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Molecular construction of NADH-cytochrome b5 reductase inhibition by flavonoids and chemical basis of difference in inhibition potential: Molecular dynamics simulation study

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ABSTRACT

NADH-cytochrome b5 reductase, a flavoprotein, plays a central role in many diverse metabolic reactions. NADH-cytochrome b5 reductase has been shown to be responsible for the generation of free radicals from heterocyclic amines. Flavonoids compounds share remarkable similarity in structure but showed differences in their cytochrome b5 reductase inhibition pattern. Our molecular dynamics simulation studies revealed that the difference in substitution at C3 position of ring C may lead to difference in interaction with enzyme. Absence of hydroxyl group substitution at C3 in luteolin facilitates the strong cation-π interaction between Lys185 and ring A, and C and π-π between Phe92 and ring A, and C along with h-bonding between Lys185 and oxo group. Ring B of luteolin showed strong π-π interaction with FAD. These interactions were found absent in quercetin and taxifolin. These results suggest that absence of hydroxyl group substitution at C3 increases the potency of flavonoid inhibitors for cytochrome b5 reductase.

Keywords: NADH-cytochrome b5 reductase, luteolin, quercetin, taxifolin, molecular dynamics simulation.

INTRODUCTION

NADH-cytochrome b5 reductase (EC 1.6.2.2), a flavoprotein, transfers the electrons from NADH to cytochrome b5 which plays a central role in many diverse metabolic reactions in liver such as in fatty acid desaturation, elongation of fatty acids, biosynthesis of cholesterol, plasmologen synthesis, prostaglandin synthesis and drug metabolism involving cytochrome P450 mixed function oxidations (Arinc, 1991, 1995). Microsomal cytochrome b5 reductase and cytochrome b5 can stimulate cytochrome P450 catalyzed monooxygenase reactions (Porter, 2002). Apart from this, cytochrome b5 reductase alone can directly catalyze the metabolism of a wide range of xenobiotics, thus affect their toxicological or therapeutic effects. NADH-cytochrome b5 reductase has been shown to be responsible for the generation of free radicals from heterocyclic amines (Maeda, 1999). Cytochrome b5 reductase using NADH reduces heterocyclic amines to free radicals which in turn catalyze the transfer of one electron to molecular oxygen, thereby producing superoxide radical.

Thus, in addition to the highly reactive DNA-adduct forming metabolites formed by cytochrome P450 isozymes through oxidative pathways, reactive oxygen species generated in the presence of cytochrome b5 reductase may contribute to the carcinogenic effects of heterocyclic amines (Hodnick and Sartorelli, 1993; Holtz et al., 2003). As the above-mentioned studies demonstrate, it appears that the microsomal cytochrome b5 reductase is a clinically and toxicologically important enzyme. Recently, celik et al (2012) demonstrated the inhibitory effects of flavonoids of variable structure on the activity of purified bovine liver cytochrome b5 (Celik and Kosar, 2012). Although, these compounds share remarkable similarity in structure but showed differences in inhibition potency. Modulation of cytochrome b5 reductase by flavonoids with physiologically relevant IC_{50} values may have implications in toxicity or bioactivity of certain drugs and carcinogens such as increased toxic effects or reduced therapeutic efficacy (Celik and Kosar, 2012).

The present study was conducted to investigate the structural requirements necessary for inhibition of cytochrome b5 reductase activity by structurally related polyphenols (luteolin, quercetin and taxifolin) and role of residues which facilitae the binding of inhibitors using molecular dynamics simulation along with molecular basis of difference in inhibitor potency. Such compounds, by modulating the activity of cytochrome b5 reductase, could have potential effects in the therapeutic effectiveness of a wide range of drugs or in the toxicities associated with certain drugs or carcinogens.

