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Complexity of the Role of Various Site-Specific and Selective Sudlow Binding Site Drugs in the Energetics and Stability of the Acridinedione Dye−Bovine Serum Albumin Complex: A Molecular Docking Approach

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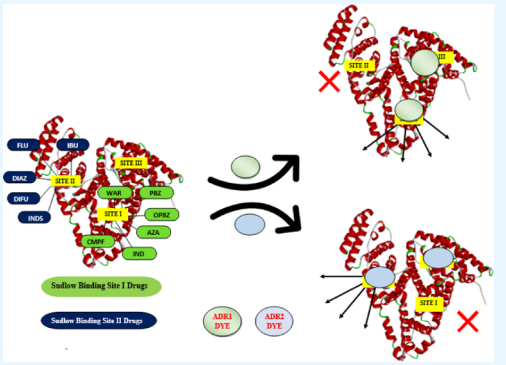
ABSTRACT: Molecular docking (Mol.Doc) techniques were employed to ascertain the binding affinity of two resorcinol-based acridinedione dyes (ADR1 and ADR2) with the widely studied globular protein Bovine Serum Albumin (BSA) in the presence of site-selective binding drugs by Autodock Vina 4.2 software. Docking of various feasible conformers of ADR1 dye with BSA was found to be energetically more favored than ADR2 dye, even though both these dyes differ in the 9th position of the basic dye structure. Analysis of dyes with BSA establishes the location of dye in all of the binding sites of BSA, predominantly through conventional and nonconventional hydrogen-bonding (HB) interactions. The coexistence of hydrophobic interactions resulted in the stability of various conformers generated. The introduction of site I and site II (Sudlow site binding drugs) into ADR1−BSA and ADR2−BSA complexes effectively destabilizes the dye−protein complex; however, the drugs do not displace ADR dyes completely from their selective binding domains. Site II binding drugs effectively destabilize the binding ability of the dye−protein complex rather than site I drugs. However, docking of site I drug 3-carboxyl-4-methyl-5-propyl-2-furanpropanic acid (CMPF) largely destabilizes the ADR1−protein complex, whereas indomethacin (INDO) enhances the binding affinity of the ADR2−protein complex. Interestingly, simultaneous docking of ADR dyes to the BSA−drug complex results in larger stability of the protein−drug complex through HB interactions rather than hydrophobic interactions. Both ADR1 and ADR2 dyes predominantly occupy the Sudlow binding sites of BSA, and the introduction of either site I or site II binding drugs does not displace the dye efficiently from the corresponding binding sites, rather the drugs are effectively displaced toward other binding domains apart from their specific site-binding domains of BSA. Through Mol.Doc techniques, we authenticate that the interactions in host−guest complex systems involving competing ligands are established in depth, wherein the dye as well as the amino acid (AA) moieties in BSA act as both HB donor and acceptor sites apart from several hydrophobic interactions coexisting toward the stability.



INTRODUCTION

Acridinedione (AD) dyes are well-known fluorophores that exhibit excellent binding affinities with serum albumin (SA).[1−3] Among various albumins, Bovine Serum Albumin (BSA) is the most widely studied protein involving fluorophores (dyes) and ligands (drugs). Our previous studies of BSA with photoinduced electron transfer (PET)- and non-PET-based AD dyes by fluorescence spectral techniques reveal the binding of dyes to several binding sites apart from Sudlow binding sites I and II. Employing fluorescence techniques, we have illustrated the role and governance of BSA in the ground and excited state properties of AD dyes[1,2] such that the dye molecule prefers to reside in a multiheterogeneous microenvironment (buried or exposed) comprising hydrophilic and hydrophobic phases. Further, spectroscopic techniques assisted by electrochemical and theoretical studies have imparted a crucial role in establishing the nature of interactions of several dyes with BSA.[4−34] Molecular docking (Mol.Doc) studies authenticate the binding domains and most preferred site of dye/drug in the protein structure. The combined theoretical and experimental approach provides substantial information on the nature of interactions in several studies involving ligands (dye or drug), which is of larger significance in the concept of biophysical chemistry at the molecular level. However, the role of competing ligands influencing the binding domains of BSA is an interesting

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and fascinating concept in the field of biochemistry and medicinal chemistry. The study involving non-steroidal anti-inflammatory drugs (NSAIDs) has been an area of ever explorable domain that has provided new significant insights into the binding parameters. The role of various types of drugs in inducing the denaturation properties of the primary, secondary, and tertiary structure of proteins is a concept that acts as a bridge between chemists and biologists in the concept of photophysical chemistry and photobiology.

Fluorescence studies of PET- and non-PET-based dyes with BSA have provided the most preferred site of binding of the dye−BSA system.[1,2] These dyes that involve in binding with BSA will either result in a decrease or increase in the absorption and emission spectra accompanied by conformational changes. These dyes are highly specific and site-selective in nature and are described as both site I and site II binding dyes. The role of drugs in dye−BSA binding characteristics is compared through the energetics and molecular interactions resulting from Mol.Doc studies. The structure of AD dyes employed in the present study is shown in Figure 1. These dyes, although similar in structure,

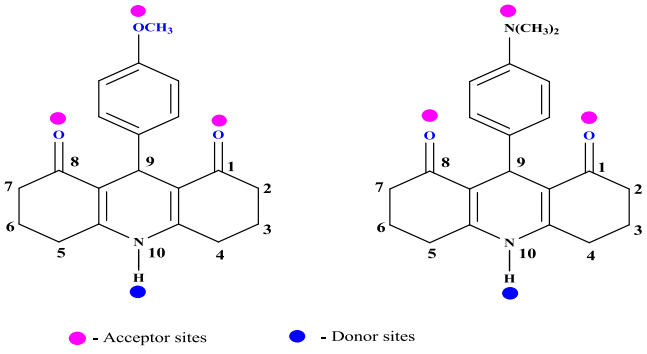

Figure 1. Structure of ADR1 and ADR2 dyes.

result in a distinct variation in the spectral properties on the introduction of BSA.[1,2] The ADR1 dye is categorized as a PET dye, whereas the ADR2 dye is a non-PET dye (in aqueous medium). Substantial information based on fluorescence emission and lifetime studies reveals the presence of dye in more than two binding domains of protein molecules. But the exact location of domains and the most preferred binding sites of ADR1 and ADR2 dyes in BSA could not be authenticated by fluorescence techniques due to uneven population distribution. Based on the above studies, we further analyzed and interpreted the simultaneous docking of site I and site II binding drugs to determine the binding stability of ADR1−BSA and ADR2−BSA systems through Mol.Doc tools. The extent of destabilization of dye−protein interactions is established by Mol.Doc studies, which accounts for several energy parameters and molecular interactions. The free energy of formation (BE) of dye−protein with drugs and that of drug−protein with ADR dyes are taken for comparison. The site I drugs employed in the present study are Warfarin (WAR), Phenylbutazone (PBZ), Oxyphenbutazone (OPBZ), Azapropazone (AZA), Indomethacin (INDO), and 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) and the site II drugs are Flufenamic acid (FLU), Ibuprofen (IBU), Diazepam (DIAZ), Diflunisal (DIFU), and Indoxyl sulfate (INDS). The structure of these drugs is well reported in the literature[35,36] and is provided in Supporting Figure S1.

RESULTS AND DISCUSSION

Binding of ADR Dyes with BSA. The energetics pertaining to the formation of a complex between ADR1 with BSA and ADR dye with BSA are provided in Supporting Tables S1 and S2, respectively. The binding energy (BE) associated with intermolecular, electrostatic, and torsional energies associated with various other molecular interaction energies attributed to several bimolecular interactions like van der Waals's energy, desolvation energy, and HB interactions is in particular accounted for the binding stability of the dye−protein complex. The 10 conformers generated for ADR1 with BSA are labeled as ADR1−BSA1−10, respectively, and are arranged in the

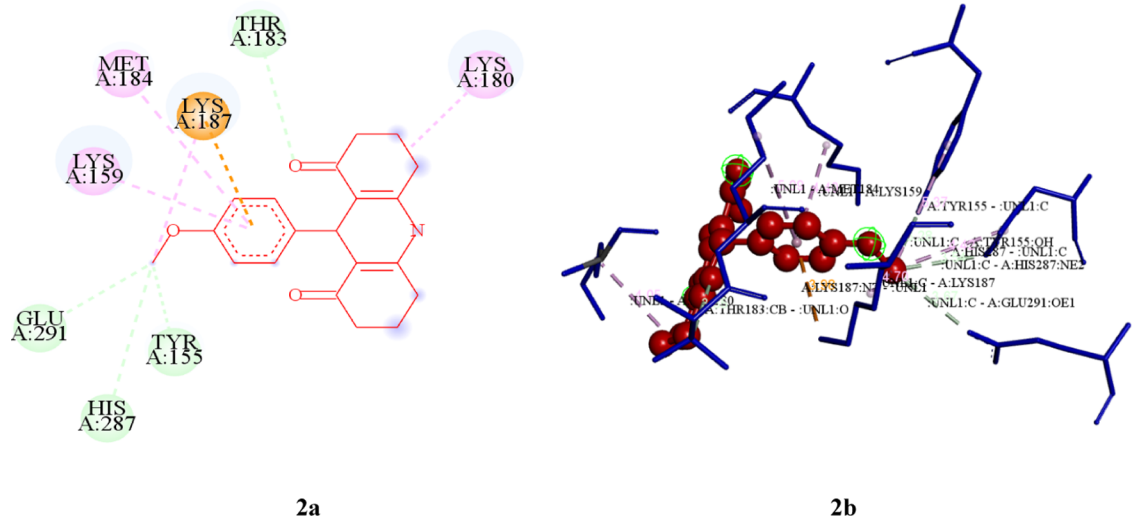

Figure 2. 2D and 3D diagram of ADR1−BSA1 visualized using Biovia Discovery Studio visualizer. Key amino acids contributing to interactions are shown in circles; DDP dye is represented in the ball and stick model in red color. Green color dotted line indicates hydrogen bonds with electronegative elements like N and O atoms; light green color dotted line indicates carbon−hydrogen bonds; light purple color indicates pi-alkyl interactions; and violet color dotted line indicates pi-sigma interaction. Light green color amino acids without interactions represent van der Waals interactions, and the red color amino acids indicate unfavorable interaction. The blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter.

