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RESEARCH ARTICLE

Determination of Phytoestrogenic Potential of Anthranoids by Molecular Docking Studies

Lutfiye Omur DEMİREZER[*,°], Mine UZUN[*], Nadire OZENVER[*], Zuhal GUVENALP[**]

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SUMMARY

The interest in phytoestrogens as potential therapeutic agents has recently risen in the field of oncology. Consumption of phytoestrogens have decreased risk of mortality due to several types of cancer. Estrogen receptor alpha and beta (ER α and β) ligand binding domains were selected to determine phytoestrogenic potential of main anthranoids. Five anthranoid skeletons and their derivatives as hydroxyanthraquinones, total 18 ligand (emodin, aloe-emodin, chrysophanol, physcion, rhein, emodin-8-O-β-D-glucopyranoside, emodin-6-O-β-D-glucopyranoside, glucofrangulin A, glucofrangulin B, frangulin A, frangulin B, anthrone, anthranol, anthraquinone, 1,8-dihydroxyanthraquinone, dianthrone skeletons and reference daidzein, genistein) -enzyme interactions were simulated by the docking program SYBYL X 2.0. Nine scoring functions for molecular docking were compared and evaluated. According to the our docking results, glucofrangulin B and emodin-8-O-β-D-glucopyranoside having similar binding affinity to ER α like reference isoflavone compounds daidzein and genistein., also emodin showed best binding affinity to ER β. Emodin having similar total score with daidzein and genistein. The distance between the two hydroxyl substituents of estradiol and tested phytoestrogens were measured. According to our results the distance between two hydroxyl substituents was found similar. Drug candidate agents as phytoestrogens may be considered because of its potential activity of anthraquinones.

Key Words: *Anthranoids, phytoestrogen, docking, drug discovery, natural compound, estrogen*

Antranoitlerin Fitoöstrojenik Potansiyelinin Moleküler Doking Çalışmaları ile Belirlenmesi

ÖZET

Son yıllarda onkoloji alanında potansiyel terapötik ajan olarak fitoöstrojenlere ilgi artmıştır. Fitoöstrojenlerin tüketimi, kanserin neden olduğu ölüm riskini azaltmıştır. Östrojen reseptör alfa ve beta (ER α ve β) ligand bağlanma bölgeleri, ana antranoitlerin potansiyel fitoöstrojenik potansiyellerini belirlemek üzere seçilmiştir. Hidroksiantrakinon yapısında beş antranoit iskeleti ve türevleri, toplam 18 ligand (emodin, aloe-emodin, krizofanol, fiskyion, rein, emodin-8-O-β-D-glukopiranozit, emodin-6-O-β-D-glukopiranozit, glukofrangulin A, glukofrangulin B, frangulin A, frangulin B, antron, antranol, antrakinon, 1,8-dihidroksiantrakinon, diantron iskeletleri ve referans daidzein, genistein)-enzim etkileşimi docking programı SYBYL X 2.0 ile simüle edilmiştir. Moleküler docking için dokuz skor fonksiyonu karşılaştırılarak değerlendirilmiştir. Docking sonuçlarımıza göre, glukofrangulin B ve emodin-8-O-β-D-glikopiranozitin, ER α'ya karşı, referans izoflavon bileşikleri daidzein ve genisteine benzer bağlanma afinitesine sahip oldukları bulunmuş ve emodin ER β ya en iyi bağlanma afinitesi göstermiştir. Emodin, genistein ve daidzein ile benzer total skora sahiptir. Östradiolün ve test edilen fitoöstrojenlerin 2 hidroksili arasındaki mesafe ölçülmüştür. Sonuçlarımıza gore 2 hidroksil arasındaki mesafe benzer bulunmuştur. Antrakinonlar potansiyel aktiviteleri nedeniyle fitoöstrojen ilaç adayı olarak düşünülebilir.

Anahtar Kelimeler: *Antranoitler, fitoöstrojen, docking, ilaç keşfi, doğal bileşik, östrojen.*

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INTRODUCTION

Estradiol, estrone, and estriol are three main endogenous estrogens and estradiol is the most potent (Clark, 1979). Estrogens alone or in combination with progestins, is traditionally prescribed to women undergoing menopausal transition to attenuate symptoms associated with menopause (Vestergaard et al., 2003), such as hot flashes, vaginal dryness, and osteoporosis (Bachmann, 1999; Rodstrom et al., 2002). Estrogens have long been recognized as being important for stimulating the growth of a large proportion of breast cancers (Rossouw et al., 2002). These side effects among consumers creates a reluctance to use HRT and induced a search for new estrogen analogues with an improved risk profile.

