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Molecular Modeling Study of Some Anthelmintic 2-phenyl Benzimidazole-1-Acetamides as *β*-tubulin Inhibitor

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ABSTRACT: The 3D QSAR analysis was performed of 2-phenyl benzimidazole-1-acetamides series for their anthelmintic activity. The calculated steric and electrostatic field descriptors were used as independent variables and Log $PT_{min,}$ Log DT_{min} values was used as dependent variables in partial least-squares regression analysis to derive the 3D QSAR models. Docking of anthelmintic 2-phenyl benzimidazole-1-acetamides with *β*-tubulin (1jff) was done using BioPredicta module of VLife MDS 3.5 software. Molecular modeling study reveals that polar, more electropositive with less bulky substitution of benzimidazole analogues are required to enhance the activity. Docking study with β-tubulin as target indicates that the observed anthelmintic activity of benzimidazole analogues may be due to its binding with β-tubulin of intestinal cell of parasite.

KEYWORDS: 2-phenyl benzimidazole-1-acetamide; Anthelmintic; QSAR; Docking; β-tubulin.

Introduction

The *β*-tubulin is of major importance in microtubules (part of cytoskeletal) and has been an attractive target in drug discovery. A number of discrete functions are ascribed to microtubules at the cellular level like formation of the mitotic spindle in cell division; maintenance of cell shape; cell motility; cellular secretion; nutrient absorption and intracellular transport[1]. The benzimidazole group of anthelmintics including thiabendazole, mebendazole and fenbendazole, are broad-spectrum anthelmintics which have an action against gastrointestinal nematodes and in some cases, at a higher concentration, actions against trematodes. Their mode of action is now known to involve binding to β-tubulin along with α-tubulin polymerizes to form microtubule structures inside the cells of nematodes[2-3]. The versatile utility of substituted benzimidazole and amide group (CONH) both have pharmacological interest, prompted us to prepare a series of benzimidazole derivatives containing amide group, and evaluate their anthelmintics activities. We have already reported synthesis and anthelmintic activity in terms of paralysis time (PTmin) and death time (DTmin) of eighteen analogues of 2-phenyl benzimidazole-1-acetamide as shown in Table 1[4].

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The benzimidazole containing anthelmintics have good anthelmintic potential but also suffer many limitations. The development of resistance would create a pressing need for new anthelmintics. Further efforts should be encouraged to ensure that novel products will be available in future. In present work, as an extension of our research, we have performed 3D QSAR analysis of our earlier reported eighteen 2-phenyl benzimidazole-1-acetamides for their anthelmintic activity. We also report the molecular docking studies of these compounds with *β*-tubulin in order to have insight into structural requirements of 2-phenyl benzimidazole-1-acetamides as potential anthelmintics so that better anthelmintic could be obtained from this series.

Experimental Procedure

3D QSAR

Several 3D-QSAR techniques such as comparative molecular field analysis (CoMFA), comparative molecular similarity analysis (CoMSIA), and *k*-nearest neighbor (*k*NN) are being used in modern QSAR research[5]. In the present study, molecular field analysis coupled with partial least squares (PLS) was applied to obtain a 3D QSAR models, PLS is frequently used as the regression method in 3D QSAR. The calculated steric and electrostatic field descriptors were used as independent variables and Log$PT_{min,}$ Log DT_{min}values was used as dependent variables in partial least-squares regression analysis to derive the 3D QSAR models.The 3D QSAR studies were carried out by using molecular design suite software version 3.5 running on pentium IV processor. Three-

dimensional structures of all compounds have been constructed using MDS 3.5 and their geometries were subsequently optimized to make the conformations having least potential energy. Energy minimizations were performed using Merck Molecular Force Field (MMFF) and MMFF charge for the atom[6] followed by considering distance-dependent dielectric constant of 1.0 and convergence criteria (rms gradient) of 0.01 kcal/mol. All compounds in the data set were aligned by template based method where a template is built by considering common substructures in the series. Highly bioactive energetically stable conformation in this class of compounds is chosen as a reference molecule, on which other molecules in the data set are aligned, considering template as a basis for the alignment. The 3D descriptors for each optimized molecule were calculated by "compute descriptor" module of the software. The predictability of the QSAR model would be good if the values of biological activity predicted by the QSAR model do not appreciably differ from the observed biological activity for the given data set. The QSAR models were evaluated using statistical measures such as nrepresents number of observations, r^2 squared correlation coefficient, q^2 is the cross-validated r^2, and Fis the *F*-statistic for the regression model.