COMPUTATIONAL METHOD

MD simulation of the complex was carried out with the GROMACS 4.5.4 package using the GROMOS96 43a1 force field (Berendsen et al., 1995; Lindah et al., 2001). The crystal structure of NADH-cytochrome b5 reductase (pdb id 1NDH) obtained from RCSB protein data bank and the structures of inhibitors generated from smile strings.The highest scoring docking conformation of cyt b5 reductase-inhibitor complex generated by Patchdock server (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005) was taken as initial conformation for MD simulation. The topology parameters of proteins were created by using the Gromacs program. The topology parameters of luteolin, quercetin and taxifolin were built by the Dundee PRODRG server (Schuttelkopf et al., 2004; Van Gunsteren el al., 1996). The complex was immersed in cubic box of simple point charge (SPC) water molecules (Van Gunsteren el al., 1998). The solvated system (enzyme, inhibitor and water) was neutralized by adding 4 Na ions. To release conflicting contacts, energy minimization was performed using the steepest descent method of 10,000 steps followed by the conjugate gradient method for 10,000 steps. MD simulation studies consist of equilibration and production phases. To equilibrate the system, the solute (protein, counterions, and inhibitor) were subjected to the position-restrained dynamics simulation (NVT and NPT) at 300 K for 300 ps. Finally, the full system was subjected to MD production run at 300 K temperature and 1 bar pressure for 10,000 ps. For analysis, the atom coordinates were recorded at every 0.5 ps during the MD simulation.

RESULTS AND DISCUSSION

The molecule of NADH-cytochrome b5 reductase is composed of two domains, the FAD binding domain (residues 2-118) and the NADH domain (residues 119-272) (Nishida et al., 1995). The highest scored docking pose for inhibitors (luteolin, quercetin and taxifolin) revealed that all the inhibitors occupied the NADH binding domain (Fig 1).

These docking poses were subjected for the molecular dynamics simulation for 10 ns. RMSD of backbone atoms with respect to the initial conformation was calculated as a function of time to assess the conformational stability of the protein during the simulations. Fig. 2(A) shows that the RMSD profiles were always less than 0.35 nm for the entire simulation which suggested the stability of enzyme-ligand complex, however, taxifolin bound form showed slight higher value of the RMSD. An initial steep rise in the RMSD for the first ~1000 ps and subsequently a constant profile was observed. These results showed that the trajectories of the MD simulations after equilibrium were reliable for post analyses. RMSD profile of ligands showed more or less constant pattern below 0.10 nm with marked fluctuation at different time interval (Fig. 1(B)). Luteolin showed sudden increase in RMSD after ~2500 ps which might be associated with the high conformational rearrangement and significant movement at binding site. Analysis of RMSD of inhibitors suggested stability of inhibitors for binding site during MD simulation.

Number of H-bonds (cut off 0.35 nm) which were formed during MD simulation between inhibitors and Cyt b5 reductase also calculated. A variable profile was observed with an average value of 0.67, 0.52 and 0.76 for luteolin, quercetin and taxifolin respectively. To elucidate the participation of surrounding residues in inhibitor binding, we individually calculated the average SR electrostatic energies and van der Waals of interaction between inhibitors, and residues (selected on the basis of 2-D plots of docking pose see fig.1) for last 4000 ps and summarized in Table 1. Van der Waals interaction energies were found dominant to support the binding of inhibitors. luteolin showed high negative value of vdWs with Phe85, Thr153, Lys185, Asp186, Pro249 and Met250. Apart of these vdWs interaction, luteolin showed very high (negative) value of electrostatic energy (-40.94 Kj/mol) with residue 185 which was found completely absent in case of quercetin and taxifolin. Quercetin showed vdWs interaction with Phe 85, Gly151, Thr153, Gln182, Asp186, Pro248, Pro249 and Met250. Quercetin binding was not favored by the electrostatic energy. Taxifolin showed more or less similar interaction as quercetin and was showed vdWs interaction with Gly151, Thr153, Gln182, Pro248, Pro249 and Met250. These results suggested that the dominant interaction of luteolin with residue Lys185 might be the one of the reason behind high inhibitory activity which was found negligible in case of rest of two. To found the other interacting residues and groups of inhibitors which participate in binding, we generated the 2D plots at the difference of 2 ns of

simulation by Discovery studio 3.1 (Accelrys, 2011). We found that ring A and C involved in cation-π intetraction with Lys185 and π-π interaction with Phe92. The π-π interaction with Phe92 showed 50% appearance in 2D interaction plots. Ring B showed strong π-π interaction with FAD. Oxo group at C4 position formed h-bonding with Lys185 along with Gln182 (Fig 3). In case of quercetin, oxo group at C4 form h-bonding with residue Thr153. Quercetin binding mostly favored by vdWs forces (Fig 4). The oxo group of taxifolin at C4 form h-bonding with residue Met250 and Ile251 (Fig 5).

