L A M P I R A N

Lampiran 1. Tabel nilai afinitas pengikatan (ΔG Binding) replikasi daun cocor bebek

Senyawa	*ΔG Binding* (kkal/mol)±SD									
	1YWN					6GL8				
	Rep 1	Rep 2	Rep 3	Rata-Rata	±SD	Rep1	Rep2	Rep 3	Rata-Rata	±SD
Native Ligand	-10,37	-10,37	-10,35	**-10,36**	**0,04**	-12,65	-12,66	-12,68	**-12,66**	**0,02**
Kalanchoside A	-7,48	-7,4	-7,43	-7,44	0,04	4022,18	4051,59	4066,37	4046,71	22,50
Kalanchoside B	-7,47	-7,44	-7,41	-7,44	0,03	-4,64	-4,66	-4,64	-4,65	0,01
Kalanchoside C	-6,58	-6,53	-6,45	-6,52	0,07	-5,71	-5,68	-5,03	-5,47	0,38
Thesiuside	-6,33	-7,33	-7,42	-7,03	0,61	-4,86	-4,61	-4,7	-4,72	0,13
Hellebrigenin	-7,32	-7,33	-7,34	-7,33	0,01	-6,32	-6,32	-6,32	-6,32	0,00
Hellebrigenin-3-acetate	-7,33	-7,25	-7,3	-7,29	0,04	-6,98	-6,95	-6,94	-6,96	0,02
Bryophyllin B	-6,48	-6,46	-6,94	-6,63	0,28	**-7,42**	**-7,48**	**-7,43**	**-7,44**	**0,03**
Myricitrin	-7,22	-7,21	-7,27	-7,23	0,03	-4,38	-4,71	-4,56	-4,55	0,17
Bersaldegenin-1,3,5- orthoacetate	-6,26	-6,26	-6,25	-6,26	0,01	-6,09	-6,09	-6,09	-6,09	0,00
Quercetin 3-O-alpha-L-rhamnopyranoside	**-7,78**	**-7,77**	**-7,78**	**-7,78**	**0,01**	-8,17	-8,17	-8,17	-5,99	0,04
Bufalin	**-7,65**	**-7,9**	**-7,65**	**-7,73**	**0,14**	**-8,17**	**-8,17**	**-8,17**	**-8,17**	**0,00**
1β-hydroxylbufalin	-6,79	-6,72	-6,72	-6,74	0,040	-6,44	-6,44	-6,46	-6,45	0,01
12β-hydroxylbufalin	**-7,55**	**-7,56**	**-7,55**	**-7,55**	**0,02**	**-7,7**	**-7,63**	**-7,63**	**-7,65**	**0,04**
Dihydroquercetin	-7,54	-7,53	-7,55	-7,54	0,01	-5,05	-5,05	-5,05	-5,05	0,00
Kontrol Negatif	-4,69	-4,69	-4,69	-4,690	0,000	-3,91	-3,93	-3,91	-3,917	0,012

Senyawa	*ΔG Binding* (kkal/mol)±SD									
	3POZ					4L6S				
	Rep 1	Rep 2	Rep 3	Rata-Rata	±SD	Rep1	Rep2	Rep 3	Rata-Rata	±SD
Native Ligand	-10,02	-9,93	-9,96	**-9,970**	**0,046**	-10,47	-10,47	-10,46	**-10,467**	**0,006**
Kalanchoside A	-3,83	-5,13	-4,35	-4,437	0,654	-3,3	-3,33	-3,31	-3,313	0,015
Kalanchoside B	-6,58	-6,57	-5,57	-6,240	0,580	-3,15	-3,17	-3,15	-3,157	0,012
Kalanchoside C	-7,76	-7,76	-7,77	-7,763	0,006	-9,27	-9,27	-9,27	-9,270	0,000
Thesiuside	-7,16	-6,94	-7,09	-7,063	0,112	-7,51	-7,52	-7,52	-7,517	0,006
Hellebrigenin	-8,64	-8,63	-8,64	-8,637	0,006	-9,85	-9,88	-9,87	-9,867	0,015
Hellebrigenin-3-acetate	-7,95	-7,95	-7,95	-7,950	0,000	-9,91	-9,93	-9,93	-9,923	0,012
Bryophyllin B	**-7,71**	**-7,73**	**-7,72**	**-7,720**	**0,010**	**-10,22**	**-10,23**	**-10,26**	**-10,237**	**0,021**
Myricitrin	-7,99	-7,97	-7,98	-7,980	0,010	-9,04	-9,04	-9,05	-9,043	0,006
Bersaldegenin-1,3,5- orthoacetate	-8,07	-8,07	-8,08	-8,073	0,006	-5,09	-5,09	-5,07	-5,083	0,012
Quercetin 3-O-alpha-L-rhamnopyranoside	**-7,84**	**-7,86**	**-7,88**	**-7,860**	**0,020**	**-10,17**	**-10,05**	**-10,27**	**-10,163**	**0,110**
Bufalin	**-9,09**	**-9,09**	**-9,08**	**-9,087**	**0,006**	**-11,59**	**-11,59**	**-11,59**	**-11,590**	**0,000**
1β-hydroxylbufalin	**-10,46**	**-10,45**	**-10,45**	**-10,453**	**0,006**	-9,65	-9,67	-9,67	-9,663	0,012
12β-hydroxylbufalin	**-8,8**	**-8,83**	**-8,81**	**-8,813**	**0,015**	-9,53	-9,55	-9,53	-9,537	0,012
Dihydroquercetin	-7,32	-7,3	-7,32	-7,313	0,012	-7,94	-7,94	-7,96	-7,947	0,012
Kontrol Negatif	-5,51	-5,5	-5,51	-5,507	0,006	-5,56	-5,57	-5,56	-5,563	0,006

Senyawa	*ΔG Binding* (kkal/mol)±SD									
	4L23					4HVB				
	Rep 1	Rep 2	Rep 3	Rata-Rata	±SD	Rep1	Rep2	Rep 3	Rata-Rata	±SD
Native Ligand	-8,83	-8,84	-8,83	**-8,833**	**0,006**	-10,56	-10,56	-10,55	**-10,557**	**0,006**
Kalanchoside A	-4,7	-4,88	-4,8	-4,793	0,090	-6,37	-6,39	-6,37	-6,377	0,012
Kalanchoside B	-4,7	-4,68	-4,65	-4,677	0,025	-6,37	-6,37	-6,37	-6,370	0,000
Kalanchoside C	-5,23	-5,28	-5,24	-5,250	0,026	-6,65	-6,69	-6,66	-6,667	0,021
Thesiuside	-6,39	-6,41	-6,39	-6,397	0,012	-7,55	-7,57	-7,55	-7,557	0,012
Hellebrigenin	-6,89	-6,9	-6,88	-6,890	0,010	-7,64	-7,68	-7,66	-7,660	0,020
Hellebrigenin-3-acetate	-7,11	-7,13	-7,13	-7,123	0,012	-8,18	-8,17	-8,17	-8,173	0,006
Bryophyllin B	-6,47	-6,49	-6,46	-6,473	0,015	-8,95	-8,98	-8,95	-8,960	0,017
Myricitrin	**-8,89**	**-8,54**	**-9,01**	**-8,813**	**0,244**	-7,32	-7,35	-7,33	-7,333	0,015
Bersaldegenin-1,3,5-orthoacetate	**-8,77**	**-8,77**	**-8,77**	**-8,770**	**0,000**	**-8,78**	**-8,78**	**-8,78**	**-8,780**	**0,000**
Quercetin 3-O-alpha-L-rhamnopyranoside	**-8,96**	**-8,82**	**-8,93**	**-8,903**	**0,074**	-7,89	-7,9	-7,9	-7,897	0,006
Bufalin	**-10,59**	**-10,59**	**-10,59**	**-10,590**	**0,000**	**-10,64**	**-10,65**	**-10,65**	**-10,647**	**0,006**
1β-hydroxylbufalin	-8,21	-8,23	-8,24	-8,227	0,015	-7,78	-7,75	-7,77	-7,767	0,015
12β-hydroxylbufalin	**-9,75**	**-9,77**	**-9,80**	**-9,773**	**0,025**	**-10,16**	**-10,18**	**-10,18**	**-10,173**	**0,012**
Dihydroquercetin	-8,34	-8,36	-8,36	-8,353	0,012	-7,51	-7,54	-7,52	-7,523	0,015
Kontrol Negatif	-4,83	-4,83	-4,83	-4,830	0,000	-5,33	-5,33	-5,34	-5,333	0,006

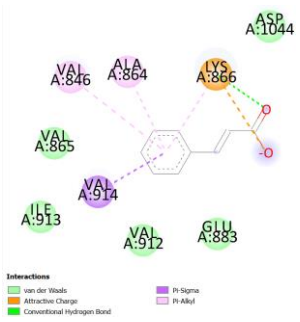
Senyawa	*ΔG Binding* (kkal/mol)±SD									
	4W4Y					3L8X				
	Rep 1	**Rep 2**	**Rep 3**	**Rata-Rata**	**±SD**	**Rep1**	**Rep2**	**Rep 3**	**Rata-Rata**	**±SD**
Native Ligand	-10,84	-10,84	-10,83	**-10,837**	**0,006**	-9,99	-9,99	-9,99	**-9,990**	**0,000**
Kalanchoside A	-2,14	-2,14	-2,16	-2,147	0,012	1,11	1,15	1,13	1,130	0,020
Kalanchoside B	-1,87	-1,88	-1,86	-1,870	0,010	1,61	1,61	1,61	1,610	0,000
Kalanchoside C	-1,89	-1,89	-1,88	-1,887	0,006	-7,32	-7,32	-7,35	-7,330	0,017
Thesiuside	2,06	2,08	2,06	2,067	0,012	-6,8	-6,83	-6,82	-6,817	0,015
Hellebrigenin	-6,06	-6,08	-6,05	-6,063	0,015	**-9,86**	**-9,86**	**-9,99**	**-9,903**	**0,075**
Hellebrigenin-3-acetate	-3,76	-3,78	-3,78	-3,773	0,012	**-8,75**	**-8,74**	**-8,75**	**-8,747**	**0,006**
Bryophyllin B	-4,8	-4,83	-4,83	-4,820	0,017	-7,23	-7,25	-7,28	-7,253	0,025
Myricitrin	-6,56	-6,57	-6,55	-6,560	0,010	-7,89	-7,88	-7,88	-7,883	0,006
Bersaldegenin-1,3,5-orthoacetate	-6,32	-6,32	-6,33	-6,323	0,006	-6,11	-6,13	-6,13	-6,123	0,012
Quercetin 3-O-alpha-L-rhamnopyranoside	-6,55	-6,57	-6,57	-6,563	0,012	-6,99	-6,99	-6,96	-6,980	0,017
Bufalin	-6,33	-6,33	-6,33	-6,330	0,000	**-8,53**	**-8,51**	**-8,54**	**-8,527**	**0,015**
1β-hydroxylbufalin	**-8,11**	**-8,13**	**-8,13**	**-8,123**	**0,012**	-7,92	-7,93	-7,95	-7,933	0,015
12β-hydroxylbufalin	**-5,67**	**-5,69**	**-5,69**	**-5,683**	**0,012**	-7,71	-7,74	-7,72	-7,723	0,015
Dihydroquercetin	**-7,19**	**-7,22**	**-7,2**	**-7,203**	**0,015**	-7,87	-7,85	-7,88	-7,867	0,015
Kontrol Negatif	-5,46	-5,46	-5,47	-5,463	0,006	-5,18	-5,18	-5,18	-5,180	0,000

Lampiran 2. Tabel Hasil Validasi Metode Penambatan.