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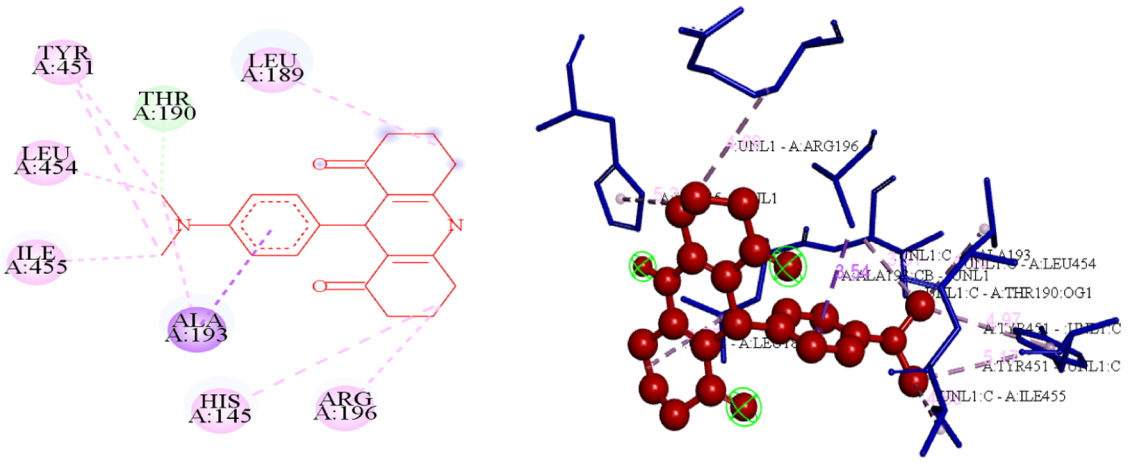

3a 3b

Figure 3. 2D and 3D diagrams of the ADR2−BSA1 conformer visualized using Biovia Discovery Studio visualizer. Key amino acids contributing to interactions are shown in circles; ADR2 dye is represented in red color. Green color dotted line indicates hydrogen bonds with electronegative elements like N and O atoms; light green color dotted line indicates carbon−hydrogen bonds; light purple color indicates pi-alkyl interactions; and violet color dotted line indicates pi-sigma interaction. Light green color amino acids without interactions represent van der Waals interactions, and the red color amino acids indicate unfavorable interactions. The blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter.

Table 1. Binding Sites and Subdomains of ADR1 with BSA

conformers	subdomains	binding sites
ADR1−BSA1	IB and IIA	III and I (Sudlow binding site)
ADR1−BSA2	IIA, IIB, IIIA	I and II (Sudlow binding sites)
ADR1−BSA3		
ADR1−BSA4		
ADR1−BSA5		
ADR1−BSA8		
ADR1−BSA6	IB, IIIA	III and II (Sudlow binding site)
ADR1−BSA10		
ADR1−BSA7	IA and IB	III
ADR1−BSA9	IIA	I (Sudlow binding site)

Table 2. Binding Sites and Subdomains of ADR2 with BSA

conformers	domains	binding sites
ADR2−BSA1	IB and IIIA	III and II (Sudlow binding site)
ADR2−BSA2	IIB and IIIA	II (Sudlow binding site)
ADR2−BSA3	IA and IIA	I (Sudlow binding site)
ADR2−BSA4	IIIA and IIIB	II (Sudlow binding site)
ADR2−BSA8		
ADR2−BSA5	IIB	
ADR2−BSA6	IIIA	II (Sudlow binding site)
ADR2−BSA7	IIA and IIB	I (Sudlow binding site)
ADR2−BSA9	IIA	I (Sudlow binding site)
ADR2−BSA10	IA	III

decreasing order of stability based on their combined energetics. In general, the BE is correlated with the free energy of formation of the dye−protein complex, which is usually represented in terms of thermodynamical parameter (ΔG).[37] Likewise, the conformers for ADR2 with protein molecule are labeled as ADR2−BSA1−10 and are arranged based on their BE values (ΔG).

The formation of dye−protein complex comprises several factors, which results in the stability of the conformers generated. In the present study, the negative value of ligand efficiency favors the stability of the complex, and the very high inhibitory constant value existing between the ligands and the guest decreases the docking efficiency, resulting in the least favored conformers. A larger negative value of intermolecular energy due to HB and electrostatic energy favors the stability of the conformers. The above parameters govern the ease of formation of the complex and play a predominant role in docking studies. This methodology has been well established in our earlier studies involving DDP dye with globular proteins[38−41] like BSA, HSA, ovalbumin, β-lactoglobulin, and myoglobin.

In the present study involving ADR1 dye docking with BSA, the presence of eight unique conformers was ascertained based on their binding sites and domains in BSA. Further, based on the energetics-associated parameters, the ligand efficiency of all 10 conformers was found to be in the range of -0.38 ± 0.6. However, the inhibitory constant values of the ADR1−BSA1 conformer were found to be the lowest compared to those of all other conformers. The variation in the ΔG value of the most stable conformer (ADR1−BSA1) with that of the least stable conformers (ADR1−BSA8−10) was >3 kcal mol^{-1}. Mol.Doc studies clearly visualize that there exist several conformers that substantially differ in energetics. The two-dimensional (2D) and three-dimensional (3D) structure of the most stable conformer ADR1−BSA1 complex is provided in Figure 2. For better resolution and clarity regarding the docking assemblies and mode of interaction, 3D structures of other ADR1−BSA complex conformers are provided in Supporting Figure S3.

Based on Mol.Doc studies, the docking of ADR1 dye with BSA is energetically most favorable and stable compared to that of ADR2 dye with BSA. The 2D and 3D structures of the most stable conformers of ADR2−BSA1 are provided in Figure 3 for better clarity regarding the energetics, and the 2D and 3D structures of other conformers of ADR2−BSA are provided in Supporting Figure S4. The formation of the most stable conformer ADR1−BSA1 has a ΔG value of −10.58 kcal mol^{-1}, whereas that of ADR2−BSA1 is −7.17 kcal mol^{-1}. The ligand efficiency, intermolecular energy, and energy associated

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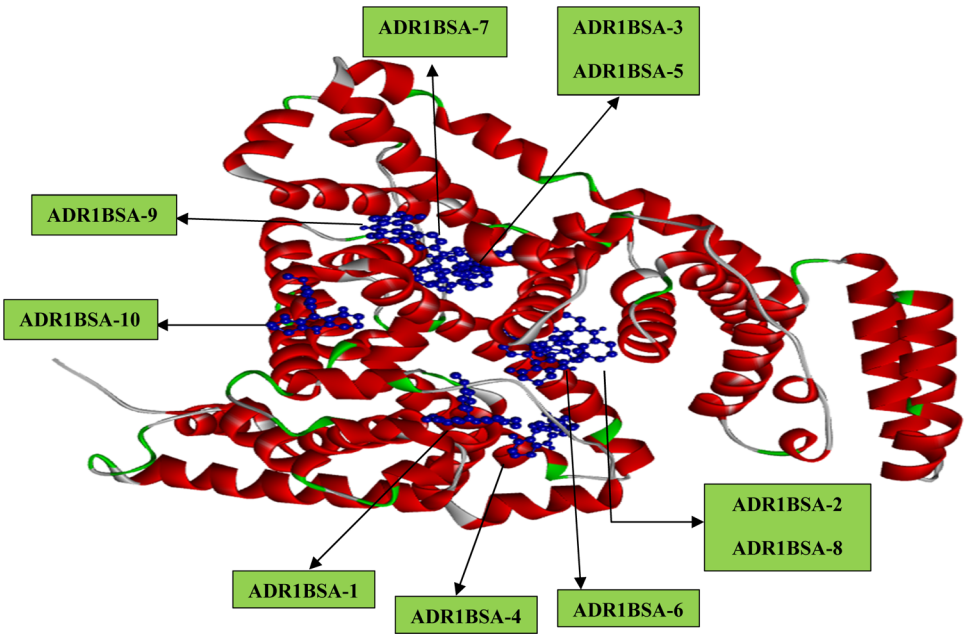

Figure 4. Ten conformations of ADR1 dye (depicted in a blue color ball and stick model) with three-dimensional structures of BSA (colored based on solid ribbon model; red color represents helix; green and white colors represent turns and coils).