In recent years, the interest in phytoestrogens as potential therapeutic agents has increased in the oncologic area. Phytoestrogens are a group of chemicals found in plants and mainly isoflavones, lignans and coumestans that can act like the hormone estrogen (De Kleijn et al., 2001). The effectiveness of phytoestrogens is associated with chemical similarity to estrogen molecules (Ganry, 2002; Sadovsky & Adler, 1998; Vincent & Fitzpatrick, 2000). Because of their chemical structure, phytoestrogens compete with endogenous estrogens for binding with estrogen receptors and reducing the hormonal effect of endogenous estrogens. On the other hand, phytoestrogens also have effects that are different from those of estrogen. The phytoestrogen can act like estrogen at low doses but block estrogen at high doses (Yildiz, 2005).

A comprehensive investigation is required for new natural compounds, with significantly better properties than the existing agents, such as higher potency. The natural compounds, anthranoids are well known as laxative (Riecken et al., 1990; Srinivas, Babykutty, Satbiadevan, & Srinivas, 2007). Recent investigations have shown that anthraquinones have the potential to inhibit cell growth, in several tumor cells such as breast (Cichewicz et al., 2004; Zhang et al., 1995; Zhang et al., 1999). *Rumex* and *Rhamnus* species have proven to be rich sources of anthranoids (Demirezer et al., 1991, 1994a, 1994b, 1994c). 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 (Cetinkaya et al., 2002; Demirezer et al., 1994; Silig et al., 2004; Suleyman et al., 1999; 2001a; 2001b; 2002; 2004) 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 et al., 1991, 1994a, 1994b, 1994c, 1997, 2001;).

In our previous studies, the comparative docking analysis of some anthraquinone derivatives on various receptors have been performed (Demirezer, 2016). There is no study about relationship between anthranoids' molecular structure and phytoestrogenic effect, we thought that anthraquinone molecules may be novel phytoestrogens because of the similarity of their molecular structures such as well-known phytoestrogen genistein and daidzein.

Over the past decades, various screening and computational approaches have been established to evaluate bioactivities of molecules. *In silico* methods have been widely used to investigate potential drug targets and understand metabolic properties as well as physicochemical characterization of drugs on the related receptors and enzymes. To reduce the cost of drug development and save time; these computer-based methods can be used to have preliminary assessment and screening before *in vitro* and *in vivo* research.

The aim of this study was to determine phytoestrogenic activities of anthranoids via docking studies using Surflex-Dock in SYBYL-X 2.0 by Tripos Associates along with database search algorithms. We selected estrogen receptor alpha and beta (ER α and β) ligand binding domains to determine phytoestrogenic potential of main anthranoids. Five anthranoid skeletons and their derivatives as hydroxyanthraquinones, total 18 ligand-enzyme interactions for ER α and total 15 ligand-enzyme interactions for ER β were simulated and compared with potential phytoestrogens daidzein and genistein.

MATERIALS AND METHODS

Target Identification

The three dimensional structures of ER α and β were obtained from Protein Data Bank (PDB) (1A52, 1QKM respectively). All solvent molecules and co-crystallized ligands were removed from the structures and proteins used for docking analysis were gained (Figure 1-2).

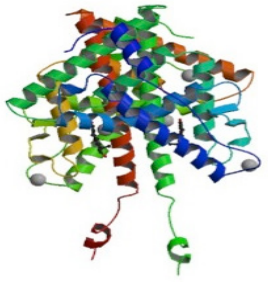


Figure 1. The structure of ER α

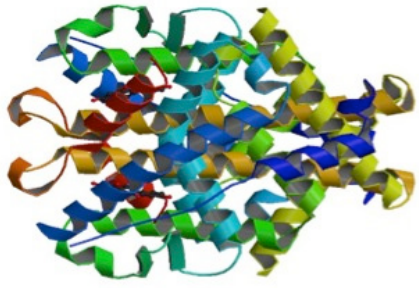
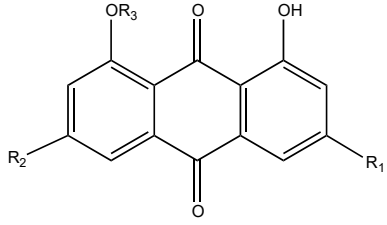


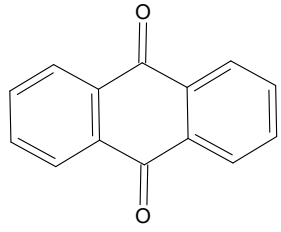
Figure 2. The structure of ER β

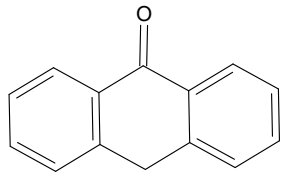
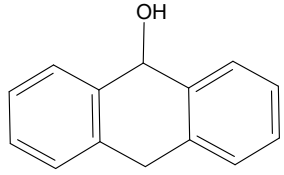
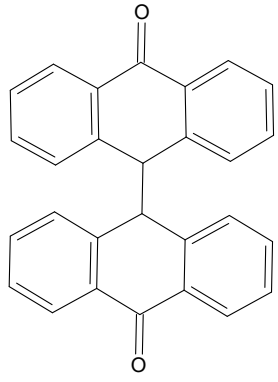
Ligand Identification

Anthranoids (emodin, aloe-emodin, chrysophanol, physcion, rhein, emodin-8-*O*-β-*D*-glucopyranoside, emodin-6-*O*-β-*D*-glucopyranoside, glucofrangulin A, glucofrangulin B, frangulin A, frangulin B, anthrone, anthranol, anthraquinone, 1,8-dhydroxyanthraquinone and dianthrone skeletons) (Table 1) and daidzein (Figure 3), genistein (Figure 4), molecules 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.