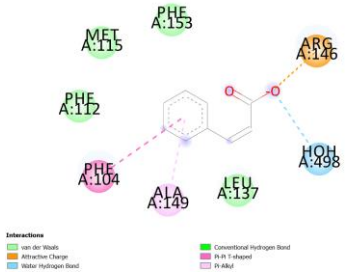
Makromolekul	RMSD (Å)			Rata-rata	*ΔGbinding* (kkal/mol)			Rata-rata±SD
	Rep 1	Rep 2	Rep 3		Rep 1	Rep 2	Rep 3	
1YWN	0,344	0,337	0,358	0,346	-10,37	-10,37	-10,35	-10,363±0,012
6GL8	0,140	0,102	0,103	0,115	-12,65	-12,66	-12,68	-12,663±0,015
3POZ	0,527	0,728	0,575	0,610	-10,02	-9,93	-9,96	-9,970±0,046
4L6S	0,342	0,350	0,342	0,342	-10,47	-10,47	-10,46	-10,467±0,006
4L23	0,000	0,001	0,000	0,000	-8,83	-8,84	-8,83	-8,833±0,006
4HVB	0,062	0,044	0,069	0,058	-10,56	-10,56	-10,55	-10,557±0,006
4W4Y	0,369	0,276	0,272	0,369	-10,84	-10,84	-10,83	-10,837±0,006
3L8X	0,552	0,550	0,548	0,550	-9,99	-9,99	-9,99	-9,990±0,000

Lampiran 3. Binding Site Kontrol negatif

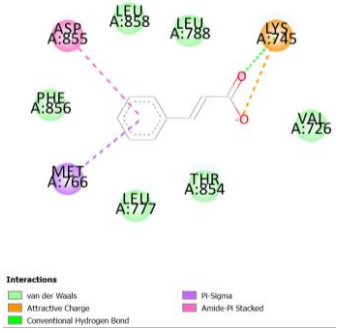
Cinnamic acid - (1YWN)



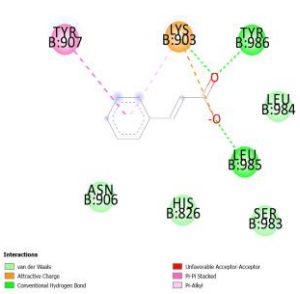
Cinnamic acid - (6GL8)



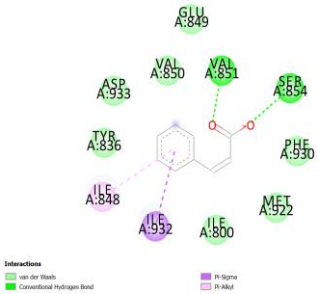
Cinnamic acid - (3POZ)



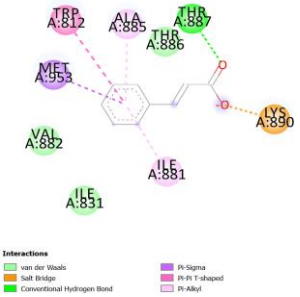
Cinnamic acid - (4L6S)



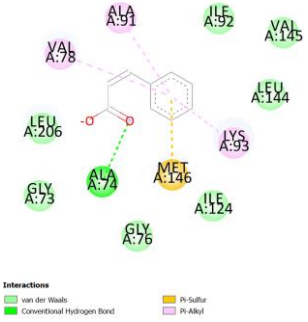
Cinnamic acid - (4L23)



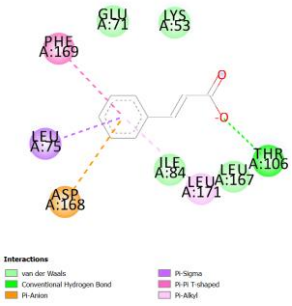
Cinnamic acid - (4HVB)



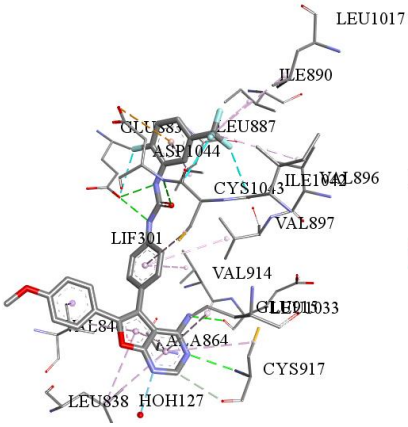
Cinnamic acid - (4W4Y)



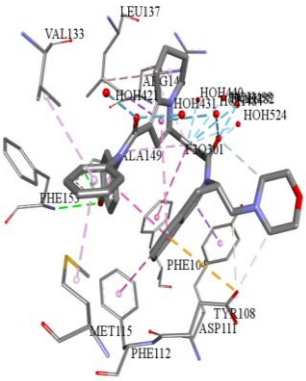
Cinnamic acid - (3L8X)



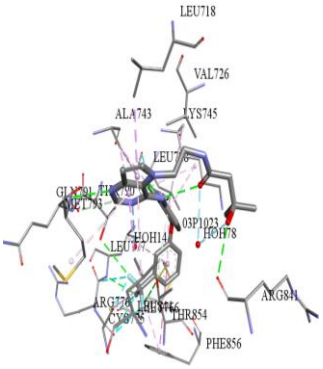
Lampiran 4. Gambar interaksi *native ligand* dan protein target terpilih



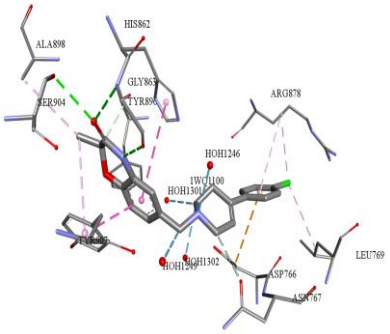
1YWN



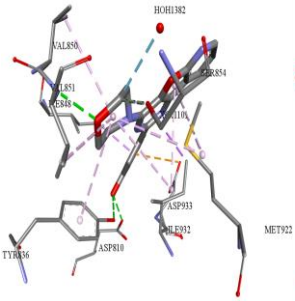
6GL8



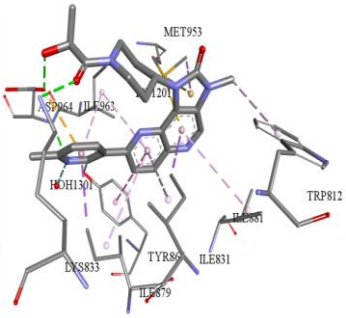
3POZ



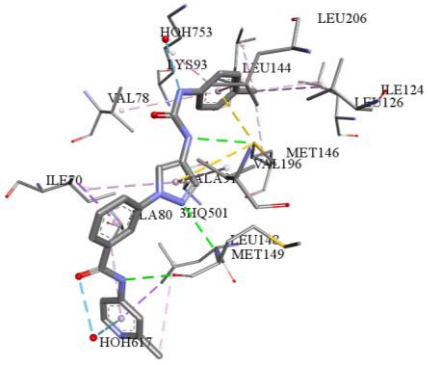
4L6S



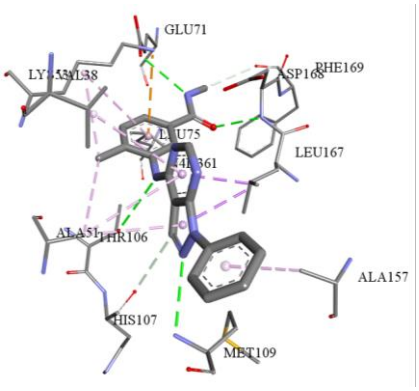
4L23



4HVB



4W4Y

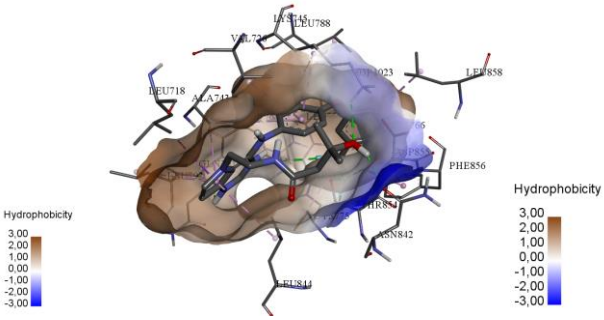


3L8X

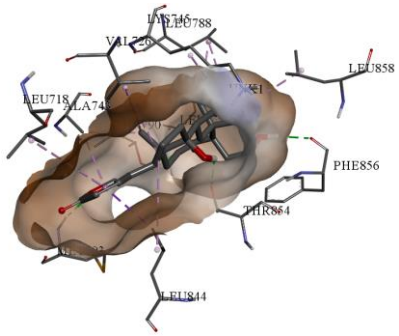
Lampiran 5. Interaksi ligan dan protein terpilih dalam ruang 3 dimensi

