid residues in enzymes with ligand inhibitor was conducted.

### 3. Result and Discussion

#### 3.1 Determination of class II HDAC Homo sapiens.
The results of the *Homo sapiens* Class II HDAC search in the NCBI sites are 91 sequences. The sequences were dowloaded in FASTA format, to conduct multiple sequence alignment for each *Homo sapiens* Class II HDAC. The highest score was chosen as the modeling sequences. The obtained sequences of each enzyme HDAC class II are presented in Table 1.

| Enzyme | Sequences code |
|--------|----------------|
| HDAC4  | P56524         |
| HDAC5  | Q9UQL6.2       |
| HDAC6  | Q9UBN7.2       |
| HDAC7  | Q8WU14.2       |
| HDAC9  | Q9UKV0.2       |
| HDAC10 | Q969S8.1       |

From the modeling results, the enzyme HDAC5 and HDAC9 structures were quite valid, but HDAC10 showing results that are less valid. HDAC10 protein structure were taken in the absence of structural data that more comparable with the results HDAC10 modeling. After HDAC structure was obtained, then the determination of the active side of the structure was done.

The 3D structure of a protein can be accessed at the database of Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB) through the website of http://rscb.org/pdb/.
3D structures of HDAC5, HDAC9, and HDAC10 were not found in the database RSCB-PDB, so it needs to conduct modeling with other software. FASTA sequences of HDAC5, HDAC9, and HDAC10 was served into its 3D models by using homology modeling software with online SWISS models (http://swissmodel.expasy.org/) (Table 2 and Figure 1).

| Protein | QMEAN4 score | Identity |
|---------|--------------|----------|
| HDAC5   | -2.72        | 76.90%   |
| HDAC9   | -2.20        | 74.54%   |
| HDAC10  | -4.43        | 39.94%   |

After HDAC structure is obtained, then the active site of the structure was determined (Figure 2).

**Figure 1.** 3D structure of *Homo sapiens* class II HDAC: a.HDAC4, b.HDAC5, c. HDAC6, d. HDAC7, e. HDAC9, and f. HDAC10

**Figure 2.** 3D structure of *Homo sapiens* class II HDAC in MOE software: a.HDAC4, b.HDAC5, c. HDAC6, d.HDAC7, e.HDAC9, and f.HDAC10

Description: purple: H-bonding, green: Hydrophobic region, and blue: Mild Polar
3.2 Determination of the Enzyme Active Side of class II HDAC Homo sapiens. Crystal protein contained in the PDB database is the protein inhibitor compound that has been shown to lower the catalytic activity. From this data, information can be found on the location of amino acid residues that become the catalytic site of enzyme. The PDB 3D structures of the HDAC4, HDAC6, and HDAC7 enzymes were found as well. Thus, the amino acid residues of the active site were obtained (Table 3). The structures of HDAC5, HDAC9, and HDAC10 were the result of homology modeling with SWISS Model.

The existence of Zn$^{2+}$ cofactor became a key factor for inhibition of class II HDAC enzymes. It is designed so that the ligand inhibitor has a group that is able to interact or bind Zn$^{2+}$ ions in order to inhibit the catalytic activity of the enzyme.

| Enzyme | Location of The Catalytic site |
|--------|--------------------------------|
| HDAC4  | Ion Zn$^{2+}$ with residu Asp196, His198, dan Asp290 |
| HDAC5  | Ion Zn$^{2+}$ with residu Asp870, His872, dan Asp964 |
| HDAC6  | Ion Zn$^{2+}$ with residu Cys5, His7, dan Cys78 |
| HDAC7  | Ion Zn$^{2+}$ with residu Asp707, His709, dan Asp801 |
| HDAC9  | Ion Zn$^{2+}$ with residu Asp820, His822, dan Asp914 |
| HDAC10 | Ion Zn$^{2+}$ dengan residu Asp172, His174, dan Asp265 |

3.3 Design of Class II HDAC Homo sapiens Inhibitor. SAHA was modified to obtain a potential drug candidate with minimal side effects for the treatment of cervical cancer. Modifications were carried out on Zinc Binding Group (ZBG) by substituting organoselenium in the group, while maintained the structure of the CAP and the linker. Organoselenium compounds have been selected in previous studies with the same modification on ZBG selenium compounds to inhibit HDAC on lung cancer cell (Figure 3). In vitro studies proved that the result of this modification provides better performance than the standard compound. Therefore, modification was followed by substituting the variation of organoselenium to obtain the possibility of better results.

