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RESEARCH ARTICLE

Molecular Docking of Anthranoids on Some Targeted Human Proteins

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Molecular Docking of Anthranoids on Some Targeted Human Proteins

SUMMARY

Natural products have been intensively investigated for years in point of isolation of natural compounds from natural sources and their biological activity studies. Biological activity studies usually routed based on their usage in folk medicine. The screening of novel bioactivities for plant natural compounds using computational methods have a crucial role to accelerate the progress of drug discovery. In this study the discovery of undiscovered biological activities of anthranoids, well known for their laxative properties, were planned. Casein kinase II subunit α (CK II α), human myosin light chain kinase member 4 (MYLK 4) and angiotensin converting enzyme (ACE) were selected from among hundreds of proteins. Five anthranoid skeletons and their derivatives as totally being 18 ligands were simulated to targets by using the docking program SYBYL X 2.0 and AutoDock Vina 4.0. Nine scoring functions for molecular docking were compared and evaluated. As a result, glucofrangulin A showed the best interaction for CK II α, glucofrangulin B for MYLK 4, frangulin A and glucofrangulin A for ACE, respectively.

Key Words: *Anthranoid, AutoDock Vina 4.0, docking, drug discovery, natural compound, SYBL-X 2.0.*

Antranoitlerin, Hedeflenmiş Bazı İnsan Proteinleri Üzerindeki Moleküler Dokingi

ÖZET

Doğal ürünler, doğal kaynaklardan madde izolasyonu ve bunların biyolojik aktivite çalışmaları nedeniyle yıllardır araştırılmaktadır. Biyolojik aktivite çalışmaları genellikle halk arasındaki kullanımlarına dayanmaktadır. Bilgisayara dayanan metotlar ile bitkilerden elde edilen doğal kaynaklı maddeler için yeni olabilecek aktivitelerin taranması ilaç keşfi açısından önemli bir role sahiptir. Bu çalışmada laksatif etkileri ile bilinen antrakinonların henüz tespit edilmemiş aktivitelerinin keşfi planlanmıştır. Kazein kinaz II α, insan myosin hafif zinciri 4 ve anjiyotensin dönüştürücü enzim birçok protein arasından hedef olarak seçilmiştir. 5 antranoit iskeleti ve türevleri olmak üzere toplam 18 ligand SYBYL X 2.0 ve AutoDock Vina 4.0 programları kullanılarak hedefler ile simüle edilmiştir. Doking sonuçlarına göre 9 skor fonksiyonu karşılaştırılmış ve değerlendirilmiştir. Sonuç olarak glukofrangulin A CK II α, glucofrangulin B MYLK 4 ve frangulin A ACE ile en iyi etkileşim gösterdiği gözlenmiştir.

Anahtar kelimeler: *Antranoit, AutoDock Vina 4.0, dokingilaç keşfi, doğal bileşik, SYBL-X 2.0.*

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INTRODUCTION

In recent years, as a preliminary study, the computational methods have taken an important role in determining the relationship of the potential active compounds to the receptors or enzymes. These methods examining prospective drug targets can also be used to test parameters such as absorption, metabolism, excretion, toxicity, physicochemical properties. Docking, modeling, similarity research, quantitative structure-activity relationships can be realized with these methods and it is possible to apply them in many fields. *In silico* methods are useful to make a preliminary assessment because of their low cost benefit in advance of *in vitro* and *in vivo* studies.

Substantially new terminology, reverse pharmacognosy (Do and Bernard, 2004) from molecules to plants, enable for identifying biological properties of natural molecules from plants natural resources that contain the active molecules by a virtual screening and provided an efficient and rapid tool for natural drug discovery.

Anthranoids naturally occurring molecules consist of three benzene rings and derived from anthracene. This group is subdivided, based on additional carbonyl and oxygenated functional groups and dimerization, into anthrone, anthranol, anthraquinone and dianthrone (Figure 1).

Rumex and *Rhamnus* species have proven to be rich sources of anthranoids (Demirezer, 1994a; Demirezer, 1994b, Demirezer, 1994c, Demirezer, 1991). In our previous studies, cytotoxic, antiinflammatory, analgesic, antipyretic, gastroprotective activities and effect on drug metabolizing enzymes of methanolic extract of *Rumex patientia* were studied and significant results were achieved (Suleyman et al., 1999; Suleyman et al., 2001a; Suleyman et al., 2001b; Suleyman et al., 2002; Cetinkaya et al., 2002; Suleyman et al., 2004; Silig et al., 2004; Demirezer and Kuruuzum, 1994; Demirezer, 2001). Also the main bioactive constituents emodin, aloe-emodin, chrysophanol, physcion, rhein and their glycosides (Table 1) were isolated from *Rumex* and *Rhamnus* in our previous studies (Demirezer, 1994a; Demirezer, 1994b, Demirezer, 1994c, Demirezer, 1991; Demirezer and Kuruuzum, 1997; Demirezer et al, 2001; Demirezer and Kuruuzum, 1997).