***Epidermal growth factor receptor* erbB1 (3POZ)**

Native Ligan

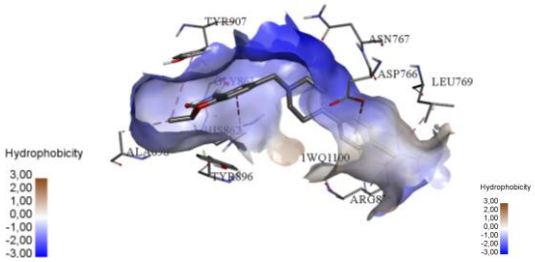


1β-hydroxylbufalin

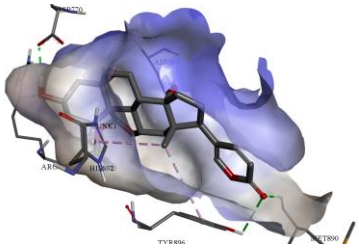


***Poly(ADP-ribose) polymerase*-1 (4L6S)**

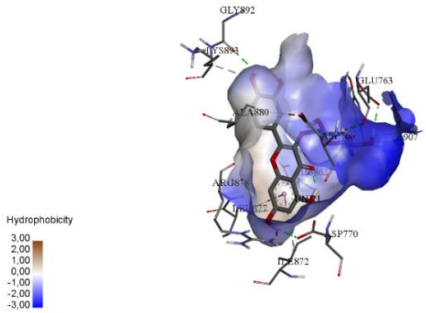
Native Ligan



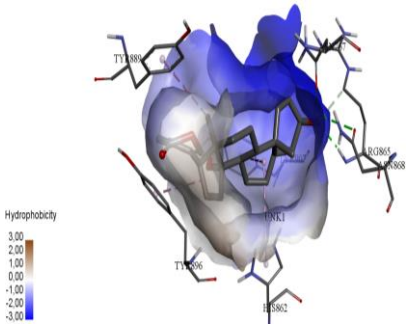
Bryophyllin B



Quercetin 3-O-alpha-L-rhamnopyranoside

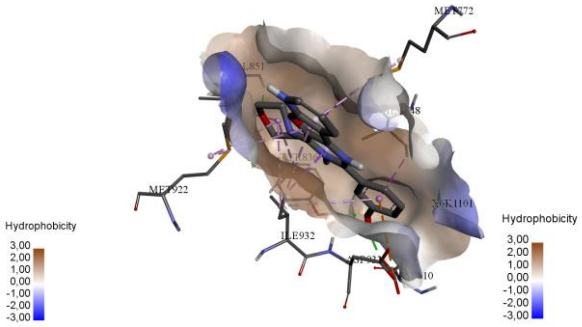


Bufalin

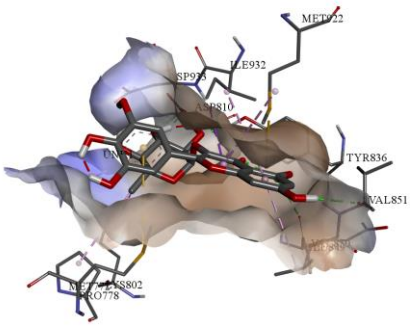


***PI3K p110-alpha* (4L23)**

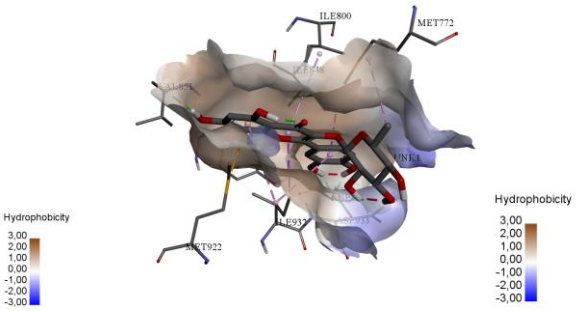
Native Ligan



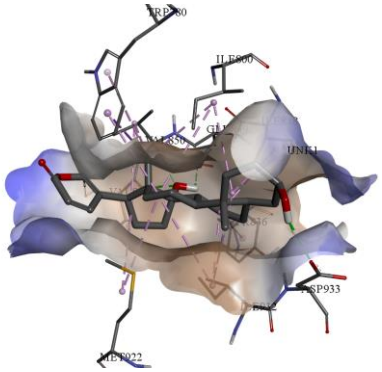
Myricitrin



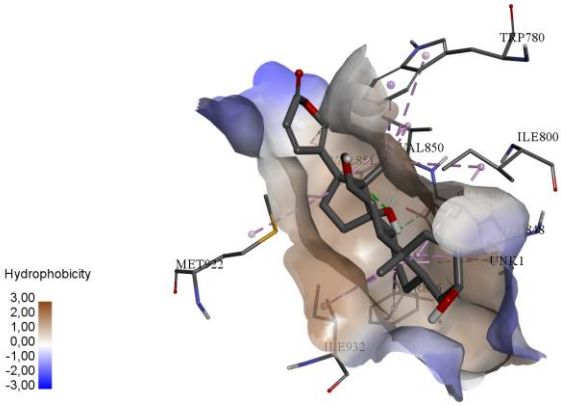
Quercetin 3-O-alpha-L-rhamnopyranoside



Bufalin

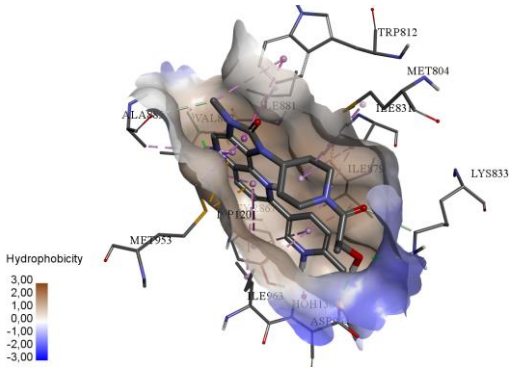


12β-hydroxylbufalin

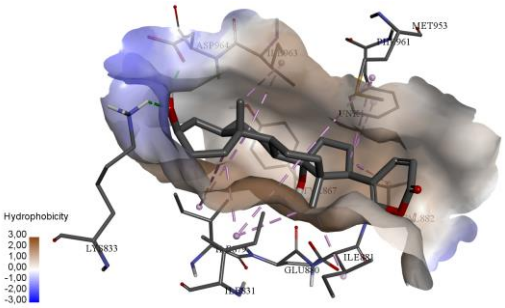


***Serine/threonine-protein kinase mTOR* (4HVB)**

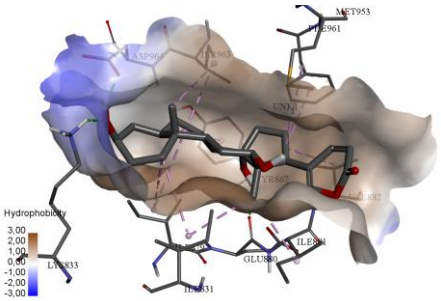
Native Ligan



Bufalin

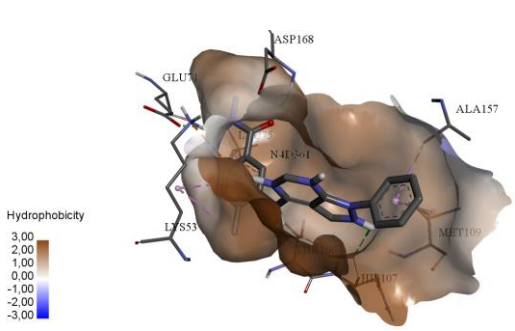


12β-hydroxylbufalin

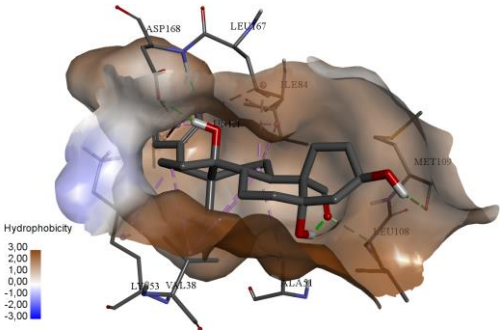


***TNF-Alpha* (3L8X)**

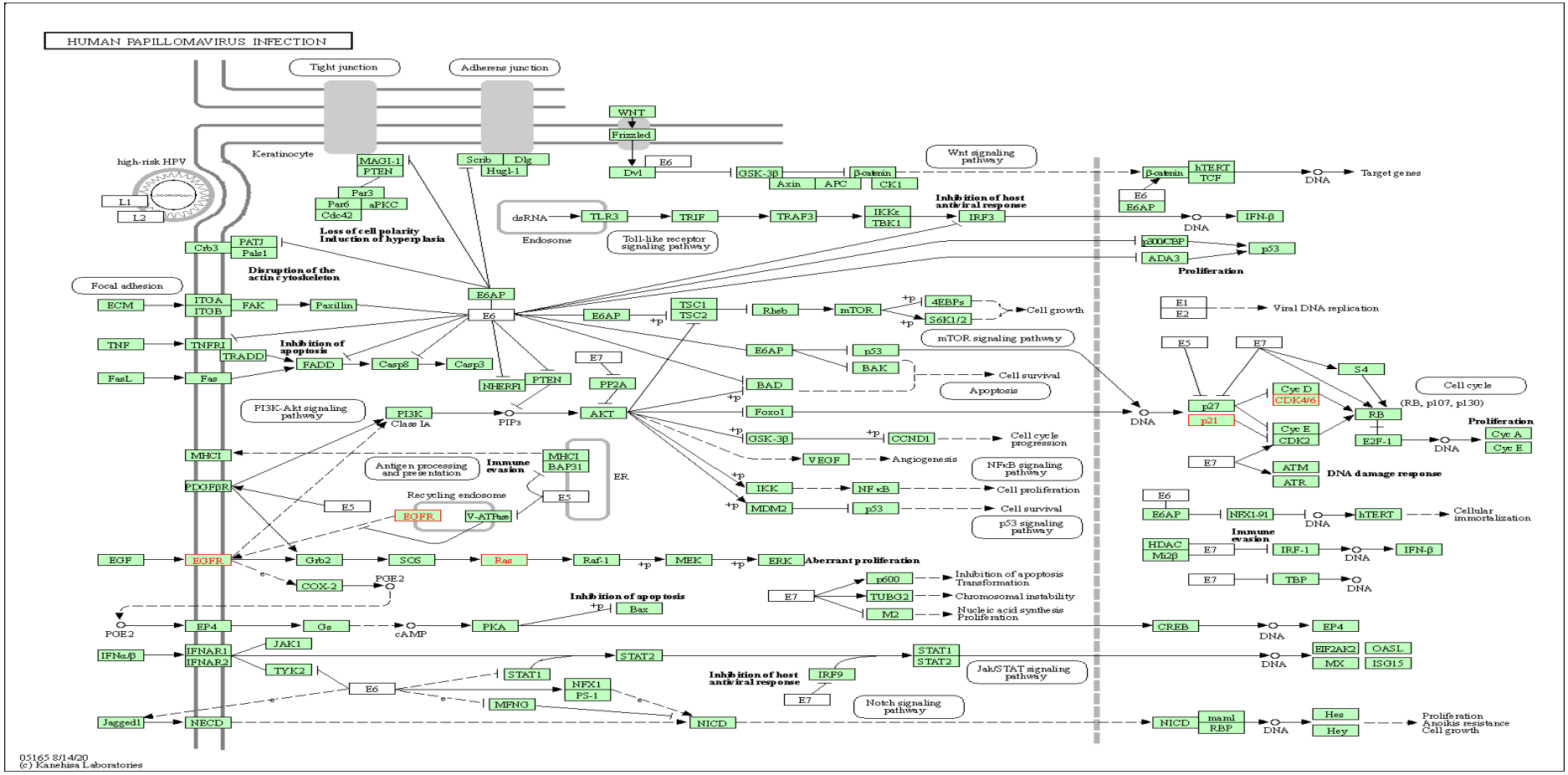
Native Ligan



Hellebrigenin



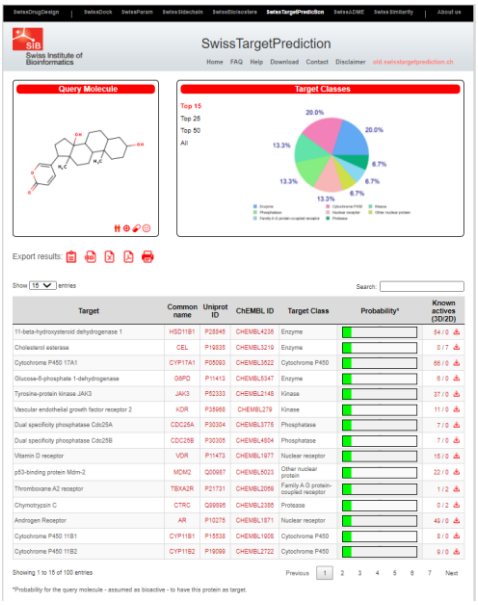