These results were found in coherence with the interaction energy profiles and revealed that those inhibitors interact with Lys185 and Phe 92 showed potent inhibitory effect. Celik et al., (2012) mentioned that a hydroxyl group substitution at C3 contributed negatively to the potency of enzyme inhibition, as shown by higher inhibitory activity of luteolin than that of the quercetin, which differs from luteolin only at ring position C3. The lack of this hydroxyl group increased the inhibitory potency of inhibitor by 10-fold as evidenced by the IC_{50} values of quercetin and luteolin (Celik and Kosar, 2012). Our results was found in the agreement with the above mentioned statement as the absence of hydroxyl group substitution at C3 facilitated the interaction of ring A and C and oxo group with Lys185 and Phe 92 in case of luteolin which lead to more efficient inhibition as compared to quercetin. On the other hand, the poor inhibitory effect of the taxifolin might be due to absence of C2-C3 double bond and the presence of a hydroxyl group substitution at C3.

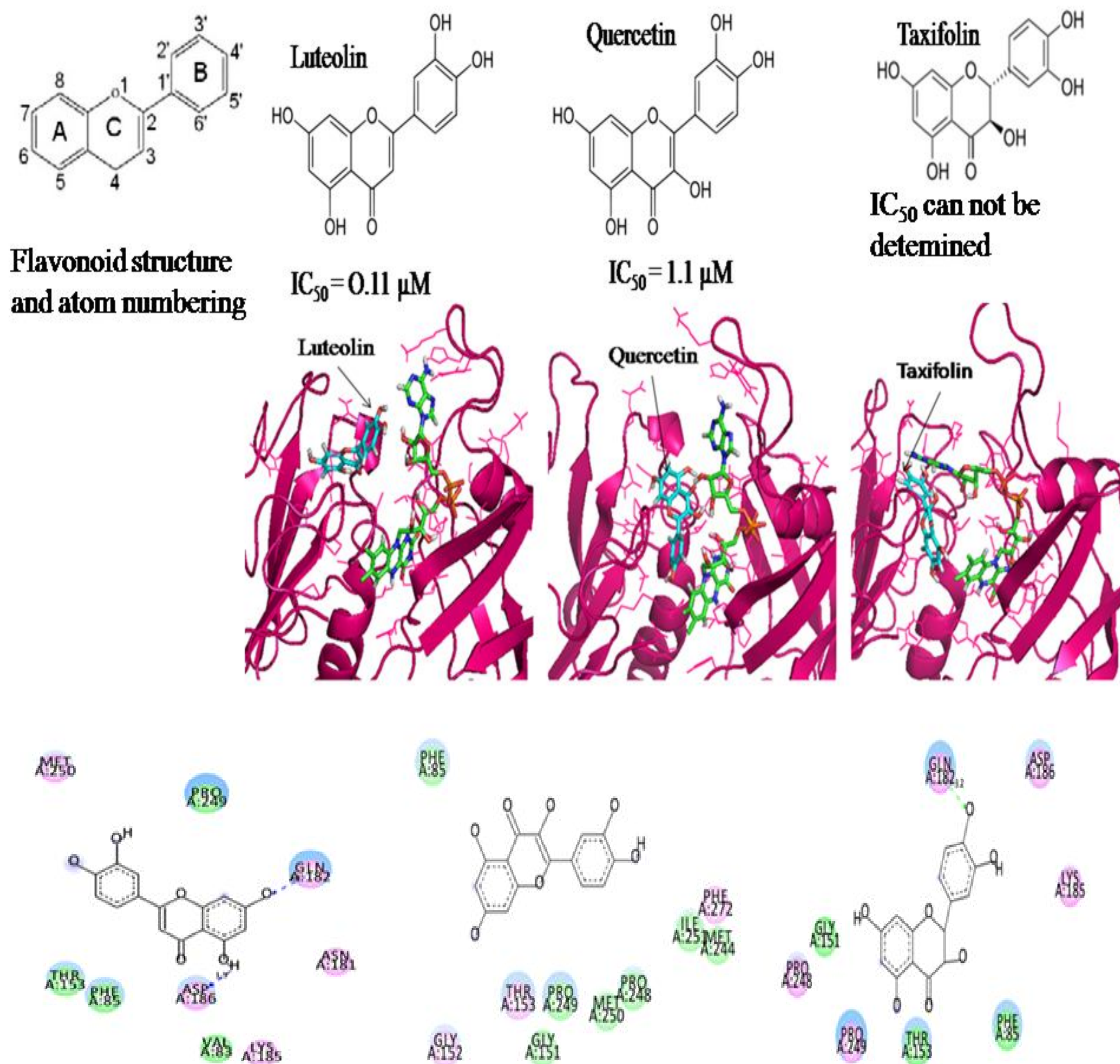

Fig. 1: Structure and binding pose of flavonoid inhibitors at NADH-cytochrome b5 reductase (IC_{50} values of inhibitors were taken from reference "Celik and Kosar, 2012").