Docking

Docking procedures aim to identify correct poses of ligands in the binding pocket of a protein and to predict the affinity between the ligand and the protein. Docking of anthelmintic 2-phenyl benzimidazole-1-acetamides with *β*-tubulin (1jff) was done using BioPredicta module of VLife MDS 3.5 software.

From BioPredicta tool open batch docking and then grid batch docking. Batch docking shows browsing of receptor, ligand (molecule) and result generated saved in output file. Molecule saved in output file as a docked ligand format with proper conformation which will further used in checking binding interaction. For checking binding interaction first open target in MDS followed by molecule which is saved as ligand dock file. From tool option click on merge molecule so that molecule and target merged together, after that cavity no 1selected from analyses cavity option. From biopredicta tool edit this complex and select ligand and target structure to check its interactions.

Results and Discussion

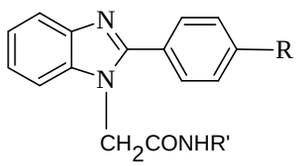
3D QSAR using logarithm of paralysis time as dependant variable

$$\text{Log PT}_{min} = 0.0003 - 340.1080\ (\pm\ 30.1267)\ S\_76 + 0.1024\ (\pm\ 0.0198)\ E\_962 - 0.4467\ (\pm\ 0.1285)\ S\_965$$

$n = 12$, $r^2 = 0.8601$, $q^2 = 0.7771$, F test = 16.3954 Model 1

The best selected model 1 describes the optimum structural feature for the anthelmintic activity. The training set of 12 molecules and test set of 4 molecules was used. The S_76, S_965 and E_962 are the steric and electrostatic field energy of interactions between probe (CH_3) and compounds at their corresponding spatial grid points of 76, 965 and 962 as shown in Fig. 1.

Table 1 Anthelmintic activity of 2-phenyl benzimidazole-1-acetamides



Compound	R	R'	Paralysis Time (min) ± SEM	Death Time (min) ± SEM
3a	NO_2	-NHphCOOH	19.29 ± 2.89**	58.25 ± 2.38**
3b	NO_2	-NHNHph	31.37 ± 1.1**	61.51 ± 1.64**
3c	NO_2	C_2H_5NH-	14.60 ± 3.53**	47.22 ± 1.03**
3d	NO_2	-$NHCH_2$ph	17.27 ± 2.81**	58.43 ±1 .15**
3e	NO_2	-$NHphNO_2$	25.31 ± 3.49**	55.12 ± 5.12**
3f	NO_2	-NHphOH	20.39 ± 1.19**	55.52 ± 2.7**
3g	NO_2	-$NHC_{10}H_7$	29.16 ± 1.12**	86.79 ± 2.95^{ns}
3h	NO_2	-NHph	17.23 ± 2.35**	63.72 ± 1.73**
3i	NO_2	-$NHphNO_2$	28.29 ± 1.73**	72.36 ± 3.44**
3j	Cl	-NHphCOOH	22.53 ± 2.17**	53.48 ± 1.62**
3k	Cl	-NHNHph	17.47 ± 1.08**	58.43 ± 1.15**

Table 1 *Contd…*

Compound	R	R'	Paralysis Time (min) ± SEM	Death Time (min) ± SEM
3l	Cl	C_2H_5NH-	26.76 ± 3.40**	51.51 ± 1.76**
3m	Cl	-$NHCH_2$ph	25.36 ± 2.95**	63.38 ± 1.72**
3n	Cl	$NHphNO_2$	19.46 ± 3.03**	58.26 ± 3.55**
3o	Cl	-NHphOH	23.58 ± 4.01**	58.71 ± 2.33**
3p	Cl	-$NHC_{10}H_7$	30.40 ± 1.77^{ns}	69.22 ± 1.13**
3q	Cl	-NHph	20.36 ± 1.59**	51.38 ± 1.09**
3r	Cl	-$NHphNO_2$	34.44 ± 2.32**	59.45 ± 1.15**
Albendazole			21.43 ± 1.16	55.44 ± 1.65

Control worms were alive up to 24 hours of the experiment.

Values are expressed as mean ± SEM, *n* = 6 and data were analyzed by ANOVA followed by Dunnet's test.