Lampiran 6. *Pathway* Kanker Serviks



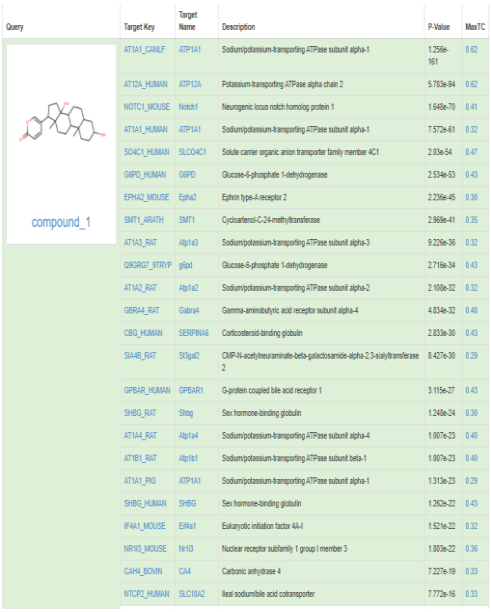
Lampiran 7. Prosedur Penambatan Molekuler

1. Pencarian target makromolekul

Pencarian target molekuler dilakukan dengan screening awal terhadap struktur 3D tiap ligan uji menggunakan *SWISSPred* dan *SEA server* selanjutnya disesuaikan yang terdapat di KEGG pathway kanker serviks.



Webserver : Swiss Target Prediction

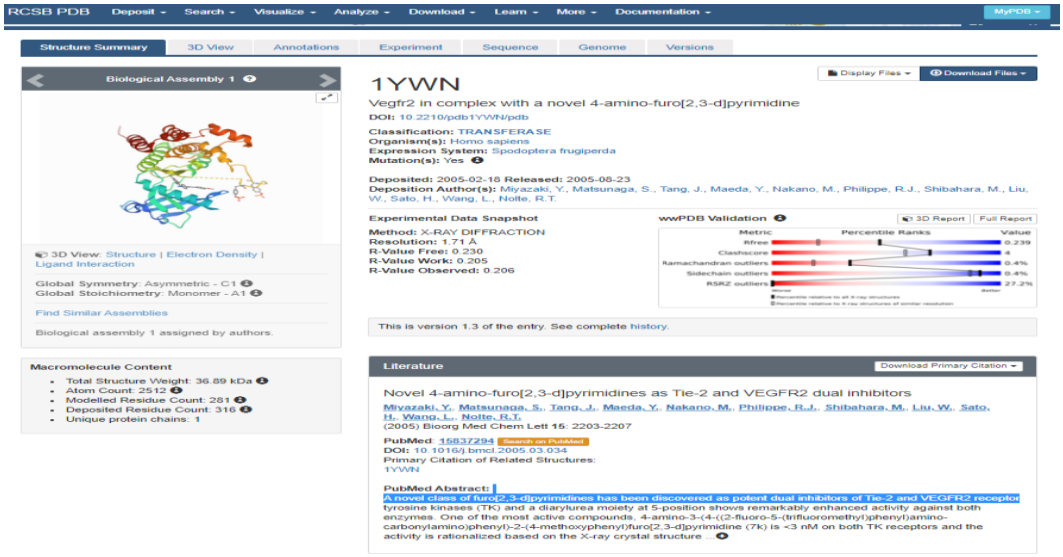


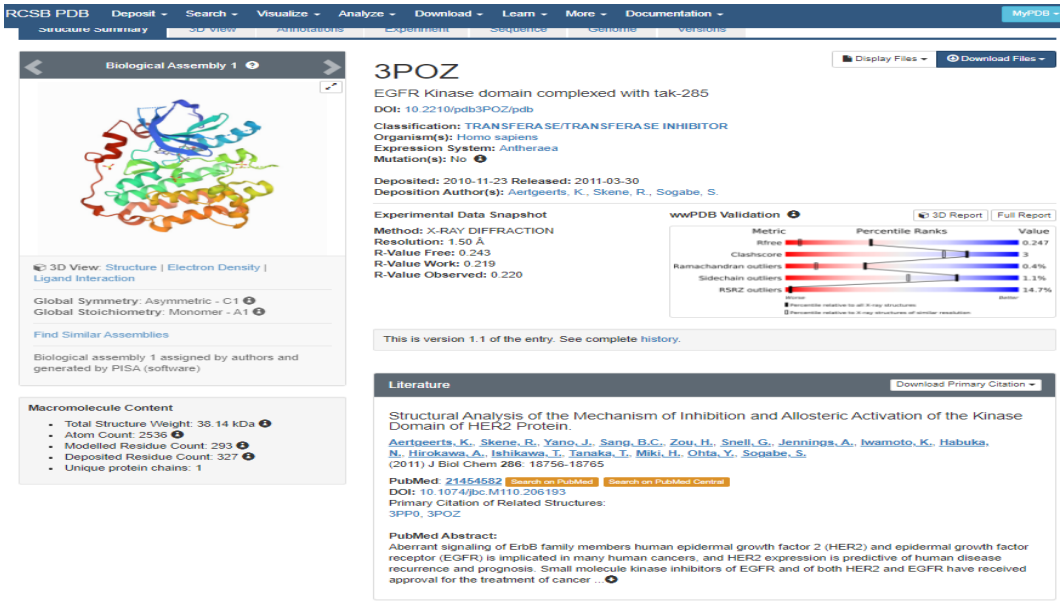
Webserver : Sea Search Server

Prediksi target molekuler terpilih	1. *VEGFR-2* 2. *Bcl-2* 3. *erbB1* 4. *PARP-1* 5. *PI3K-Alpha* 6. *mTOR* 7. *ERK-1* 8. *TNF-α*

2. Pengunduhan makromolekul target

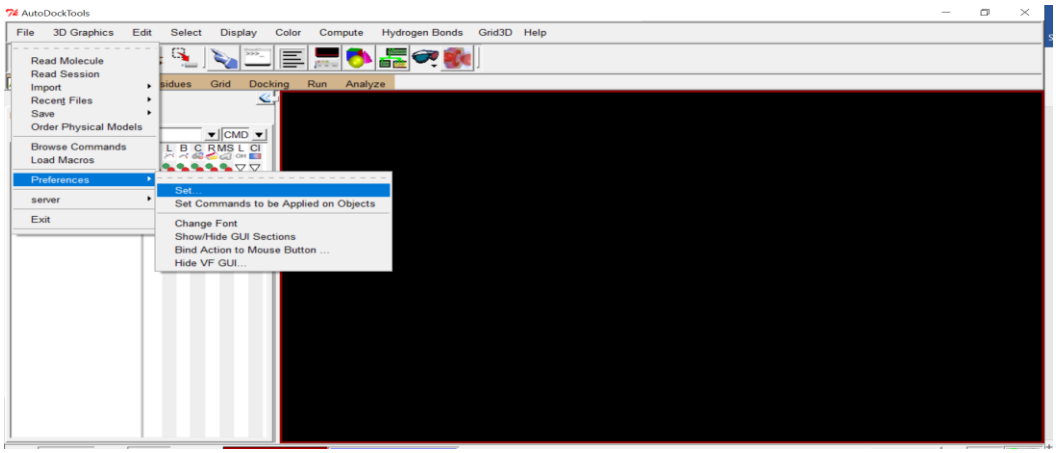
Pengunduhan makromolekul melalui (http://www.rcsb.org/pdb/). Identitas molekul tersebut yaitu *VEGFR-2* dengan kode PDB 1YWN, *Bcl-2* dengan kode 6GL8, *erbB1*dengan kode 3POZ, *PARP-1* dengan kode 4L6S, *PI3K-Alpha* dengan kode 4L23, *mTOR* dengan kode 4HVB, *ERK-1* dengan kode 4W4Y, *TNF-α* dengan kode 3L8X. Data makromolekul diunduh dalam format .pdb dan disimpan dalam folder untuk masing-masing protein.