Anthranoids are natural compounds having diverse biological activities. The best known effect of anthranoids are laxative effect (Srinivas et al., 2007; Riecken et al., 1990). Recent investigations have shown that anthraquinones have the potential to inhibit cell growth, in several tumor cells such as breast (Zhang et al, 1995; Zhang et al., 1999; Cichewicz et al., 2004), lung (Cichewicz et al, 1994; Chen et al., 2009; Ko et al., 2011), servical (Srinivas et al., 2003), prostate (Cha et al., 2005), colon, CNS, glioma (Cichewicz et al., 2004; Duncan et al., 2004), hepatoma (Shieh et al., 2004) and leukemia cell lines (Chen et al., 2002; Lin et al., 2003). Emodin, aloe- emodin and rhein have been shown to activate apoptotic cell death in different tumor cells and the mitochondrial-dependent pathway is suggested to be the main apoptotic process (Duncan et al., 2004; Shieh et al., 2004; Chen et al., 2002; Lin et al., 2003; Lin et al., 2006; Wang et al., 2007).

Most of the drug targets as well as the drug metabolizing enzymes (DMEs) are proteins, which are mostly membrane bound proteins that are involved in signal transduction. By using structure and sequence similarity search algorithms, protein structure, sequence and genomic sequence databases may be used to conduct computational researches.

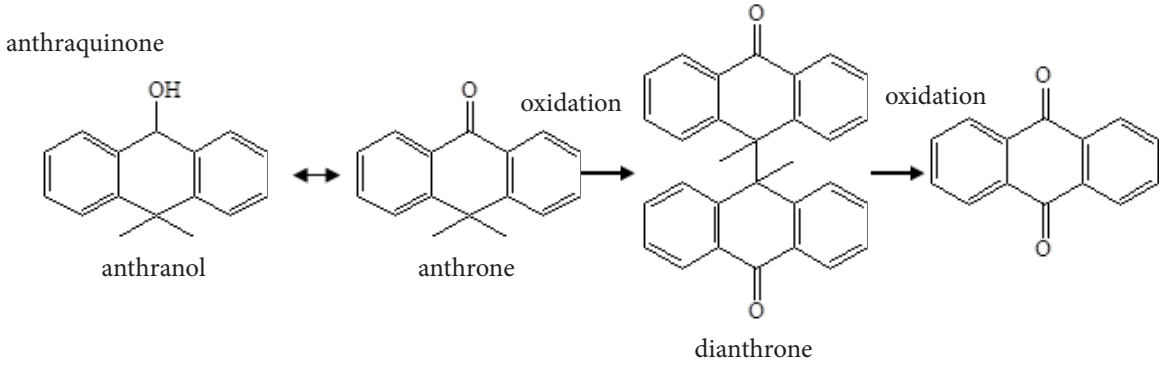
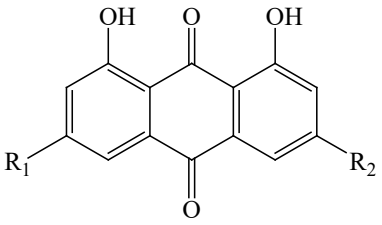


Figure 1. Oxidation Steps of Anthranoids

Table 1. Anthranoids as Ligands Interacting with Targets



Compound	R_1	R_2	R_3
1,8-dihydroxyanthraquinone	H	H	H
Chrysophanol	CH_3	H	H
Aloe-emodin	CH_2OH	H	H
Emodin	CH_3	OH	H
Rhein	COOH	H	H
Physcion	CH_3	OCH_3	H
Emodin-6-glucoside	CH_3	O-β-D-glucose	H
Emodin-8-glucoside	CH_3	OH	β-D-glucose
Frangulin A	CH_3	O-α-L-rhamnose	H
Frangulin B	CH_3	O-β-D-apiose	H
Glucofrangulin A	CH_3	O-α-L-rhamnose	β-D-glucose
Glucofrangulin B	CH_3	O-β-D-apiose	β-D-glucose

The aim of this study is to determine novel bioactivities for anthranoids via docking studies using AutoDock Vina 4.0 and Surflex-Dock in SYBYL-X 2.0 by Tripos Associates along with database search algorithms such as PDB sum, RCSB PDB, SDSC Biology Workbench.

MATERIAL AND METHODS

Target Identification

The three dimensional structures of proteins complexed with some ligands similar to anthraquinones, casein kinase II subunit α (CK II α), myosin light chain kinase member 4 (MYLK4) and angiotensin converting enzyme (ACE), were obtained from Protein Data Bank (PDB) (3q9w, 2x4f, 1o86, respectively) (Battistutta et al., 2012; Muniz et al., 2010; Natesh et al., 2003). All solvent molecules and co-crystallized ligands were removed from the structures and proteins used for docking analysis were gained. 3D structures of the proteins as targets gained from 3q9w, 2x4f, 1o86 pdb files were shown in Figure (2-4).

Ligand Identification

The pdb files of emodin and rhein two aglycones of anthraquinones were obtained from files belong to 3q9w and 3r2a respectively in Protein Data Bank. Aloe-emodin, chrysophanol, physcion the other aglycones of anthraquinones; emodin-8-glucoside, emodin-6-glucoside, glucofrangulin A, glucofrangulin B, frangulin A, frangulin B glycosides of anthraquinones; anthrone, anthranol, anthraquinone, 1,8-dhydroxyanthraquinone and dianthrone skeletons were constructed by using Discovery Studio 3.5 Client programme. The 3D structures of these compounds were drawn and then converted into pdb format by using this programme.

Docking Receptors with Derivatives Using Autodock-Vina.