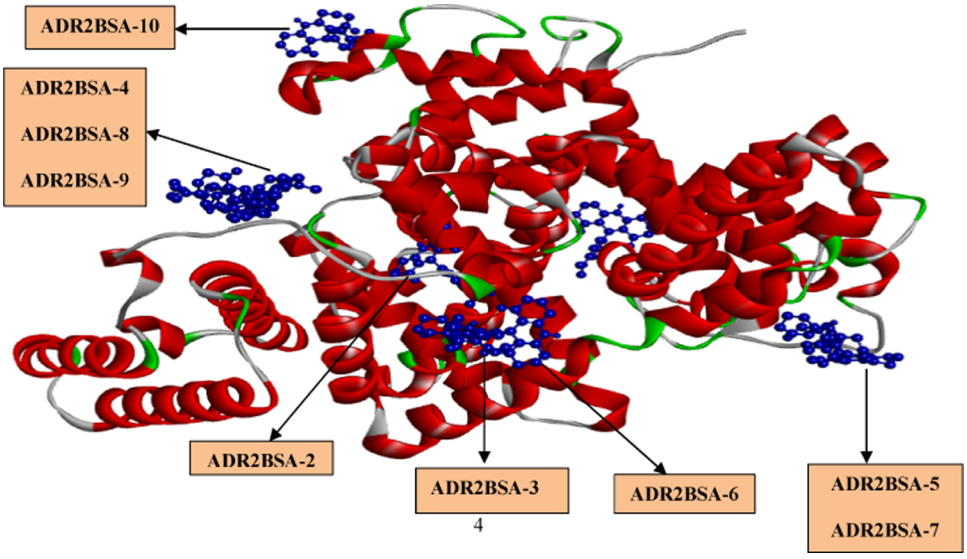

Figure 5. Ten conformations of ADR2 dye (depicted in a blue color ball and stick model) with three-dimensional structures of BSA (colored based on a solid ribbon model; red color represents helix; green and white colors represent turns and coils).

with various molecular interactions of the ADR2−BSA1 conformer is lower than that of the ADR1−BSA1 conformer. Mol.Doc studies reveal that the ligand efficiency values are lower in the case of ADR2 dye (−0.24 ± 0.05) than the ADR1 dye. Further, on evaluation and analysis of the various conformers generated for dyes with BSA, we ascertain that both ADR1 and ADR2 dyes are confined to all six major binding domains of the protein molecule, which supports the fluorescence lifetime distribution of dye confined to several domains of varying phases (hydrophilic and hydrophobic). Interestingly, unlike the interaction of specific site-selective drugs and ligands with BSA, ADR1 and ADR2 dyes predominantly reside in site I and site II (Sudlow binding sites) of BSA, but they also exist in site III of BSA, which is not a favorable binding site of site-specific drugs as mentioned in the literature pertaining to drug interactions with BSA. The illustration and compilation of the various conformers of ADR dye with BSA corresponding to the binding domains are provided in Tables 1 and 2. The overall schematic representation of the conformers generated for ADR1 and ADR2 dyes located in the various subdomains of BSA is provided in Figures 4 and 5, respectively.

Molecular Interactions of the Dye−Protein Complex. *ADR1−BSA Conformers.* HB interactions accompanied by several hydrophobic interactions (pi-alkyl, alkyl, pi-sigma, pi−pi) exist in all of these conformers generated. However, the largest stability of the ADR1−BSA complex is attributed to several HB and hydrophobic interactions. The number of HB (both conventional and nonconventional) and hydrophobic interactions for ADR1−BSA1 is four and eight, respectively. The molecular interaction table representing various interactions

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Table 3. Molecular Interaction Parameters of All Conformers of ADR1−BSA

conformation	binding energy	hydrogen-bonding interaction donor −acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance	other interactions
ADR1−BSA1	−10.58	GLU291 (C···O)	3.67	pi-alkyl		
		TYR155 (C···OH)	3.28	MET184	5.09	
		HIS287 (C···N)	3.54	LYS159	4.40	
		THR183 (C···O)	3.35	TYR155	5.32	
				HIS287	4.42	
				alkyl		
				LYS180	4.05	
				LYS187	4.70	
				pi-sigma	2.94	
				LYS187	3.98	
ADR1−BSA2	−9.92	ARG208 (O···HN)	3.05	pi-alkyl		
				ALA209	4.43	
				VAL481	4.57	
				alkyl		
				ARG208	4.10	
				ALA209	3.74	
				ALA212	3.59	
				ALA349	4.85	
				VAL481	4.60	
				LEU480	3.84	
				LEU346	4.59	
				pi-sigma		
				LEU480	3.84	
				LEU346	3.83	
ADR1−BSA3	−9.87	SER343 (O···O)	2.49	pi-alkyl		
		SER343 (N···O)	2.76	TRP213	5.36	
		SER453	3.88	TRP213	5.20	
		SER343	4.14	TRP213	3.93	
				LEU480	4.73	
				LEU197	5.14	
				alkyl		
				LEU454	5.12	
				LEU197	5.15	
				LEU197	4.70	
				LEU210	5.03	
				ARG483	4.54	
				ARG198	4.47	
ADR1−BSA4	−9.37	SER453 (O···O)	2.61	pi-alkyl		
		ASP450	3.36	VAL342	4.43	
				LEU197	5.26	
				TRP213	4.32	
				alkyl		
				LEU210	4.44	
				VAL342	3.98	
				LEU480	4.22	
				VAL481	3.79	
				LEU346	4.49	
				pi−pi T-shaped		
				TRP213	4.02	
				TRP213	5.68	
ADR1−BSA5	−8.74	SER343 (O···O)	2.85	pi-alkyl		
		SER343 (N···O)	2.71	TRP213	5.30	
		SER453	4.00	TRP213	4.02	
		SER343	4.14	TRP213	5.25	
				LEU480	4.71	
				LEU197	5.19	
				alkyl		
				LEU454	5.12	
				LEU197	5.07	
				LEU197	4.71	

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Table 3. continued

conformation	binding energy	hydrogen-bonding interaction donor −acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance	other interactions
				LEU210	4.94	
				ARG483	4.64	
				ARG198	4.40	
ADR1−BSA6	−8.73	THR190 (C···O)	2.89	pi-alkyl		
				ARG458	4.58	
				TYR458	5.28	
				alkyl		
				LYS431	4.57	
				LUE454	5.14	
				ILE455	4.25	
				ARG458	4.20	
				ALA193	3.83	
				ALA196	4.18	
ADR1−BSA7	−8.48	SER104 (C···O)	3.26	pi-alkyl		
				TYR84	5.12	
				alkyl		
				PRO110	5.43	
ADR1−BSA8	−7.91	ARG208 (O···H)	3.05	pi-alkyl		
				ALA209	4.43	
				VAL481	4.57	
				alkyl		
				ARG208	4.12	
				ALA209	3.78	
				ALA212	3.24	
				VAL481	4.49	
				LEU480	3.84	
				LEU346	4.55	
				pi-sigma		
				LEU480	3.88	
				LEU346	3.84	
ADR1−BSA9	−7.75	LYS242 (O···H)	1.95	pi-alkyl		
		GLU207 (N···O)	3.35	ILE202	5.16	
		GLU207 (C···O)	3.22	LYS242	5.28	
				HIS246	4.22	
				alkyl		
				CYS245	3.81	
				ILE202	4.11	
				LYS242	4.09	
ADR1−BSA10	−6.34	GLU424 (H···O)	2.31	pi-alkyl		LEU189(2.72)
		HIS145 (N···O)	2.68	ARG458	4.84	
		ALA193 (C···O)	03.06	ARG196	4.51	
		ALA454 (C···O)	3.06	ALA193	3.93	
				alkyl		
				ALA193	3.79	
				ARG458	4.71	
				ARG196	3.88	
				pi-cation		
				ARG458	4.49	

existing between ADR1 dye and BSA is tabulated in detail (Table 3), mentioning the HB donor and acceptor groups. In the ADR1−BSA1 conformer, four different AAs are involved in HB interactions with the dye molecule. They are HIS, THR, TYR, and GLU located at the 155th, 183rd, 287th, and 291st position of the protein sequence. Interestingly, all of these AAs are polar in nature, which are more favorable toward HB. Further, the AAs that are involved in hydrophobic interactions of the ADR1−BSA1 conformer are also polar in nature (LYS). Likewise, the second and third most stable conformers, namely, ADR1−BSA2 and ADR1−BSA3 are largely stabilized through HB by AAs like Arg and Ser, which are also polar in nature. However, AAs like VAL, ALA, TRP, and LEU are categorized as nonpolar in nature compared to other AAs that are involved in hydrophobic interactions in the ADR1−BSA2 and ADR1−BSA3 conformers. A detailed investigation of the molecular interaction of other conformers clearly reveals that HB interactions exist through polar AAs only. The above information could not be established through fluorescence studies such that the theoretical studies complement our photophysical studies. Docking studies illustrate that the dye and AA moieties in BSA act as the HB donor and acceptor, respectively.