Table 1. Anthraquinones 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-*O*-*β*-*D*-glucopyranoside	CH_3	O-β-*D*-glucose	H
Emodin-8-*O*-*β*-*D*-glucopyranoside	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
Antraquinone			

Anthrone	
Anthranol	
Dianthrone	

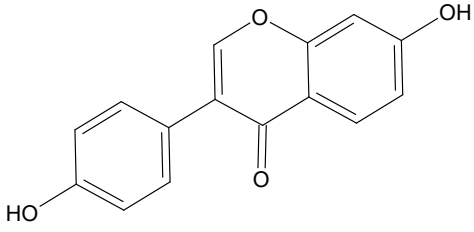


Figure 3. The structure of daidzein

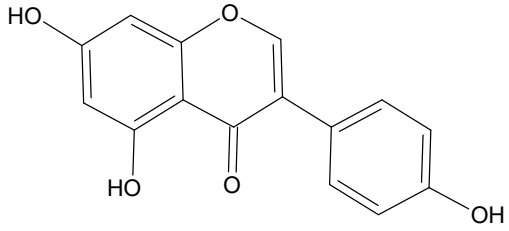


Figure 4. The structure of genistein

Docking ER α and ER β with Ligands 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. From 3 to 9 docking runs were performed.

RESULTS

In this study we selected estrogen receptor alpha and beta (ER α and β) ligand binding domains to determine phytoestrogenic potential of main anthranoids.

The macromolecules ER α and ER β receptors, five anthranoid skeletons and their derivatives as hydroxyanthraquinones, totally 18 ligand (genistein, daidzein, anthrone, anthranol, dianthrone, 1,8-dihydroxyanthraquinone, anthraquinone skeletons and emodin, aloe-emodin, chrysophanol, rhein, physcion, emodin-8-*O*-**β**-*D*-glucopyranoside, emodin-6-*O*-**β**-*D*-glucopyranoside, glucofrangulin A and B, frangulin A and B) (Table 1) were subjected to docking analysis. Daidzein (Figure 3) and genistein (Figure 4) were used as reference molecules. The structures of the enzymes were obtained from the Protein Data Bank. (Figure 1-2.)

All docking simulations, 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 2-3). The possible interactions were simulated by the docking program SYBYL-X 2.0.

Table 2. Docking results of ligands and ER α

LIGAND	T_score	Polarity_ score	D_score	PMF_ score	G_score	Chem_ score	C_score	Hydrogen Bond Interactions
Antraquinone	3.06	1.88	-183.797	-32.12	-102.408	-17.562	4	ARG515 (2.23 Å), ARG515 (2.15 Å)
Anthrone	3.18	2.07	-217.38	-30.451	-95.252	-19.908	3	ARG515 (2.01 Å), ARG515 (2.08 Å)
Anthranol	2.13	1.16	-91.22	-26.524	-96.459	-28.909	4	GLY521 (2.07 Å)
Dianthrone	4.28	1.79	-283.924	-37.426	-183.757	-27.214	4	ARG515 (2.19 Å), ARG515 (1.19 Å)
Emodin	3.77	1.9	-173.55	-17.546	-112.609	-25.115	2	THR347 (2.01 Å), HIS524 (1.94 Å)
Rhein	3.44	3.78	-102.431	-58.244	-99.207	-16.585	2	THR460 (2.22 Å), LYS520 (2.16 Å), LYS520 (1.99Å), SER518 (2.09 Å) GLU385 (2.07 Å), CYS381 (2.73 Å), ARG515 (2.49 Å)
Chrysophanol	3.54	1.83	-185.572	-22.527	-128.738	-28.592	3	GLY521 (2.02 Å),HIS524 (1.99 Å)
Aloe-emodin	4.82	2.53	-142.599	-33.014	-181.308	-26.207	3	GLU353 (2.59 Å), GLU353 (1.99 Å), ARG394 (2.10 Å)
Physcion	3.31	2.21	-288.953	-42.557	-117.474	-14.74	2	SER518 (2.37 Å), HIS516 (2.68 Å), ARG515 (1.79 Å), ARG515 (2.15 Å), THR460 (2.14 Å)
Emodin-6-*O*-β-*D*-glucopyranoside	5.64	4.42	-205.647	-103.904	-136.753	-11.317	4	CYS381 (2.04 Å), THR460 (1.81 Å), ARG515 (2.05 Å) ARG515 (2.56 Å), ARG515 (1.96 Å), HIS516 (2.70 Å), HIS516 (2.18 Å)
Frangulin A	3.37	3.8	-288.275	-90.161	-119.834	-12.385	2	GLU380 (1.83 Å), HIS377 (1.85 Å), HIS377 (2.60 Å), CYS381 (1.98 Å), HIS516 (2.65 Å), ARG515 (1.96 Å)
Frangulin B	4.07	4.21	-220.153	-78.313	-131.391	-9.957	2	CYS381 (2.15 Å), ARG515 (1.98 Å), ARG515 (1.90 Å), SER456 (1.87 Å), THR460 (2.01 Å)