AutoDock Vina (Trott and Olson, 2010; Goodsell et al., 1996; Archana et al., 2010) 1.0 beta 0.2 software was used in our study to search the potential targets of Rumex and Rhamnus anthraquinones; emodin, aloe- emodin, chrysophanol, physcion, rhein and their glycosides. 'Auto-Dock Tools' was used to

preoare proteins and ligands for docking. For protein preparation, the receptor PDB file was loaded to docking simulation with atomic charges, solvation parameters and polar hydrogens. Pre-calculated grid maps are required for AutoDock Vina. These grids should contain the potential binding region of the targets we are interested in. The size of the grid box was set to 20, 20 and 20 ° (x, y and z). The gap between the grid points is set to 1.0 angstrom. Following the simulation was completed, ligand-enzyme interactions were analyzed and binding distance were measured. Docking studies were perfomed in triplicate.

Docking Receptors with Anthranoid Derivatives Using Surflex-Dock in SYBYL-X 2.0 by Tripos Associates

The docking study was also performed using Surflex-Dock in SYBL-X 2.0 by Tripos Associates. 3D structures of all compounds were constructed using the SYBYL sketcher module. The structures were minimized using the steepest descent conjugated gradient method until the gradient was 0.05 kcal/mol, max iterations: 1,000 with the Tripos force field with the Gasteiger Huckel charge. The simulation system was built on the crystal structures of 3q9w, 2x4f and 1o86, which were obtained from the Protein Data Bank. At the commencement of docking, all the water and ligands were removed and the random hydrogen atoms were added. Docking calculations using Surflex-Dock for 3q9w, 2x4f and 1o86 were performed through protomol generation by ligand. The parameters used were threshold 0.5 and bloat 0.

Molecular docking simulations were conducted with this software suite. From 3 to 9 docking runs were performed. Grid parameters were set as mentioned earlier and spacing between grid points was 1.0 A°. After the simulations were complete, the docked structures were analyzed and the binding distance between the donors and acceptors were measured for the best conformers.

RESULTS

In this study, nine scoring functions for molecular docking, binding energies, C-Score (Consensus Score integrating a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score), Polar score (the contribution of the polar interactions to the total score), D-score (for charge and van der Waals interactions between the protein and the ligand), PMF-score (Potential of Mean Force, PMF indicating the Helmholtz free energies of interactions for protein- ligand atom pairs), G-score (shows hydrogen bonding, complex (ligand-protein), and internal (ligand-ligand) energies), Chem-score (points for hydrogen bonding, lipophilic contact, and rotational entropy, along with an intercept term) and T score (total score indicating binding affinity (kcal/mol)) and H-bond interactions of anthranoids were determined (Table 2-4). The possible interactions were simulated by the docking program SYBYL-X 2.0 and AutoDock Vina 4.0. The structures of the enzymes were obtained from the Protein Data Bank.

The proteins having high percentage similarity and those keeping residues important in interactions were chosen for docking with anthranoids. In this process more than a hundred proteins were examined and only ten maintained residues important in interaction.

As being one of these ten proteins having high similarity to CK II α and keeping the residues important in interaction, human myosin light chain kinase member 4 was chosen as a target in addition to CK II α ligand binding domain and ACE. Therefore other commonly existed anthranoids may interact with all these proteins at the sites in which different kinds of ligands have interaction.

The main anthranoid skeletons anthrone, anthranol, dianthrone, 1,8-dihydroxyanthraquinone and anthraquinone- were docked to chosen targets.

The macromolecules CK II α, MYLK 4, ACE (Figure (2-4)) and the ligands (emodin, aloe-emodin, chrysophanol, rhein, physcion, emodin-8-glucoside, emodin-6-glucoside, glucofrangulin A and B, frangulin A and B) (Table 1) were subjected to docking analysis.

From this study, docked energy scores were analyzed in detail in effort to know CK II α, MYLK 4 and ACE inhibitors. All 18 compounds had binding energies ranging from – 10.50 to -6.30 kcal/mol according to AutoDock Vina (Table 1). We decided initially to determine total scores of the main skeletons of anthranoids which are anthrone, anthranol, dianthrone and anthraquinone (Figure 1).

Binding energies of tested anthraquinones were well enough low and the best surflex dock scores and also total score belonging to glucofrangulin A for CK II α. It has -9.7 kcal/mol binding energy, 6.16 polarity score, -691.025 D-score, -77.697 PMF score, -204.206 G-score, -35.519 Chem- score, 5 C score and 8.84 T score (Table 2). It was observed that, in the active site of CK II α, the residues Leu 45 (1,92 A°), Leu 45 (2,03 A°), Asn 161 (1,87 A°), Asn 161 (1,91 A°), Tyr 50 (2,01 A°) seem to play crucial role in binding with ligands.

MYLK 4 has specific effect on lung and breast cancer (Greenman et al., 2007). Our results of Human MYLK 4 family were identical with CK II α. Binding energies of anthraquinone derivatives were lower than other anthranoid skeletons. Glucofrangulin B showed the best docking scores in plenty of parameters. T score of glucofrangulin B was observed with 8.85 as highest (Table 3).

In the active site of MYLK 4, the residues Val 183 (2,03 Aº), Glu 181 (1,71 Aº) Leu 112 (2,12 Aº), Gly 114 (1,98 Aº), Gly 114 (1,80 Aº), Lys 135 (2,22 Aº) was observed to play prominent role in binding with ligand glucofrangulin B.