***P* < 0.05 was considered significant when compared with albendazole, ns – non significant.

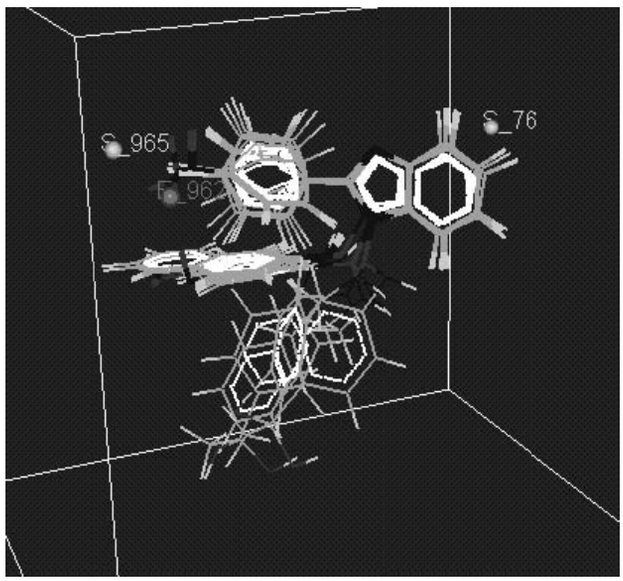


Fig. 1 Show point of 3D QSAR model 1.

The descriptor values and their correlation matrix are shown in Table 2 and Table 3 respectively.

Observed and predicted activity are reported in Table 4 and graphically presented in Fig. 2. Residual values are low indicating that predicting ability of model is good**.** Model suggests that, steric descriptor S_76 and S_965 with negative coefficients represent less bulky substituent is favorable whereas electrostatic field descriptor E_962 with positive coefficient indicates that electropositive groups are favorable for anthelmintic activity.

Table 2 Selected 3D descriptor values for model 1

Compound	S_76	E_962	S_965
3a	-0.00615	-10	-0.35068
3c	-0.00579	-9.71064	-0.36418
3d	-0.00627	-10	-0.28587
3e	-0.00645	-10	-0.49692
3f	-0.00634	-10	-0.46101
3g	-0.00642	-10	-0.65955
3h	-0.00644	-10	-0.40425
3i	-0.00684	-10	-0.4907
3j	-0.00608	-6.63779	-0.33791
3l	-0.0058	-6.64199	-0.27932
3m	-0.00612	-7.92078	-0.32053
3n	-0.00644	-10	-0.48692
3o	-0.0064	-10	-0.46258
3p	-0.00648	-10	-0.69122
3q	-0.00643	-10	-0.38576
3r	-0.00682	-10	-0.46132

Table 3 Correlation matrix of log PT_{min} and descriptor for model 1

	Log PT_{min}	E_962	S_76	S_965
Log PT_{min}	1.000000			
E_962	0.314411	1.000000		
S_76	-0.281699	0.664931	1.000000	
S_965	-0.544583	0.502591	0.669162	1.000000

Table 4 Observed, predicted activity and residuals for model 1

Compound	Observed	Predicted	Residual
3a	1.285332	1.223617	0.061715
***3c**	1.164353	1.137872	0.026481
3d	1.237292	1.236841	0.000451
3e	1.403292	1.393015	0.010277
***3f**	1.309417	1.336501	-0.02708
3g	1.464788	1.452737	0.012051
3h	1.236285	1.34686	-0.11058
***3i**	1.451633	1.52084	-0.06921
3j	1.352761	1.540553	-0.18779
3l	1.427486	1.418379	0.009107
3m	1.404149	1.41497	-0.01082
3n	1.289143	1.384466	-0.09532

Table 4 *Contd…*

Compound	Observed	Predicted	Residual
3o	1.372544	1.36033	0.012214
3p	1.482874	1.489668	-0.00679
3q	1.308778	1.333497	-0.02472
***3r**	1.537063	1.500233	0.03683

* Test set compound

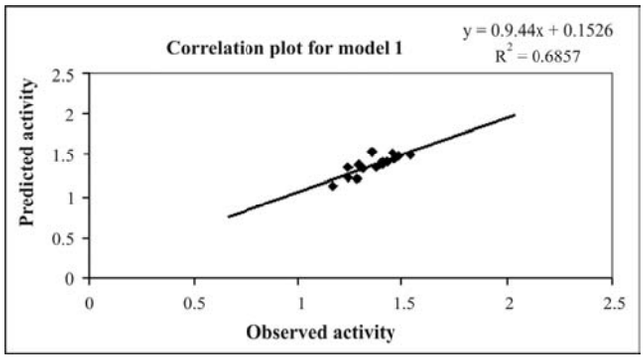


Fig. 2 Plot of predicted against observed activity for model 1.