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Table 4. Molecular Interaction Parameters of All Conformers of ADR2 Dye with BSA

conformation	binding energy	hydrogen-bonding interaction donor −acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance	other interactions
ADR2−BSA1	−7.17	Thr190 (CH···O)	3.53	pi-alkyl		
				Tyr451	4.97	
				Tyr451	5.17	
				His145	5.20	
				alkyl		
				Arg196	4.69	
				Leu189	4.53	
				Leu454	4.10	
				Ile455	4.86	
				Ala193	3.91	
				pi-sigma		
				Ala193	3.54	
ADR2−BSA2	−5.69	Glu382 (NH···O)	2.21	pi-alkyl		
		Ser488 (O···CH)	3.05	Leu386	4.90	
				alkyl		
				Pro484	4.86	
				pi-sigma		
				Ser488	3.46	
ADR2−BSA3	−5.62	Glu284 (CH···O)	3.13	pi-alkyl		
				His18	3.88	
				Pro281	5.06	
				Leu283	5.43	
				alkyl		
				Leu282	3.80	
				Leu283	3.96	
				Pro281	4.39	
ADR2−BSA4	−5.47	Val497 (NH···O)	3.93	pi-alkyl		
		Pro498 (O···CH)	3.74	Lys499	5.39	
				alkyl		
				Ala500	4.32	
				Ala500	4.49	
				Val497	5.26	
ADR2−BSA5	−5.42	Thr305 (NH···O)	2.26	pi-alkyl		
		Leu301 (CH···O)	3.78	Phe373	4.07	
				Arg336	5.12	
				alkyl		
				Leu304	4.09	
				Pro303	4.44	
				Arg336	3.46	
				Leu301	3.52	
				Leu301	4.73	
ADR2−BSA6	−5.42	Tyr410 (CH···O)	3.68	pi-alkyl		
		Ser488 (CH···O)	3.67	Leu386	4.93	
		Arg409 (O···CH)	3.00	Tyr410	5.12	
		pi-donor		alkyl		
		Arg409	3.55	Leu406	5.12	
ADR2−BSA7	−5.34	Glu299 (NH···O)	2.24	pi-alkyl		
				Pro303	5.35	
				alkyl		
				Leu304	4.48	
				Leu304	3.68	
				Pro303	5.07	
				Leu301	4.70	
				Ile297	4.75	
				unfavorable donor−donor		
				Leu301	2.34	
ADR2−BSA8	−5.25	Pro498 (O···NH)	2.54	pi-alkyl		
		Glu470 (O···CH)	3.64	Tyr496	4.58	
				Val497	5.37	

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Table 4. continued

conformation	binding energy	hydrogen-bonding interaction donor −acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance	other interactions
				alkyl		
				Val497	3.90	
				Lys499	3.91	
ADR2−BSA9	−5.22			pi-alkyl		
				Lys499	5.17	
				alkyl		
				Lys533	5.49	
				Ala500	4.00	
ADR2−BSA10	−4.83	Lys76 (O···NH)	3.07	alkyl		
				Arg81	4.31	
				Lys76	4.37	
				Lys76	5.05	

Table 5. Energetics of All Drugs with the ADR1−BSA Complex[a]

conformer	binding energy	ligand efficiency	inhibitory constant	vDW energy + H-bond energy + desolvation energy	intermolecular energy	BSA subdomain	binding site	difference in BE
BSAADR1	−10.58	−0.44	17.63	−10.89	−11.17	IB, IIA	III and I	0
BADR1WAR1	−7.0	−0.3	7.4	−8.16	−8.49	IB	III	3.58
BADR1PBZ1	−7.12	−0.31	6.07	−8.55	−8.61	IB, IIIA	III and II	3.46
BADR1OPB1	−7.3	−0.3	4.44	−8.91	−9.09	IA	III	3.28
BADR1INDO1	−7.21	−0.29	5.19	−8.31	−8.7	IA	III	3.37
BADR1AZA1	−6.14	−0.28	31.57	−7.1	−7.03	IA	III	4.44
BADR1CMPF1	−4.29	−0.25	722.38	−4.75	−6.67	IB, IIIA	III and II	6.29
BADR1FLU1	−6.59	−0.33	14.78	−7.98	−8.08	IB, IIIA	III and II	3.99
BADR1INDS1	−5.72	−0.41	64.56	−6.13	−6.61	IB and IIA	III and I	4.86
BADR1DIFU1	−6.6	−0.37	14.45	−6.89	−7.8	IIIA	II	3.98
BADR1IBU1	−6.92	−0.46	8.53	−7.55	−8.41	IB	III	3.66
BADR1DIAZ1	−6.56	−0.33	15.63	−7.83	−8.05	IIA	I	4.02

[a]Binding sites I and II are Sudlow binding sites.

Table 6. Energetics of All Drugs with the ADR2−BSA Complex[a]

conformer	binding energy	ligand efficiency	inhibitory constant	vDW energy + H-bond energy + desolvation energy	intermolecular energy	HSA subdomain	binding site	difference in BE
ADR2−BSA	−7.17	−0.29	5.57	−7.77	−7.76	IB and IIIA	III and II	0
BADR2WAR1	−6.94	−0.3	8.19	−8.29	−8.43	IA	III	0.23
BADR2PBZ1	−7.12	−0.31	6.06	−8.6	−8.61	IIA	I	0.05
BADR2OPB1	−6.3	−0.26	23.92	−8.16	−8.09	IA	III	0.87
BADR2INDO1	−9.09	−0.36	216.94	−9.93	−10.58	IB, IIIA, IIIB	III and II	−1.92
BADR2AZA1	−6.22	−0.28	27.66	−7.04	−7.11	IIA and IIB	I	0.95
BADR2CMPF1	−4.81	−0.28	297.11	−5.67	−7.2	IIIA	II	2.36
BADR2FLU1	−6.12	−0.31	32.85	−7.99	−7.61	IB, IIA IIB, IIIB	III, I and II	1.05
BADR2INDS1	−5.73	−0.41	63.02	−6.09	−6.63	IIIA and IIIB	II	1.44
BADR2DIFL1	−6.64	−0.37	13.64	−6.95	−7.83	IB	III	0.53
BADR2IBU1	−6.93	−0.46	8.34	−7.58	−8.42	IIIA	II	0.24
BADR2DIAZ1	−6.41	−0.32	19.97	−7.29	−7.9	IIA, IIB, IIIA	I and II	0.76

[a]Binding sites I and II are Sudlow binding sites.

Further, a closer analysis of the AAs that are involved in molecular interaction is located in all of the binding sites of protein, such that ADR1 dye resides in all of the domains with variation in their binding energies and dock at several binding sites of BSA, which supports our earlier elucidation on the presence of eight unique conformers (Figure 4).

ADR2−BSA Conformer. As observed in the case of the ADR1−BSA complex, HB interactions accompanied by several hydrophobic interactions also coexist in all of the conformers of

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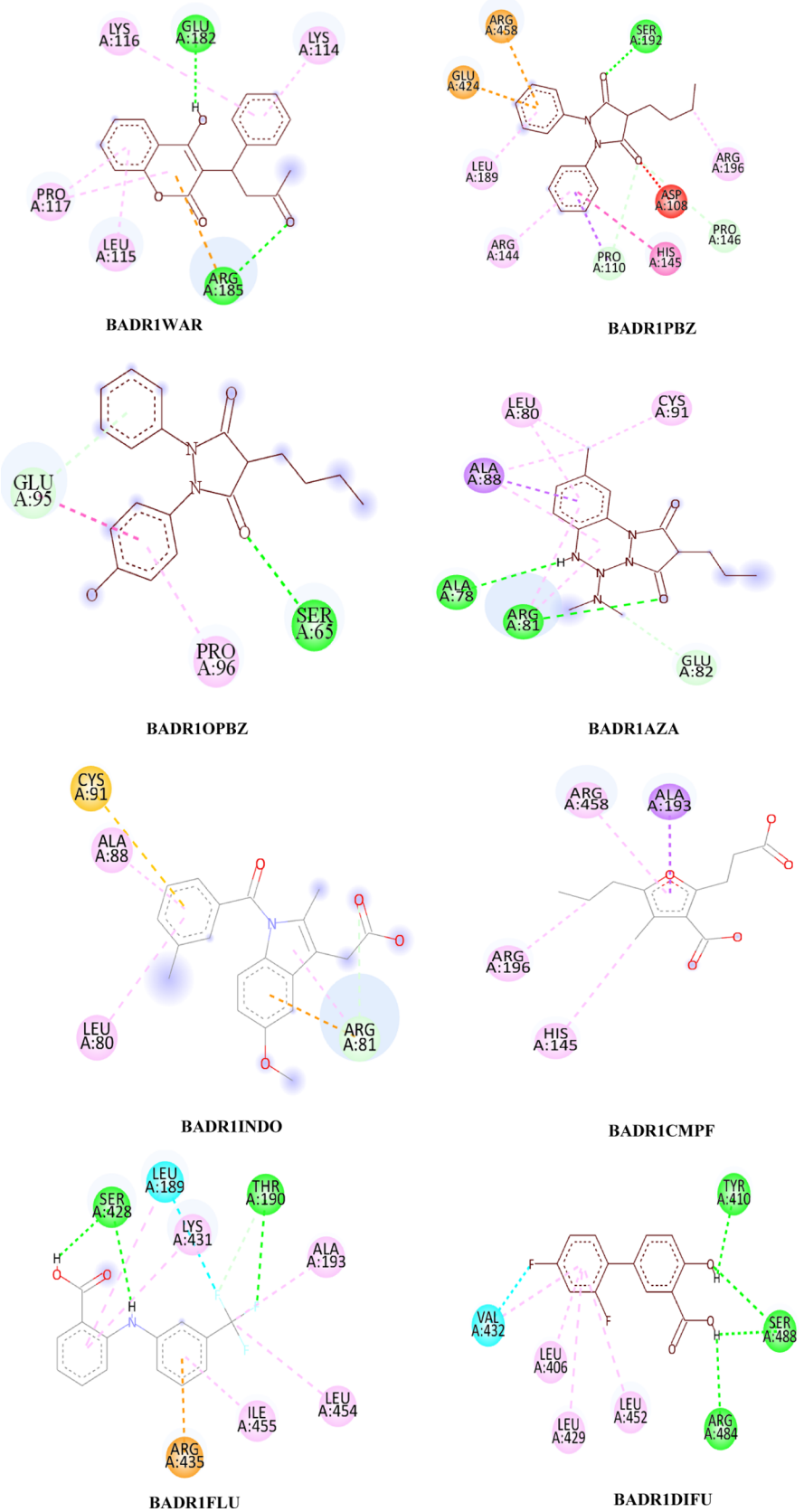