The docking results for ACE showed us glycoside derivatives of anthranoids had lower binding energies and the best docking scores (Table 4) than aglycons. Frangulin A and glucofrangulin A showed the significant interaction with ACE. Both of them binding energy is -9.1 and total score of frangulin A and glucofrangulin A is 7.76 and 7.59, respectively. Frangulin A interaction with amino acids of ACE were Glu 162 (2,04 A), Glu 162 (2,34 A), Ala 354 (2,05 A), Ala 354 (2,12 A), Glu384 (1,87 A), Asp 415 (2,15 A), Lys 454 (1,70 A), Asp 453 (2,04 A), Asp 453 (2,05 A), Asp 453 (2,45 A) and glucofrangulin A interaction with amino acids of ACE were Tyr 520 (2,20 A), Gln 281 (1,95 A), Thr 282 (1,88 A), Glu 376 (2,43 A), Glu 376 (2,02 A), Asp 377 (2,08 A), Asp 377 (2,44 A), Asp 377 (1,89 A), Glu 162 (2,60 A), Ala 354 (1,83 A) Ala 354 (2,59 A).

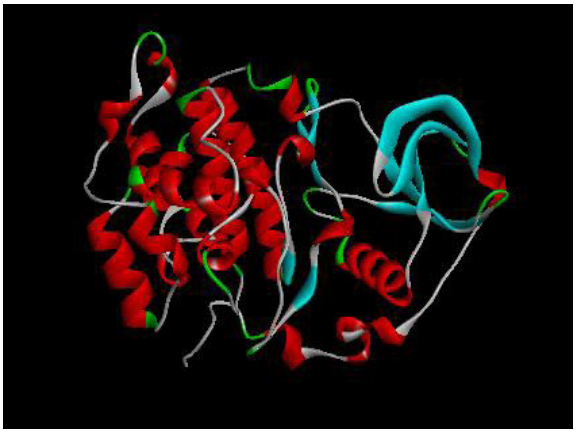


Figure 2. 3D Structure of CK II α

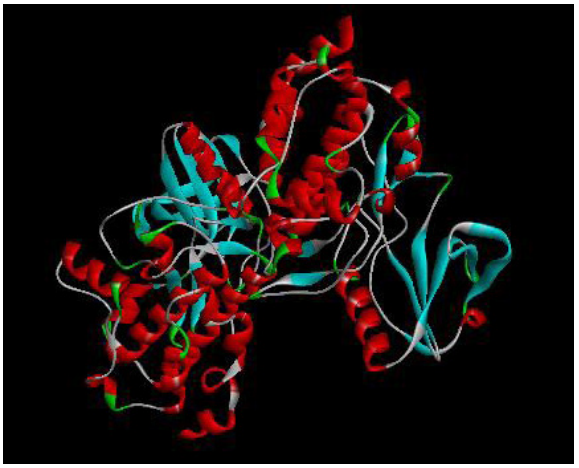


Figure 3. 3D Structure of MYLK 4

Figure 4. 3D Structure of ACE

Table 2. Docking Scores of Anthranoids on CK II α

	Compounds	Binding Energy (AutoDock Vina)	T-score	Polarity score	D- score	PMF-score	G-score	Chem- score	C-score	H-Bond Interactions	Docking Run
1	**Anthraquinone**	-9,6	2,72	0	-127,775	-39,537	-116,474	-30,215	4	-	000
2	**1,8 dihydroxy anthraquinone**		3.20	2.97	-267.388	-70.031	-72.722	-18.505	3	Tyr26(1.86 A) Lys76 (2.01 A) Arg155 (1.93 A) Arg155 (2.26 A)	045
3	**Anthrone**	-9,6	3,35	0	-149,755	-25,399	-128,145	-31,306	4	-	003
4	**Anthranol**	-9,3	3,21	0	-133,453	-27,293	-123,747	-28,863	4	-	006
5	**Dianthrone**	-6,3	3,19	2,04	-677,303	-41,172	-96,130	-27,998	4	Arg80 (1,98 A) Arg80 (1,97 A)	009
6	**Emodin**	-10,5	3,56	1,33	-228,829	-55,168	-142,601	-25,281	4	Lys68 (2,73 A) Asp175 (2,41 A) Asp175 (1,97 A)	012
7	**Rhein**	-10	4,38	1,08	-150,772	-56,219	-191,123	-31,850	4	Asp175 (1,93 A) Val116 (2,17 A)	015
8	**Chrysophanol**	-9,5	3,90	3,54	-356,842	-69,342	-83,383	-20,715	3	Asn189 (2,29 A) Arg155 (2,08 A) Lys76 (1,95 A) Tyr26 (1,91 A)	018
9	**Aloe-emodin**	-9	4,05	0,76	-167,628	-49,835	-156,980	-24,544	5	Asp175 (1,96 A)	021
10	**Physicon**	-8	2,96	0	-349,914	-42,223	-169,862	-29,319	4	-	024
11	**Emodin-6-glucoside**	-10,3	5,41	1,88	-407,860	-62,895	-214,525	-27,401	5	Asn118 (2,56 A) Asn118 (2,03 A) Asn118 (2,37 A) Asp120 (2,09 A) His160 (2,34 A)	027
12	**Frangulin A**	-9,8	5,75	3,15	-454,348	-89,070	-171,353	-27,700	3	Asp175 (1,97 A) Asp175 (2,65 A) His160 (2,70 A) Asp120 (1,86 A) Asp120 (2,04 A)	030

