LAMPIRAN

Lampiran 1. Perhitungan Rendemen

Berat kering : 1.200 gram

Total Volume ekstrak (EB) : 8.820 ml

Dilakukan fraksinasi 800 ml ekstrak dengan 800 ml klorform, dihasilkan:

Volume fraksi kloroform (FKB) : 1.234 ml (dievaporasi sebanyak 1000 ml)

Volume ekstrak (EB) : 1000 ml

Setelah dikenatalkan, maka:

Berat fraksi kloroform (FKB) : 3,27 gram

Berat ekstrak (EB) : 22,21 gram

Konsentrasi serbuk kering total : $\frac{1200\ g}{8820\ ml}$ = 0,136 g/ml

Konsentrasi serbuk kering sebelum fraksi : 0,136 g/ml x 800 ml = 108,84 g

Perhitungan Rendemen

a. Faraksi kloroform bandotan

Konsentrasi serbuk kering setelah fraksi : $\frac{108{,}84\ g}{1234\ ml}\ x\ 1000\ ml$ = 88,2 g

% rendemen FKB : $\frac{3{,}27\ g}{88{,}20\ g} x100\% = 3{,}7\ \%$

b. Ekstrak etanol

Konsentrasi serbuk kering : $\frac{1200\ g}{8820\ ml}\ x\ 1000\ ml$ = 136 g

% rendemen EB : $\frac{22{,}21\ g}{136\ g} = 16{,}33\%$

Lampiran 2. Perhitungan Rf Kromatografi Lapis Tipis

$$\text{Nilai Rf} = \frac{\text{Jarak elusi bercak (cm)}}{\text{Jarak elusi fase gerak (cm)}}$$

Nilai Rf bercak 1 = 1,0/8,0 = 0,125

Nilai Rf bercak 2 = 1,1/8,0 = 0,137

Nilai Rf bercak 3 = 1,25/8,0 = 0,156

Nilai Rf bercak 4 = 1,45/8,0 = 0,181

Nilai Rf bercak 5 = 1,6/8,0 = 0,200

Nilai Rf bercak 6 = 2,1/8,0 = 0,260

Nilai Rf bercak 7 = 2,3/8,0 = 0,287

Nilai Rf bercak 8 = 2,5/8,0 = 0,312

Nilai Rf bercak 9 = 2,7/8,0 = 0,337

Nilai Rf bercak 10 = 2,8/8,0 = 0,350

Nilai Rf bercak 11 = 3,0/8,0 = 0,375

Nilai Rf bercak 12 = 3,9/8,0 = 0,487

Nilai Rf bercak 13 = 4,25/8,0 = 0,531

Nilai Rf bercak 14 = 5,7/8,0 = 0,587

Nilai Rf bercak 15 = 5,1/8,0 = 0,637

Nilai Rf bercak 16 = 7,4/8,0 = 0,925

Lampiran 3. Tabel dan perhitungan viabilitas sel HeLa setelah pemberian FKB

No.	Kadar (µg/ml)	Absorbansi				Viabilitas sel (%)				
		A1	A2	A3	Rata-rata	V1	V2	V3	Rata-rata	SD
1	1.5625	0.787	0.861	0.972	**0.873**	84.34	93.01	106.02	**94.45**	10.91
2	3.125	0.763	0.868	0.892	**0.841**	81.52	93.83	96.64	**90.66**	8.04
3	6.25	0.761	0.809	0.826	**0.799**	81.29	86.91	88.91	**85.70**	3.95
4	12.5	0.697	0.739	0.760	**0.732**	73.79	78.71	81.17	**77.89**	3.76
5	25	0.561	0.578	0.522	**0.554**	57.85	59.84	53.28	**56.99**	3.36
6	50	0.152	0.148	0.143	**0.148**	9.92	9.45	8.87	**9.41**	0.53

	Absorbansi			Rata-rata
	A1	A2	A3	
Kontrol sel	0.937	0.912	0.913	0.921
Kontrol media	0.067	0.067	0.068	0.067

