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ORIGINAL PAPER

Cancer-relevant biochemical targets of cytotoxic *Lonchocarpus* flavonoids: A molecular docking analysis

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Abstract A molecular docking investigation has been carried out on cytotoxic prenylated flavonoids from *Lonchocarpus haberi* with cancer-relevant chemotherapeutic targets known to be inhibited by flavonoids. Two molecular docking programs, Molegro and ArgusDock, were used to compare the binding energies of *Lonchocarpus* flavonoids with other flavonoids, inhibitors, or known ligands, to aromatase (CYP 19), fatty acid synthase (FAS), xanthine oxidase (XO), cyclooxygenases (COX-1 and COX-2), lipoxygenase (LOX-3), ornithine decarboxylase (ODC), protein tyrosine kinase (PTK), phosphoinositide 3-kinase (PI3K), protein kinase C (PKC), topoisomerase II (ATP binding site), ATP binding cassette (ABC) transporter, and phospholipase A_2 (PLA). The *Lonchocarpus* flavonoids examined in this study exhibited docking energies comparable to or stronger than other flavonoids that had been previously shown to be effective inhibitors of these enzymes. Furthermore, prenylated flavonoids, such as the *Lonchocarpus* flavonoids and xanthohumol, generally showed greater binding energies than the non-prenylated flavonoids. We conclude, therefore, that the *Lonchocarpus* flavonoids possibly owe their cytotoxic activity by inhibition of one or more of these enzymes.

Keywords Cancer · Flavonoids · *Lonchocarpus haberi* · Molecular docking

Introduction

It is estimated that approximately one out of three Americans will develop cancer at some point during their lifetime [1]. In 2008, approximately 1.5 million individuals in the United States were diagnosed with cancer, and an estimated half million deaths were attributed to this disease [1]. Chemotherapy is one of the four major approaches to cancer treatment, (along with surgery, radiotherapy, and immunotherapy), and a number of potential chemotherapeutic targets for cancer have been identified [2–15].

Phytochemicals such as vincristine and paclitaxel are clinically important cancer chemotherapeutic agents, and higher plants continue to be promising sources of new antitumor compounds [16, 17]. Flavonoids are ubiquitous polyphenolic phytochemicals and these materials have shown promising anticancer and cancer chemopreventive activities [18–23]. Flavonoids consist of two benzene rings with a three-carbon connecting group and are derived from flavone [24]. This group is subdivided, based on additional oxygen-containing heterocyclic rings and oxygenated functional groups, into chalcones, flavones, flavonols, flavanones, anthocyanins, and isoflavonoids (Fig. 1) [24]. *Lonchocarpus* species have proven to be rich sources of flavonoids [25–35] many of which have exhibited promising cytotoxic activity [36–38].

Estrogens are known to be important in the development of breast cancer, and a majority of postmenopausal women with breast cancer have estrogen-receptor-positive tumors. One pharmacological approach to treat endocrine-related breast cancers is to block estrogen synthesis. Aromatase is a key cytochrome P450 enzyme that catalyzes aromatization of androgens to estrogens. Therefore, this enzyme has been the target for the design of inhibitors [39]. The flavonoids rotenone, chrysin and apigenin (see Fig. 2) have been shown to be potent inhibitors of aromatase [40].

The primary enzyme responsible for the synthesis of fatty acids is fatty acid synthase (FAS) and, compared to normal human tissues, this enzyme is expressed in high

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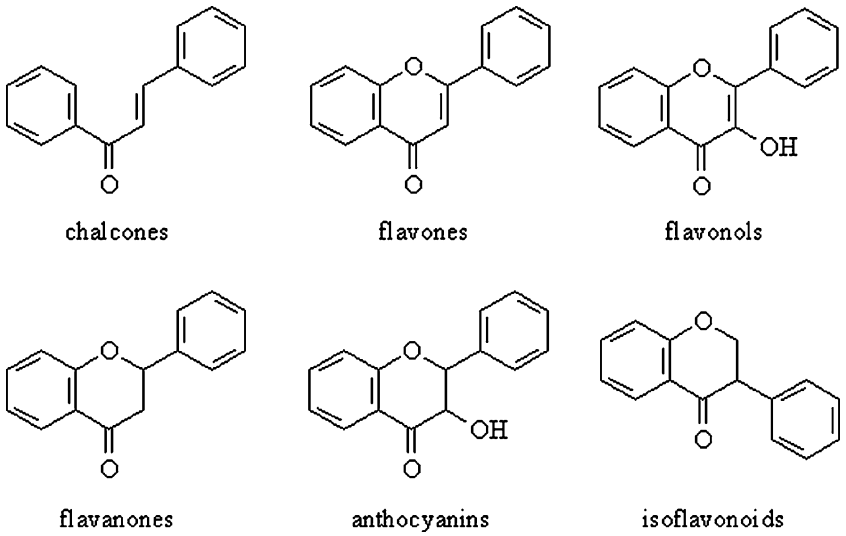
Fig. 1 Flavonoid classes



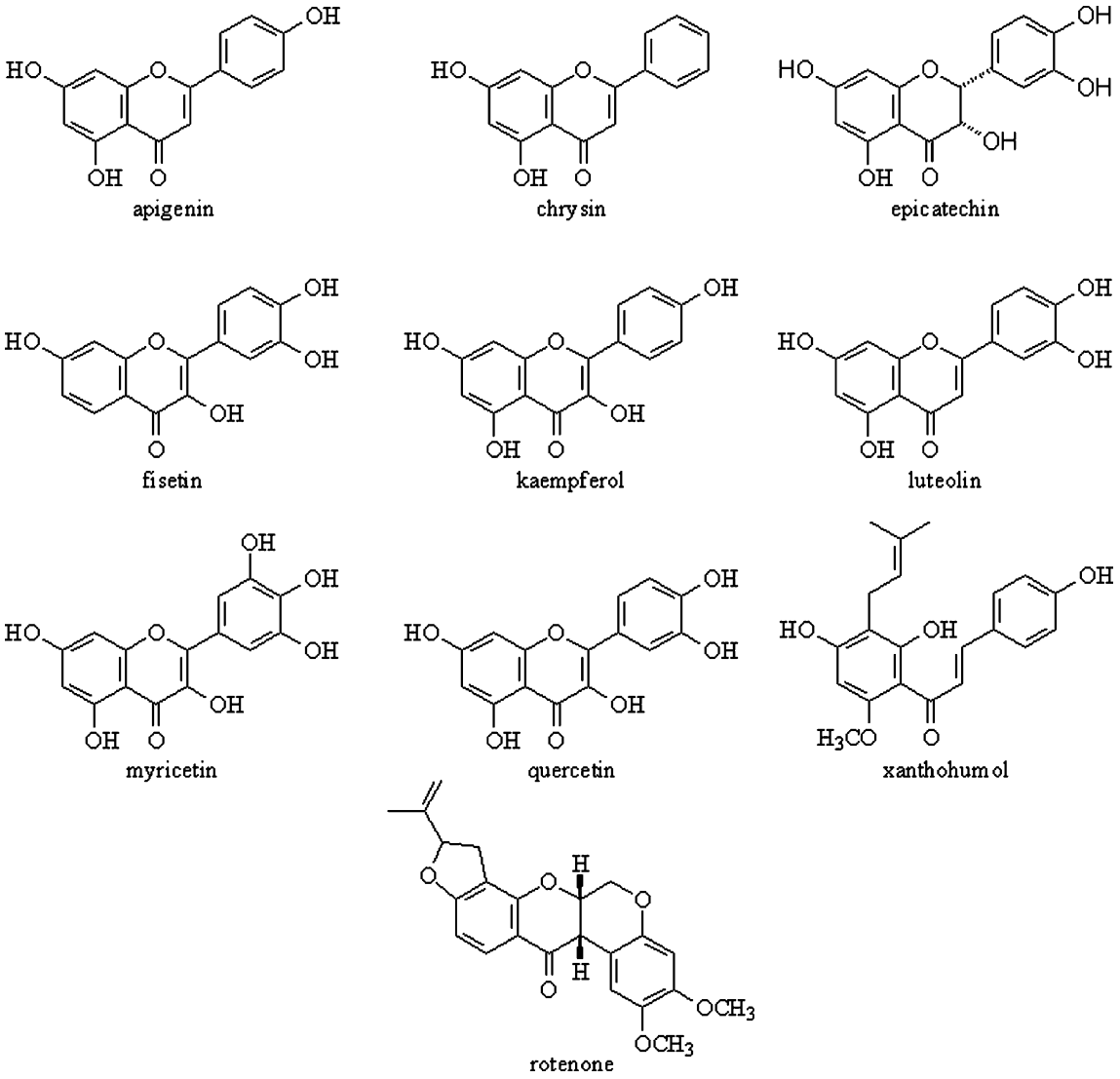


Fig. 2 Flavonoids discussed in this work

levels in many common tumor cancers, including breast cancer [41, 42]. A number of flavonoids have shown a dose-response correlation between fatty acid synthesis, inhibition of cell growth, and induction of apoptosis in tumor cells [43].