13	**Frangulin B**	-9,9	5,86	2,96	-383,443	-52,452	-215,369	-31,838	3	Gln123 (2,63 A) Asp 120 (1,94 A) Asn118 (2,49 A) Asn118 (2,37 A) Asn118 (2,52 A) Asn118 (1,98 A) Leu45 (1,93 A)	033
14	**Emodin-8-glu-coside**	-9,4	6,18	3,07	-449,035	-73,024	-204,327	-26,181	3	Asn161 (1,90 A) His160 (1,87 A) Asp175 (1,90 A)	036
15	**Glucofrangulin A**	-9,7	8,84	6,16**	-691,025	-77,697	-204,206	-35,519	5	Asn118 (2,01 A) Leu 45 (1,92 A) Leu 45 (2,03 A) Asn 161 (1,87 A) Asn 161 (1,91 A) Tyr 50 (2,01 A)	039
16	**Glucofrangulin B**	-7,8	6,78	2,58	-595,281	-60,123	-255,517	-19,247	2	Asn118 (2,71 A) Asp175 (2,44 A) Asp175 (2,09 A) Asp175 (1,81 A) Lys68 (2,68 A) Tyr50 (2,09 A)	042
17	**CX-4945**	-7,1	4.16	0.91	-244.813	-58.632	-172.396	-31.957	4	Tyr50 (1.99 A) Ser51(2.34 A)	048

Table 3. Docking Scores of Anthranoids on MYLK 4

	Compounds	Binding Energy (AutoDock Vina)	T-score	Polarity-score	D-score	PMF-score	G-score	Chem- score	C-score	H-Bond Interactions	Docking_Run
1	**Anthraquinone**	-8,9	2,90	1,18	93,293	-20,914	-106,855	-29,008	3	Lys135 (1,99 A)	001
2	**1,8 dihydroxy anthraquinone**		4.39	2.27	-4.810	-33.351	-141.547	-25.111	4	Glu181 (1.87 A) Val183 (1.95 A) Val183 (2.71 A)	046
3	**Anthrone**	-8,7	3,92	1,13	31,178	-23,838	-127,359	-26,313	4	Val183 (1,98 A)	004
4	**Anthranol**	-8,6	3,76	1,27	22,508	-21,983	-136,427	-24,498	3	Glu181 (2,56 A) Val183 (1,89 A)	007
5	**Dianthrone**	-7,5	2,51	0	110,853	14,628	-145,219	-23,423	3	-	010
6	**Emodin**	-9,4	6,13	3,62	-85,070	-32,035	-149,148	-30,359	3	Gly186 (1,98 A) Val183 (1,86 A) Val183 (2,14 A) Glu181 (1,90 A)	013
7	**Chrysophanol**	-9,2	4,75	2,23	-72,942	-31,838	-150,769	-26,914	5	Val183 (2,01 A) Ile246 (2,05 A)	019
8	**Aloe-emodin**	-9,3	5,68	4,09	25,069	-31,831	-143,517	-36,474	4	Gly186 (2,02 A) Leu112 (2,08 A) Lys135 (2,02 A) Ile246 (2,03 A)	022
9	**Rhein**	-10	5,09	3,20	0,236	-34,722	-197,387	-30,213	5	Tyr182 (2,74 A) Tyr182 (2,44 A) Val183 (2,21 A) Asp184 (2,22 A) Leu112 (2,09 A)	016
10	**Physicon**	-8,7	5,69	3,36	-107,864	-27,367	-171,803	-28,926	5	Glu181 (1,86 A) Val183 (2,20 A) Val183 (2,28 A) Val183 (1,96 A) Gly186 (2,61 A)	025
11	**Emodin-6-glucoside**	-8,5	6,53	4,85	46,023	-20,121	-192,324	-31,734	2	Ile111 (1,99 A) Ile111 (2,10 A) Gly186 (2,54 A) Val183 (2,12 A) Val183 (2,42 A) Val183 (2,02 A) Glu181 (1,73 A)	028
12	**Emodin-8-glucoside**	-7,2	6,08	2,07	-17,846	-4,286	-213,755	-15,209	2	Val183 (2,15 A) Val183 (1,94 A) Glu181 (2,20 A)	037

13	**Frangulin A**	-9	7,24	5,56	-29,428	-29,276	-186,423	-28,847	3	Leu112 (1,90 A) Gly186 (2,63 A) Val183 (1,98 A) Ile111 (1,99 A) Val183 (2,28 A) Val183 (2,05 A) Glu181 (1,77 A)	031
14	**Frangulin B**	-9,2	6,67	5,82	8,534	-33,501	-185,483	-28,913	2	Glu181 (1,79 A) Val183 (2,05 A) Val183 (2,28 A) Val183 (1,94 A) Gly186 (2,60 A) Ile111 (2,01 A) Gly186 (1,98 A)	034
15	**Glucofrangulin A**	-7	5,90	5,05	69,893	-35,071	-206,813	-29,523	4	Ile111 (1,85 A) Leu112 (2,01 A) Val 183 (2,00 A) Leu188 (2,21 A) Gly186 (2,54 A) Gly186 (1,98 A)	040
16	**Glucofrangulin B**	-6,4	8,85	5,94	32,077	15,559	-287,634	-19,319	3	Val183 (2,03 A) Glu181 (1,71 A) Leu112 (2,12 A) Gly114 (1,98 A) Gly114 (1,80 A) Lys135 (2,22 A)	043
17	**CX-4945**	-8,1	5.03	2.03	74.760	-22.950	-184.228	-31.134	4	Glu181 (2.80 A) Val183 (2.07 A)	049