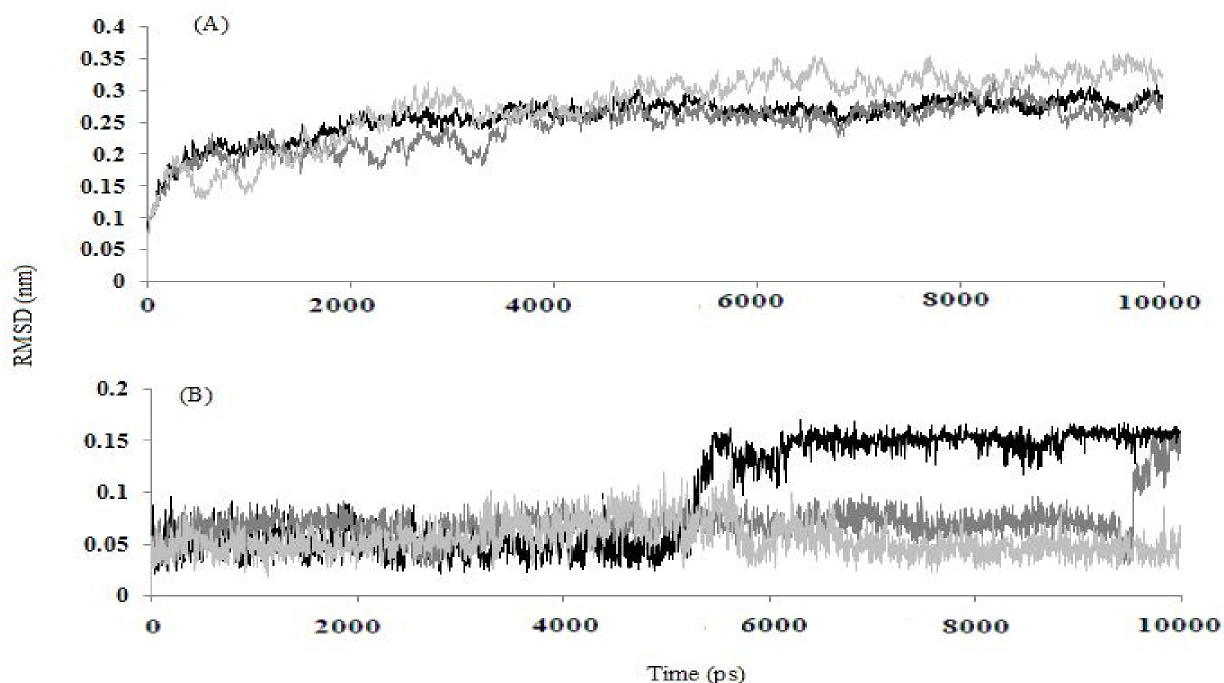

Fig. 2: (A) RMSD profile of NADH-cytochrome b5 reductase backbone atoms (black-luteolin bound, dark grey-quercetin bound, light grey-taxifolin bound), (B) RMSD profile of flavonoid inhibitors (black-luteolin, dark grey-quercetin, light grey-taxifolin).

Fig. 3: 2D presentation of interaction of luteolin with cytochromr b5 reductase residues at differentime of MD simulation.