Reactive oxygen species have been implicated in both tumor promotion and progression [44, 45], and prooxidant enzymes such as cyclooxygenases, xanthine oxidase and lipoxygenases are induced or activated by tumor promoters [23]. Inhibition of these enzymes, therefore, would inhibit tumor cell proliferation. Flavonoids have been shown to be particularly effective at inhibiting xanthine oxidase [46], cyclooxygenases [47], and lipoxygenases [48].

Ornithine decarboxylase is the first and the rate-limiting enzyme in the biosynthesis of polyamines (putrescine, spermidine, and spermine) [49]. The functions of these polyamines is not entirely clear, but accumulation seems to be essential for the growth, proliferation, and differentiation of mammalian cells, and this suggests that uncontrolled regulation of ornithine decarboxylase may lead to tumorigenesis and tumor growth [50, 51]. Ornithine decarboxylase is overexpressed in a number of tumors [52–54] including breast cancer [55]. The flavonoids quercetin [56] and apigenin [57] have been shown to inhibit ornithine decarboxylase in tumorigenesis models.

Protein tyrosine kinases are a group of enzymes that catalyze the phosphorylation of tyrosine on substrate proteins, which then plays a key role in signal transduction pathways and in many human malignancies [58]. A number of flavonoids have shown potent inhibitory activity against protein tyrosine kinase [59].

Protein kinase C (PKC) is involved in a wide range of cellular activities, including tumor promotion [60], and a number of flavonoids have been shown to inhibit the activity of this enzyme. Thus, for example, fisetin, quercetin, myricetin, and luteolin, have been shown to be potent inhibitors of PKC [61, 62]. Furthermore, apigenin, fisetin and luteolin have been shown to competitively block the ATP binding site on the catalytic unit of PKC [61, 63].

Phosphoinositide 3-kinase (PI3K) plays an essential role in survival, proliferation, differentiation, motility, and cytoskeleton organization in cancer cells [64]. Flavonoids have been shown to be effective at inhibiting this signal transduction enzyme [62, 65, 66]. Myricetin, luteolin, apigenin [67], fisetin [68], quercetin, and naringenin [69], for example, have shown in-vitro PI3K inhibitory activity. X-ray crystallographic studies have shown than quercetin and myricetin bind to the ATP binding pocket of PI3K [70].

Topoisomerases are essential enzymes that catalyze modifications to the tertiary structure of DNA. There are two well-characterized classes of human topoisomerases. Topoisomerase I acts by breaking and religating one DNA strand [71], while topoisomerase II involves double-strand breaking [72]. Genistein has been shown to inhibit both topoisomerase I and II [73] as well as inhibit topoisomerase II-catalyzed ATP hydrolysis [74].

Resistance of cancer cells to chemotherapy is often mediated by overexpression of P-glycoprotein, a plasma membrane ATP-binding cassette (ABC) transporter, which extrudes cytotoxic drugs at the expense of ATP hydrolysis [75]. Some flavonoids have been reported to possess potent inhibitory activity against the drug exporting function of P-glycoprotein [76], and this modulation by flavonoids may be through high-affinity binding directly to nucleotide-binding domains or inhibition of ATPase activity [77, 78].

Genes encoding for phospholipase A_2 are overexpressed in breast tumor [79] as well as prostate tumor tissue [80], and phospholipase A_2 generated lipid biomediators can facilitate tumor progression [81]. Inhibition of phospholipase A_2 has been shown to be effective in decreasing cancer growth [82], and the flavonoid quercetin has been found to be an effective inhibitor of phospholipase A_2 [20].

We have found four prenylated (appended with a 3-methyl-2-buten-1-yl moiety) flavonoids (Fig. 3), isolated from the bark extract of *Lonchocarpus haberi*, to exhibit in-vitro cytotoxic activity against Hep G2, PC-3, and Hs 578 T human tumor cell lines with LC_{50} values around 30–90 μg/mL [83]. In this work, we have used molecular docking methods to examine potential binding of these flavonoids to cancer-relevant molecular targets: aromatase (CYP 19), fatty acid synthase (FAS), xanthine oxidase (XO), cyclooxygenases (COX-1 and COX-2), lipoxygenase (LOX-3), ornithine decarboxylase (ODC), protein tyrosine kinase (PTK), phosphoinositide 3-kinase (PI3K), protein kinase C (PKC), topoisomerase II (ATP binding site), ATP binding cassette (ABC) transporter, and phospholipase A_2 (PLA).

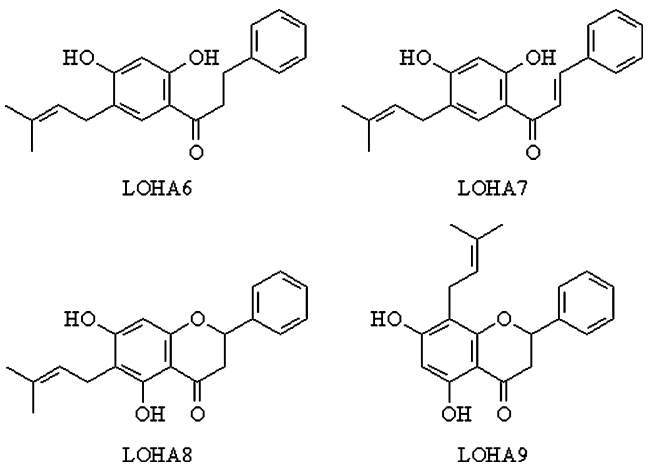


Fig. 3 Cytotoxic prenylated flavonoids from *Lonchocarpus haberi*

Computational methods

Molecular docking analysis

Protein-ligand docking studies were carried out based on the crystal structures of cytochrome P450 CYP1A (PDB: 2hi4) [84], cytochrome P45019 (PDB: 2fr7) [85] three models of human aromatase (CYP19, PDB: 1og5 [86], 1dt6 [87], 1tqa [88]) and a recent crystal structure of human aromatase (CYP19A1, PDB: 3eqm) [89]; fatty acid synthase (PDB: 1xkt [90] and 2px6 [91]), xanthine oxidase (PDB: 1vdv [92], 1fo4 [93], and 1n5x [94]), cyclooxygenase-1 (PDB: 1eqg and 1eqh) [95], cyclooxygenase-2 (PDB: 3pgh and 4cox) [96], lipoxygenase-3 (PDB: 1jnq [97], 1n8q [98], and 1no3 [99]); ornithine decarboxylase (PDB: 1d7k [100] and 2on3 [101]), protein tyrosine kinase (PDB: 1m14 [102], 1m17 [102], and 1xkk [103]), protein kinase C (PDB: 1zrz [104], 1xjd [105], and 1byg [106]), phosphoinositide 3-kinases (PDB: 1w2c [107], 1w2d [107], 1e8w [108], 1e90 [108], 1e8y [108], and 1e8z [108]); topoisomerase II ATP binding site (PDB: 1qzr [109] and 1zxm [110]), ATP-binding cassette transporter (PDB: 1mv5 [111], 2cbz [112], and 1l2t [113]), and phospholipase A_2 (PDB: 1kpm [114], 2b00 [115], 1kqu [116], 1pod [117], 1db4 [118], and 1bbc [119]). All solvent molecules and the co-crystallized ligands were removed from the structures. Molecular docking calculations for all compounds with each of the proteins were undertaken using Molegro Virtual Docker 2.3 [120, 121], with a sphere large enough to accommodate the cavity centered on the binding sites of each protein structure in order to allow each ligand to search. Different orientations of the ligands were searched and ranked based on their energy scores. As a check of docking accuracy, a comparison was carried out using ArgusLab 4.0.1 [122].

Results

A number of representative flavonoids (Fig. 2) in addition to the cytotoxic *Lonchocarpus haberi* flavonoids (Fig. 3) were examined for potential binding into each protein target using both the Molegro and the ArgusLab docking algorithms. The lowest-energy binding energies are summarized for the protein targets in Tables 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12. For comparison purposes, the docked energies of the co-crystallized ligands were also determined.

Discussion

The molecular docking results presented in this study reveal that the cytotoxic *Lonchocarpus haberi* flavonoids have strong binding affinities for the cancer-relevant

Table 1 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into cytochrome P450[a]