Table 4. Docking Scores of Anthranoids on ACE

	Compounds	Binding Energy (AutoDock Vina)	T-score	Polarity score	D-score	PMF-score	G-score	Chem- score	C-score	H Bond Interactions	Docking Run
	Anthraquinone	-7,1	1,58	1,28	-35,474	-24,176	-68,231	-14,991	2	Arg522 (2,25 A) Arg522 (1,98 A)	002
2	**1,8 dihydroxy anthraquinone**		3.51	3.51	-31.358	-50.463	-111.566	-17.482	2	His353 (2.88 A) Glu162 (1.92 A) Ala354 (2.09 A) Ala354 (2.23 A) Ala354 (1.86 A)	047
3	**Anthrone**	-7,2	0,99	1,04	-77,296	-37,305	-60,298	-15,562	4	Ala354 (2,05 A)	005
4	**Anthranol**	-7,5	2,80	2,15	-45,903	-67,536	-93,038	-23,440	4	Arg522(1,87 A) Glu411(1,89 A)	008
5	**Dianthrone**	-9,8	2,73	0,15	-125,874	-44,144	-149,577	-27,354	4	Thr372 (2,24 A) Gln281 (2,17 A)	011
6	**Emodin**	-8,1	5,25	4,08	-153,025	-102,694	-107,868	-18,323	4	Gln281 (2,29 A) Glu411 (2,26 A) His 383 (2,85 A) Glu384 (1,99 A) Ala354 (2,03 A)	014
7	**Chrysophanol**	-7,8	3,07	3,29	-138,132	-91,372	-83,470	-23,407	4	Glu384 (2,20 A) Glu384 (1,97 A) Ala356 (2,03 A) Ala356 (1,98 A)	020
8	**Aloe-emodin**	-7,9	3,89	4,52	-45,765	-98,641	-151,340	-22,181	4	Glu384 (1,95 A) His383 (2,40 A) Asp415 (2,18 A) Asp415 (2,05 A)	023
9	**Rhein**	-8,7	4,06	4,88	-45,623	-64,102	-155,376	-19,987	3	Glu384 (1,97 A) Ala354 (1,90 A) Glu162 (2,17 A) Glu162 (2,03 A) Thr166 (1,88 A)	017
10	**Physicon**	-7,8	3,38	2,61	-266,238	-112,784	-83,570	-16,778	3	Lys511 (2,43 A) Gln281 (1,87 A) Ala354 (2,01 A)	026
11	**Emodin-6-glucoside**	-8,8	4,74	5,74	-180,964	-177,319	-152,315	-21,492	4	Ala354 (2,13 A) Ala356 (1,81 A) Glu384 (1,83 A) His353 (1,87 A) His513 (2,19 A) Tyr523 (2,11 A) Gln281 (2,33 A) Gln281 (2,50 A) Gln281 (1,93 A) Thr282 (2,66 A)	029

12	**Emodin-8-glucoside**	-8,7	5,49	5,27	-172,299	-186,358	-291,030	-22,992	4	Ala356 (2,47 A) Glu384 (1,94 A) Glu384 (2,45 A) His 383 (2,61 A) Glu 411 (2,01 A) Asp 415 (2,18 A) Asp 415 (2,14 A)	038
13	**Frangulin A**	-9,1	7,76	9,14	-269,601	-127,059	-236,890	-27,137	4	Glu162 (2,04 A) Glu162 (2,34 A) Ala354 (2,05 A) Ala354 (2,12 A) Glu384 (1,87 A) Asp415 (2,15 A) Lys 454 (1,70 A) Asp453 (2,04 A) Asp453 (2,05 A) Asp453 (2,45 A)	032
14	**Frangulin B**	-8,1	6,04	6,41	-172,134	-159,588	-169,613	-17,218	4	Lys511 (2,19 A) Lys511 (2,36 A) Lys511 (1,89 A) Asp415 (2,00 A) Lys454 (1,83 A) Asp453 (1,94 A) Asp453 (2,14 A)	035
15	**Glucofrangulin A**	-9,1	7,59	7,94	-387,376	-206,494	-251,398	-21,402	3	Tyr520 (2,20 A) Gln281 (1,95 A) Thr282 (1,88 A) Glu376 (2,43 A) Glu376 (2,02 A) Asp377 (2,08 A) Asp377 (2,44 A) Asp377 (1,89 A) Glu162 (2,60 A) Ala 354 (1,83 A) Ala 354 (2,59 A)	041
16	**Glucofrangulin B**	-10,2	6,50	8,61	-247,536	-202,701	-290,507	-19,272	4	Thr372 (2,49 A) Asp377 (2,12 A) Glu162 (1,83 A) Glu162 (2,00 A) Gln281 (1,95 A) Gln281 (2,55 A) Asp415 (2,21 A) Lys454 (2,03 A) Asp453 (2,08 A) Asp453 (2,50 A) Asp453 (2,20 A)	044
17	**Captopril**	-5	2.35	2.81	-371.471	-72.819	-122.203	-16.498	5	Lys454 (1.92 A) Asp453 (2.02 A) Asp453 (2.51 A)	053

DISCUSSION

Recently drug discovery studies focused on preliminary assessment such as *in silico* methods for reducing the cost and saving time. In this study, we tried to find (check and correct the word) eventual novel binding targets, interacted with *Rhamnus* and *Rumex* anthranoids, which may have potential therapeutic activities.