3D QSAR using logarithm of death time as dependant variable

Log DT $_{min}$ = 0.0001-325.2820 (±3.8725) S_219 + 0.0581 (± 0.0119)

E_1152+0.0102(±0.0046) E_1072

n = 12, r^2 = 0.8956, q^2 = 0.7849, F test = 22.8748 Model 2

The best selected model 2 describes the optimum structural feature for the anthelmintic activity. The S_219, E_1152 and E_1072 are the steric and electrostatic field energy of interactions between probe (CH_3) and compounds at their corresponding patial grid points of 219, 1152 and1072 as shown in Fig. 3.

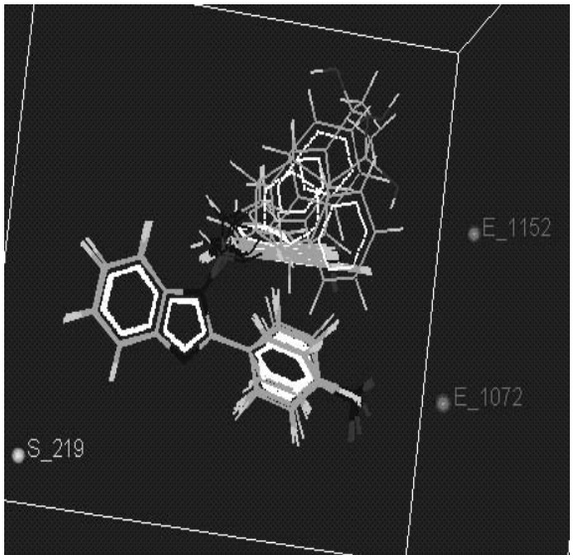


Fig. 3 Show point of 3D QSAR model 2.

The descriptor values and their correlation matrix are shown in Table 5 and Table 6 respectively.

Observed and predicted activity are reported in Table 7 and graphically presented in Fig. 4. Residual values are low indicating that predicting ability of model is good**.** Model suggests that, steric descriptor S_219 with negative coefficients represent less bulky substituent is favorable whereaselectrostatic field descriptorsE_1152 and E_1072 with positive coefficient indicatethat electropositive groups are favorable for anthelmintic activity.

Table 5 Selected 3D descriptor values for model 2

Compound	S_219	E_1152	E_1072
3a	-0.00557	-0.2626	-7.12234
3c	-0.00527	0.072348	-3.91652
3d	-0.00576	-0.2137	-9.3922
3e	-0.0057	-0.88562	-3.58641
3f	-0.00562	-0.60369	-3.92688
3g	-0.00578	0.978656	-3.62584
3h	-0.00562	0.37823	-3.83229
3i	-0.00576	-0.67572	-4.56663
3j	-0.00533	0.350326	-3.13164
3l	-0.0053	0.060281	-2.61954
3m	-0.00561	0.041246	-3.14431
3n	-0.00565	-1.03216	-3.44729
3o	-0.0056	-0.70762	-3.9798
3p	-0.00576	0.860398	-3.46578
3q	-0.00556	0.291985	-3.65376
3r	-0.00571	-0.75121	-4.4859

Table 6 Correlation matrix of log DT $_{min}$ and descriptor for model 2

	Log DT $_{min}$	E_1072	E_1152	S_219
Log DT $_{min}$	1.00000			
E_1072	-0.011358	1.000000		
E_1152	0.489007	0.168510	1.00000	
S_219	-0.704281	0.329597	0.022254	1.00000

Table 7 Observed, predicted activity and residuals for model 2

Compound	Actual	Predicted	Residue
3a	1.765296	1.723696	0.0416
***3c**	1.677151	1.678123	-0.00097
3d	1.767379	1.765954	0.001425
3e	1.741309	1.765982	-0.02467
3f	1.742332	1.754519	-0.01219