Ligand	Molegro						ArgusDock					
	2hi4	2fr7	1og5	1dt6	1tqa	3eqm	2hi4	2fr7	1og5	1dt6	1tqa	3eqm
Xtal[b]	−25.21	—	**−26.51**	—	—	**−25.84**	−14.40	—	−13.07	—	—	−12.97
Apigenin	−23.75	−43.48	−19.05	−20.52	−18.67	−21.65	−10.15	−10.57	−9.72	−9.16	−10.91	−10.23
Chrysin	−24.58	−41.68	−19.01	−19.37	−19.66	−22.96	−10.17	−10.67	−11.57	−8.68	−10.92	−9.50
Epicatechin	−23.60	−46.10	−19.85	−20.34	−19.91	−18.95	−8.86	−5.30	−8.72	−8.21	−8.61	−7.79
Fisetin	−24.60	−45.56	−21.42	−20.64	−20.46	−22.01	−9.93	−5.70	−9.27	−8.02	−9.61	−8.43
Kaempferol	−24.63	−43.96	−20.12	−21.96	−18.97	−21.99	−9.41	−5.60	−9.84	−8.59	−9.18	−8.52
LOHA6	−29.83	−49.29	−25.33	**−23.69**	−25.37	**−25.92**	**−14.91**	**−13.88**	−14.14	−11.73	**−14.55**	**−13.28**
LOHA7	**−30.83**	**−49.41**	**−25.36**	−23.47	**−25.39**	−25.65	−14.48	−8.39	**−14.39**	**−11.87**	−14.12	−12.69
LOHA8	−22.03	−46.06	−23.25	−22.99	−24.59	−9.49	−12.45	−7.32	−12.60	−10.89	−11.87	−11.74
LOHA9	−21.06	−44.58	−23.16	−21.61	−22.59	−7.83	−13.46	−8.02	−12.64	−11.36	−12.54	−12.02
Luteolin	−23.57	−45.70	−20.50	−20.28	−20.34	−22.86	−9.22	−9.46	−8.86	−8.70	−9.49	−8.45
Myricetin	−24.14	−48.82	−22.33	−21.63	−21.00	−20.58	−8.72	−6.93	−7.80	−8.42	−8.11	−7.86
Quercetin	−24.50	−45.05	−21.61	−22.37	−20.49	−22.71	−8.86	−8.43	−9.21	−8.21	−8.75	−8.89
Rotenone	14.19	−42.14	−21.56	−25.46	−21.06	−5.10	−6.19	−7.29	−10.91	−9.02	−11.27	−6.15
Xanthohumol	−23.35	**−51.72**	**−25.42**	**−27.42**	**−27.16**	−19.89	−13.94	−7.95	−12.86	−10.82	−13.22	−11.17

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 2hi4 was the inhibitor α-naphthoflavone [84]. The crystallized ligand for 1og5 was the *anti*-coagulant warfarin [86]. The crystallized ligand for 3eqm was androstenedione [89]. The other protein structures did not contain a co-crystallized ligand.

Table 2 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into fatty acid synthase[a]

Ligand	Molegro		ArgusDock	
	1xkt	2px6	1xkt	2px6
Orlistat[b]	−20.23	−19.48	**−16.97**	−14.18
Apigenin	−20.05	−17.86	−9.48	−9.61
Chrysin	−20.05	−17.57	−10.67	−9.94
Epicatechin	−19.89	−19.91	−8.26	−8.94
Fisetin	−19.77	−20.20	−9.43	−8.99
Kaempferol	−21.05	−18.53	−9.62	−9.10
LOHA6	−22.12	−22.70	**−14.05**	−14.08
LOHA7	**−23.14**	**−22.89**	−13.91	**−14.38**
LOHA8	−18.63	−19.60	−11.25	−11.57
LOHA9	−20.75	−18.10	−11.88	−13.45
Luteolin	−20.78	−20.03	−9.71	−9.48
Myricetin	−22.16	−20.77	−8.32	−8.79
Quercetin	−21.28	−20.34	−9.15	−8.97
Rotenone	−20.54	−20.94	−8.96	−10.16
Xanthohumol	**−24.46**	−22.55	−12.34	−12.62

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 2px6 was the drug Orlistat [91]. Orlistat was docked into both protein structures.

Table 4 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into xanthine oxidase[a]

Ligand	Molegro			ArgusDock		
	1vdv	1fo4	1n5x	1vdv	1fo4	1n5x
Xtal[b]	−31.20	−18.68	−28.29	−8.67	−10.56	−10.35
Apigenin	−23.84	−25.68	−26.13	−9.93	−10.57	−10.39
Fisetin	−27.84	−27.74	−27.13	−9.36	−8.81	−9.27
Kaempferol	−26.84	−27.56	−25.12	−9.28	−8.88	−9.01
LOHA6	−31.17	−30.22	−24.84	−12.12	−12.97	**−12.44**
LOHA7	**−34.07**	**−32.67**	**−29.18**	**−12.18**	**−13.15**	−12.16
LOHA8	−25.70	−16.33	−16.75	−11.06	−11.52	−11.45
LOHA9	−30.97	−21.88	−17.54	−11.67	−11.04	−10.96
Luteolin	−27.59	−26.14	−26.17	−9.76	−10.09	−10.08
Quercetin	−28.55	−28.11	−27.20	−9.95	−9.51	−9.73
Xanthohumol	−29.11	−24.48	−25.34	−11.15	−11.19	−11.20

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1vdv was the synthetic inhibitor Y-700 [92]. The crystallized ligand for 1fo4 was salicylic acid [93]. The crystallized ligand for 1n5x was the inhibitor TEI-6720 [94].

[c] Ligand did not dock.

molecular targets that were examined in this analysis, consistent with previous observations that flavonoids had been shown to inhibit these enzymatic targets. In many cases the *L. haberi* flavonoids were found to have the best docking energy out of all of the ligands docked. Out of the four *L. haberi* flavonoids studied, LOHA6 and LOHA7 were found to consistently have the lower binding energies, but LOHA8 and LOHA9 still had notably strong binding results.

The docking energies for flavonoid ligands docked into cytochrome P450 (PDB: 2hi4, 2fr7, 1og5, 1dt6, 1tqa, 3eqm) showed LOHA6 and LOHA7, along with xanthohumol, to be the strongest binding ligands (Table 1). The Molegro program calculated LOHA7 to generally have the strongest docking energy for the *L. haberi* flavonoids while ArgusDock showed LOHA6 to have the greater

Table 3 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into cyclooxygenases 1 and 2[a]

Ligand	Molegro				ArgusDock			
	COX-1		COX-2		COX-1		COX-2	
	1eqg	1eqh	3pgh	4cox	1eqg	1eqh	3pgh	4cox
Xtal[b]	−19.77	−23.31	−23.02	**−31.43**	−12.94	**−14.48**	**−14.04**	−12.41
Apigenin	−20.81	−19.83	−21.54	−24.76	−9.09	−10.12	−9.90	−10.52
Fisetin	−21.71	**−23.39**	−22.50	−25.74	−9.52	−8.46	−9.46	−9.51
Kaempferol	−19.65	−19.32	−22.24	−25.76	−9.25	−8.48	−9.11	−9.61
LOHA6	−20.59	−20.31	**−26.22**	−26.71	**−14.46**	**−14.32**	**−13.81**	−14.24
LOHA7	**−24.09**	**−21.19**	−25.46	**−27.25**	−13.36	−13.42	−13.63	**−14.96**
LOHA8	−16.65	−14.75	−14.54	−24.48	−12.41	−11.01	−12.65	−11.45
LOHA9	−19.16	−9.36	−17.19	−21.57	−11.01	−11.13	−13.02	−11.90
Luteolin	−21.86	−22.86	−23.25	−26.71	−9.40	−9.64	−9.43	−9.65
Quercetin	−20.71	−21.22	−24.19	**−27.69**	−8.78	−8.58	−9.16	−9.20
Xanthohumol	−21.26	−18.10	−22.23	**−29.27**	−12.17	−12.18	−12.54	−12.30

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1eqg was the inhibitor ibuprofen [95]. The crystallized ligand for both 1eqh and 3pgh was the inhibitor flurbiprofen [95, 96]. The crystallized ligand for 4cox was the non-selective inhibitor indomethacin [96].

Table 5 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into lipoxygenase-3[a]

Ligand	Molegro			ArgusDock		
	1jnq	1n8q	1no3	1jnq	1n8q	1no3
Xtal[b]	−15.45	−17.18	−13.85	−9.11	−8.82	−8.26
Apigenin	−21.72	−1.77	−5.17	−8.32	c	c
Fisetin	−22.62	c	c	−8.46	c	c
Kaempferol	−21.42	c	**−9.28**	−8.63	c	−8.11
LOHA6	**−28.04**	**−8.23**	**−2.08**	**−14.05**	**−12.98**	**−12.71**
LOHA7	−26.91	c	−0.09	−13.33	−8.15	−10.82
LOHA8	−10.46	c	c	−11.98	c	c
LOHA9	−6.52	c	c	c	c	c
Luteolin	−23.56	c	−3.88	−8.32	c	c
Quercetin	−22.87	c	−2.16	−9.06	c	c
Xanthohumol	**−30.72**	c	c	−11.85	c	c

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1jnq was (−)-epigallocatechin [97]. The crystallized ligand for 1n8q was protocatechuic acid [98].The crystallized ligand for 1no3 was 4-nitrocatechol [99].

[c] Ligand did not dock.

Table 6 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into ornithine decarboxylase[a]

Ligand	Molegro		ArgusDock	
	1d7k	2on3	1d7k	2on3
Xtal[b]	−22.38	−14.37	−9.56	−7.24
Apigenin	−23.08	−21.65	−9.53	−8.67
Fisetin	−22.44	−20.45	−7.90	−7.80
Kaempferol	−23.39	−22.07	−8.28	−8.38
LOHA6	−22.16	−23.49	−11.81	**−10.81**
LOHA7	**−25.11**	**−24.41**	**−12.12**	−10.57
LOHA8	−23.00	−22.14	−11.07	**−10.81**
LOHA9	−23.67	−21.89	−9.85	−9.57
Luteolin	−24.93	−23.82	−8.25	−8.21
Quercetin	−24.02	−24.46	−8.50	−7.95
Xanthohumol	**−28.21**	**−27.61**	−10.02	−10.64

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1d7k was LLP [2-lysine-(3-hydroxy-2-methyl-5-phosphonooxymethyl-pyridin-4-ylmethane] [100]. The crystallized ligand for 2on3 was 1-amino-oxy-3-aminopropane [101].