Existing knowledge of known interactions of anthranoids with some surface receptor molecules were gathered via extensive literature search. Based on these researches anthraquinone derivatives were mostly observed as agents interacting with various targets like enzymes or receptors.

We selected CK II α, MYLK 4 and ACE from among hundreds of proteins.

The main skeletons of anthranoids which are anthron, anthranol, dianthrone, anthraquinone and 1,8 dihydroxyanthraquinone were searched. We decided to search on anthraquinone derivatives due to the fact that reduction form of anthraquinones, which are anthrone, anthranol and dianthrones are instable (Figure 1). In position 1 and 8 position hydroxylated anthraquinone skeleton showed higher T score than nonsubstituted thereby we searched 1,8- dihydroxyanthraquinone derivatives present in *Rhamnus and Rumex* species. We investigated 1,8 dihydroxyanthraquinone derivatives, the contents of *Rumex* and *Rhamnus* species, due to their high T scores.

Docking results of MYLK 4 and CK II α were found similar to each other. On the contrary, the docking results of ACE showed diversity.

We discussed the effect of the number and position of the hydroxyl and sugar groups on the binding affinity. The binding affinity of the ligands is found to increase with the increase in the number on hydroxyl and sugar moiety in the molecule.

T-score of anthraquinone - O-glycosides were higher than aglycones and also usually higher when the number of sugar α sugar having molecules. Two sugar molecules containing structures were mostly bond with six hydrogen on four amino acids of CK II α. The structures having one sugar molecule, mostly showed interaction with three amino acids and various hydrogen bondings. It was seen that, the more the number of sugar, the higher the total score of molecule.

Glucofrangulin A having a joint H bond interaction with Tyr 50 on CK II α like CX-4945 showed the best surflex T-score with CK II α (Figure 5).

CK II α regulates numerous cellular processes, such as cell cycle progression, apoptosis and transcription (Papinutto et al., 2012; Litchfield, 2003). From this viewpoint some anthraquinone molecules may be considered as potential drug candidates to treat some important diseases like cancer.

The relevance of CK II α as a molecular target in cancer has led to the development of CK II α inhibitors for clinical use. CX-4945 with known antitumor efficacy in breast, pancreatic and prostate xenograft mouse models (Siddiqui-Jain et al., 2010) is the first small-molecule inhibitor of CK II (Figure 6) to progress to human clinical trials.

Rhein and glycosylated anthraquinone derivatives showed higher T scores than CX-4945. Two sugar molecule containing compounds had better binding affinity to the CK II α. Glucofrangulin A and the others having the better binding affinity than reference CX-4945 may be significant for development of new agents inhibiting CK II α.

MYLK 4 has specific effect on lung and breast cancer (Greenman et al., 2007). According to our MYLK 4- anthranoids interaction results, substituting or multiplying sugar molecules on the 1,8- dihydroxyanthraquinone skeleton increased binding affinity. Glucofrangulin B having joint H bond interactions with Glu181 and Val183 like CX-4945 showed the best docking scores in plenty of parameters (Figure 7).

Due to similarity of CK II α with MYLK 4, we had docking analysis of CX-4945 as a reference to the MYLK 4 (Figure 8). Anthraquinone derivatives usually had high T score. Glucofrangulin B having the best binding affinity also showed the highest T score of all. Those having higher T score may have a function on MYLK 4 and a potential to treat some related diseases.

To have a better interaction with angiotensin-converting enzyme, 1,8-dihydroxyanthraquinone molecule has to contain a methyl group on the third position, a hydroxyl group binding with sugar on the sixth position. Frangulin A and glucofrangulin A showed the significant interaction with ACE.

Glucofrangulin A and frangulin A contain a methyl pentose (rhamnose) instead of apiose on the sixth position. In this respect it can be said that rhamnose containing molecules have lower binding energies and better interactions with ACE than apiose having molecules. ACEs are a first line of therapy for hypertension, heart failure, myocardial infarction and diabetic nephropathy (Natesh et al., 2003; Hyun et al., 2009).

Captopril is a widely used ACE inhibitor (Figure 9) for treatment of arterial hypertension and cardiovascular diseases (Cushman and Ondetti, 1991; Brooks et al., 1997; Peng et al., 2005). All anthraquinone derivatives showed higher T score than the reference Captopril. Frangulin A showing joint H bond interactions with Lys454 and Asp453 like Captopril (Figure 10) and subsequent to glucofrangulin A having the highest binding affinity to ACE may be used as an agent to treat related diseases.