Emodin-8-*O*-*β*-*D*-glucopyranoside	6.39	3.79	-299.44	-47.999	-238.018	-32.237	4	LEU346 (1.89 Å), GLU353 (1.68 Å), ARG394 (2.10 Å), LEU387 (2.15 Å)
Glucofrangulin A	3.17	2.66	-491.159	-66.103	-318.993	-25.921	4	LYS529 (2.05 Å), LYS529 (2.04 Å), THR347 (2.73 Å), LEU346 (1.82 Å), ALA350 (2.24 Å), GLU353 (1.87 Å)
Glucofrangulin B	6.55	5.65	-307.605	-105.904	-180.151	-7.167	2	GLU423 (2.54 Å), LYS520 (1.90 Å), HIS516 (2.77 Å), HIS516 (2.28 Å), ARG515 (1.88 Å), ARG515 (2.29 Å), ARG515 (2.00 Å), CYS381 (1.97 Å)
1,8-Dihydroxyan-traquinone	3.84	1.9	-97.793	-24.894	-122.11	-26.962	3	HIS524 (2.00 Å), GLY521 (2.04 Å)
Daidzein	6.07	4.94	-93.763	-50.168	-159.02	-34.932	4	HIS524 (2.13 Å), GLY521 (2.09 Å) GLU353 (2.00 Å), GLU353 (2.21Å), ARG394 (1.95 Å)
Genistein	6.72	5.76	-97.029	-54.032	-169.012	-36.198	4	HIS524 (2.09 Å), GLY521 (2.06 Å), LEU346 (2.08 Å), GLU353 (2.00 Å), GLU353 (2.21 Å), ARG394 (1.93 Å)

Table 3. Docking results of ligands and ER β

LIGAND	T_score	Polarity score	D_score	PMF_ score	G_score	Chem_ score	C score	Hydrogen Bond Interactions
Antraquinone	2.98	0	36.504	-13.379	-120.389	-30.047	2	-
Anthrone	2.95	0	73.008	-4.428	-116.854	-29.808	2	-
Anthranol	3.05	0	63.142	-3.553	-115.99	-27.367	1	-
Emodin	4.74	1.34	-59.335	-23.179	-156.87	-29.457	3	GLU305 (1.92 Å)
Rhein	4.08	0	-23.504	-32.159	-161.176	-26.464	2	-
Chrysophanol	3.7	1.02	-52.022	-39.989	-158.325	-32.801	5	ALA302 (2.55 Å),LEU298 (1.99 Å)
Aloe-emodin	4.36	2.2	8.105	-32.211	-168.108	-32.234	3	GLU305 (2.01 Å)
Physcion	3.75	0	-84.841	-31.897	-147.068	-29.545	1	-
Emodin-6-*O*-β-D-glucopyranoside	3.7	2.37	-53.94	-45.533	-307.1	-35.431	4	LEU298 (2.75 Å), GLU305 (1.67 Å), GLU305 (1.99 Å), GLU305 (2.44 Å)
Frangulin A	4.08	2.26	-100.4	-41.737	-297.7	-38.167	4	LEU339 (2.64 Å), ARG346 (1.97 Å), ARG346 (2.57 Å), GLU305 (2.12 Å), GLU305 (1.97 Å) GLU305 (1.72 Å)

Frangulin B	4.21	2.95	-71.82	-28.987	-297.1	-35.505	5	LEU298 (1.95 Å), ALA302 (2.41 Å), GLU305 (2.00 Å), GLU305 (2.27 Å), ARG346 (2.53 Å) ARG346 (2.44 Å)
Emodin-8-*O*-β-*D*-glucopyranoside	1.77	2.35	-68.62	-40.645	-276.3	-34.331	4	LEU298 (1.78 Å), GLU305 (2.47 Å), GLU305 (1.87 Å), ARG346 (1.68 Å), LEU339 (1.81 Å)
1,8-Dihydroxyanthraquinone	3.66	1.33	-8.286	-39.937	-151.8	-31.118	5	GLU305 (1.94 Å)
Daidzein	5.25	3.48	26.151	-40.247	-197.8	-37.164	5	GLY472 (2.03 Å), ARG346 (2.74 Å), ARG346 (1.85 Å), GLU305 (2.34 Å), GLU305 (2.08 Å)
Genistein	5.28	3.7	13.49	-53.922	-190.8	-37.349	5	GLY472 (1.99 Å), ARG346 (2.67 Å), ARG346 (1.77 Å), GLU305 (2.15 Å), GLU305 (2.09 Å)