Table 7 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into protein tyrosine kinase[a]

Ligand	Molegro			ArgusDock		
	1m14	1m17	1xkk	1m14	1m17	1xkk
Xtal[b]	c	−25.68	−26.56	c	−8.51	−12.45
Apigenin	−17.07	−19.17	−19.97	−8.34	−8.36	−8.84
Epicatechin	−17.93	−20.40	−21.00	−7.27	−7.26	−8.59
Fisetin	−17.99	−20.65	−21.44	−7.55	−7.76	−8.10
Kaempferol	−17.63	−20.28	−20.23	−7.73	−7.55	−8.23
LOHA6	−20.20	−20.63	−22.24	**−10.74**	**−11.45**	**−11.95**
LOHA7	**−22.58**	**−21.69**	−24.87	−10.73	−11.39	−11.78
LOHA8	−18.34	−19.55	**−26.36**	−9.34	−9.35	−11.72
LOHA9	−17.45	−18.26	−19.22	−8.92	−9.01	−10.20
Luteolin	−18.26	−20.34	−21.69	−7.94	−8.06	−8.82
Myricetin	−19.80	**−23.02**	−23.57	−7.76	−7.98	−8.04
Quercetin	−19.02	−22.17	−21.66	−8.03	−8.31	−8.73
Xanthohumol	**−22.60**	−22.56	−24.79	−9.18	−9.93	−10.22

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1m17 was the 4-anilinoquinazoline inhibitor Erlotinib [102]. The crystallized ligand for 1xkk was GW572016 (Lapatinib) [103].

[c] There was no co-crystallized ligand for 1m14.

Table 8 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into protein kinase C[a]

Ligand	Molegro			ArgusDock		
	1zrz	1xjd	1byg	1zrz	1xjd	1byg
Xtal[b]	−27.90	−36.06	−25.06	−10.50	−11.01	−10.80
Apigenin	−17.77	−19.69	−22.34	−9.58	−9.08	−9.41
Fisetin	−18.88	−21.73	−23.73	−8.50	−8.46	−8.46
Kaempferol	−18.61	−21.09	−24.09	−8.34	−8.79	−7.98
LOHA6	**−22.88**	−22.14	−25.88	**−11.75**	**−12.65**	−12.05
LOHA7	−21.48	**−23.75**	**−26.32**	−11.62	−12.39	−12.26
LOHA8	−20.72	−18.34	−23.82	−11.22	−9.90	−10.23
LOHA9	−20.19	−19.66	−20.37	−11.18	−10.03	**−12.43**
Luteolin	−18.63	−21.58	−23.28	−8.97	−8.41	−8.94
Quercetin	−19.56	−23.00	−24.37	−8.64	−8.35	−8.91
Xanthohumol	−21.74	**−23.96**	**−27.68**	−10.62	−10.61	−11.18

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1zrz was the bis(indolyl)maleimide inhibitor BIM1 [104]. The crystallized ligand for 1xjd and for 1byg was staurosporine [105, 106].

Table 9 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into the ATP binding site for phosphoinositide 3-kinases[a]

Ligand	Molegro						ArgusDock					
	1w2c	1w2d	1e8w	1e90	1e8z	1e8y	1w2c	1w2d	1e8w	1e90	1e8z	1e8y
Xtal[b]	−26.85	−28.77	−24.78	−25.04	−31.19	c	−9.49	−7.17	−8.62	−11.07	−8.87	c
Apigenin	−17.28	−17.71	−22.10	−22.04	−21.32	−16.11	−9.51	−9.10	−9.55	−9.43	−8.89	−9.63
Fisetin	−19.72	−19.36	−23.50	−22.15	−21.28	−17.38	−9.13	−9.19	−8.91	−8.90	−8.71	−9.41
Kaempferol	−18.41	−19.91	−22.99	−22.24	−21.08	−17.21	−8.53	−9.00	−8.21	−8.68	−8.24	−9.06
LOHA6	**−20.42**	−20.72	**−25.59**	−22.47	−23.06	−20.46	**−13.47**	−12.55	**−13.36**	−12.73	**−12.76**	**−12.36**
LOHA7	−20.35	**−20.91**	−25.29	**−22.58**	**−23.50**	**−20.60**	−11.80	**−12.64**	−12.43	**−12.89**	−12.15	−11.66
LOHA8	−19.53	−20.60	−19.51	−21.12	−18.43	−18.59	−11.22	−11.11	−11.20	−10.98	−10.16	−10.81
LOHA9	−18.18	−18.73	−22.07	−19.44	−19.74	−16.91	−11.23	−11.92	−11.94	−11.76	−11.05	−10.94
Luteolin	−19.08	−20.32	−23.79	−24.01	−22.10	−16.74	−9.09	−8.95	−9.19	−9.06	−8.45	−9.25
Myricetin	**−21.60**	**−21.61**	**−26.10**	**−25.04**	−23.75	−19.27	−8.72	−8.28	−8.39	−11.07	−8.37	−8.43
Quercetin	−19.50	−20.61	−24.78	−23.90	−22.81	−17.13	−9.20	−9.05	−8.62	−8.82	−8.94	−9.35
Xanthohumol	−19.97	−20.30	−23.91	−24.14	**−26.40**	**−22.20**	−11.36	−10.98	−11.25	−11.50	−11.17	−10.89

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1w2c was ANP (adenine phosphoaminophosphonate) [107]. The crystallized ligand for 1w2d was ADP [107]. The crystallized ligand for 1e8w was quercetin [108]. The crystallized ligand for 1e90 was myricetin [108]. The crystallized ligand for 1e8z was staurosporine [108].

[c] There was no co-crystallized ligand for 1e8y.

Table 10 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into the ATP binding site of topoisomerase II[a]

Ligand	Molegro		ArgusDock	
	1qzr	1zxm	1qzr	1zxm
ANP[b]	−46.84	−43.82	−7.71	−9.25
Apigenin	−21.85	−22.74	−9.40	−9.62
Chrysin	−21.30	−21.51	−8.78	−9.10
Epicatechin	−23.45	−24.21	−7.50	−7.85
Fisetin	−23.07	−23.57	−9.41	−8.27
Kaempferol	−22.44	−22.99	−7.86	−7.94
LOHA6	**−26.25**	−25.78	−10.46	−12.13
LOHA7	−25.97	**−26.69**	**−10.95**	**−12.18**
LOHA8	−24.99	−25.88	−9.48	−9.04
LOHA9	−23.35	−24.11	−10.58	−9.89
Luteolin	−23.33	−24.53	−9.03	−9.18
Myricetin	−25.49	−26.82	−7.56	−7.64
Quercetin	−24.18	−24.88	−8.28	−7.88
Rotenone	−23.66	−25.18	−7.96	−8.89
Xanthohumol	**−29.73**	**−28.59**	−9.48	−9.23

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand was adenine phosphoaminophosphonate [109, 110].

Table 11 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into ATP-binding cassette transporter[a]

Ligand	Molegro			ArgusDock		
	1mv5	2cbz	1l2t	1mv5	2cbz	1l2t
ADP[b]	−31.36	−26.33	−36.97	−8.71	−8.16	−8.54
ATP[b]	−30.48	−22.94	−44.10	−8.05	−8.32	−7.27
Apigenin	−19.47	−13.98	−22.42	−8.64	−7.32	−8.81
Epicatechin	−19.76	−16.47	−24.36	−7.48	−6.76	−7.89
Fisetin	−20.30	−15.61	−22.07	−7.88	−6.24	−7.91
Kaempferol	−20.06	−15.53	−22.61	−7.62	−6.28	−7.87
LOHA6	−22.77	**−20.77**	−25.80	**−11.44**	**−8.22**	−10.86
LOHA7	**−23.55**	−20.03	**−25.97**	−11.16	−7.33	**−10.94**
LOHA8	−20.90	−16.27	−22.69	−10.56	−6.59	−10.51
LOHA9	−20.65	−17.07	−21.61	−10.01	−7.19	−8.42
Luteolin	−20.50	−16.13	−22.90	−8.36	−7.03	−8.81
Myricetin	−20.73	−16.55	−23.45	−8.15	−6.60	−7.93
Quercetin	−21.05	−17.40	−23.04	−8.53	−7.10	−8.30
Xanthohumol	**−28.30**	−20.59	**−27.42**	**−11.69**	−7.37	−9.64

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The protein structure 1mv5 contained both ADP and ATP as crystallized ligands [111]. ATP was the crystallized ligand for both 2cbz [112] and 1l2t [113].