Figure 6. continued

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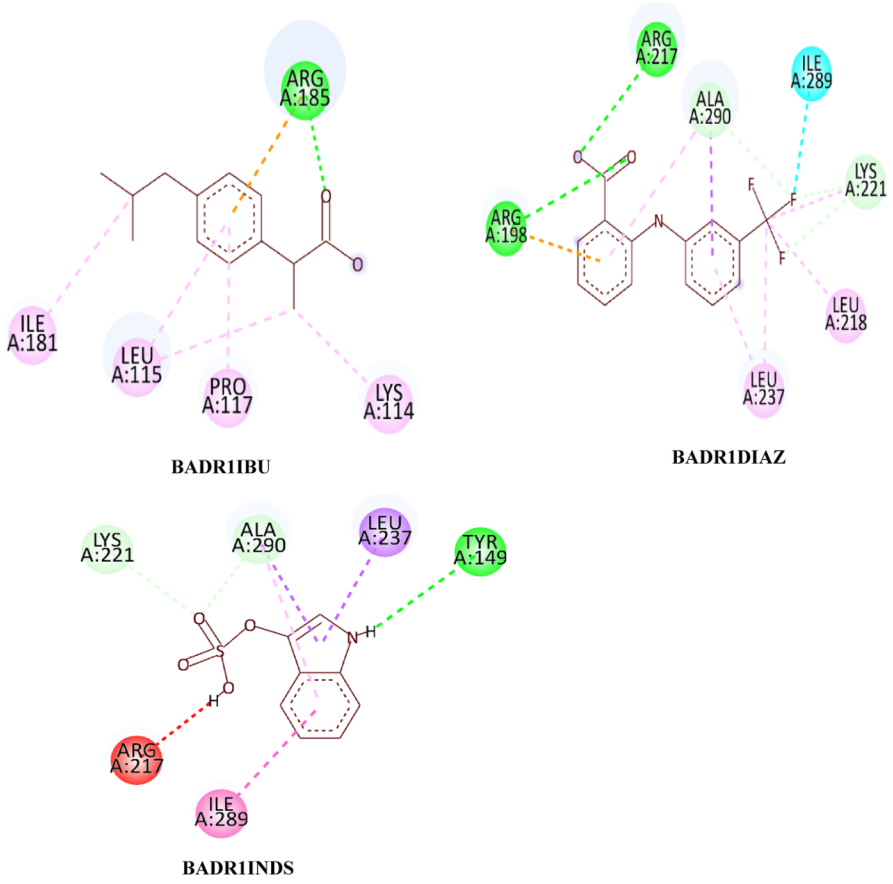

Figure 6. 2D diagram of **BADR1WAR, BADR1PBZ, BADR1OPBZ, BADR1AZA, BADR1INDO, BADR1CMPF, BADR1FLU, BADR1DIFU, BADR1IBU, BADR1DIAZ**, and **BADR1INDS** visualized using Biovia Discovery Studio visualizer. Green color dotted line indicates hydrogen bonds with electronegative elements like N and O atoms; light green color dotted line indicates carbon−hydrogen bonds; light purple color indicates pi-alkyl interactions; and violet color dotted line indicates pi-sigma interactions. Light green color amino acids without interactions represent van der Waals interactions, and red color interaction represents unfavorable interactions. The blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter.

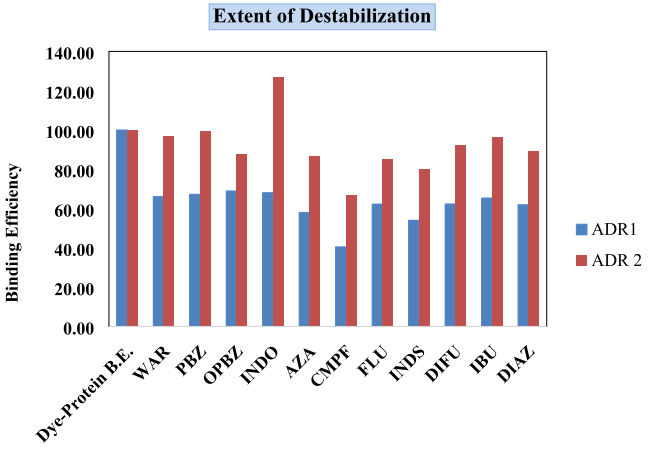

Figure 7. Pictorial representation of destabilization of dye−protein complex after the addition of site I and site II binding drugs.

ADR2−BSA. However, the number of HB interactions is fewer in all of the conformers of ADR2−BSA compared to that of the ADR1−BSA complex. The most stable conformer ADR2−BSA1 possesses only one HB (nonconventional) interaction through THR190 AA, whereas nine different hydrophobic interactions coexist through several polar and nonpolar AAs present in BSA. The molecular interaction table of the ADR2−BSA conformer is provided in Table 4, which comprises the HB donor and acceptor moieties. In all of the conformers of ADR2−BSA, the AAs that are involved in HB interactions with dye are GLU, SER, VAL, PRO, LEU, TYR, ARG, and LYS. All of these AAs are either polar or nonpolar in nature and act as the HB acceptor only, wherein the ADR2 dye acts as the HB donor. The nature of this interaction was not observed in the case of ADR1 dye, even though both these dyes have similar structural features except for the nature of the functional group in the para position of the phenyl ring attached at the 9th position (Figure 1). Interestingly, in all other conformers of the ADR2−BSA complex, HB accompanied by hydrophobic interactions coexist through the AAs like VAL, ALA, TRP, TYR, HIS, SER, PRO, ILE, and LEU. A comparative investigation of the molecular interactions of all of the conformers of both ADR1 and ADR2 dyes with BSA confirms that the stability of the conformers is attributed to both HB as well as hydrophobic interactions. There is no origin of unfavorable interactions in the case of ADR2 dye docking with BSA, whereas only one unfavorable site of binding, which is energetically unfavorable, ceases to exist in the case of ADR1 dye.

Further, to ascertain the extent of binding stability of these dyes in various binding domains of BSA, we incorporated a competitive ligand that possesses enormous binding affinity toward specific domains. Drugs are classified as potent competing ligands, which can stabilize or destabilize the

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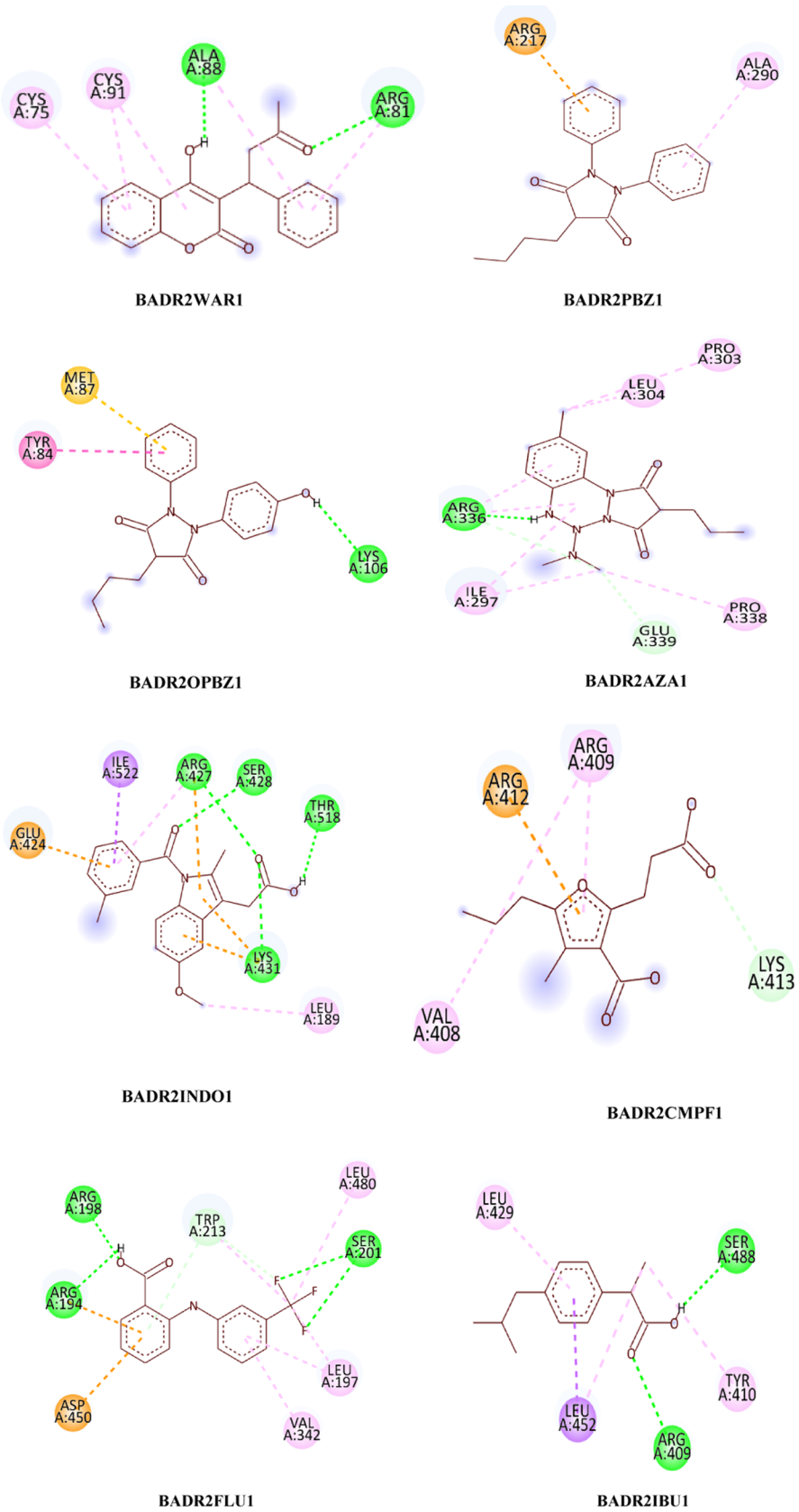