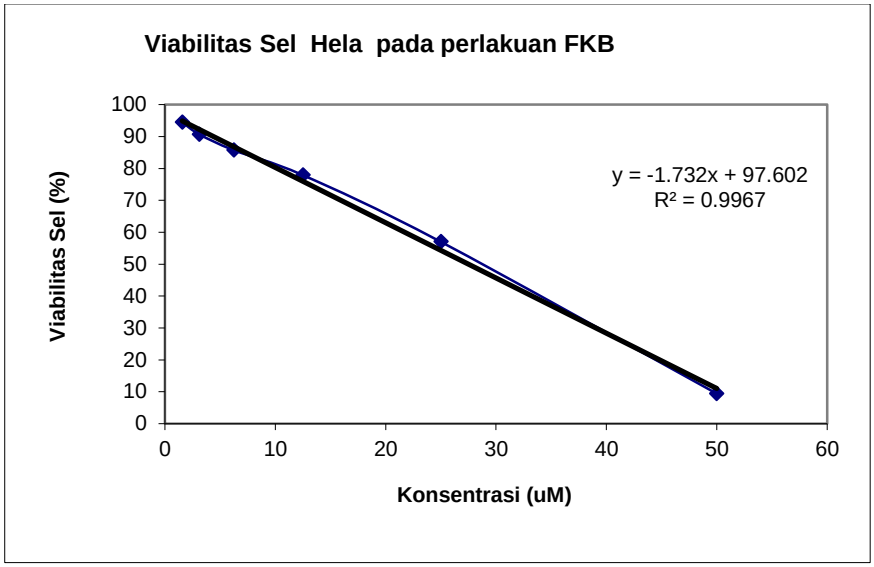
Pengukuran dengan ELISA *reader* menghasilkan nilai absorbansi. Nilai absorbansi yang dihasilkan dikonversi menjadi viabilitas sel (%sel hidup) dengan rumus:

$$\% \text{ sel hidup} = \frac{Absorbansi\ sampel - Absorbansi\ kontrol\ media}{Absorbansi\ kontrol\ sel - Absorbansi\ kontrol\ media} \text{ X } 100\%$$

Viabilitas sel HeLa setelah pemberian FKB sebagai berikut:

1. % sel hidup kadar 1.5625 µg/ml $= \frac{0.873-0.067}{0.921-0.067}$ x 100% = 94.45%
2. % sel hidup kadar 3.125 µg/ml $= \frac{0.841-0.067}{0.921-0.067}$ x 100% = 90.66%
3. % sel hidup kadar 6.25 µg/ml $= \frac{0.799-0.067}{0.921-0.067}$ x 100% = 85.70%
4. % sel hidup kadar 12.5 µg/ml $= \frac{0.732-0.067}{0.921-0.067}$ x 100% = 77.89%
5. % sel hidup kadar 25 µg/ml $= \frac{0.554-0.067}{0.921-0.067}$ x 100% = 56.99%

6. % sel hidup kadar 12.5 µg/ml $= \frac{0.148-0.067}{0.921-0.067}$ x 100% = 9.41%



Dibuat regresi linier untuk menentukan nilai IC_{50} FKB sehingga didapatkan persamaan garis y = -1.732x + 97.602 dengan y sebagai %sel hidup dan x sebagai konsentrasi.

y = -1.732x + 97.602

50 = -1.732x + 97.602

1.732x = 97.602 – 50

x = 27.48 µg/ml

Lampiran 4. Tabel dan perhitungan viabilitas sel HeLa setelah pemberian 5-FU

No.	Kadar (µg/ml)	Absorbansi				Viabilitas sel (%)				
		A1	A2	A3	Rata-rata	V1	V2	V3	Rata-rata	SD
1	1.69	0.711	0.793	0.831	**0.778**	75.43	85.04	89.49	**83.32**	7.19
2	3.38	0.676	0.779	0.795	**0.750**	71.33	83.40	85.27	**80.00**	7.57
3	9.76	0.759	0.733	0.773	**0.755**	81.06	78.01	82.70	**80.59**	2.38
4	19.53	0.533	0.707	0.646	**0.629**	54.57	74.96	67.82	**65.78**	10.35
5	39.06	0.478	0.491	0.457	**0.475**	48.13	49.65	45.67	**47.82**	2.01
6	78.125	0.268	0.253	0.246	**0.256**	23.52	21.76	20.94	**22.08**	1.32