Table 12 Molegro and ArgusDock binding energies (kcal mol^{-1}) of best binding poses for ligands into phospholipase A_2[a]

Ligand	Molegro						ArgusDock					
	1kpm	2b00	1kqu	1pod	1db4	1bbc	1kpm	2b00	1kqu	1pod	1db4	1bbc
Xtal[b]	−19.25	−29.80	−29.80	c	−31.01	c	−12.23	−11.70	−13.84	c	−11.32	c
Apigenin	−25.67	−19.14	−19.14	−22.42	−19.44	−18.42	−9.61	−9.33	−9.08	−8.48	−9.49	−8.07
Fisetin	−25.39	−20.85	−20.85	−21.98	−21.89	−20.64	−8.49	−8.51	−7.93	−7.53	−8.72	−8.28
Kaempferol	−26.30	−19.74	−19.74	−22.14	−21.38	−20.82	−7.92	−8.66	−7.76	−7.66	−8.86	−7.69
LOHA6	−27.72	−24.11	−26.27	**−27.46**	−26.53	**−23.99**	**−13.81**	−12.58	**−12.64**	−10.78	**−12.26**	−11.00
LOHA7	**−28.45**	**−25.08**	**−27.25**	−27.44	**−27.00**	−23.75	−13.39	**−12.90**	−11.01	**−10.96**	−12.12	−10.48
LOHA8	−24.32	−21.98	−22.92	−20.21	−22.85	−19.96	−12.65	−11.61	−11.14	−9.25	−9.91	**−11.11**
LOHA9	−25.96	−22.44	−22.48	−20.32	−22.76	−20.22	−12.17	−11.89	−11.86	−9.43	−10.46	−10.18
Luteolin	−26.44	−21.61	−20.68	−22.94	−18.80	−18.93	−9.06	−8.78	−8.49	−8.53	−8.58	−8.20
Quercetin	−27.19	−22.14	−22.41	−23.49	−21.31	−21.59	−8.41	−8.74	−8.34	−7.70	−8.85	−8.84
Xanthohumol	−26.77	**−27.46**	−25.70	**−28.75**	**−27.18**	−23.10	−11.15	−12.46	−10.11	−9.73	−11.05	−9.97

[a] The values above in bold show the lowest docking energy for the *L. haberi* flavonoids. Other notable low-binding-energy ligands are also indicated in bold.

[b] The crystallized ligand for 1kpm was vitamin E [114]. The crystallized ligand for 2b00 was glycholate [115]. The crystallized ligand for 1kqu was 6-phenyl-4(R)-(7-phenyl-heptanoylamino)-hexanoic acid [116]. The crystallized ligand for 1db4 was indole [118].

[c] There were no crystallized ligands for 1pod or 1bbc.

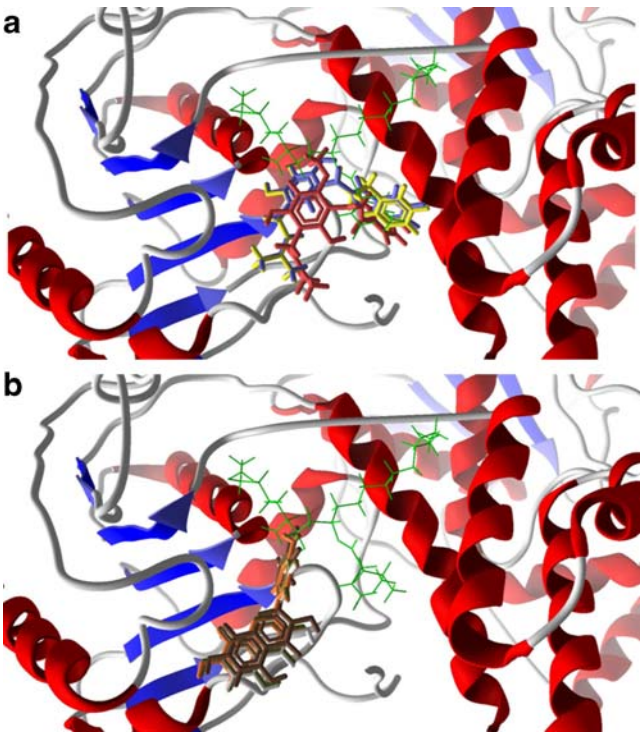


Fig. 4 Molegro overlay of the docked structures of LOHA6 (yellow), LOHA7 (blue), and xanthohumol (red), **a**, in the active site of fatty acid synthase (PDB: 2px6). Overlays of the non-prenylated flavonoids apigenin, kaempferol, luteolin, and quercetin are shown in **b**. The co-crystallized ligand (Orlistat) is shown as a green wire structure

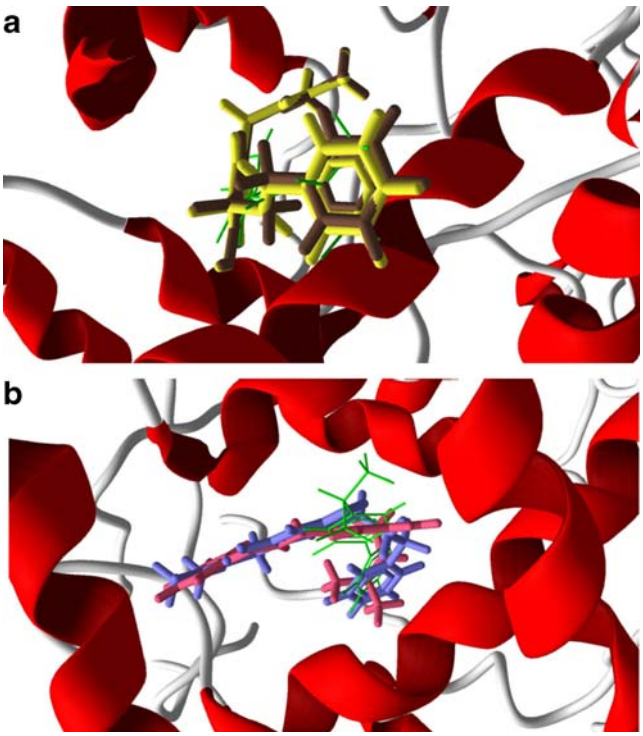


Fig. 5 Molecular docking of LOHA6 (brown) and LOHA7 (yellow) in the active site of COX-1 (PDB: 1eqh, **a**); docking of LOHA6 (violet) and LOHA7 (magenta) in the active site of COX-2 (PDB: 3pgh, **b**). The co-crystallized ligand (flurbiprofen) in each case is shown as a green wire figure

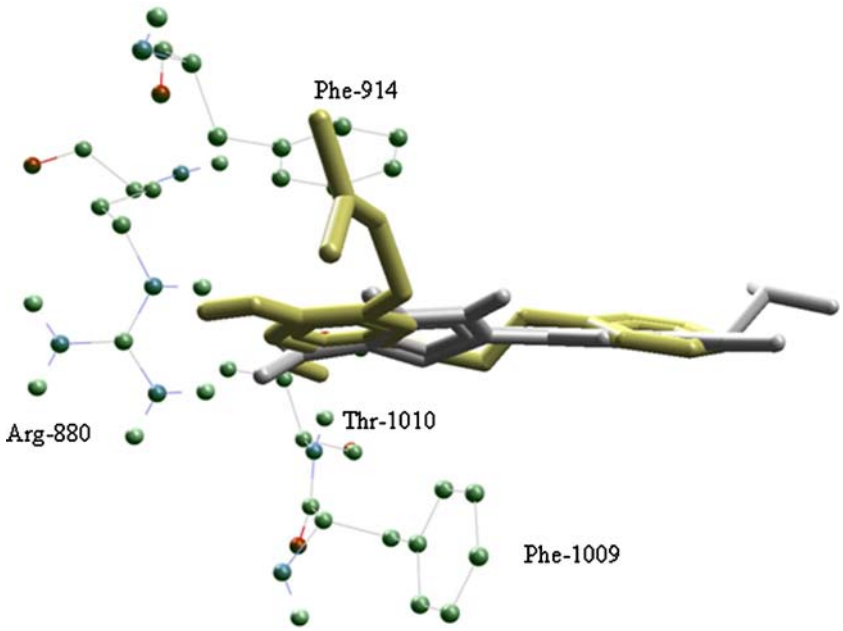


Fig. 6 Key intermolecular contacts between the docked ligands LOHA7 (tan) or the synthetic inhibitor TEI-6720 (grey) with Arg-880, Phe-914, Phe-1009, and Thr-1010, in the active site of xanthine oxidase (PDB: 1n5x)

docking energy. Both docking methods, however, revealed LOHA6 and LOHA7 to be close in energy and comparable in binding energy to the co-crystallized ligands. The co-crystallized ligand (4-androstene-3,17-dione) in human aromatase (PDB: 3eqm) forms hydrogen bonds to Met-374, Arg-115, and the heme cofactor in addition to hydrophobic interactions with Phe-134, Trp-224 and Ile-133. The docked orientations of both LOHA6 and LOHA7 share the same binding site with comparable ligand-protein interactions: hydrogen-bonding to Arg-115 and Met-374, hydrophobic interactions with Ile-133, Phe-134, and Trp-224, and electrostatic interactions with the heme cofactor. This binding site is somewhat different from that found by Paoletta and co-workers [123], however. In their docking studies, the ligands were in proximity to Asp-309, Thr-310, Pro-429, His-475, Ser-478, and Leu-479.