![Figure 3. Modification of SAHA ligand. The area within blue circle is the Zinc Binding Group (ZBG).](image)

Total ligands that have been successfully made are 1763, and 2 standard ligand compounds of SAHA and TSA.

3.4 Docking.

Molecular docking simulation process was iterated once on each of the six class II HDAC Homo sapiens enzymes with the same 1165 ligands. Docking process takes 18-50 hours, depending on the number of ligands and the computer processor specifications.

The selected 18 best ligands are HA27, UA28, MB25, UA30, A24, HB29, LA30, SA27, ZA28, UA30, KA7, HB28, CC27, A1A27, ZA25, EB25, IB25, and BA24. Six others were taken from the best ligand structure approach that has a good interaction with the six class II HDAC Homo sapiens enzymes and remain within the below standard criteria of $\Delta G_{\text{binding}}$ value. Those six ligands are B1B25, BB24, BC7, HB20, JA27, and LA26. The binding free energy ($\Delta G_{\text{binding}}$) of the best ligand with HDAC enzymes is presented in Table 4, while the inhibition constant is in Table 5, respectively.
Table 4. The binding free energy ($\Delta G_{\text{binding}}$) of the best ligand with HDAC enzymes

| Ligand | HDAC4 | HDAC5 | HDAC6 | HDAC7 | HDAC9 | HDAC10 |
|--------|-------|-------|-------|-------|-------|--------|
| SAHA   | -29.420 | -39.175 | -12.562 | -45.617 | -25.340 | -11.079 |
| TSA    | -53.710 | -4.057 | -1.032 | -32.101 | -17.760 | 27.276 |
| AIA27  | -26.044 | -49.282 | -14.273 | -64.810 | -30.377 | -89.697 |
| A24    | -4.146 | -59.975 | -14.969 | -59.968 | -36.873 | -10.939 |
| BIB25  | -42.219 | -58.684 | -1.628 | -42.561 | -42.004 | -14.847 |
| BA24   | -39.919 | -60.331 | -17.748 | -24.563 | -34.751 | 63.416 |
| BB24   | -40.248 | -58.882 | -13.248 | -31.459 | -31.459 | -82.618 |
| BC7    | -24.517 | -42.294 | -17.277 | -44.085 | -27.217 | -99.316 |
| CC27   | -26.108 | -36.836 | -13.487 | -58.605 | -33.903 | -12.392 |
| EB25   | -3.825 | -59.831 | -16.090 | -3.794 | -32.282 | -13.166 |
| HA27   | -54.414 | -58.829 | -17.251 | -41.632 | -27.043 | 11.455 |
| HB20   | -25.480 | -37.405 | -18.655 | -31.388 | -26.977 | -98.902 |
| HB28   | -32.076 | -46.794 | -2.436 | -36.847 | -45.414 | 11.430 |
| HB29   | -31.434 | -51.708 | -26.456 | -0.375 | -29.787 | -38.785 |
| IB25   | -44.025 | -60.535 | -15.989 | -33.678 | -38.057 | -12.633 |
| JA27   | -22.347 | -60.990 | -17.564 | -46.531 | -45.342 | -1.163 |
| KA7    | -34.164 | -36.715 | -18.564 | -38.382 | -22.533 | -98.975 |
| LA26   | -33.382 | -40.031 | -17.985 | -33.393 | -0.271 | -98.266 |
| LA30   | -31.023 | -43.625 | -25.909 | -43.167 | -3.162 | -19.152 |
| MB25   | -47.377 | -57.618 | -19.077 | -52.978 | -38.898 | -88.693 |
| SA27   | -31.655 | -4.733 | -25.688 | -53.560 | -28.638 | -88.903 |
| UA28   | -57.837 | -75.258 | -12.381 | -99.786 | -63.495 | 0.641 |
| UA29   | -28.383 | -70.755 | -15.962 | -42.443 | -23.721 | -55.034 |
| UA30   | -46.946 | -75.085 | -14.086 | -57.256 | -2.646 | -50.433 |
| ZA25   | -27.777 | -58.900 | -15.961 | -17.130 | -44.571 | -14.146 |
| ZA28   | -40.935 | -32.213 | -25.553 | -2.508 | -25.874 | -33.987 |

Note: The number in bold is the lowest free energy value

3.5 Interaction Analysis of HDAC with The Best Ligand.
After getting the value of $\Delta G_{\text{binding}}$, molecular docking results, the next step is to analyze the interaction between class II HDAC Homo sapiens enzymes with the ligand inhibitor. From the results of molecular docking database, the selected the file-browser menu would determine the ligand interactions. After the minimization of energy, visualization of the interaction between the enzyme and the ligand was obtained.