	Absorbansi			Rata-rata
	A1	A2	A3	
Kontrol sel	0.937	0.912	0.913	0.921
Kontrol media	0.067	0.067	0.068	0.067

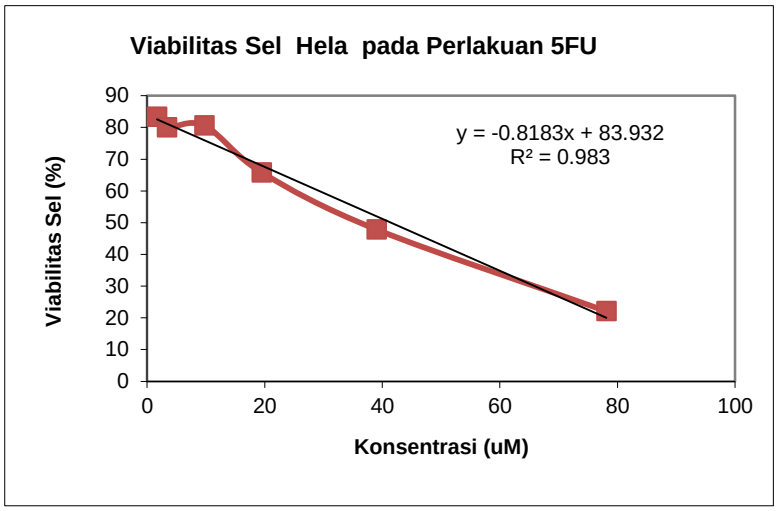
Pengukuran dengan ELISA *reader* menghasilkan nilai absorbansi. Nilai absorbansi yang dihasilkan dikonversi menjadi viabilitas sel (%sel hidup) dengan rumus:

$$\% \text{ sel hidup} = \frac{Absorbansi\ sampel - Absorbansi\ kontrol\ media}{Absorbansi\ kontrol\ sel - Absorbansi\ kontrol\ media} \text{ X } 100\%$$

Viabilitas sel HeLa setelah pemberian 5-FU sebagai berikut:

1. % sel hidup kadar 1.69 µg/ml $= \frac{0.778 - 0.067}{0.921 - 0.067}$ x 100% = 83.32%
2. % sel hidup kadar 3.38 µg/ml $= \frac{0.750 - 0.067}{0.921 - 0.067}$ x 100% = 80.00%
3. % sel hidup kadar 9.76 µg/ml $= \frac{0.755 - 0.067}{0.921 - 0.067}$ x 100% = 80.59%
4. % sel hidup kadar 19.53 µg/ml $= \frac{0.629 - 0.067}{0.921 - 0.067}$ x 100% = 65.78%
5. % sel hidup kadar 39.06 µg/ml $= \frac{0.475 - 0.067}{0.921 - 0.067}$ x 100% = 47.82%

6. % sel hidup kadar 78.125 µg/ml = $\frac{0.256-0.067}{0.921-0.067}$ x 100% = 22.08%



Dibuat regresi linier untuk menentukan nilai IC_{50} FKB sehingga didapatkan persamaan garis lurus y = -0.8183x + 83.932 dengan y sebagai %sel hidup dan x sebagai konsentrasi.

y = -0.8183x + 83.932

50 = -0.8183x + 83.932

0.8183x = 83.932– 50

x = 41.46 µg/ml

Lampiran 5. Desain uji sitotoksik kombinasi FKB dan 5-FU

Uji kombinasi FKB dan 5-FU pada konsentrasi masing-masing ½ IC_{50}, 3/8 IC_{50}, ¼ IC_{50} dan 1/8 IC_{50} dimana IC_{50} FKB adalah 27,48 µg/ml dan 5-FU adalah 41.46 µg/ml.