In this study, docking scores were analyzed in detail in effort to know ER α and ER β inhibitors. According to our results, glucofrangulin B and emodin-8-*O*-β-*D*-glucopyranoside having similar binding affinity to ER α like reference isoflavone compounds daidzein (Figure 3) and genistein (Figure 4), and they showed better scores than the other ligands while emodin having the best binding affinity to ER β. T score values of ligands with ER α and ER β have shown in Figure 5 and 6.

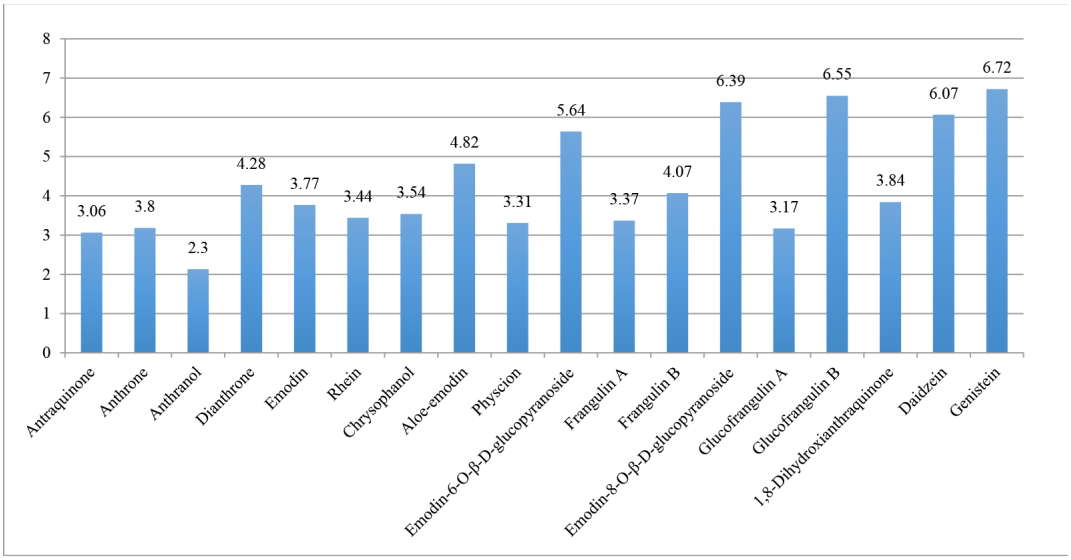


Figure 5. T score values of ligands with ER α

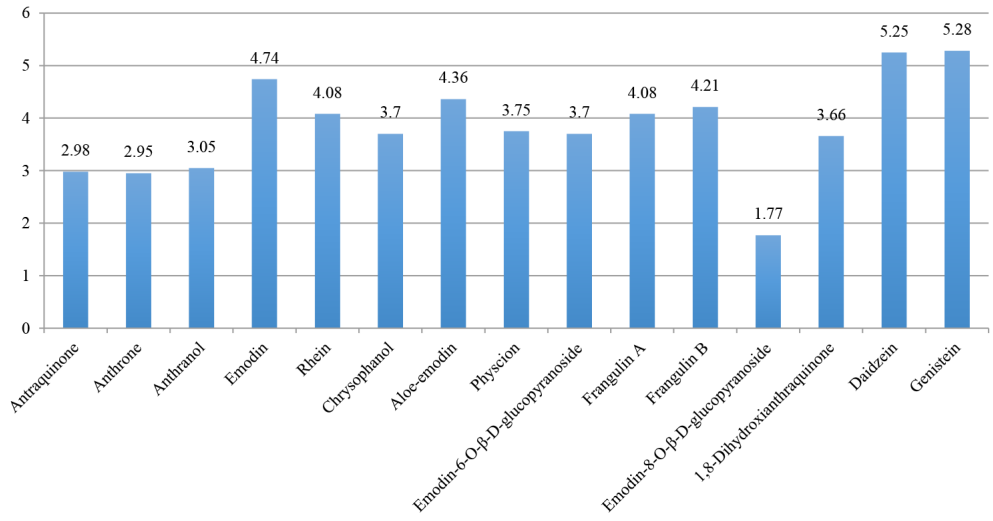


Figure 6. T score values of ligands with ER b

After genistein (6.72) for ERα glucofrangulin B (6.55) and emodin-8-*O*-*b*-*D*-glucopyranoside (6.39) showed the highest total score. It was observed that, in the active site of ER α, the residue Glu 423 (2.54 Å), Lys 520 (1.90 Å), His 516 (2.77 Å), His 516 (2.28 Å) seem to play crucial role in binding with glucofrangulin B and has 5.65 polarity score, -307.605 D-score, -105.904 PMF score, -180.151 G-score, -7.167 Chem- score, 2 C score. The residues Leu 346 (1.89 Å), Glu 353 (1.68 Å), Arg 394 (2.10 Å), Leu 387 (2.15 Å) were observed to play prominent role in binding with ligand emodin-8-*O*-*b*-*D*-glucopyranoside in the active site of ER α. It has 3.79 polarity score, -299. 