In HDAC4, the interaction was formed between Zn$^{2+}$ cofactor ligand compounds with oxygen atoms at hydroxyl groups that bound to the amide group (Figure 4). The Zn$^{2+}$ cofactor coordination complex bonding with oxygen atoms also bind 3 amino acids (His198, Asp196, and Asp290), which is the enzyme active site residues of HDAC4. Zn$^{2+}$ coordination number in these bonds is four. Zn$^{2+}$ electronic charges on oxygen have shown empty p orbitals (n acceptor). Then, the interaction between His872 side chains with a hydroxyl group attached to the Selenium was observed as well.
active sides on the outside, so it should be easier to give a pose that generate the interaction. However, hydrogen bonds. 
backbone of Gly1005 was able to donate a proton to the oxygen atom on ZBG fragment and form covalent bond between the Zn$^{2+}$ cofactor with oxygen atoms in the carboxylic group of ZBG, also with the active site amino acid residues (His872, Asp870, and Asp964) (Figure 5). Then H in the amine backbone of Gly1005 was able to donate a proton to the oxygen atom on ZBG fragment and form hydrogen bonds. 

The HDAC6 enzyme interaction with ligand CC27 shows that it does not bind Zn$^{2+}$ cofactor, but provide hydrogen bonding interactions with residues Cys78 which became one of the amino acid residues in the enzyme active site (Figure 6). Secondary amine ligands were donating protons to the backbone chain of Cys78 residue. In the hydrophobic stamp or linker, there is no interaction between the ligand with the enzyme.

HDAC6 enzyme has a smaller structure than other class II HDAC enzymes. In addition, there are active sides on the outside, so it should be easier to give a pose that generate the interaction. However, there is also possibility of interactions with the standard SAHA and HDAC6. The Zn$^{2+}$ on enzyme did not show any interaction with the standard compound.

### Table 5. Inhibition constant value of the best ligand with HDAC enzymes

| Ligand | HDAC4 | HDAC5 | HDAC6 | HDAC7 | HDAC9 | HDAC10 |
|--------|-------|-------|-------|-------|-------|--------|
| SAHA   | 21.4463 | 28.5574 | 9.1573 | 33.2534 | 18.4721 | 8.0762 |
| TSA    | 39.1529 | 2.9574 | 0.7523 | 23.4006 | 12.9465 | -19.8834 |
| A1A27  | 18.9853 | 35.9251 | 10.4046 | 47.2445 | 22.1439 | 65.3863 |
| A24    | 3.223 | 43.7199 | 10.9119 | 43.7148 | 26.8793 | 7.9742 |
| B1B25  | 30.7763 | 42.7788 | 1.1868 | 31.0257 | 30.6196 | 10.8230 |
| BA24   | 29.0997 | 43.9794 | 12.9377 | 17.9057 | 25.3324 | -46.2283 |
| BB24   | 29.3395 | 42.9232 | 9.6574 | 22.9326 | 22.9326 | 60.2260 |
| BC7    | 17.8721 | 30.8310 | 12.5944 | 32.1366 | 19.8404 | 72.3983 |
| CC27   | 19.0319 | 26.8523 | 9.8316 | 42.7212 | 24.7142 | 9.0334 |
| EB25   | 2.7883 | 43.6150 | 11.7291 | 2.7657 | 23.5326 | 9.5976 |
| HA27   | 39.6661 | 42.8845 | 12.5754 | 30.3484 | 19.7135 | -8.3503 |
| HB20   | 18.5741 | 27.2671 | 13.5989 | 22.8809 | 19.6654 | 72.0965 |
| HB28   | 23.3824 | 34.1114 | 1.7758 | 26.8603 | 33.1054 | -8.3321 |
| HB29   | 22.9144 | 37.6935 | **19.2856** | 0.2734 | 21.7138 | 28.2731 |
| IB25   | 32.0929 | 44.1281 | 11.6555 | 24.5502 | 27.7424 | 9.2091 |
| JA27   | 16.2903 | 44.4598 | 12.8036 | 33.9197 | 33.0529 | 0.8478 |
| KA7    | 24.9045 | 26.7641 | 13.5326 | 27.9793 | 16.4259 | 72.1497 |
| LA26   | 24.3344 | 29.1814 | 13.1105 | 24.3425 | 0.1976 | 71.6329 |
| LA30   | 22.6148 | 31.8013 | 18.8869 | 31.4674 | 2.3050 | 13.9612 |
| MB25   | 34.5364 | 42.0017 | 13.9065 | 38.6193 | 28.4218 | 64.6545 |
| SA27   | 23.0755 | 3.4502 | 18.7258 | 39.0436 | 20.8762 | 64.8075 |
| UA28   | **42.1614** | **54.8608** | 9.0254 | **72.7409** | **46.2859** | -0.4673 |
| UA29   | 20.6903 | 51.5782 | 11.6358 | 30.9396 | 17.2919 | 40.1181 |
| UA30   | 34.2222 | 54.7346 | 10.2683 | 41.7379 | 1.9289 | 36.7641 |
| ZA25   | 20.2486 | 42.9363 | 11.6351 | 12.4872 | 32.4909 | 10.3120 |
| ZA28   | 29.8404 | 23.4823 | 18.6273 | 1.8283 | 18.8613 | 24.7755 |