Konsentrasi untuk uji kombinasi

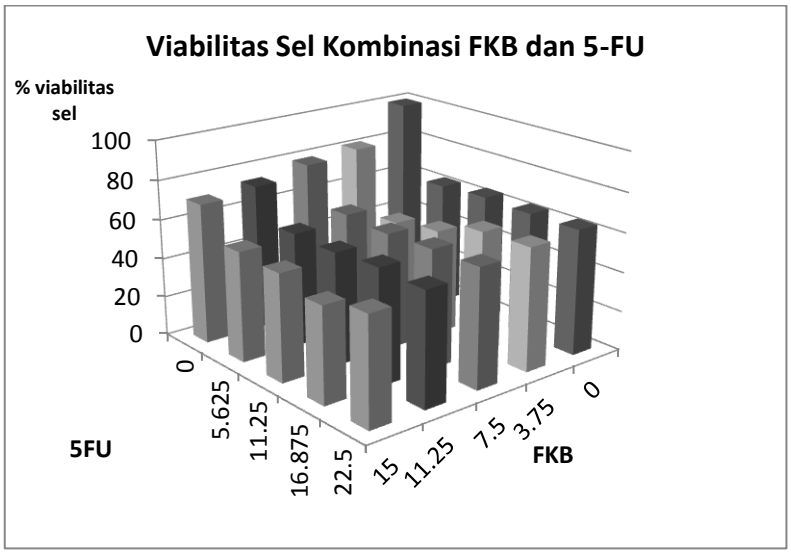
Nomor konsentrasi	% IC_{50}	Konsentrasi (µg/ml)	
		FKB	5-FU
1	½ IC_{50}	15	22.5
2	3/8 IC_{50}	11.25	16.875
3	¼ IC_{50}	7.5	11.25
4	1/8 IC_{50}	3.75	5.625

Maping *96 well plate* untuk uji kombinasi

<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td rowspan="4" colspan="3">Seri konsentrasi 1-4 FKB</td><td rowspan="4" colspan="3">Seri konsentrasi 1-4 FKB + 5-FU konsentrasi 1</td><td rowspan="4" colspan="3">Seri konsentrasi 1-4 FKB + 5-FU konsentrasi 3</td><td colspan="3">2 IC_{50} FKB</td></tr>
<tr><td>B</td><td colspan="3">IC_{50} FKB</td></tr>
<tr><td>C</td><td colspan="3">2 IC_{50} 5-FU</td></tr>
<tr><td>D</td><td colspan="3">IC_{50} 5-FU</td></tr>
<tr><td>E</td><td rowspan="4" colspan="3">Seri konsentrasi 1-4 5-FU</td><td rowspan="4" colspan="3">Seri konsentrasi 1-4 FKB + 5-FU konsentrasi 2</td><td rowspan="4" colspan="3">Seri konsentrasi 1-4 FKB + 5-FU konsentrasi 4</td><td colspan="3" rowspan="2">Kontrol sel</td></tr>
<tr><td>F</td></tr>
<tr><td>G</td><td colspan="3" rowspan="2">Kontrol media</td></tr>
<tr><td>H</td></tr>
</table>

Viabilitas sel HeLa pada perlakuan kombinasi FKB dan 5-FU

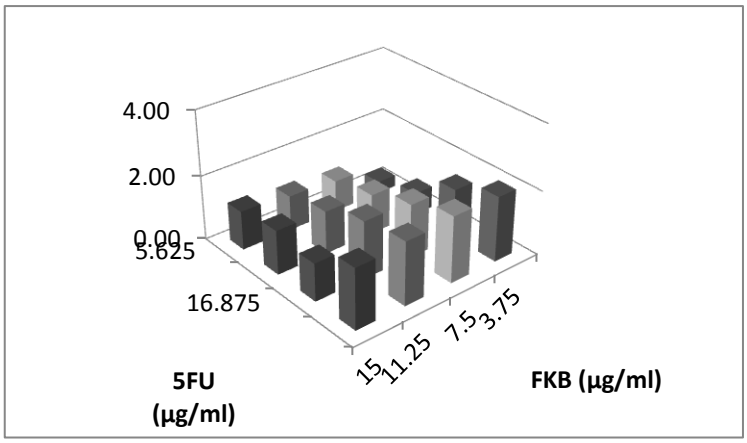
Senyawa		Viabilitas perlakuan 5-FU (%sel hidup)				
		0	5.625	11.25	16.875	22.5
Viabilitas perlakuan FKB (%sel hidup)	0	100	63.20	64.63	63.78	63.71
	3.75	81.79	49.92	53.45	61.37	62.15
	7.5	79.17	60.82	59.09	59.87	60.11
	11.25	73.97	57.76	56.85	58.41	57.12
	15	71.12	55.79	54.26	48.35	54.23