44 D- score, -47.999 PMF score, -238.018 G-score, -32.237 Chem- score, 4 C score (Table 2). Interactions of daidzein, genistein, glucofrangulin B and emodin-8-*O*-*b*-*D*-glucopyranoside ligands with ERα can be seen in Figure 7-10.

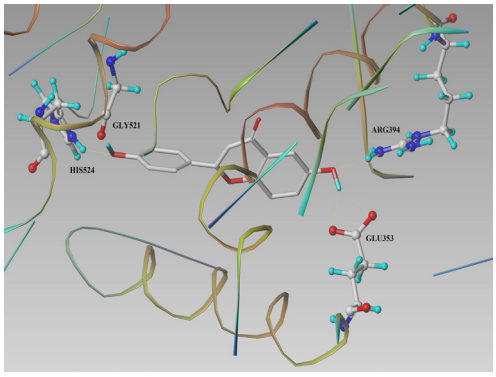


Figure 7. Interaction of daidzein with ER α

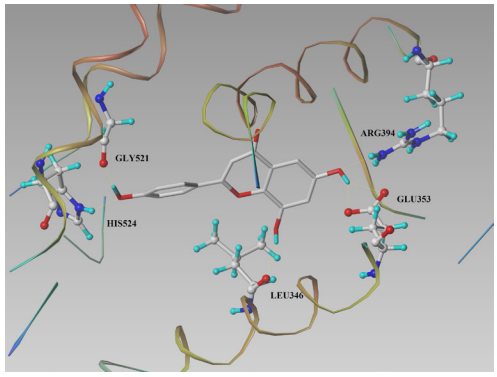


Figure 8. Interaction of genistein with ER α

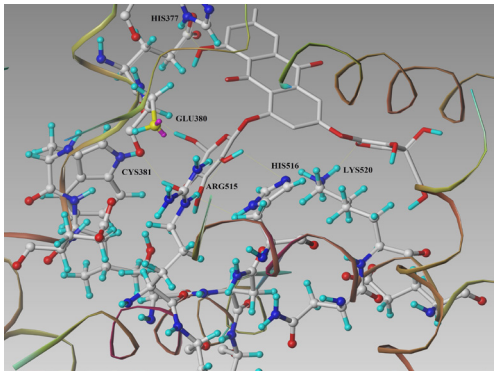


Figure 9. Interaction of glucofrangulin B glucopyranoside with ER α

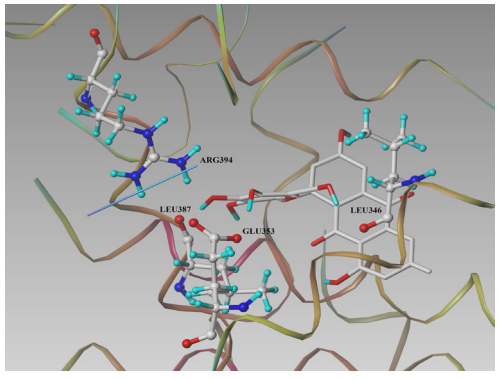


Figure 10. Interaction of emodin-8-*O*-β-*D* with ER α

On the other hand, emodin and aloe emodin showed the best docking scores with ER β in plenty of parameters. T score of emodin was observed with 4.74 as highest and aloe-emodin with 4.36 after genistein (5.28) In the active site of ERβ, the residue Glu305 (1.92 Å) and Glu305 (2.01 Å) were observed to play prominent role in binding with ligand emodin and aloe-emodin respectively (Table 3). Interactions of daidzein, genistein, and emodin ligands with ER β can be seen in Figure 11-13.