Note: The number in bold is the highest inhibition constant value.

From the visualization of HDAC5 interaction with ligand IB25, there is visible presence of a covalent bond between the Zn$^{2+}$ cofactor with oxygen atoms in the carboxylic group of ZBG, also with the active site amino acid residues (His872, Asp870, and Asp964) (Figure 5). Then H in the amine backbone of Gly1005 was able to donate a proton to the oxygen atom on ZBG fragment and form hydrogen bonds.
Visualization of the interaction between the enzyme HDAC7 with ligand CC27 showed five covalent bond coordination between Zn$^{2+}$ cofactor with three residues of the active sites (Asp707, His709, and Asp801) (Figure 7). His843 side chain residues showed interaction with the hydrophobic cap. Whereas His670 side chain interacts with the carbonyl of ZBG. Then, there is also a hydrogen bonding interaction between the H bonded to nitrogen with side chain residue of Asp707.

**Figure 4.** Visualization of enzyme HDAC4 enzyme with ligand HA27

**Figure 5.** Visualization of HDAC5 enzyme interaction with ligand IB25

**Figure 6.** Visualisation of enzyme HDAC6 interaction with ligand CC27

**Figure 7.** Visualisation of enzyme HDAC7 with ligand CC27 interaction
Next on the visualization of the interaction between the ligand HB28 and HDAC9 enzyme, the cofactor Zn$^{2+}$ formed four bonds coordination with oxygen atoms at hydroxyl groups with three active site residues (Asp820, His822, and Asp914) (Figure 8). Then the active site of the side chain residues of Asp820 and Asp914 was also forming hydrogen bonding interactions with the hydroxyl groups on the H atom in ZBG, while the side chains of the active site residues His822 also forms a hydrogen bond interactions with the cation H in the aromatic group.

Figure 8. Visualisation of enzyme HDAC9 with ligand HB28

At HDAC10, KA7 ligand formed coordination bond between Zn$^{2+}$ cofactor with the active site residues (His172, His174, and Asp265). Thus, the oxygen atoms in the hydroxyl group were attached to an amide (Figure 9).

Figure 9. Visualisation of enzyme HDAC10 with ligandd KA7

4. Conclusion

The designed compounds were organoselenium-modified version of SAHA at the ZBG. This modification resulted in 1763 ligands, and then advanced to the stage of molecular docking. From the analysis of $\Delta G_{binding}$ between the ligand inhibitor with a class II HDAC enzymes Homo sapiens as well as analysis and visualization of interactions, 24 best ligands were obtained. The interaction analysis between the class II HDAC Homo sapiens enzyme with the ligand inhibitor shows the covalent bond coordination between the Zn$^{2+}$ cofactor and oxygen atom in the carboxylate groups on ZBG, also with amino acid residues active side.

Acknowledgement

The authors would like to thanks Hibah BOPTN Ditjen Dikti No. 0542/UN2.R12/HKP.05.00/2015 for providing research grant. Thanks also goes to Directorate of Research and Community Engagement-University of Indonesia (DRPM-UI) for the sponsorship in project “Pelaksanaan Hibah Penyelenggaraan Pelatihan Penulisan Artikel Untuk Jurnal Internasional Tahun Anggaran 2015”, No.: 2774/UN2.R12/HKP.05.00/2015. Usman Sumo Friend Tambunan, Ridla Bakri, and Arli Aditya Parikesit supervised this research. Titin Aryani and Ratih Dyah Puspitasari worked on the technical
details. Djati Kerami gave important suggestion to improve our pipeline. All authors were credited for writing this manuscript.

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