Flavonoid docking into fatty acid synthase (PDB: 1xkt and 2px6) also had LOHA6 and LOHA7 as the best binding ligands along with xanthohumol (Table 2). These prenylated flavonoids occupied the same locations at the active site (Orlistat binding site) of fatty acid synthase (Fig. 4). Key interactions between the docked ligands (LOHA6, LOHA7, and xanthohumol) were hydrogen-bonding interactions with Ser-2308, His-2481, and Tyr-2343, and hydrophobic interactions with Glu-2251 and Ile-2250. The Molegro binding energies for these three ligands were very similar. ArgusDock binding energies for LOHA6 and LOHA7 were also very similar, but lower in energy than xanthohumol by around 1.7 kcal mol^{-1}. Molecular docking of the known fatty acid synthase inhibitors luteolin, quercetin, kaempferol, and apigenin [29] revealed these flavonoid ligands to bind less strongly than the prenylated flavonoids LOHA6, LOHA7, and xanthohumol. In addition, the non-prenylated flavonoids occupied a somewhat different binding site than the prenylated ligands (Fig. 4).

Interestingly, the Molegro docking energies for the cyclooxygenase 1 (PDB: 1eqg and 1eqh) and cyclooxygenase 2 (PDB: 3pgh and 4cox) show the flavonoid ligands to generally show selectivity for COX-2 over COX-1 averaging around 4 kcal mol^{-1} (Table 3). ArgusDock energies show a similar trend, with the exception of LOHA6, but the energy differences are smaller, on the order of 0.5 kcal mol^{-1}. Both LOHA6 and LOHA7 showed excellent binding, comparable to the co-crystallized ligands, to the cyclooxygenases. Figure 5 shows the docked poses of LOHA6 and LOHA7 in the active sites of COX-1 and COX-2, along with the co-crystallized ligand, flurbiprofen. Key interactions in the docked orientations of

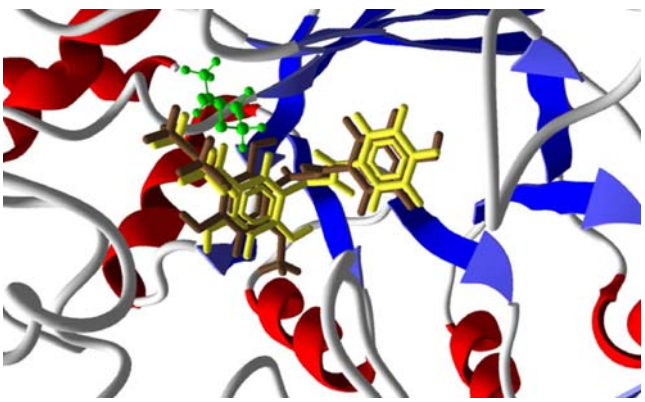


Fig. 7 Overlay of docked LOHA7 (yellow) and xanthohumol (brown) in the binding site of ornithine decarboxylase (PDB: 2on3). The co-crystallized ligand, 1-amino-oxy-3-aminopropane, is shown as a green ball and stick figure

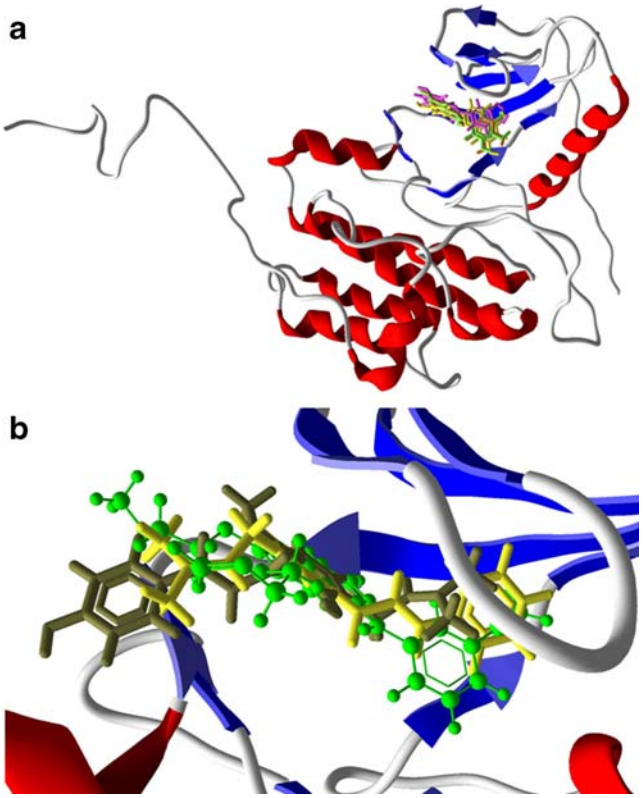


Fig. 8 Protein tyrosine kinase (PDB: 1m14) with overlays of the docked flavonoids apigenin, fisetin, kaempferol, myricetin, and quercetin, showing the consistency of flavonoid docking (**a**). Overlays of LOHA7 (yellow), xanthohumol (brown), and the co-crystallized ligand Erlotinib (green ball and stick) in the binding site of 1m17 (**b**)

LOHA6 and LOHA7 in COX-1 were hydrogen-bonding to Ser-530 and Tyr-385 and a hydrophobic pocket formed by Trp-387 and Ala-527 (that accommodated the prenylated phenyl groups of LOHA6 and LOHA7). These docked orientations were slightly different than those observed for flavonoid docking to COX-1 reported by Wu and co-workers [124]. In the previous work, the flavonoids docked at the gate of the active site whereas the present work shows the ligands to dock directly into the active site (see Fig. 5). The lowest-energy poses for LOHA6 and LOHA7 in COX-2 is largely hydrophobic made up of Tyr-355, Ala-527, Val-523, Val-349, Leu-352, Val-116, and Arg-120. The flexibility of the prenylated flavonoids apparently allow these materials to adopt better conformations upon docking to COX-2 than more rigid molecules such as indigocarpan [125]. Maia and co-workers had observed similar behavior with prenylated xanthones docking to transthyretin [126].

The tightest binding ligand for xanthine oxidase (PDB: 1vdv, 1fo4, 1n5x) as revealed in this study was LOHA7 (Table 4). Molegro calculations showed about 3 kcal mol^{-1} better binding for LOHA7 than the next best ligand, LOHA6, and comparable in binding energy to the synthetic co-crystallized inhibitors (Y-700 [92] and TEI-6720 [84]). The docked structure of LOHA7 in the binding site of xanthine oxidase shows similar spatial requirements and molecular contacts compared to the synthetic co-crystallized inhibitor, TEI-6720 (Fig. 6). There are key hydrogen bonding interactions between the ligands and Arg-880 and Thr-1010, face-to-face π–π interactions between the aromatic rings of the ligands and Phe-914, and edge-to-face π–π interactions between Phe-1009 and the aromatic rings of the ligands (see Fig. 6). These interactions are consistent to those previously reported by Omar and co-workers [127] who had carried out a molecular docking analysis of more than 100 flavonoid structures. These workers also found that hydrophobic interactions due to the prenyl groups decreased binding free energies. Lin and co-workers have carried out molecular docking studies on a number of phenylpropanoids [128] and coumarins [129] and these compounds also show docking to XO involving hydrogen bonding with Arg-880 and Thr-1010 as well as π–π interactions between the aromatic ring of the ligands and Phe-914.

Flavonoid ligands generally did not dock well into two of the lipoxygenase-3 crystal structures (PDB: 1n8q and 1no3) where the co-crystallized ligand was a simple substituted catechol (see Table 5). On the other hand, the

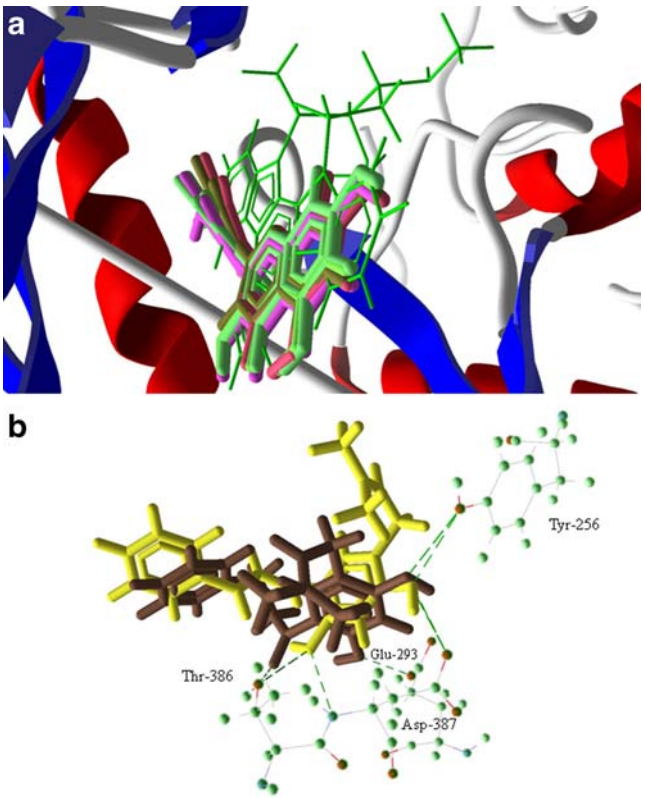


Fig. 9 Protein kinase C (PDB: 1xjd) with overlays of docked flavonoids apigenin, fisetin, kaempferol, luteolin, and quercetin, along with co-crystallized ligand staurosporine (**a**). Docked positions of LOHA6 and LOHA7 in the active site of protein kinase C (PDB: 1zrz) showing key hydrogen-bonded contacts (**b**)

crystal structure PDB: 1jnq, which was co-crystallized with the flavonoid epigallocatechin, did allow flavonoid ligands to dock well with this protein crystal structure. Apparently, the binding site for LOX-3 is flexible enough so that the structures 1n8z and 1no6 have binding sites that are too small to dock the flavonoid ligands well. Nevertheless, LOHA6 did show docking into all three crystal structures of lipoxygenase-3 examined. In addition, LOHA6, along with LOHA7 and xanthohumol, all docked into 1jnq with stronger binding energies than the co-crystallized ligand, epigallocatechin, or quercetin, a known flavonoid inhibitor of LOX [48].