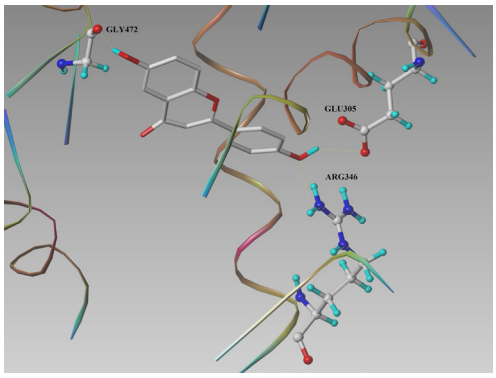


Figure 11. Interaction of daidzein with ER β

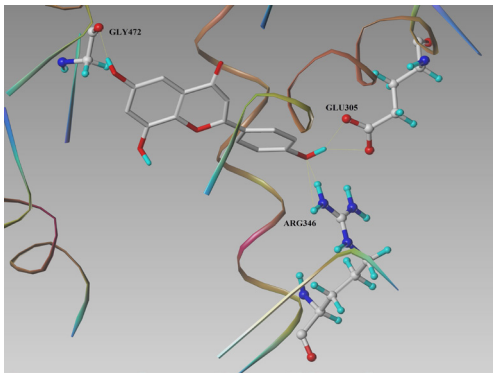


Figure 12. Interaction of genistein with ER β

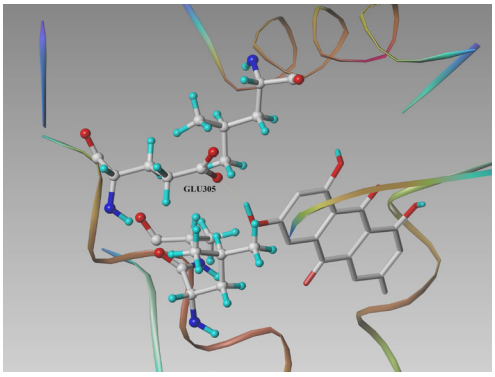


Figure 13. Interaction of Emodin with ER β

The presence of phenolic ring and hydroxyl group and the distance between these two function is important for phytoestrogenic effect. The structural features first proposed as being essential to estrogen activity are two hydroxyl groups separated by a specific distance (11.5 Å) in estradiol (Litwack, 1984).

In our study, we measured the distance between the two hydroxyl of estradiol, genistein, daidzein and potential phytoestrogen emodin and aloe-emodin. According to our results the distance between 3 and 17 located hydroxyl of estradiol was 12.11 Å (Figure 14). Daidzein and genistein give also similar results 12.18 Å and 12.57 Å respectively (Figure 15 and 16). The distance between 1st and 6th hydroxyl substituents of emodin was 9.04 Å (Figure 17) and 1st and 8th hydroxyl substituents of emodin was 6.28 **Å.** The distance between 3rd and 8th hydroxyl substituents of aloe-emodin was 8.60 **Å** (Figure 18).

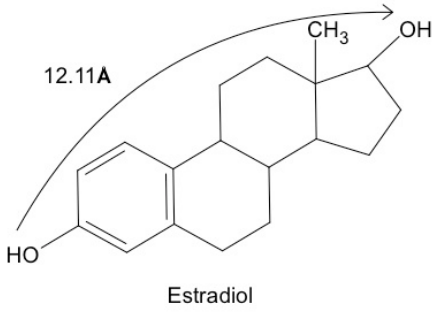


Figure 14. Distance between phenolic ring and hydroxyl group in estradiol

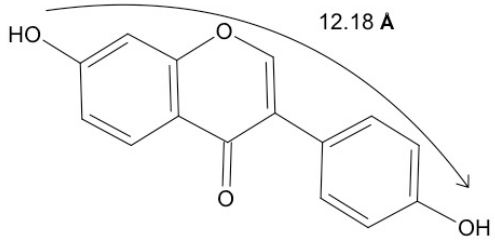


Figure 15. Distance between phenolic ring and hydroxyl group in daidzein

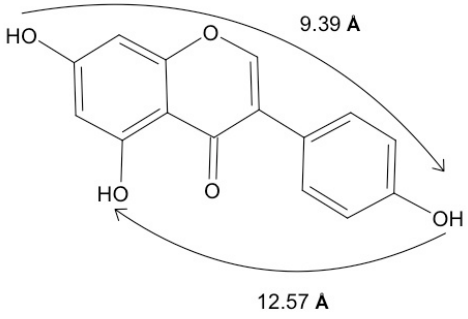


Figure 16. Distance between phenolic ring and hydroxyl group in genistein

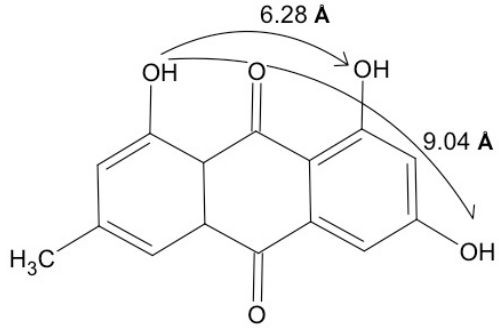


Figure 17. Distance between phenolic ring and hydroxyl group in emodin

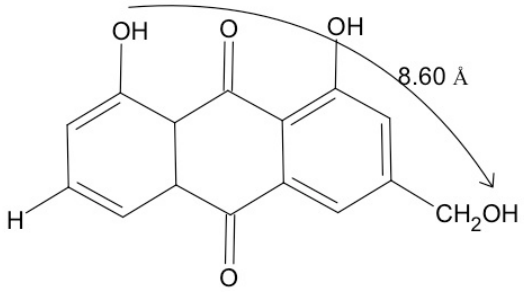


Figure 18. Distance between phenolic ring and hydroxyl group in aloe-emodin

DISCUSSION

ERα and ERβ have distributed in different tissue, ligands that have the capacity to selectively activate or inhibit these two ERs would be useful in elucidating the biology of these two receptors and might assist in the development of phytoestrogen pharmaceuticals with improved tissue selectivity. We have developed several ligands that showed ERα and/or ERβ selectivity.