Figure 8. continued

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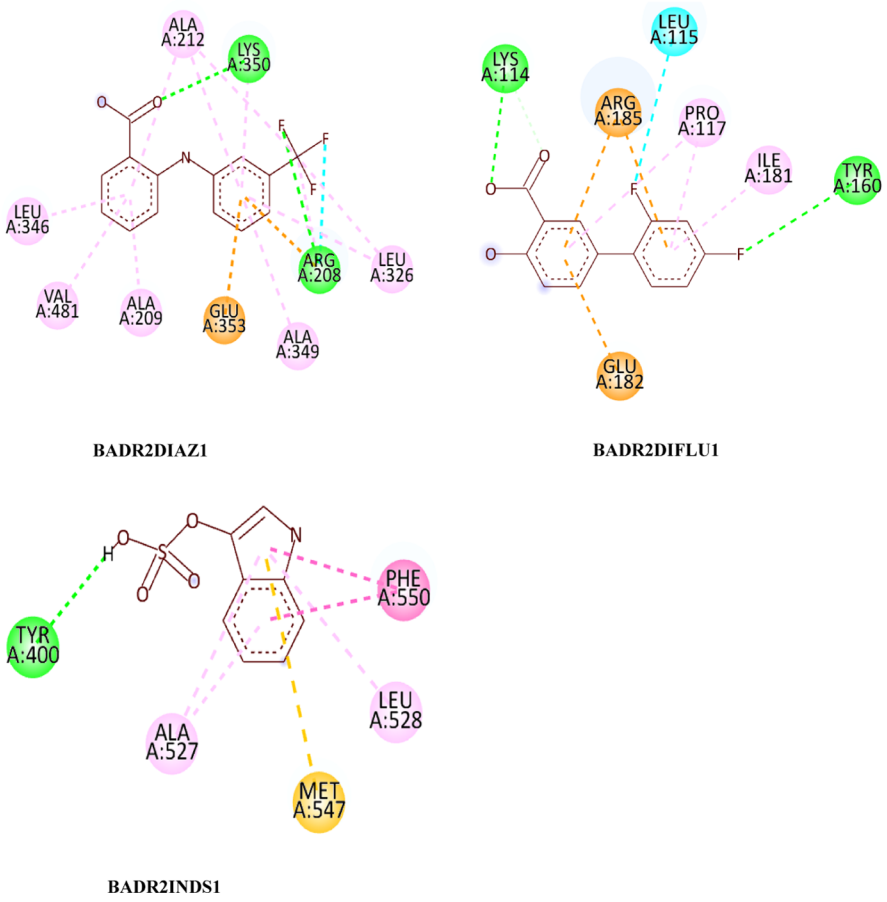

Figure 8. 2D diagram of **BADR2WAR, BADR2PBZ, BADR2OPBZ, BADR2AZA, BADR2INDO, BADR2CMPF, BADR2FLU, BADR2DIFU, BADR2IBU, BADR2DIAZ,** and **BADR2INDS** visualized using Biovia Discovery Studio visualizer. Green color dotted line indicates hydrogen bonds with electronegative elements like N and O atoms; light green color dotted line indicates carbon−hydrogen bonds; light purple color indicates pi-alkyl interactions; and violet color dotted line indicates pi-sigma interaction. Light green color amino acids without interactions represent van der Waals interactions and red color interaction represents unfavorable interactions. The blue halo surrounding the interacting residues represents the solvent-accessible surface that is proportional to its diameter.

Table 7. Combined Forces of Interaction of a Ternary System Comprising Drug ADR1−BSA

												hydrogen-bonding sites	
conformer	conventional HB	nonconventional hydrogen bonding	pi-alkyl	alkyl	pi-sigma	pi−pi	pi-cation	pi-anion	unfavorable (acceptor−acceptor)	halogen	pi-sulfur	donor	acceptor
ADR1−BSA		4	4	2	1							1	3
BADR1WAR1	2		5				1					1	3
BADR1PBZ1	1	2	2	1	1	1	1	1	1			2	
BADR1OPB1	1	2	1			1						1	3
BADR1INDO1		1	4				1				1	1	5
BADR1AZA1	2	1	4	3	1							3	
BADR1CMPF1			2	1	1							2	4
BADR1FLU1	3	1	3	2			1			1		2	5
BADR1INDS1	1	2	2		2	1						2	3
BADR1DIFU1	4		4						1	1		2	5
BADR1IBU1	3		2	3			1					1	2
BADR1DIAZ1	2	3	2	3	1		1			1		2	

interactions existing in proteins. All of the drugs used in the present study are classified as NSAIDs that bind specifically to the binding sites in the presence of competing ligands.

Binding Stability of ADR1−BSA in the Presence of Site I and Site II Drugs. To ascertain the binding stability and the energetics of ADR1 with BSA, 11 specific site-selective drugs were employed in our study, of which six site I-specific binding drugs (WAR, PBZ, OPBZ, AZA, INDO, and CMPF) and five site II binding drugs (IBU, DIAZ, DIFU, FLU, and INDS) were docked to the dye−protein complex. These drugs are generally referred to as Sudlow binding site drugs and have been well established in the literature confined to drug−protein binding

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Table 8. Combined Forces of Interaction of a Ternary System Comprising Drug−ADR2−BSA

conformer	conventional hydrogen bonding	nonconventional hydrogen bonding	pi-alkyl	alkyl	pi-sigma	pi−pi	pi-cation	pi-anion	unfavorable (acceptor−acceptor)	halogen	pi-sulfur	hydrogen-bonding sites: donor	hydrogen-bonding sites: acceptor
ADR2−BSA		1	3	5	1							1	3
BADR2WAR1	2		5									1	3
BADR2PBZ1			1				1					2	
BADR2OPB1	1					1					1	1	3
BADR2INDO1	4		2	1	1		2	2				1	5
BADR2AZA1	1	2	3	4	1							3	
BADR2CMPF1		1	1	2		1						2	4
BADR2FLU1	4	2	2	3		2	1	1				2	5
BADR2INDS1	1		3			2					1	2	3
BADR2DIFL1	2	1	4				1	2		1		2	5
BADR2IBU1	2		1	2	1							1	2
BADR2DIAZ1	2		8	3			1	1				2	

characteristics.[35,36] The structure of site I and site II selective drugs is provided in Supporting Figure S1. The drugs chosen in the present study satisfy the Lipinski rule of five PSA.[37] The well-known site I and site II binding drugs possess both HB donor and acceptor sites along with rotatable single bond characteristics that enable the drug molecule to dock in various positions of the protein molecule (helix/sheet/coils/turns) are shown in Figures 4 and 5.

The presence of two competing ligands, which are generally classified as guest molecules (fluorophore and drug), with protein is explored in depth through variations in the pattern of binding stability (increase or decrease) and the various energetics involved in the ease of formation of the ADR1−BSA complex vs ADR2−BSA site I and site II binding drugs by employing Mol.Doc studies. The energetics of the drug−dye−protein complex is tabulated in Tables 5 and 6. Further, we also probe the role of drug binding with the dye−protein complex and vice versa based on its most probable docking domains and sites. The energetically most preferred active binding sites of various subdomains of the protein for which the site I and site II drugs reside along with dye in the various subdomains of BSA is discussed in depth pertaining to the AAs involved in bimolecular interactions. The role and influence on the extent of displacement of the dye by drugs and vice versa are confirmed and elucidated by theoretical studies. The exact location of ADR1 dye and drug in the domains of BSA is established from docking studies, along with the most favorable nature of molecular interactions, resulting in the stability of the complex. The extent of stabilization or destabilization of the dye−protein complex in the presence of drugs is explored based on the BE, along with intermolecular energy and other energies attributed to molecular interactions. A detailed outcome on the simultaneous docking of drugs with ADR1−BSA is provided below.

(i) Site II drugs efficiently destabilize the binding affinity of the ADR1−BSA complex compared with site I drugs.

(ii) Compared to all of the drugs considered in our study, CMPF (site I binding drug) destabilizes the binding affinity of the dye−protein complex by 60%, whereas all other drugs result in a decrease in the stability of the dye−protein complex by 40−45% only. A pictorial representation of the extent of destabilization of dye−protein complex is provided in Figure 7.

(iii) Except AZA and CMPF, all of the site I binding drugs almost exhibit a similar proportion of destabilization of the dye−protein complex.

(iv) Apart from CMPF, INDS (site II) has better efficiency in destabilizing the dye−protein interaction.

(v) There is no direct dye−drug interaction when docked simultaneously with protein, and the presence of dye−protein and drug−protein is only observed. The 2D representation of ADR1 dye in the presence of drugs is provided in Figure 6, and the 3D images are provided in Supporting Information S5.

(vi) Site I-specific drugs do not displace ADR1 dye from the Sudlow binding sites, rather the dye governs the most probable location of the drugs predominantly to non-Sudlow binding sites of BSA.

(vii) Site II-specific drugs selectively reside in their characteristic binding domains in the presence of dye and also displace ADR1 dye from binding sites I and III of BSA.

A comparison of the simultaneous docking of the drug with ADR2 dye was also carried out, and the outcome resulted in an entirely different approach of the action of drugs on the displacement of dye from the binding domains of BSA. A pictorial representation of the extent of destabilization of dye−protein complex is provided in Figure 7.

(i) Site I drugs effectively destabilize the binding stability of dye−protein complex compared with site II drugs.

(ii) Even though site I drugs destabilize the dye−protein complex, the site I-specific drug INDO alone enhances the stability of the dye−protein complex by 26%, whereas all other drugs decrease the energetics of formation of the dye−protein complex. This phenomenon was not observed in the case of ADR1 dye.