Table 7 *Contd…*

Compound	Actual	Predicted	Residue
***3g**	1.93847	1.899327	0.039143
3h	1.804276	1.809632	-0.00536
3i	1.859499	1.7871	0.072399
***3j**	1.728191	1.723717	0.004474
3l	1.711892	1.700344	0.011548
3m	1.801952	1.795074	0.006878
***3n**	1.76537	1.743912	0.021458
3o	1.768712	1.739483	0.029229
3p	1.840232	1.887897	-0.04767
3q	1.710794	1.789516	-0.07872
3r	1.774152	1.768243	0.005909

* Test set compound

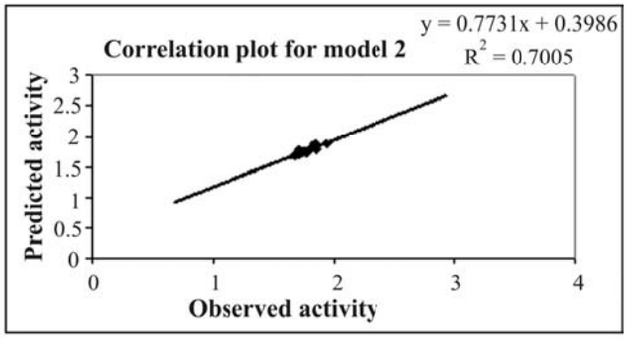


Fig. 4 Plot of predicted activity against observed activity for model 2.

Docking

The docking of the title compounds yielded fitness scores ranging from 5.229483- 4.927926 shown in Table 8. From the results of docking it is found that the title compounds have good interaction with *β*-tubulin. From the dock score, compounds **3l** and **3p** were found to have highest negative dock score as -5.229483 and -5.276368, respectively. It means that these formed most stable drug-receptor complex amongst other compounds. All the docked compounds were analyzed for various types of interactions like hydrogen bonding, hydrophobic bonding and Van der Waals (vdw) interactions. The docking study indicate that compounds bind with *β*-tubulin by forming hydrogen bond interaction with amino acid residues PRO63 and VAL62, hydrophobic interaction with amino acid residues VAL62, ARG64, LEU125 and GLN128 whereas vdw interaction with amino acid residues GLU3, HIS28, VAL62, PRO63, ARG64 and GLN128 as shown in Fig. 5-9.

Table 8 Docking score of compounds 3a-r

Sr. No.	Compound	Docking score (Kcal/mol)
1	**3a**	-5.114849
2	**3c**	-5.020557
3	**3d**	-4.913989
4	**3e**	-5.031891
5	**3f**	-5.182813
6	**3g**	-4.954057
7	**3h**	-4.996791
8	**3i**	-5.106370
9	**3k**	-5.500761
10	**3l**	-5.229483
11	**3m**	-4.927926
12	**3n**	-5.114200
13	**3o**	-5.098299
14	**3p**	-5.276368
15	**3q**	5.043057
16	**3r**	-5.089952

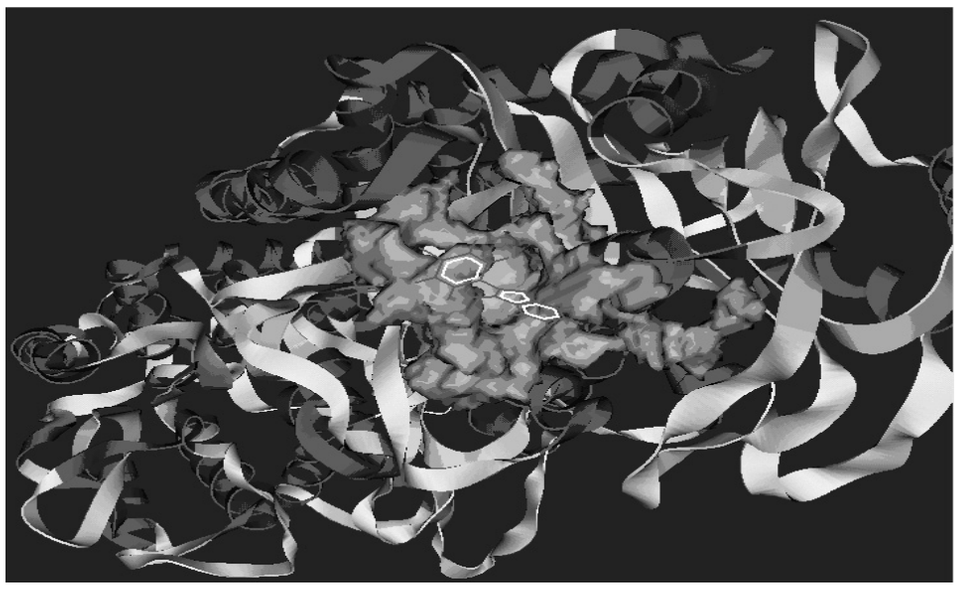


Fig. 5 Top posed dock of compound 3p in cavity no 1.