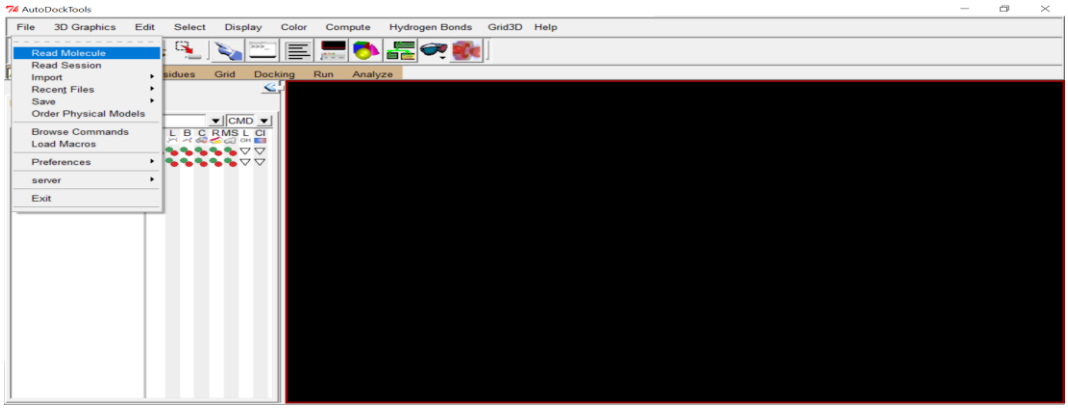


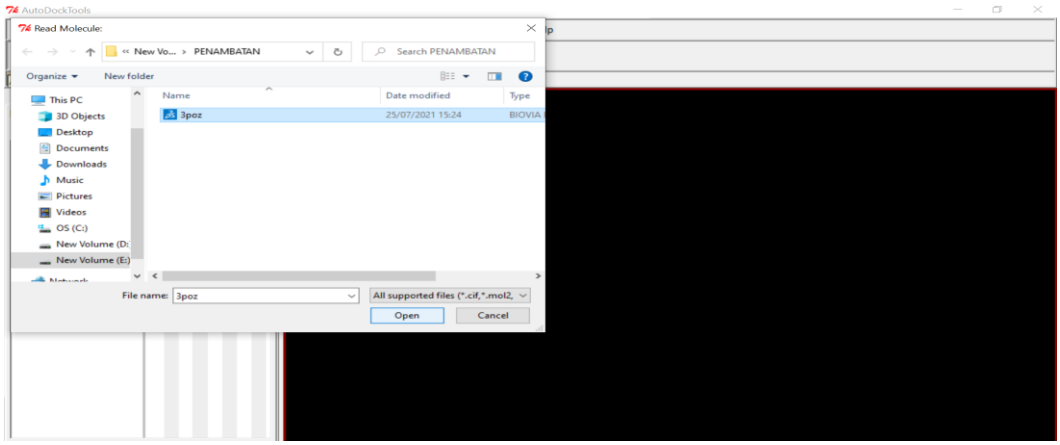
3. Pemisahan dan preparasi makromolekul dengan *native ligand*.

Pemisahan makromolekul protein dari pelarut dan ligan atau residu non standar dengan autodock tools. Disimpan dalam format .pdb.