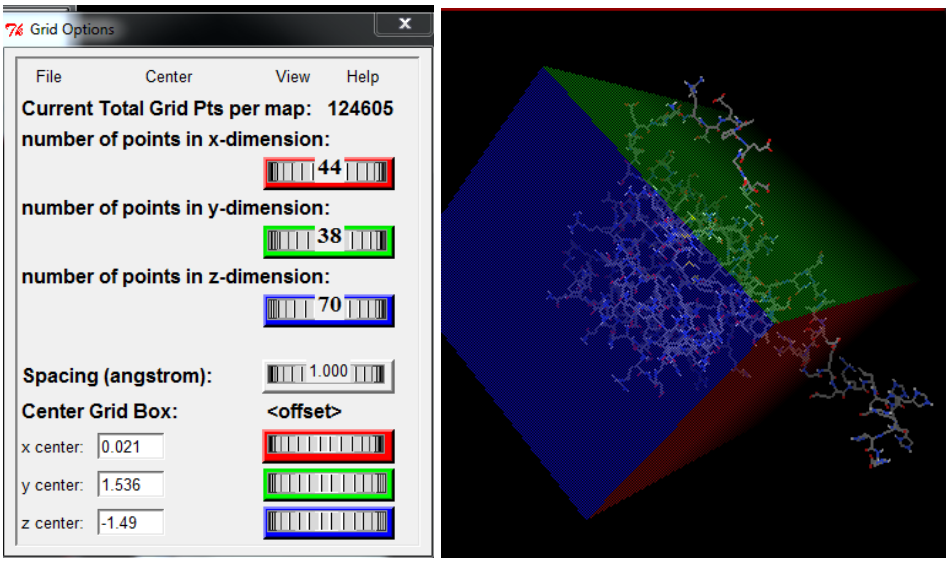
Nilai CI pada Kombinasi FKB dan 5-FU

CI		5-FU			
		5.625	11.25	16.875	22.5
FKB	3.75	0.36	0.68	1.51	2.02
	7,5	0.97	1.23	1.66	2.05
	11.25	1.08	1.33	1.78	1.96
	15	1.20	1.37	1.17	1.86

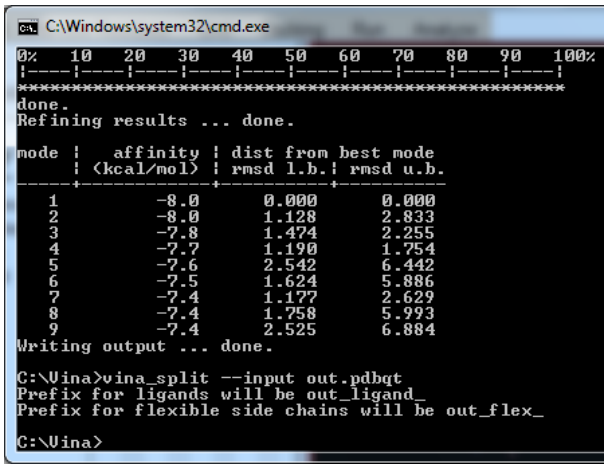
Diagram CI Kombinasi FKB dan 5-FU

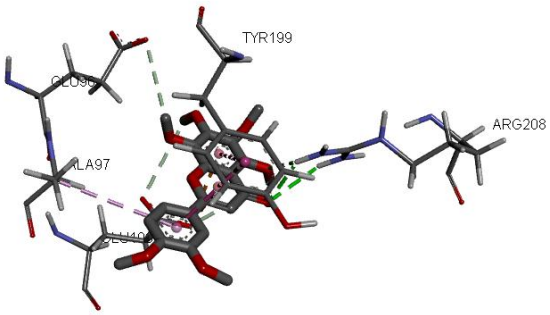


Lampiran 6. Gambaran score docking, nilai RMSD dan viasualisasi hasil docking dan interaksi antara Nobiletin dan protein Bcl-xL