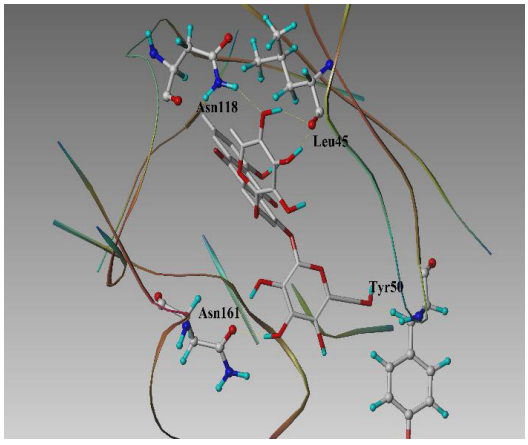


Figure 5. Interaction of Glucofrangulin A with CK II α

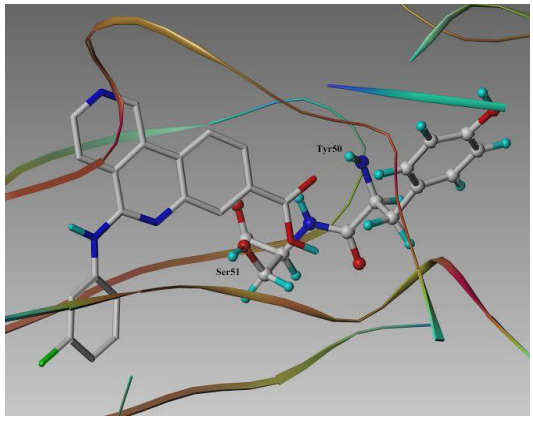


Figure 6. Interaction of CX-4945 with CK II α

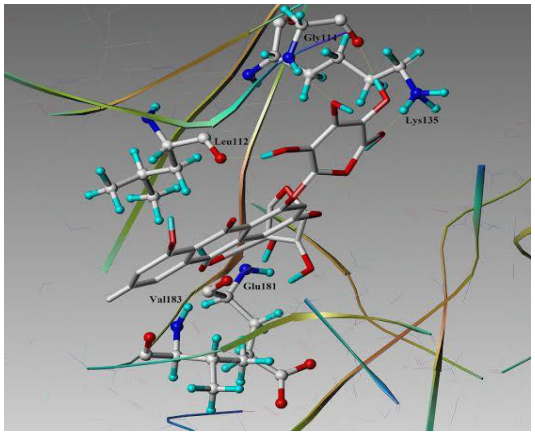


Figure 7. Interaction of Glucofrangulin B with MYLK 4

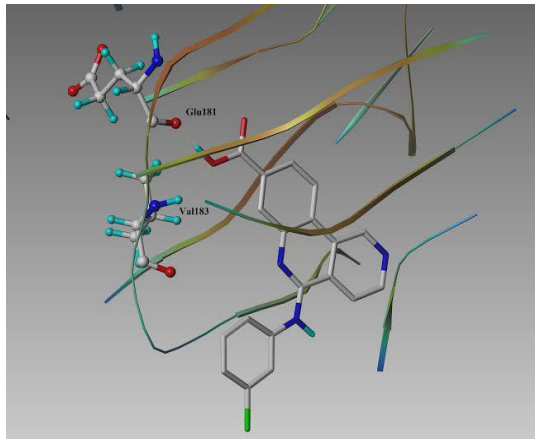


Figure 8. Interaction of CX-4945 with MYLK 4

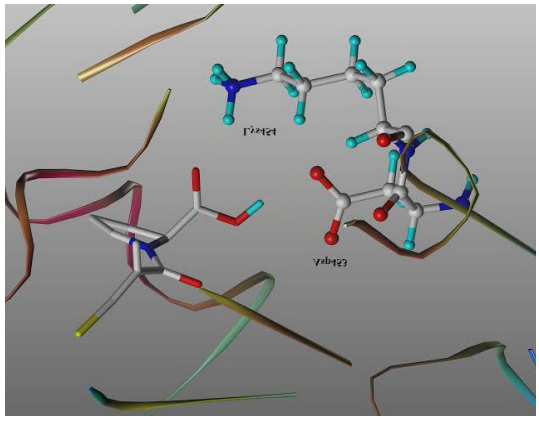


Figure 9. Interaction of Captopril with ACE

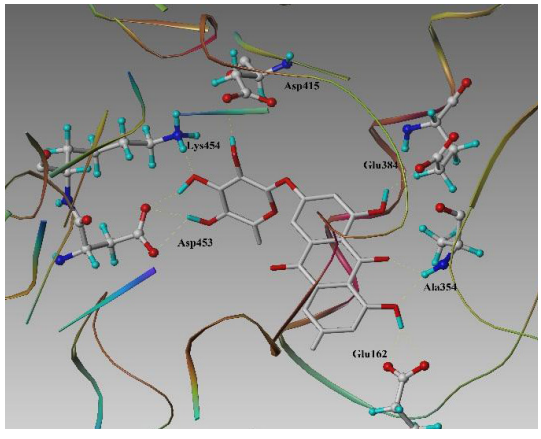


Figure 10. Interaction of Frangulin A with ACE

CONCLUSION

We have tested nine scoring functions on 18 scoring functions we have tested binding energies, protein-ligand complexes and evaluated several aspects of their performance. (Please rewrite the sentence) We have tested nine scoring functions including binding energies of protein-ligand complexes and evaluated their performance from a different viewpoint. Among all the C-Score, Polar score, D- score, PMF-score, G-score, Chem-score, ChemScore H-bond interactions and T-score. Comparative docking analysis of commonly existed anthranoids on some receptors or enzymes suggested that these ligands can be alternative sources for some diseases and agonists or antagonists for the receptors or enzymes. Therefore, these studies have not only an initial but also a vital role in development of potential drug candidates.

Further studies are needed to be considered 1,8- dihydroxy anthraquinone derivatives as novel therapeutic agents for the treatment of cancer, hypertension, heart failure, myocardial infarction and diabetic nephropathy.

Our results obtained may be helpful in developing of new series of drugs in future.

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