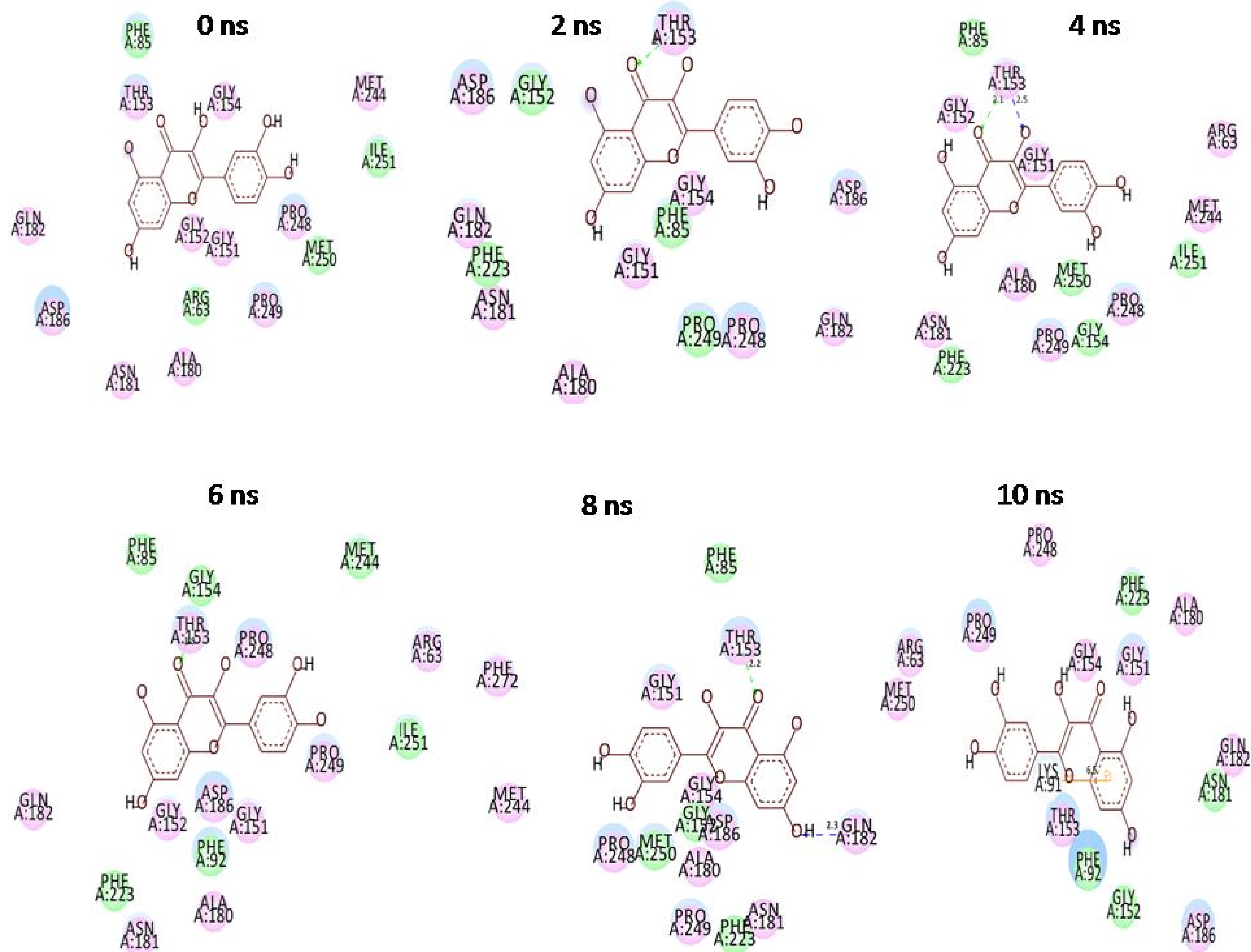

Fig. 4: 2D presentation of interaction of quercetin with cytochromr b5 reductase residues at differentime of MD simulation.

Table. 1: Account of van der Waals and electrostatic interaction energies between flavonoids and surrounding residues.

Residue	Inhibitor	vdWs (Kj/mol)	Electrostatic (Kj/mol)
Phe85	Luteolin	-10.22	-0.37
	Quercetin	-3.26	-0.62
	Taxifolin	-1.04	-0.07
Gly151	Luteolin	-0.62	0.028
	Quercetin	-15.59	0.99
	Taxifolin	-16.62	1.20
Thr153	Luteolin	-7.10	0.30
	Quercetin	-16.17	-12.92
	Taxifolin	-17.43	-0.18
Gln182	Luteolin	-1.47	-1.60
	Quercetin	-6.26	1.13
	Taxifolin	-5.10	-0.91
Lys185	Luteolin	-9.55	-40.94
	Quercetin	-0.11	0.003
	Taxifolin	0.00	0.00
Asp186	Luteolin	-10.14	1.38
	Quercetin	-8.22	4.85
	Taxifolin	-0.15	0.015
Pro249	Luteolin	-11.41	-0.14
	Quercetin	-18.28	0.15
	Taxifolin	-16.59	-2.28
Met250	Luteolin	-8.14	-0.03
	Quercetin	-8.30	0.21
	Taxifolin	-14.2	-10.7