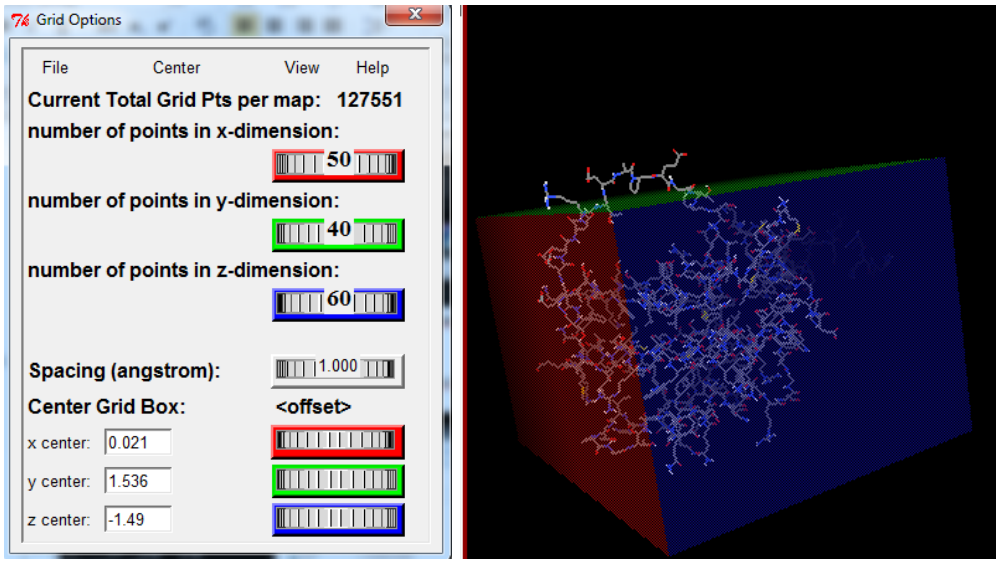
Nilai grid box dan luas area *docking*



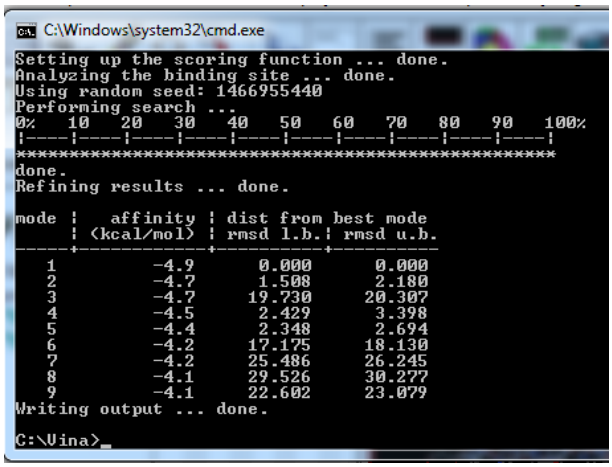


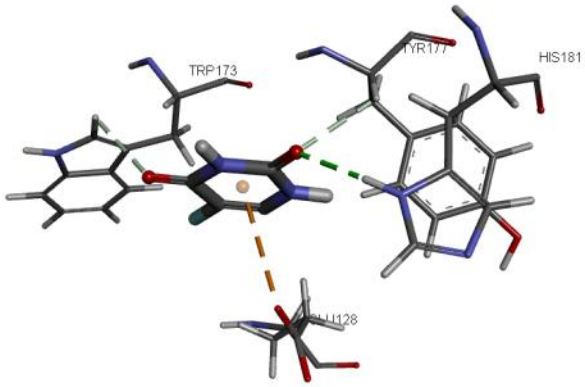
Nilai *score docking*, RMSD dan visualisasi hasil docking

Lampiran 7. Gambaran score docking, nilai RMSD dan viasualisasi hasil docking dan interaksi antara 5-Fluorourasil dan protein Bcl-xL