(iii) The drugs CMPF (site I) and INDS (site II) exhibit better destabilization efficiency than other site-specific drugs. The 2D representation of ADR2 dye in the presence of drugs is provided in Figure 8, and the 3D images are given in Supporting Information S6.

(iv) The site I drug PBZ does not influence the energetics and the binding efficiency of dye−protein complex. In other words, the role of PBZ in the displacement of the dye is not significant.

(v) The drugs WAR, IBU, and INDO exhibit a similar pattern of destabilization of dye−protein complex.

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Table 9. Molecular Interaction Parameters of All Conformers of All Drugs with the ADR1−BSA Complex

conformation	binding energy	hydrogen-bonding interaction donor−acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance	other interactions
BADR1WAR1	−7.0	GLU185 (N···O)	3.18	pi-alkyl		
		ARG182 (H···O)	1.98	LYS116	5.39	
				LYS114	4.73	
				LEU115	5.21	
				PRO117	4.23	
				PRO117	4.94	
				pi-cation		
				LYS187	2.94	
BADR1PBZ1	−7.12	SER1902 (O···O)	2.73	pi-alkyl		unfavorable (A···A)
		PRO146 (C···O)	3.65	LEU189	5.23	ASP108 2.29
		PRO110 (C···O)	3.68	ARG144	4.91	
				alkyl		
				ARG196	3.37	
				pi-sigma		
				PRO110	3.70	
				pi−pi T-shaped		
				HIS145	5.61	
				LEU480	3.84	
				LEU346	4.59	
				pi-sigma		
				LEU480	3.84	
				LEU346	3.83	
				pi-cation		
				GLU424	4.45	
				ARG458	4.40	
BADR1OPB1	−7.3	SER65 (N···O)	3.61	pi-alkyl		
		SER65 (C···O)	3.25	PRO96	5.37	
		GLU95	3.55	amide pi-stacked		
				GLU95	4.36	
BADR1INDO1	−7.21	ARG81 (C···O)	3.62	pi-alkyl		
				ALA88	4.16	
				LEU80	4.89	
				ARG81	4.29	
				ARG81	4.54	
				pi-cation		
				ARG81	3.69	
				Pi-sulfur		
				CYS91	4.60	
BADR1AZA1	−6.14	ALA78 (O···H)	2.72	pi-alkyl		
		ARG81(NH···O)	2.90	ALA88	5.08	
		GLU82 (C···OE2)	2.89	LEU80	4.85	
				ARG81	3.70	
				ARG81	4.38	
				alkyl		
				LEU454	5.12	
				LEU197	5.07	
				LEU197	4.71	
				LEU210	4.94	
				ARG483	4.64	
				ARG198	4.40	
BADR1CMPF1	−4.29		2.89	pi-alkyl		
				ARG458	4.44	
				HIS145	4.32	
				alkyl		
				HIS196	4.08	
				pi-sigma		
				ALA193	3.44	
BADR1FLU1	−6.59	THR190(O···F)	3.28	pi-alkyl		
		SER428(H···O)	2.04	ILE455	5.12	
		SER428(H···O)	2.56	LYS431	5.20	

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Table 9. continued

conformation	binding energy	hydrogen-bonding interaction donor−acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance	other interactions
		THR190(C···F)	3.39	LEU189	5.06	
				alkyl		
				ALA193	3.75	
				LEU454	4.73	
				pi-cation		
				ARG435	3.36	
				halogen		
				LEU189	3.34	
BADR1DIAZ1	−5.72	ARG217 (NH2···O)	2.83	pi-alkyl		
		ARG198(NH1···O)	3.04	ALA209	4.66	
		ALA209(C···F)	3.27	LEU237	4.96	
		LYS221(C···F)	3.29	alkyl		
		LYS221(C···F)	3.21	LEU237	5.47	
				LEU218	5.37	
				LYS221	5.35	
				pi-sigma		
				ALA290	3.64	
				pi-cation		
				ARG198	3.59	
				halogen		
				ILE289	3.12	
BADR1DIFU1	−6.6	TYR410(H···OH)	2.11	pi-alkyl		SER488(2.30)
		SER488 (H···O)	2.16	LEU452	5.29	
		SER488 (O···O)	2.87	LEU429	5.40	
		ARG484 (H···O)	2.62	LEU406	5.34	
				VAL432	5.04	
				halogen		
				VAL432	3.57	
BADR1IBU1	−6.92	ARG185 (NH2···O)	3.02	pi-alkyl		
		ARG185 (N···O)	3.32	LEU115	5.03	
		ARG185 (NH2···O)	3.24	PRO117	4.57	
				alkyl		
				ILE181	4.59	
				LEU115	4.63	
				LYS114	3.87	
				pi-cation		
				ARG185	3.98	
BADR1INDS1	−5.72	TYR149 (H···OH)	2.96	pi-alkyl	4.32	
		LYS221	3.13	ALA290	4.78	
		ALA290	3.24	ILE289		ARG217(1.98)
				pi-sigma		
				LEU115	4.00	
				ALA290	3.77	
				amide pi-stacked		
				ILE289	4.67	

Based on the extent of destabilization of dye−protein complex by various drugs, we establish that CMPF results in a larger extent of destabilization of the dye−protein complex. The order of destabilization of the ADR1−BSA complex by drugs is of the order CMPF > AZA > WAR > PBZ > INDO > OPBZ in the case of site I drugs, whereas in the case of site II drugs INDS has the maximum efficiency in destabilizing the dye−protein complex. Apart from INDS, all other site II drugs exhibit a similar proportion of destabilization of the dye−protein complex.

Likewise, in the case of ADR2−BSA, the order of destabilization by site I drugs is as follows CMPF > AZA > OPBZ > PBZ > INDO. Interestingly, the order of destabilization of ADR1−BSA by site I drugs is entirely different from that of the ADR2−BSA system. Irrespective of the nature of dyes, site II binding drugs follow a similar pattern of displacement of dye from the hydrophobic and hydrophilic pockets of BSA.

To establish a variation in the binding of AAs of BSA with ADR1 and ADR2 dyes in the presence of these drugs, a detailed account on the molecular interaction table was formulated and analyzed.

The introduction of drugs into the dye−protein complex results in a greater number of hydrophobic interactions like pi−pi, pi-cation, and pi-anion, and unfavorable interactions also arise on the introduction of certain drugs. Except for CMPF and INDO, all other drugs are involved in a conventional HB with protein molecules, which was not observed in the case of the ADR1−BSA system. A larger extent of destabilization of the ADR1−BSA complex by CMPF is presumably attributed to the

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Table 10. Molecular Interaction Parameters of All Conformers of All Drugs with the ADR2−BSA Complex

conformation	binding energy	hydrogen-bonding interaction donor−acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance
BADR2WAR1	−6.94	ALA88 (H···O)	1.37	pi-alkyl	
		ARG88 (NH1···O)	2.94	CYS75	4.62
				CYS91	4.35
				ALA88	4.86
				ARG81	4.27
				CYS91	4.73
BADR2PBZ1	−7.12	-		pi-alkyl	
				ARG217	3.35
				pi-cation	
				ALA290	4.42
BADR2OPB1	−6.3	LYS (H···O)	2.34	pi-sulfur	
				MET87	4.55
				pi−pi T-shaped	
				TYR84	4.79
BADR2INDO1	−9.09	ARG427 (NH2···O)	2.95	pi-alkyl	
		SER428 (O···O)	2.90	ARG427	5.01
		LYS431 (N···O)	2.78	LYS431	5.21
		THR518 (H···O)	1.93	alkyl	
				LEU189	4.49
				pi-sigma	
				ILE522	3.83
				pi-cation	
				ARG427	4.75
				LYS431	3.75
				pi-anion	
				GLU424	4.87
				LYS431	3.69
BADR2AZA1	−6.22	ARG336 (H···O)	2.06	pi-alkyl	
		ARG336 (C···O)	3.13	ARG336	3.97
		GLU339 (C···OE2)	3.17	ARG336	4.41
				ILE297	5.06
				alkyl	
				PRO303	4.73
				LEU304	4.73
				ILE297	4.77
				PRO338	3.19
BADR2CMPF1	−4.81	LYS413 (C···O)	3.03	pi-alkyl	
				ARG409	4.40
				alkyl	
				ARG409	4.83
				VAL408	4.34
				pi-cation	
				ARG412	4.13
BADR2FLU1	−6.12	ARG198 (NH···O)	2.77	pi-alkyl	
		ARG194 (H···O)	2.24	LEU197	5.00
		SER201 (O···F)	3.28	VAL342	4.81
		SER201 (O···F)	3.43	alkyl	
		TRP213 (C···F)	3.14	LEU480	4.37
		TRP213 (pi-donor hydrogen bonding)	3.69	TRP213	4.71
				LEU197	4.47
				pi-cation	
				ASP450	4.91
				pi-anion	
				ARG194	3.44
				pi−pi T-shaped	
				TRP213	5.10
				TRP213	4.77
BADR2INDS1	−5.73	TYR400 (H···O)	2.25	pi-alkyl	
				ALA527	4.25
				ALA527	5.42
				LEU528	4.57

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Table 10. continued

conformation	binding energy	hydrogen-bonding interaction donor−acceptor amino acid−dye	bond distance	hydrophobic interactions	bond distance
				pi-sulfur	
				MET547	5.45
				pi−pi stacked	
				PHE550	3.91
				PHE550	4.67
BADR2DIFL1	−6.64	LYS114 (NZ···O)	2.61	pi-alkyl	
		TYR160 (OH···F)	3.32	PRO117	5.33
		LYS114 (C···O)	2.88	PRO114	4.27
				ILE181	5.33
				ARG185	4.92
				pi-cation	
				GLU182	4.90
				pi-anion	
				ARG185	4.84
				ARG185	4.22
				halogen	
				LEU115	3.36
BADR2IBU1	−6.93	ARG409 (H···O)	2.05	pi-alkyl	
		SER488 (NE···O)	2.92	LEU429	4.99
				alkyl	
				TYR410	5.17
				LEU452	4.63
				pi-sigma	
				LEU452	3.90
				pi-alkyl	
BADR2DIAZ1		LYS350 (N···O)	2.74	LEU346	4.52
		ARG208 (NH···F)	3.53	VAL481	4.91
				ALA209	4.51
				ALA212	4.89
				ALA212	5.03
				LYS350	5.32
				ALA349	4.58
				LEU326	4.96
				alkyl	
				ALA212	3.84
				LEU326	4.94
				ARG208	5.18
				pi-cation	
				GLU353	4.08
				pi-anion	
				GLU353	3.66
				halogen	
				ARG208	3.28

complete binding of the drug to the hydrophobic pockets of the protein molecule, even though CMPF has several HB acceptor sites. This was established based on the nonexistence of any HB interaction existing between dye−protein or drug−protein complex. Tables 7 and 8 present evidence for the combined forces of interaction of a ternary system comprising drug−dye−protein.