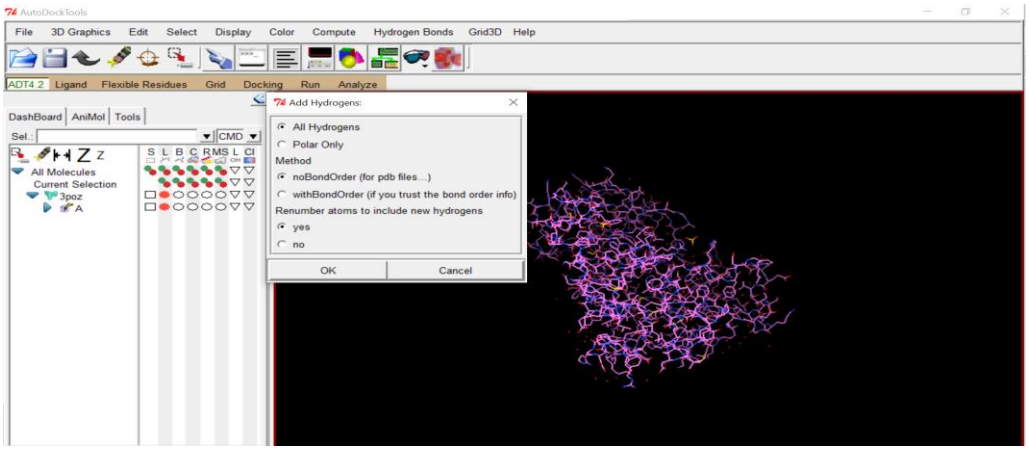


Set alamat folder makromolekul-*File-Preference-Set*

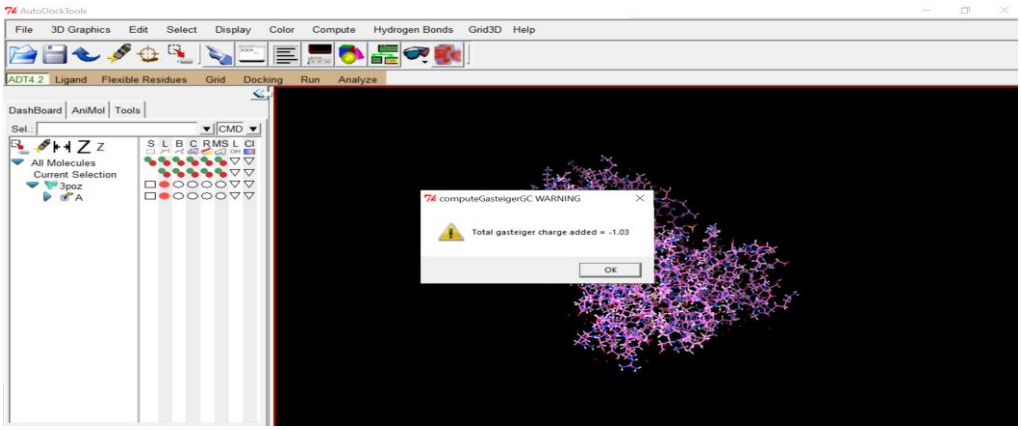




Klik *File-Open Molecule*-Dipih makromolekul 3POZ

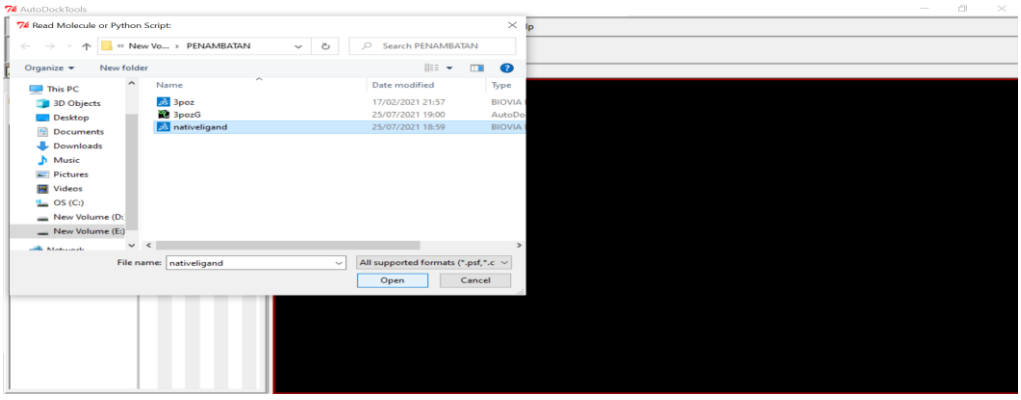


Edit-Add hydrogen-Add-Ok

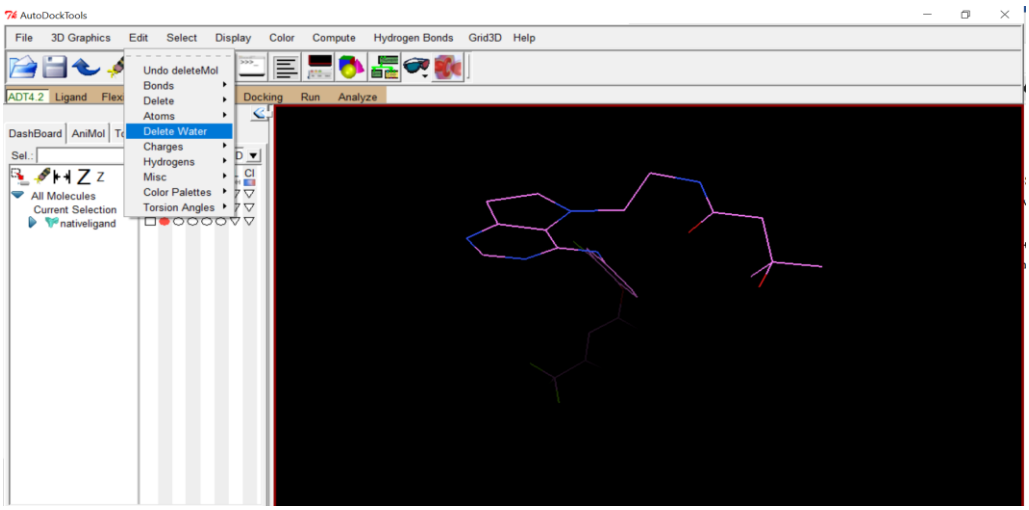


Edit-Charges-Compute gasteiger-Ok

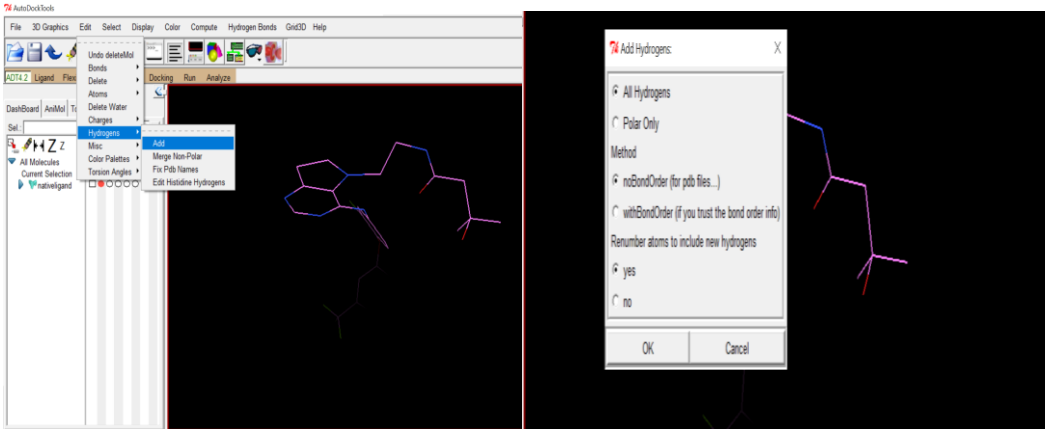
- **Preparasi *native ligand***



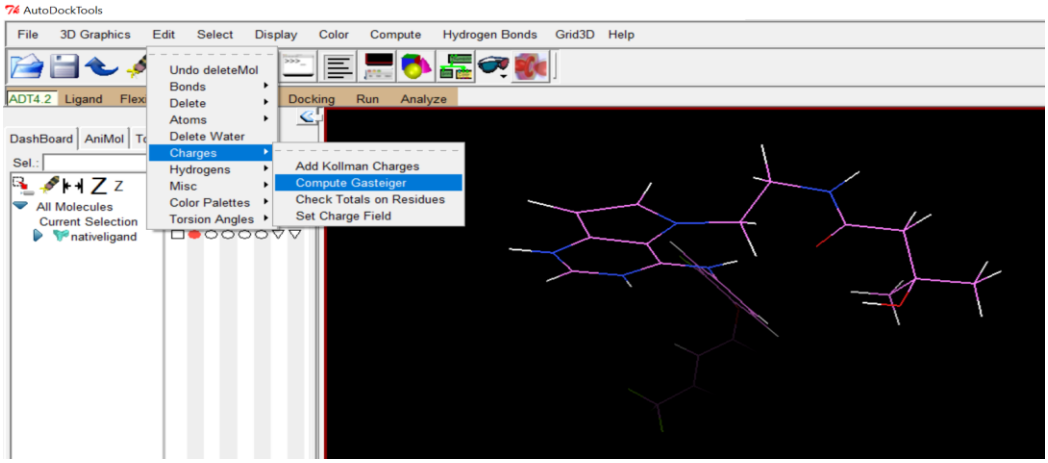
Membuka file *native ligand-Read molecul*-dipilih *native ligand*



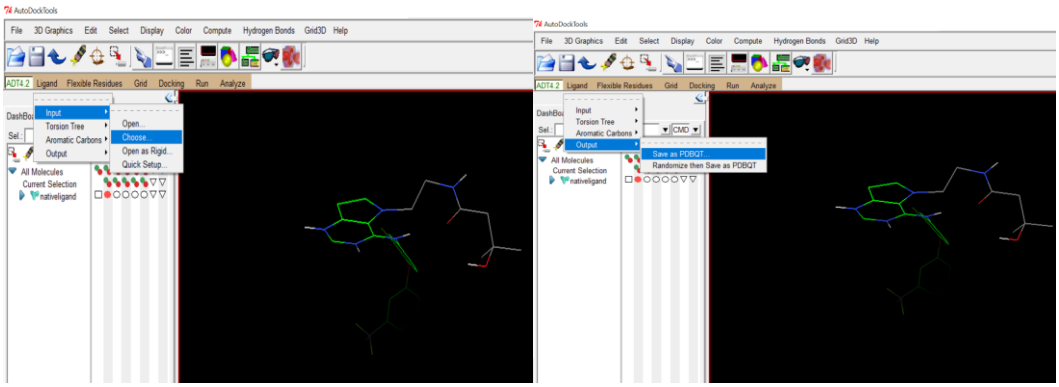
Edit-Delete water



Edit-Hydrogens-Add-Ok



Edit-Charges-Compute Gasteiger

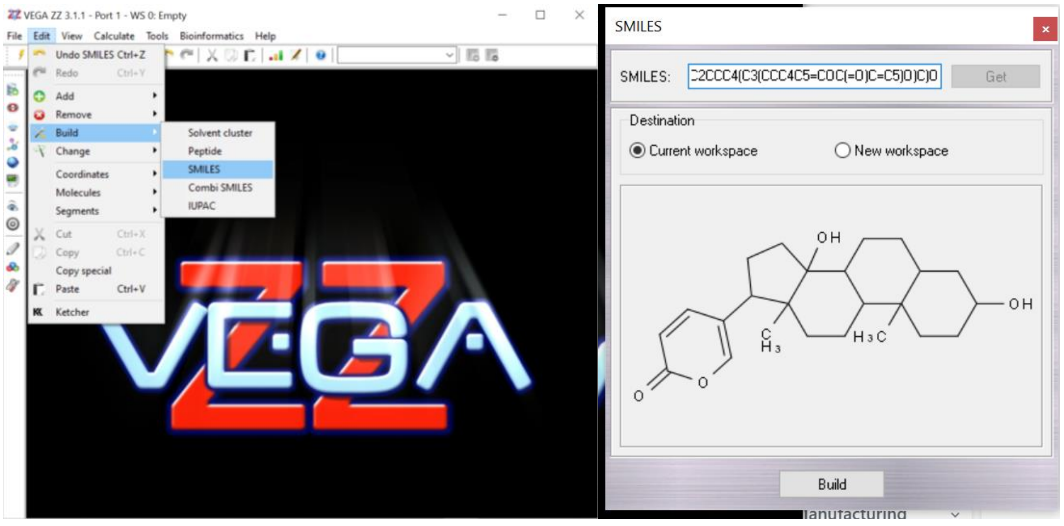


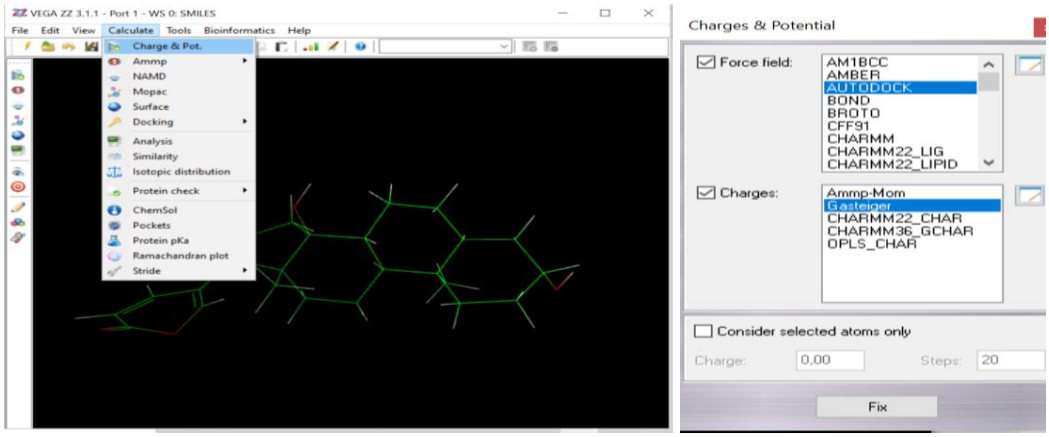
Ligand-Output-Save as PDBQT

4. Penyiapan Struktur 3 dimensi Ligan Uji

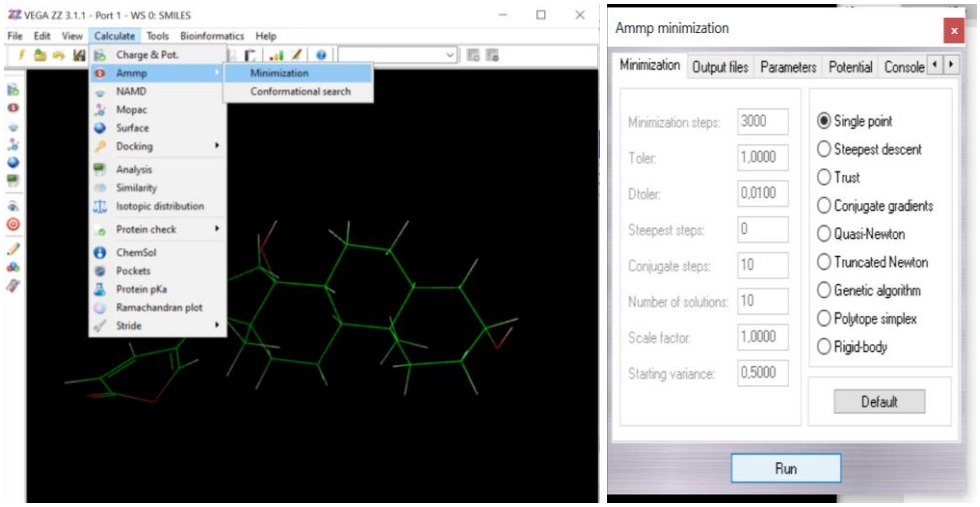
Struktur ligan uji disiapkan dengan SMILES dari pubchem.

Struktur ligan yang telah dibuat dioptimasi dengan VegaZZ





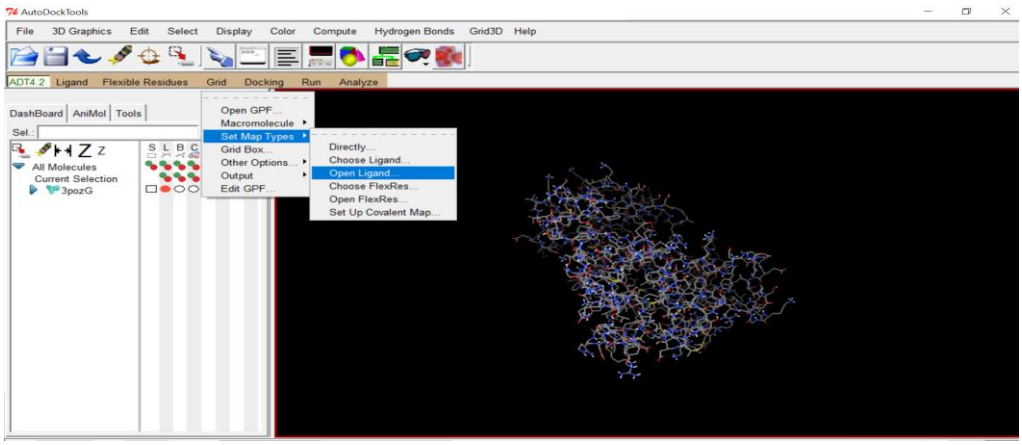
Pilih-*Calculate-Charge&Pot.-Pilih forcefield AUTODOCK-Charge Geisteger-Fix*