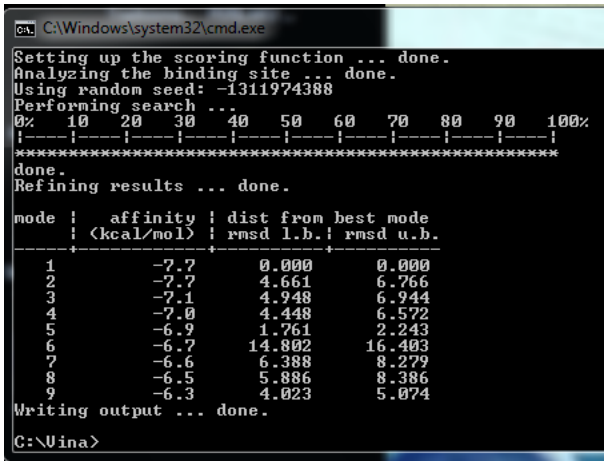
Nilai grid box dan luas area *docking*





Nilai *score docking*, RMSD dan visualisasi hasil docking

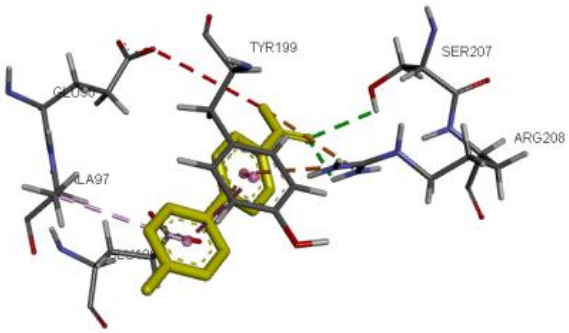
Lampiran 8. Gambaran score docking, nilai RMSD dan viasualisasi hasil docking dan interaksi antara Ligan asli 4FC dan TH1 pada protein Bcl-xL



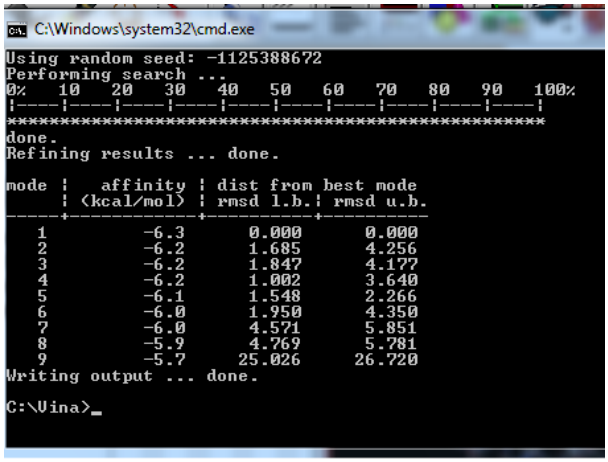
```
C:\Windows\system32\cmd.exe
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1311974388
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -7.7      0.000      0.000
   2        -7.7      4.661      6.766
   3        -7.1      4.948      6.944
   4        -7.0      4.448      6.572
   5        -6.9      1.761      2.243
   6        -6.7     14.802     16.403
   7        -6.6      6.388      8.279
   8        -6.5      5.886      8.386
   9        -6.3      4.023      5.074
Writing output ... done.

C:\Vina>
```



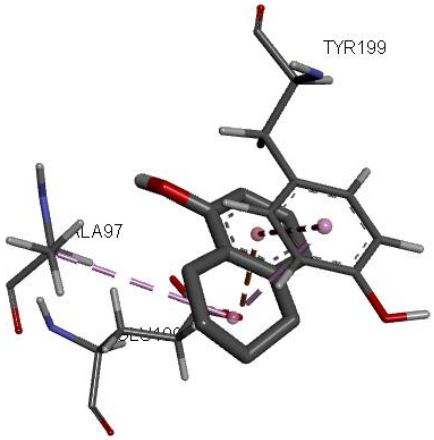
Nilai *score docking*, RMSD dan visualisasi hasil docking ligan asli 4FC



```
C:\Windows\system32\cmd.exe
Using random seed: -1125388672
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
**************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -6.3      0.000      0.000
   2        -6.2      1.685      4.256
   3        -6.2      1.847      4.177
   4        -6.2      1.002      3.640
   5        -6.1      1.548      2.266
   6        -6.0      1.950      4.350
   7        -6.0      4.571      5.851
   8        -5.9      4.769      5.781
   9        -5.7     25.026     26.720
Writing output ... done.

C:\Vina>_
```



Nilai *score docking*, RMSD dan visualisasi hasil docking ligan asli TH1

Lampiran 9. Determinasi Herba Bandotan

UNIVERSITAS GADJAH MADA
FAKULTAS FARMASI
Sekip Utara, Yogyakarta 55281 Telp.(0274) 543120 Fax.(0274) 543120 Email:farmasi@ugm.ac.id.