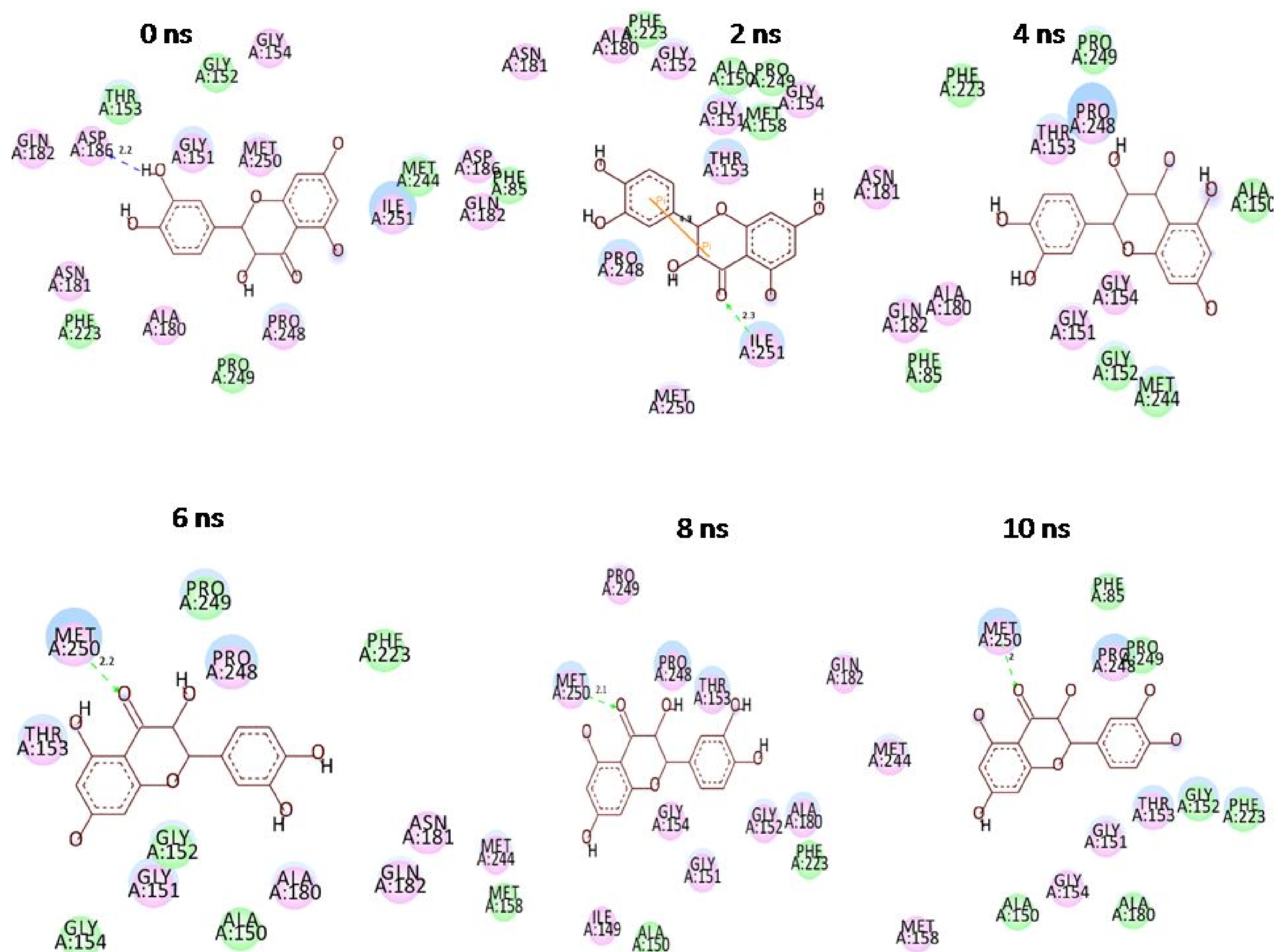

Fig. 5: 2D presentation of interaction of taxifolin with cytochromr b5 reductase residues at differentime of MD simulation.

CONCLUSION

NADH-cytochrome b5 reductase plays a central role in many diverse metabolic reactions. Flavonoids compounds share remarkable similarity in structure but showed differences in their cytochrome b5 reductase inhibition pattern. Our molecular dynamics simulation studies revealed that the difference in substitution at C3 position of ring C may lead to difference in interaction with enzyme. Absence of hydroxyl group substitution at C3 in luteolin facilitates the strong cation-π interaction between Lys185 and ring A, and C and π-π between Phe92 and ring A, and C along with h-bonding between Lys185 and oxo group. Ring B of luteolin showed strong π-π interaction with FAD. These interactions were found absent in quercetin and taxifolin. These results suggest that absence of hydroxyl group substitution at C3 increases the potency of flavonoid inhibitors for cytochrome b5 reductase.

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