The flavonoid ligands that docked best into ornithine decarboxylase (PDB: 1d7k, 2on3) were LOHA7 and xanthohumol (Table 6, Fig. 7). In most cases xanthohumol docked better than LOHA7. However, in all cases LOHA7 and xanthohumol had lower docking energies than the co-crystallized ligands. The known flavonoid inhibitors for ornithine decarboxylase are quercetin and apigenin [56, 57], and both LOHA7 and xanthohumol showed generally better docking energies than either of those inhibitors by about 1–2 kcal mol^{-1}. Key ligand-protein interactions for both LOHA7 and xanthohumol were Lys-69, Asp-332, and Asp-361 (hydrogen bonding); His-197 (electrostatic); and Tyr-389 (π–π interactions). Xanthohumol was additionally stabilized by hydrogen bonding to Phe-170 and Ser-200, which were not possible in the case of LOHA7.

Flavonoid ligands generally docked well to protein tyrosine kinase (PDB: 1m14, 1m17, and 1xkk) (Table 7, Fig. 8). ArgusDock consistently predicted LOHA6 to be the strongest binding ligand, slightly better than LOHA7, whereas Molegro favored LOHA7 over LOHA6. Xanthohumol was also shown by both docking programs to be a relatively tight binding ligand. The binding site for the prenylated flavonoid ligands in tyrosine kinase is largely hydrophobic (Ala-719, Val-702, Gly-772, Leu 820, and Leu-694).

As was the case for protein tyrosine kinase, molecular docking indicates protein kinase C (PDB: 1zrz, 1xjd, 1byg) to be a good target for flavonoids, especially the prenylated flavonoids (Table 8). The non-prenylated flavonoids generally dock in the same orientation (Fig. 9a). LOHA6, LOHA7, and xanthohumol are more flexible and tend not to adopt similar orientations, but they do dock into the active site of PKC forming hydrogen-bonded interactions with Thr-386, Asp-387, Glu-293, and Tyr-256 (Fig. 9b). Both Molegro and ArgusDock showed the prenylated flavonoids to dock better than the known flavonoid inhibitors, fisetin, luteolin, and quercetin [61, 62]; on average 2.6 kcal mol^{-1} stronger binding energies.

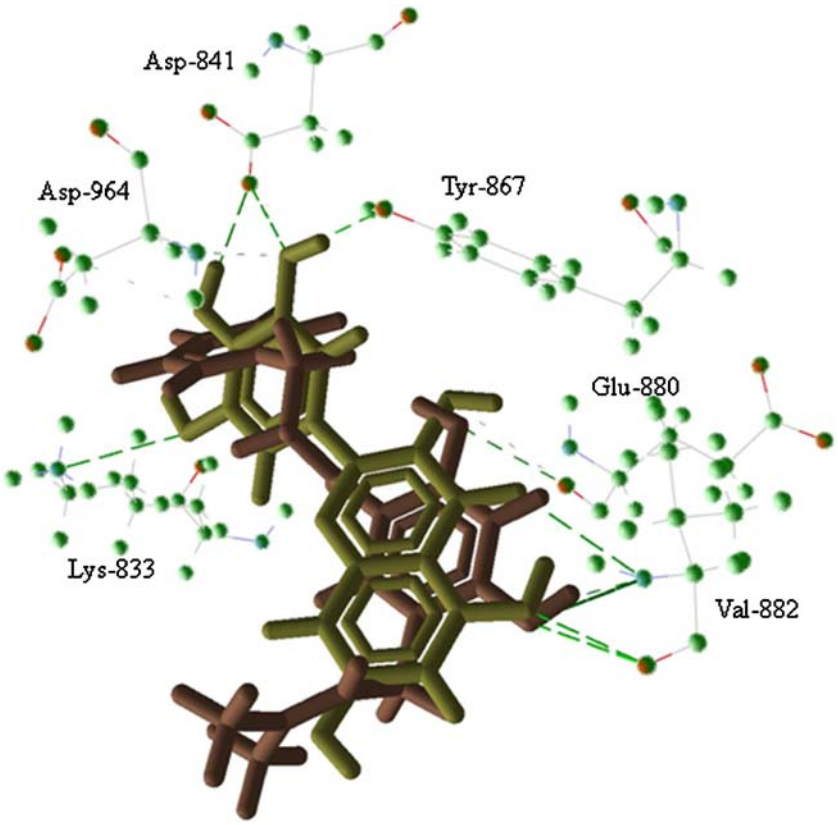


Fig. 10 Overlay of myricetin and LOHA6 in the ATP binding site of phosphoinositide 3-kinase (PDB: 1e90) showing key hydrogen-bonding interactions between the ligands and the protein

Of the *L. haberi* flavonoids, both LOHA6 and LOHA7 docked best into the ATP binding site of phosphoinositide 3-kinases (PDB: 1w2c, 1w2d, 1e8w, 1e90, 1e8z, 1e8y). The binding energies for these two flavonoids were similar for each of the docking programs (Table 9). Other flavonoids that showed strong binding energies were myricetin, a known inhibitor [67] and binding ligand [70] for PI3K, and xanthohumol. These ligands show hydrogen-bonding interactions with Lys-833, Asp-841, Tyr-867, Glu-880, Val-882, and Asp-964 at the ATP binding site of the protein (Fig. 10).

The ATP binding site of topoisomerase II has been identified as a binding site for flavonoids [74] and both Molegro and ArgusDock show flavonoids to generally dock well to this target (Table 10). Of the flavonoids examined, the prenylated flavonoids LOHA6, LOHA7, and xanthohumol docked with the lowest docking energies into the ATP binding site of topoisomerase II (PDB: 1qzr, 1zxm). A consistent feature of the docking of these prenylated flavonoids is that either a phenyl group or the prenyl group fits into a hydrophobic pocket (that normally binds the purine of ATP) formed by Phe-121 and Asn-70 of structure 1qzr (Phe-142 and Asn-91 of 1zxm) (Fig. 11).

Flavonoids, both non-prenylated and prenylated, showed tight docking into the ATP binding sites of ATP-binding cassette (ABC) transporters (PDB: 1mv5, 2cbz, and 1l2t) (see Table 11 and Fig. 12). Consistent with docking into other ATP binding sites (above), the prenylated ligands LOHA6, LOHA7, and xanthohumol, generally docked with stronger binding energies than the non-prenylated ligands. Badhan and Penny had found that prenylation of flavonoids resulted in more stronger binding to human P-glycoprotein transporter ABCB1, a member of the ATP binding cassette transporters [130], which can be attributed to the positive contribution of hydrophobicity in these materials [131]. The docking orientations of LOHA6, LOHA7, and xanthohumol, were very similar (Fig. 12c) with the prenylated phenyl group in a pocket surrounded by hydrogen-bonding amino acids (Thr-1046, Gly-1041, and Ser-1045 of PDB: 1l2t) and the non-prenylated phenyl group fitting into a hydrophobic sandwich formed by Tyr-1011, which formed a face-to-face π–π complex with each phenyl group, and the R-group of Gln-145.

Both LOHA6 and LOHA7 docked very well into phospholipase A_2 (PDB: 1kpm, 2b00, 1kqu, 1pod, 1db4, 1bbc) as did xanthohumol (Table 12). Phospholipase A_2 is known to be inhibited by the flavonoid quercetin [20]. Both LOHA6 and LOHA7 exhibited very similar docking orientations (Fig. 13) and generally docked more favorably than quercetin. The binding site in x-ray structure PDB: 1kpm shows a hydrophobic pocket made up of Leu-2, Ile-19, Phe-5 (which forms an edge-to-face π–π interaction with the prenylated phenyl groups of the ligands), and Trp-31 (which serves as a trap-door providing a face-to-face π–π interaction with the non-prenylated phenyl groups of the ligands) (see Fig 13b). The other crystal structures of PLA_2 do not have Trp-31, but the Phe-5 and Leu-2 interactions are conserved. The hydrophobic trap-door in 1kqu, 1db4, and 1pod is served by Val-30 and Phe-23; Val-30 is replaced by Gly in 1bbc and 2b00 but Phe-23 remains.