SURAT KETERANGAN
No.: UGM/FA/2449/M/03/02

Kepada Yth. :
Sdri/Sdr. Titi Komalasari
NIM. 20130350110
Fakultas Kedokteran dan Ilmu Kesehatan UMY
Di Yogyakarta

Dengan hormat,

Bersama ini kami sampaikan hasil identifikasi sampel tumbuhan yang Saudara kirimkan ke Departemen Biologi Farmasi, Fakultas Farmasi UGM, adalah :

No.Pendaftaran	Jenis	Suku
06	*Ageratum conyzoides* L.	Asteraceae

Demikian, semoga dapat digunakan sebagaimana mestinya.

Yogyakarta, 10 Juni 2016

Mengetahui,
Dekan
Fakultas Farmasi UGM

Prof. Dr. Subagus Wahyuono, M.Sc.,Apt
NIP. 195307081977021001

Ketua
Departemen Biologi Farmasi

Dr.rer.nat. Triana Hertiani, M.Si., Apt.
NIP. 197306091998032003

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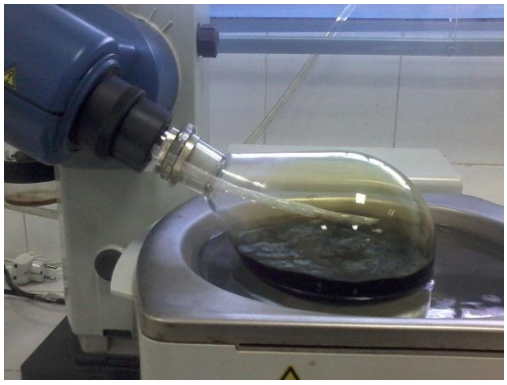
Lampiran 10. Foto Penelitian

Pengeringan

Penyerbukan simplisia

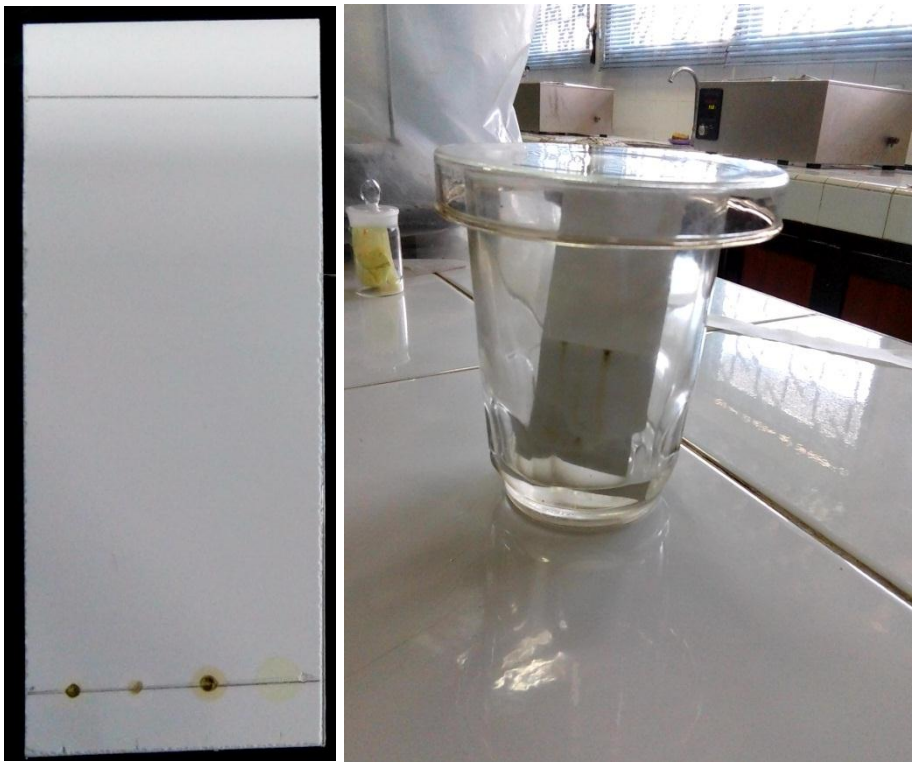
Fraksinasi

Hasil Fraksinasi



Evaporasi di evaporator

Evaporasi dengan waterbath



Plat KLT dan proses elusi

Maping 96-well plate untuk kombinasi