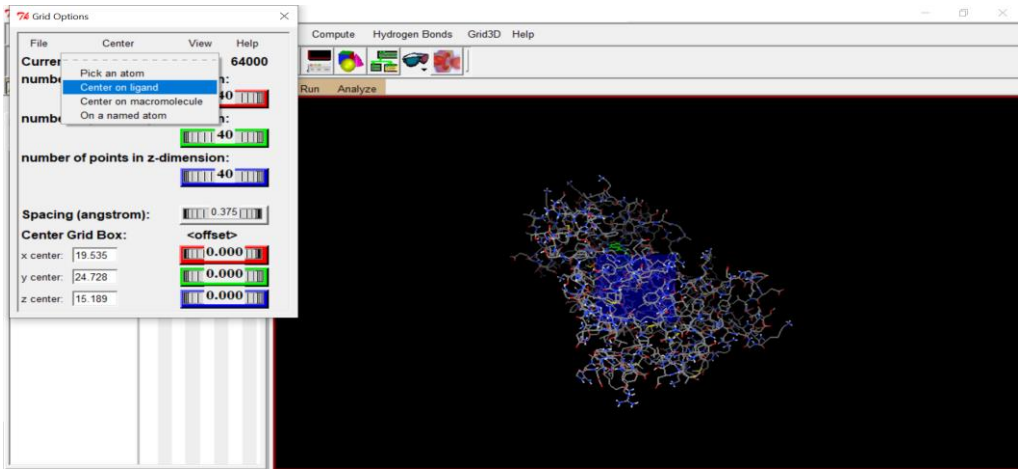
Pilih - *Calculate-Ammp-Minimization*

5. Penentuan *grid box* dari makromolekul.

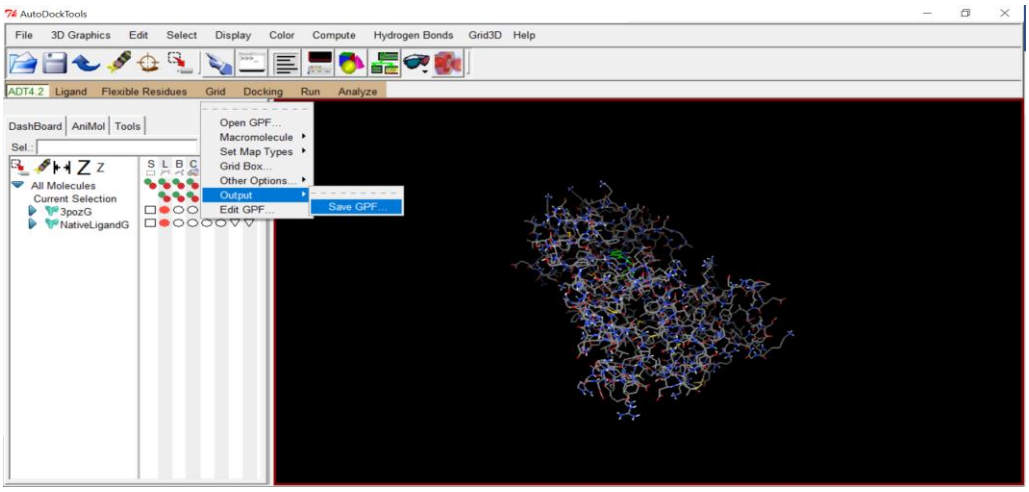
Grid box mengatur ruang bergerak ligan pada target



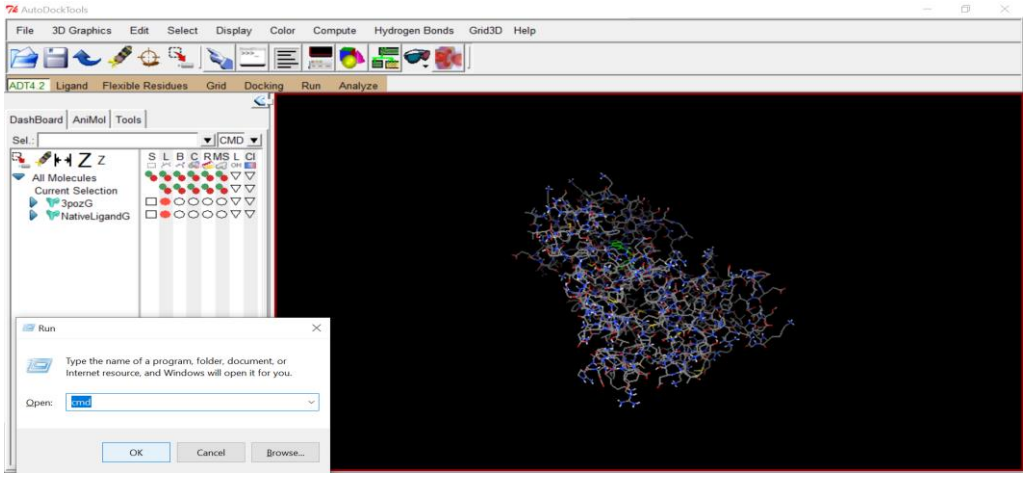
Grid- Macromolecul-Open-Set Map Types-Open Ligand



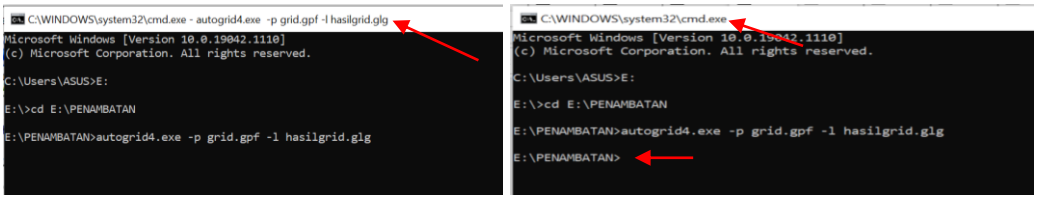
Dipilih *grid-Gridbox-Center on Ligand-Close saving current-gpf.*



Diklik *Grid-Output-Save gpf.*

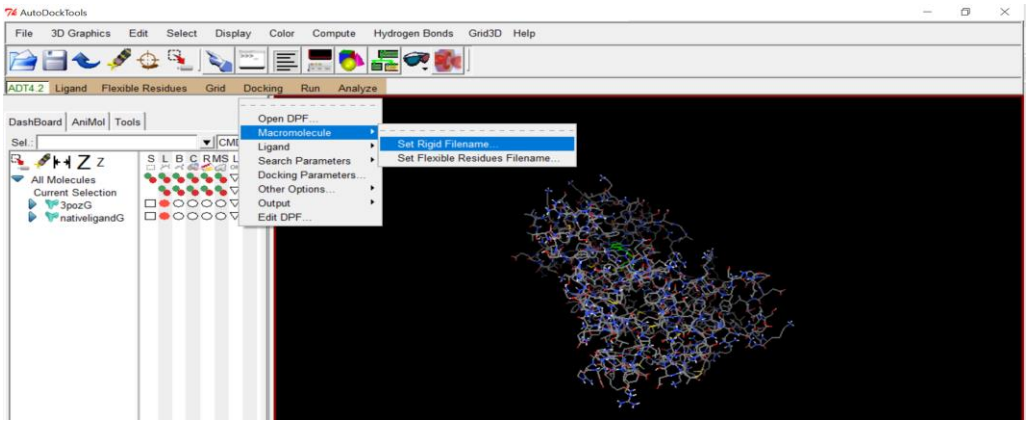


Diklik *windows+R*- kemudian diketik cmd-Ok

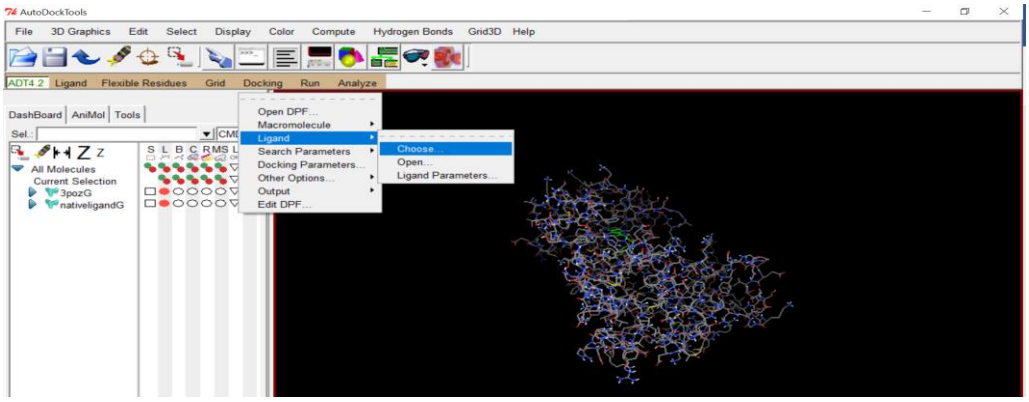


Proses grid selesai ditandai dengan kembali pada folder awal dan cmd.exe

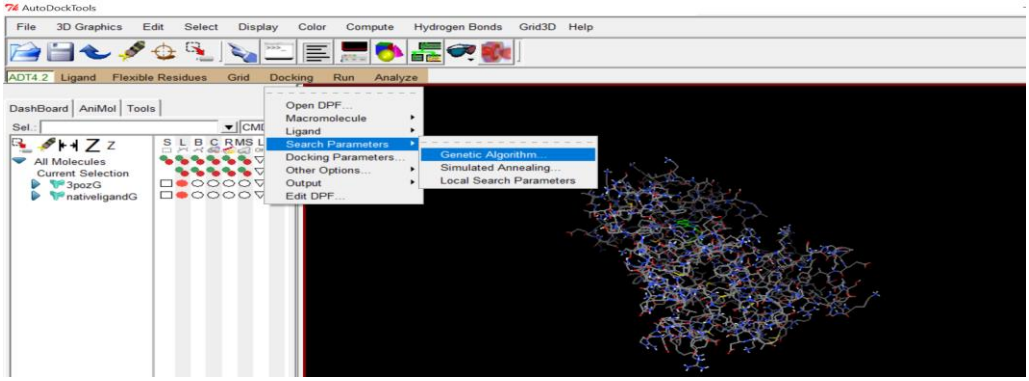
6. Validasi metode penambatan molekuler.