Compounds that bind to the estrogen receptor exhibit remarkable variability in composition and stereochemistry. Anthraquinone derivatives were mostly observed as agents interacting with various targets like enzymes or receptors. We tried to identificate novel binding targets which are ERα and ERβ, interacted with anthranoids.

In recent years, *in silico* methods are preferred as preliminary studies to discover new activities of known molecules. According to the docking results, glucofrangulin B and emodin-8-*O*-β-*D* glucopyranoside having similar binding affinity to ER α like reference isoflavone compounds daidzein and genistein, and they showed better scores than the other ligands. On the other hand emodin and aloe-emodin having the best binding affinity to ER β. T score values of ligands with ER α and ER β have shown in Figure 5 and 6.

The most extensively studied phytoestrogens are those belonging to the isoflavones class, such as genistein and daidzein (found mostly as a glycoside), or as free aglycones (Wiseman, 2000). There are several *in-silico* studies on the binding patterns of these

isoflavones to the estrogen receptor. (Dhananjaya et al., 2012; Gupta et al., 2014) Several conclusions were drawn from the relation of the structure of these flavonoids to their binding affinities, the most important being that the diaryl structure is common to all flavonoids, with a minimum of one hydroxyl substituent on each of the two A and B aromatic rings. The optimal patterns of hydroxylation on these aromatic rings for binding to the ERs are at positions 7 and 4' of A and B rings, respectively. Two adjacent hydroxyls on ring A or B such as in 7,8- or 3',4'- dihydroxyflavone, decrease binding activity. Increasing the number of hydroxyl substituents on A and B rings above four, as in 3,3',4',5,7- pentahydroxyflavone, also decreases activity (Miksicek, 1995).

Anthranoids are naturally occurring molecules and consists 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. Several functional groups may be attached to this basic skeleton, mainly 1,6,8 hydroxyl groups. When the results were analyzed, it was found that the effect of anthranoids too close but less than genistein and daidzein. These results can be depending on the three aryl ring instead of two and three hydroxyl substituent. Because phytoestrogen effect decreases with more than two hydroxyl substituent. o-hydroxyl groups also decreases phytoestrogen effect. Anthraquinone molecules have not o-hydroxyl substituent but have two adjacent hydroxyls on 1st and 8th position.

Emodin, aloe-emodin, and glycosides of emodin which are emodin-8-*O*-β-D glucopyranoside and glucofrangulin B structures are superior to the rest anthranoid structures. Chrysophanol has only two adjacent hydroxyl and also less effect than emodin derivatives. The reason of decreasing in estrogenic capability of physcion may be blocking the hydroxyl substituent with methoxy groups. To have a better interaction with ER α and ER β, 1,8-dihydroxyanthraquinone molecule needs a hydroxyl on the sixth position. When compared to the results of aloe-emodin and emodin, it was observed that emodin has better binding affinity to ERs because of its hydroxyl positions.

T score, high to low ranking can be given as genistein, daidzein, glucofrangulin B and emodin-8-*O*-β-*D* glucopyranoside for ER α respectively. Without exception, all this vast variety of chemical compounds, which demonstrate estrogen-like activity, has in common an aromatic ring with at least one phenolic hydroxyl group.[, #1094]

According to our results, the distance between the hydroxyls of estradiol, genistein and daidzein was found around 12 **Å and** has been observed to be very close to each other. The distance between the hydroxyl of the tested potential phytoestrogen anthraquinones were closer than reference substances genistein and daidzein and also estradiol.

CONCLUSION

Phytoestrogens are plant-derived chemicals with estrogen-like activities, which could have a beneficial role in humans against estrogen deficiency.

In this study the relationship between estrogen receptors and anthranoids was determined for the first time by molecular docking method. Comparative docking analysis of commonly existed anthranoids on ER α and ER β suggested that these ligands can be alternative sources for some diseases related to estrogen. Anthranoids exhibited the highest estrogenic relative potency but its potency was less than phytoestrogens genistein and daidzein. Therefore this studies have not only an initial but also a vital role in development of potential drug candidates. Further studies are needed to be considered anthraquinone derivatives as novel phytoestrogens. Our results may be helpful in developing of new series of phytoestrogens in future.

It is the first report on the relationship between phytoestrogenic effects and molecular structure of main anthranoids by molecular docking method. After all docking simulations the results showed that anthraquinone molecules may be novel phytoestrogen candidates.

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