Summary and conclusions

Flavonoids have been shown to be potential anticancer agents by virtue of binding to some key targets such as aromatase (CYP 19), fatty acid synthase (FAS), xanthine oxidase (XO), cyclooxygenases (COX-1 and COX-2),

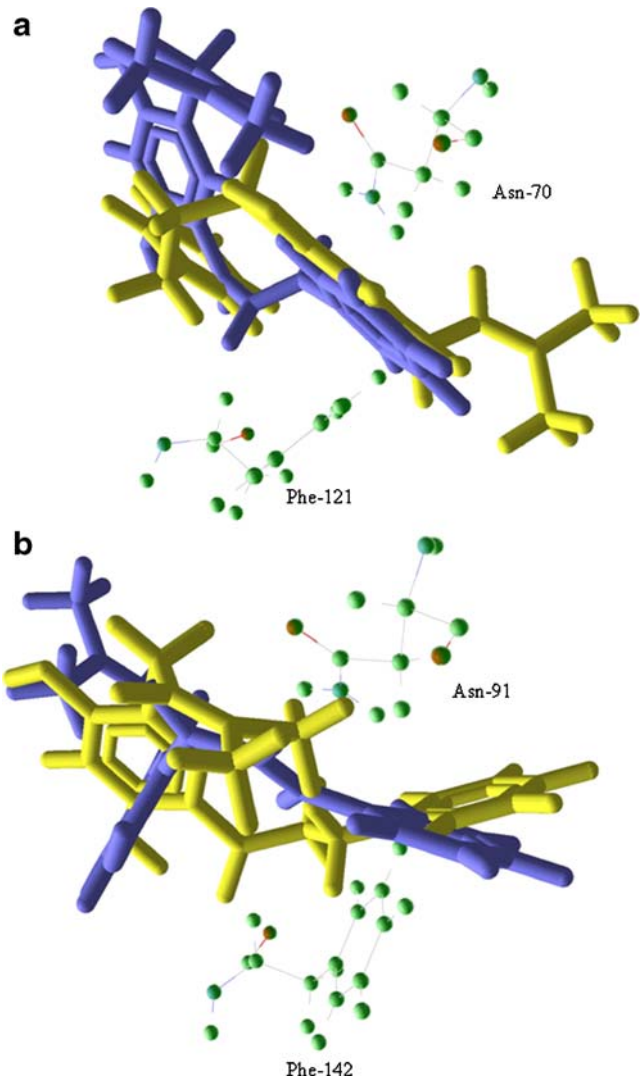


Fig. 11 LOHA6 (yellow) and LOHA7 (blue) docked into the ATP binding sites of yeast topoisomerase II, PDB: 1qzr (**a**) and human topoisomerase IIα, PDB: 1zxm (**b**) showing the hydrophobic binding pocket

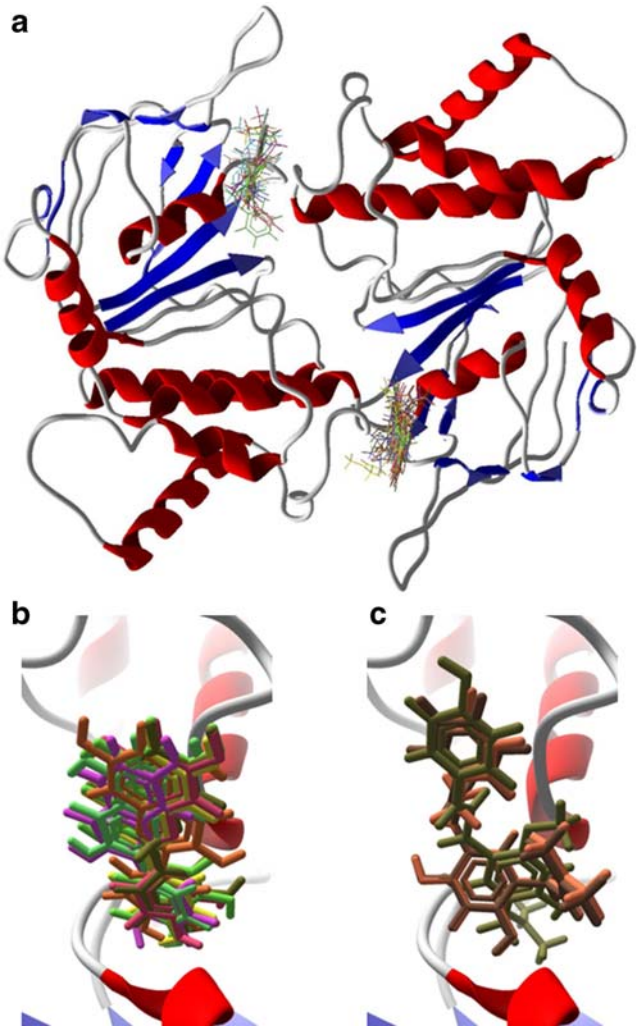


Fig. 12 Bacterial ABC transporter cassette (PDB: 1l2t) with overlays of all docked flavonoid ligands into the ATP binding site (**a**) as well as close-up views of overlays of the non-prenylated flavonoids (**b**) and the prenylated flavonoids (**c**)

lipoxygenase (LOX-3), ornithine decarboxylase (ODC), protein tyrosine kinase (PTK), phosphoinositide 3-kinase (PI3K), protein kinase C (PKC), topoisomerase II (ATP binding site), ATP binding cassette (ABC) transporter, and phospholipase A_2 (PLA). Flavonoids isolated from *Lonchocarpus haberi* as well as other representative flavonoids have been studied using molecular docking methods. Based on the molecular docking studies presented here, the consistently best docking *L. haberi* flavonoid ligands were LOHA6 and LOHA7, and these results are consistent with the slightly greater cytotoxicities observed for LOHA6 and LOHA7 compared to LOHA8 and LOHA9 [84]. It may be that the greater flexibility of the chalcone derivatives (LOHA6 and LOHA7) allows for better binding that the more rigid flavanones (LOHA8 and LOHA9). The prenylated *L. haberi* flavonoids generally docked with stronger binding energies than non-prenylated flavonoids and are comparable to the *Humulus lupulus* chalcone xanthohumol. The hydrophobic nature of the prenyl groups presumably facilitates interaction with hydrophobic regions in the flavonoid binding sites in the target proteins. We conclude, therefore, that the *L. haberi* flavonoids might owe their cytotoxic activity to inhibition of one or more of these enzymes. Aromatase, fatty acid synthase, cyclooxygenase, xanthine oxidase, lipoxygenase, and ornithine decarboxylase were seen to be the better of the molecular targets for the *L. haberi* flavonoids. In each of these target proteins the *L. haberi* flavonoids either showed lower or comparable binding energies compared to the crystallized ligands or known flavonoid inhibitors. Cytotoxicities of the *L. haberi* flavonoids, which ranged from 100 to 300 μM [69], were generally lower that those reported for xanthohumol (average around 14 μM) [132–135] or for other representative flavonoids such as apigenin [136–139], fisetin [139], or luteolin [139–141]. Non-prenylated chalcones [142, 143] have shown cytotoxic activities comparable to LOHA6 and

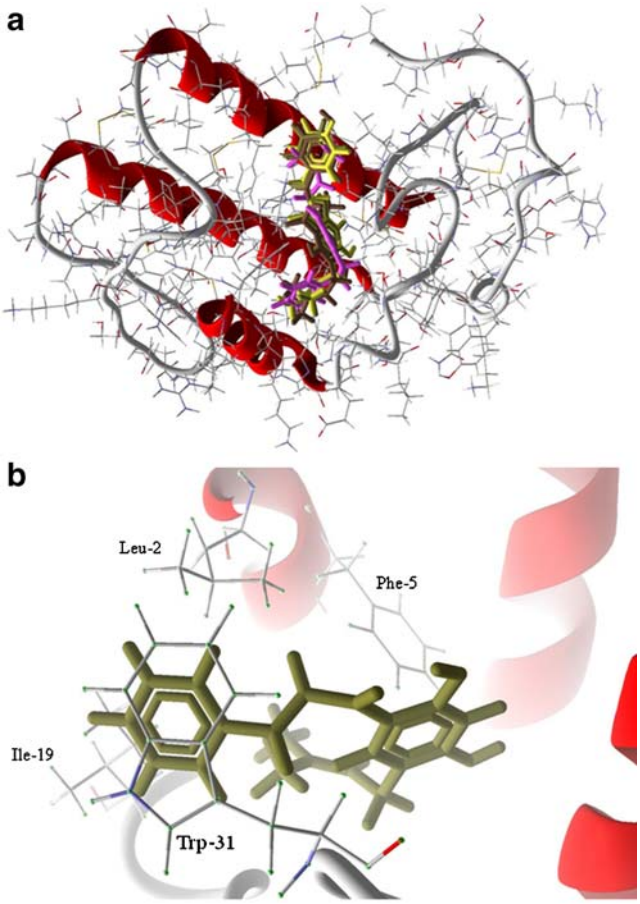


Fig. 13 Overlay of docked prenylated flavonoids into phospholipase A_2 (PDB: 1kqu) (**a**). Major hydrophobic interactions in the binding site of PLA_2 (PDB: 1kpm) shown with docked ligand LOHA7 (**b**)

LOHA7, whereas the prenylated chalcones derricin and lonchocarpin, isolated from *Lonchocarpus sericeus*, showed greater cytotoxities [37]. Thus, the cytotoxic activities of *L. haberi* flavonoids cannot be attributed to binding energies alone. We anticipate that additional promising antineoplastic flavonoids can be obtained from other *Lonchocarpus* species.

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