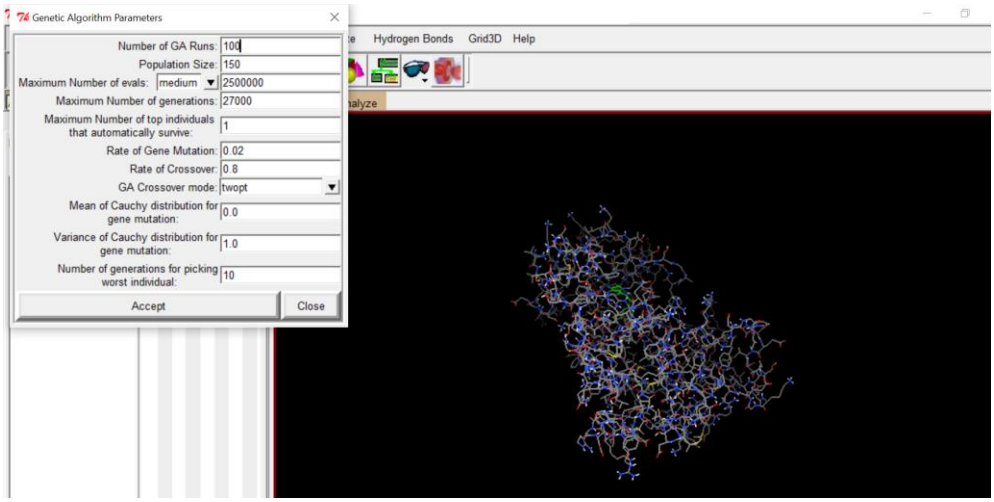
Docking- macromolecule- Set Rigit Filename



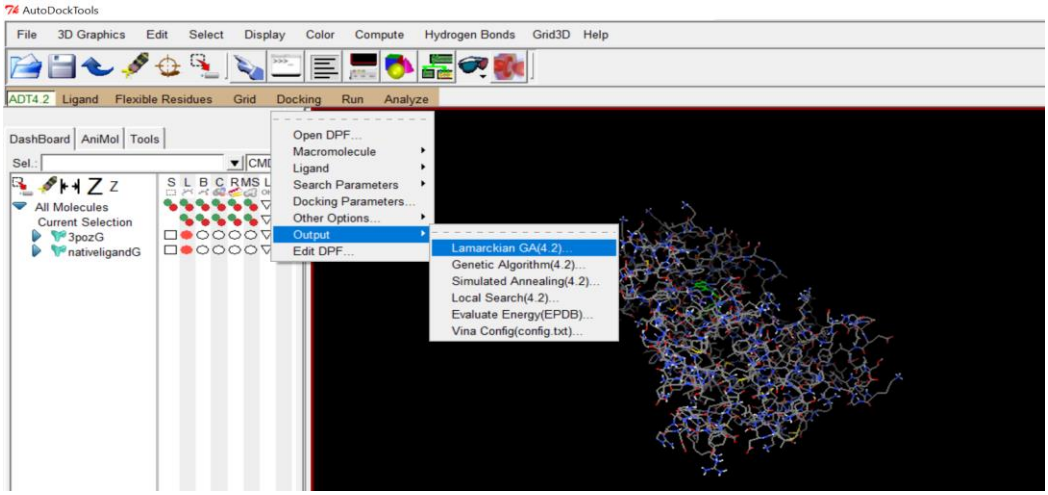
Docking-Ligand-Choose



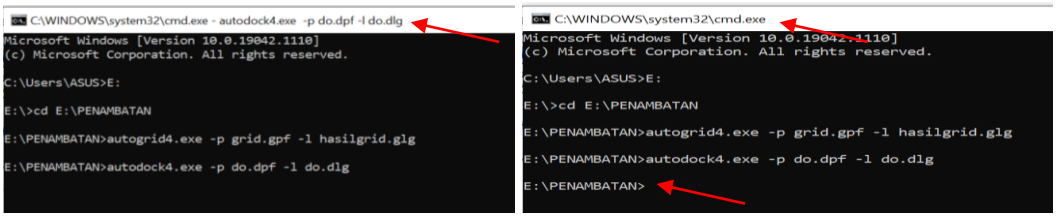
Docking-Search Parameters-Genetic Algorithm



Running di setting pada 100x dan *population size* 150.



Docking-Output-Lamarcian GA- dan file disimpan dengan format dpf.



Proses penambatan selesai ditandai dengan kembali pada folder awal dan cmd.exe

7. Analisis hasil penambatan molekuler

*do - Notepad
File Edit Format View Help

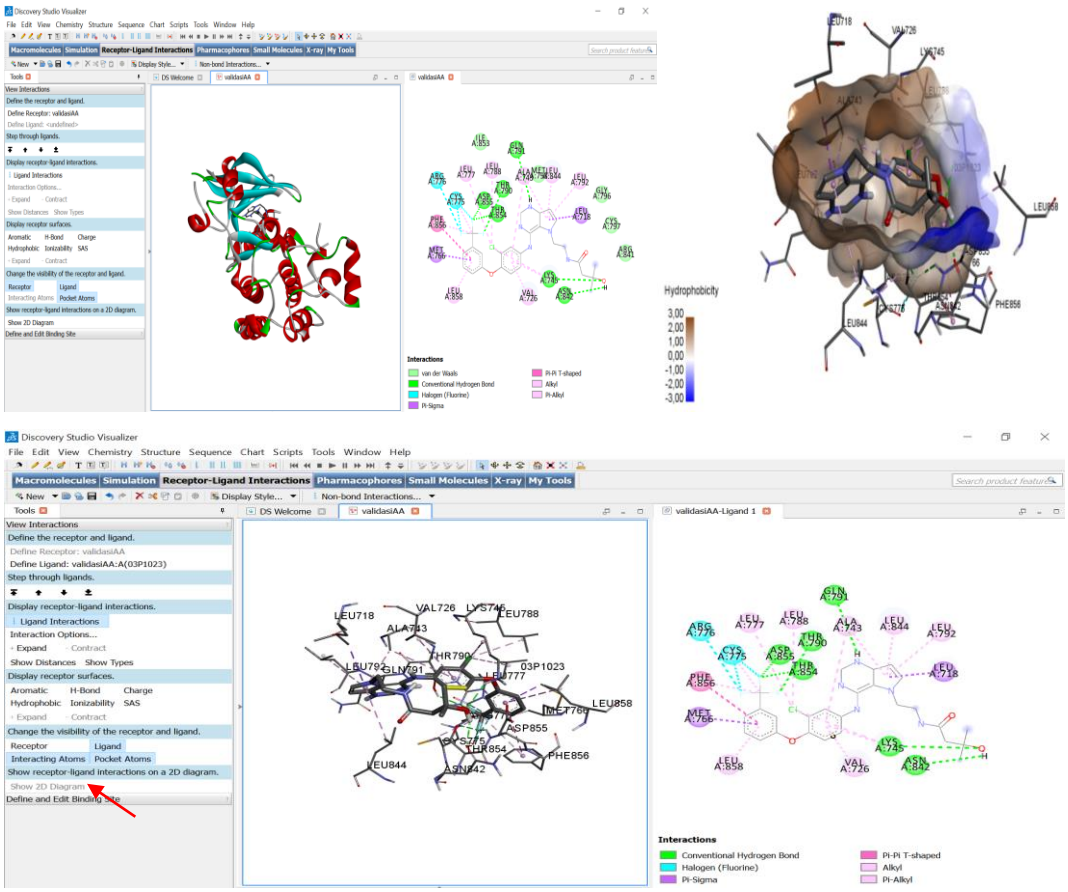
CLUSTERING HISTOGRAM

Clus-ter Rank	Lowest Binding Energy	Run	Mean Binding Energy	Num in Clus	Histogram (5 10 15 20 25 30 35)
1	-10.02	49	-8.94	37	#####################################
2	-9.39	23	-8.29	18	##################
3	-9.06	76	-9.06	1	#
4	-8.82	64	-8.82	1	#
5	-8.68	37	-8.01	4	####
6	-8.48	46	-7.61	5	#####
7	-8.48	45	-7.49	2	##
8	-8.46	30	-8.46	1	#
9	-8.02	13	-7.75	3	###
10	-7.92	61	-7.58	8	########
11	-7.36	54	-7.36	1	#
12	-7.21	56	-7.21	1	#
13	-7.09	18	-6.90	7	########
14	-6.94	58	-6.39	2	##
15	-6.88	9	-6.88	1	#
16	-6.54	44	-6.54	1	#
17	-6.45	63	-6.45	1	#
18	-6.44	36	-6.44	1	#
19	-6.11	83	-6.11	1	#
20	-6.09	27	-6.09	1	#
21	-5.78	1	-5.64	2	##
22	-5.38	94	-5.38	1	#

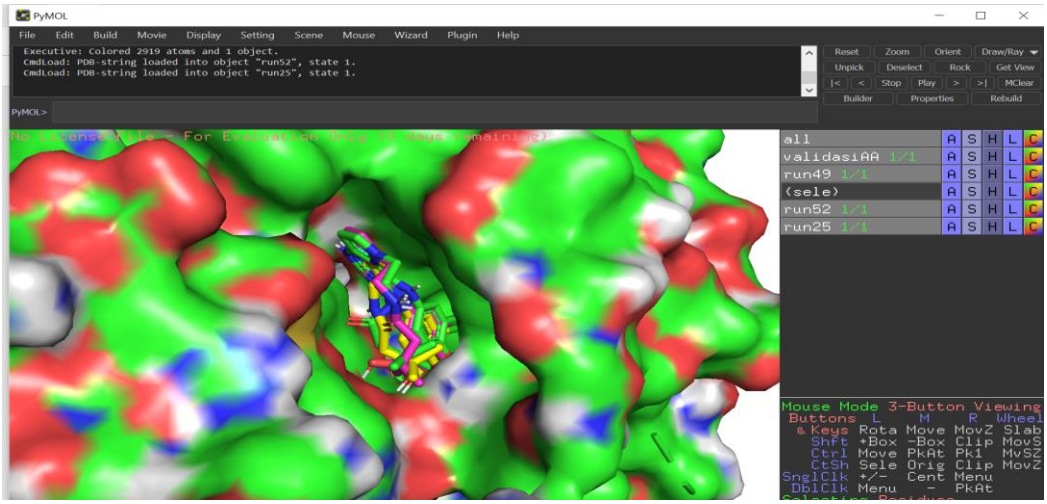
Number of multi-member conformational clusters found = 10, out of 100 runs.

Energi binding terbaik dipilih pada *Num in Clus* terbanyak dan pada hasil *run.*

8. Visualisasi dengan *PyMOL* dan *Discovery Studio Visualizer*



Visualisasi dapat dilakukan secara 2D untuk mengetahui ikatan yang terjadi: Klik show 2D Diagram-muncul 2D interaksi dan jenis ikatan yang terjadi



Pada PyMOL-dipilih-*Surface*