In the case of the ADR2−BSA system, the presence of CMPF drastically decreases the binding affinity of the dye−protein complex, whereas INDO enhances the stability of the complex. CMPF promotes a pronounced hydrophobic effect on the dye−protein complex, whereas INDO promotes stability of the complex through HB interactions between dye and protein, which was not observed in the docking of the dye with protein in the absence of drugs. Interestingly, in the case of the ADR2−BSA system, both site I and site II drugs promote the formation of HB between dye and protein. Overall, site II binding drugs promote the formation of HB between dye and protein rather than site I drugs.

The AAs that are involved in molecular interactions in the presence of drugs are provided in Tables 9 and 10. Further, we establish that there exists no direct dye−drug bonding in the presence of protein molecules.

BSA−Drug Binding. The role of several drugs binding with BSA has been well explored in the literature through experimental and computational methods.[42−56] These methods have provided an immense source of information regarding the bimolecular interactions, binding affinity, and binding sites that are crucial for protein−ligand interactions.[57] Herein, we have carried out the Mol.Doc method in analyzing the binding stability and the energetics of BSA site I and BSA site II drugs. A comparison of the energetics and molecular interaction of

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Table 11. Comparison of BE of BSA Site I and BSA Site II Complexes with ADR1 and ADR2 Dyes in kcal mol^{-1}

drugs	BE of BSA–drug complex	BE of BSA site I and BSA site II complexes with ADR1 dye	difference in BE	BE of BSA site I and BSA site II complexes with ADR2 dye	difference in BE
WAR	−7.66	−8.36	0.7	−8.58	0.92
PBZ	−7.46	−8.43	0.97	−7.79	0.33
OPBZ	−7.48	−10.2	2.72	−8.64	1.16
AZA	−9.24	−7.93	−1.31	−8.73	−0.51
INDO	−8.0	−7.96	−0.04	−8.6	0.6
CMPF	−6.01	−8.57	2.56	−9.69	3.68
FLU	−6.45	−8.23	1.78	−8.16	1.71
INDS	−5.89	−8.78	2.89	−9.71	3.82
DIFU	−6.63	−8.91	2.28	−8.82	2.19
IBU	−7.02	−9.29	2.27	−9.73	2.71
DIAZ	−7.0	−9.87	2.87	−10.67	3.67

protein–drug complexes in the presence of ADR1 and ADR2 dyes was performed to establish the exact role of dye in protein–drug complex. The energetics of BSA with site I and site II drugs, BSA drug with ADR1 dye, and BSA drug with ADR2 dye are provided in Supporting Table S3. It is evident that the addition of ADR1 dye with site I drug bound to BSA is more stabilized than site II drugs except for INDO, which alone exhibits a decrease in stability. Interestingly, ADR2 dye stabilizes the site II binding drugs with protein rather than site I drug, except in the case of AZA. Moreover, the energetics provides an interesting pattern of information through docking studies. BSA site I drug complex binding stability is more enhanced than BSA site II drugs, and this binding stability is further stabilized by the presence of ADR1 dye (PET dye). On the contrary, ADR2 dye selectively destabilizes the site I binding drugs bound to the protein to a significant extent than site II drugs. The 2D images of BSA–drug complex is given in Supporting Figure S6.

An in-depth analysis of the binding site of BSA with site I and site II drugs provides significant information on the probable location of drugs in the various domains of BSA. Hydrophobic interactions are the most predominant interactions in the case of site I drug binding with BSA, and hydrophobic forces like pi-alkyl, and pi–pi interactions determine the binding stability. However, apart from hydrophobic forces, conventional HB interactions play a predominant role in the binding stability of site II drugs–BSA interaction. Detailed information regarding the binding sites is provided in Supporting Table S4. Even though the name specifies in the literature as specific and site-selective binding drugs, all of these drugs were found to be residing in all of the binding sites of BSA through docking studies.

A distinct pattern of binding nature and binding interactions prevails in the introduction of ADR1 dye to the BSA–drug complex. Docking of ADR1 dye to the protein–drug complex results in an increase in the number of HB interactions existing in the system, which clearly elucidates that ADR1 dye promotes stability through the presence of several HB interactions existing in the drug–protein system. However, in the absence of dye, hydrophobic forces govern the binding stability of drug–protein complex, which was a key factor influencing the binding interactions existing in the presence of competing host–guest systems. The energetics and molecular interactions of BSA–drug with ADR1 dye are provided in Supporting Tables S5 and S6, and the 2D images are given in Supporting Figure S7.

However, in the case of ADR2 dye, both HB interactions and hydrophobic interactions coexist. The energetics and molecular interactions of BSA–drug with ADR1 dye is provided in Supporting Tables S7 and S8, and the 2D images are given in Supporting Figure S8. The role of hydrophobic interactions predominates over HB interactions, which presumably results in a variation in the binding characteristics of the ADR1–BSA complex with drugs over the ADR2–BSA complex in the presence of drugs. A comparison of BE of BSA site I and BSA site II complex with ADR1 and ADR2 dye is given in Table 11.

CONCLUSIONS

Molecular docking is used as an efficient and reliable tool in ascertaining the forces that govern the binding stability of dye–protein system in the absence and presence of drugs. The competitive influences through hydrogen bonding accompanied by several hydrophobic interactions resulting in variation in the energetics and stability of the dye–protein system are ascertained accurately. This tool employed in ligand systems that exhibits lower solubility in water provides an easier and reliable approach toward the binding sites and domains of the protein molecule in which the fluorophore and drug are docked. Further, the presence of two similar dyes exhibiting contrasting binding approaches is authenticated by Mol.Doc studies.

EXPERIMENTAL SECTION

Molecular Docking (Mol.Doc) Techniques.

(i) The structures of ADR1 and ADR2 dyes and drugs employed in the present study were drawn and optimized using Chemsketch and saved in the MDL-mol format and converted to the pdb format using the open babel molecular converter program. The SMILES format was generated using Chemsketch, and their properties were calculated using the Molinspiration tool.

(ii) Molecular docking studies of ADR dye with BSA.

The crystal structure of BSA was retrieved from the protein databank (PDB) (http://www.rcsb.org/pdb, PDB ID: 4F5S, A Chain) (Supporting Figure S2). The water molecules and complexes were removed during the binding interaction studies. The globular protein preparation[58] was carried out using Autodock software version 4.2. The polar hydrogen and Kollman charges were added and saved in the pdbq format. The structures of PET dye, non-PET dye, site I binding drugs, and site II binding drugs were uploaded, and the center node and torsional bonds were selected and saved in the pdbqt format. In grid preparation, the ligands were saved in the pdbqt format, and later grid spacing was set as 0.560 Å with the grid box size of 126 Å × 126 Å × 126 Å, which covers the entire protein; the initial search was carried out as reported in our previous work. A Lamarckian genetic algorithm was applied in docking studies. Ten (10) genetic algorithm (GA) runs were performed with the following parameters: population size of 150, maximum number of 2.5×10^6 energy evaluations, and maximum number of 27,000 generations; other parameters were default. The region of the most populated of the first 10 clusters was selected as the probable binding region, which is universally accepted. The resulting conformations were clustered using a root-mean-square deviation (RMSD) of 2.0 Å, and the clusters were ranked in the order of increasing BE of the lowest BE conformation in each cluster. Energies were calculated based on the autodock scoring function.[59] Finally, all ten conformations were selected and saved in the pdb format. ADR1–BSA and ADR2–BSA

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complexes formed were visualized using Biovia Discovery Studio visualizer[60] and analyzed for HB, hydrophobic, and van der Waals interactions as well as for any unfavorable interactions existing during the complex formation. Further docking with drugs, we have taken the most stable conformer of ADR1−BSA and ADR2−BSA complexes, and the same procedure has been followed.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c07111.

Structure of site I and site II binding drugs; Bovine Serum Albumin; the 2D and 3D structures of the unique conformers of ADR1−BSA and ADR2−BSA; the 2D structures of ADR1−BSA and ADR2−BSA complexes with site I and site II binding drugs; the 2D structures of BSA with site I and site II binding drugs and BSA site I and BSA site II complexes with ADR1 and ADR2 dyes are provided in (Figures S1−S9); the energetics pertaining to the formation of a complex between ADR1 with BSA and ADR2 dye with BSA; BSA with site I and site II binding drugs and the molecular interaction parameters of BSA site I and BSA site II complexes with ADR1 and ADR2 dyes are provided in (Tables T1−T8) (PDF)

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Notes

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