Fig. 6 Interactions of compound 3p with *β*-tubulin.

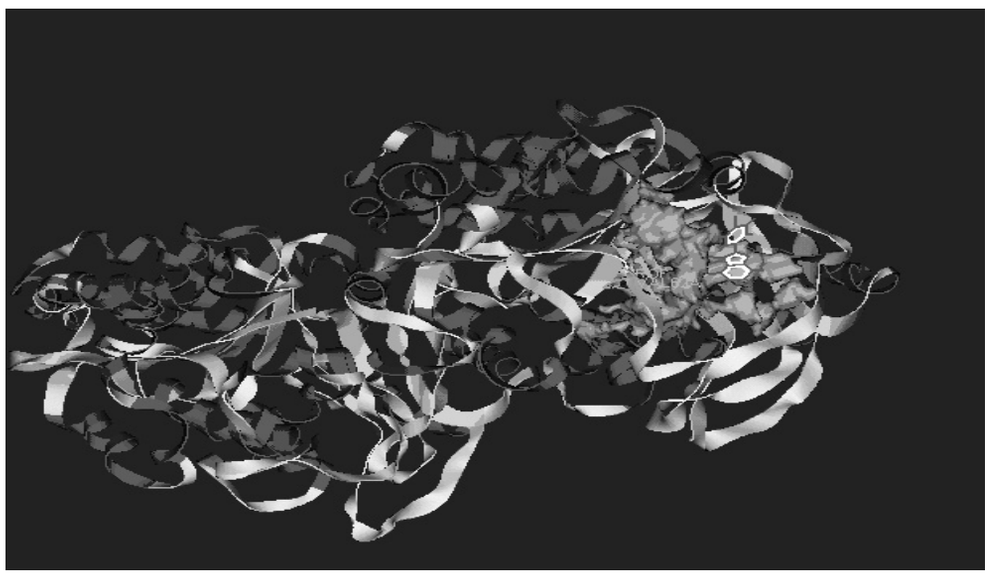


Fig. 7 Hydrogen bond interaction of compound 3p with *β*-tubulin.

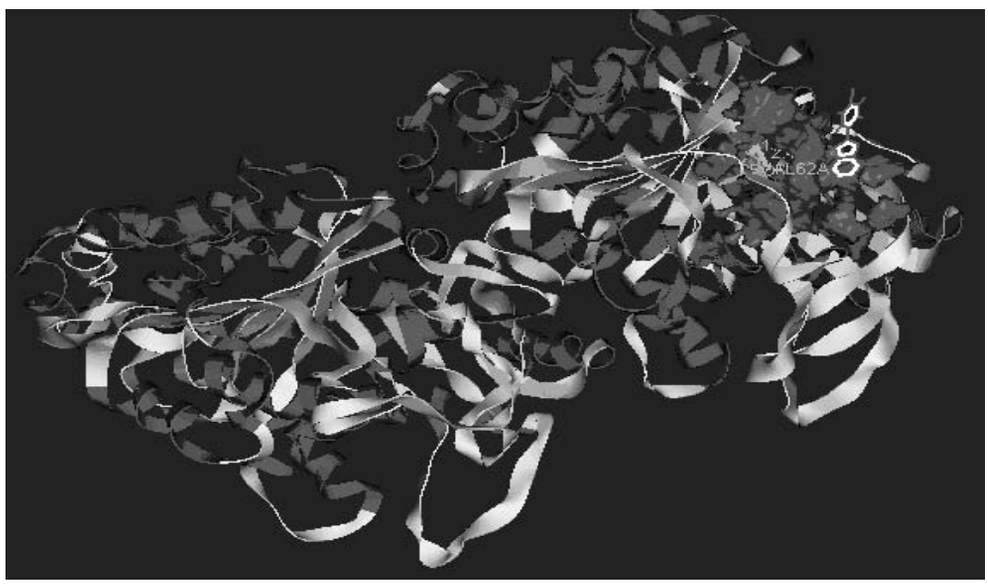


Fig. 8 Hydrophobic interaction of compound 3p with *β*-tubulin.

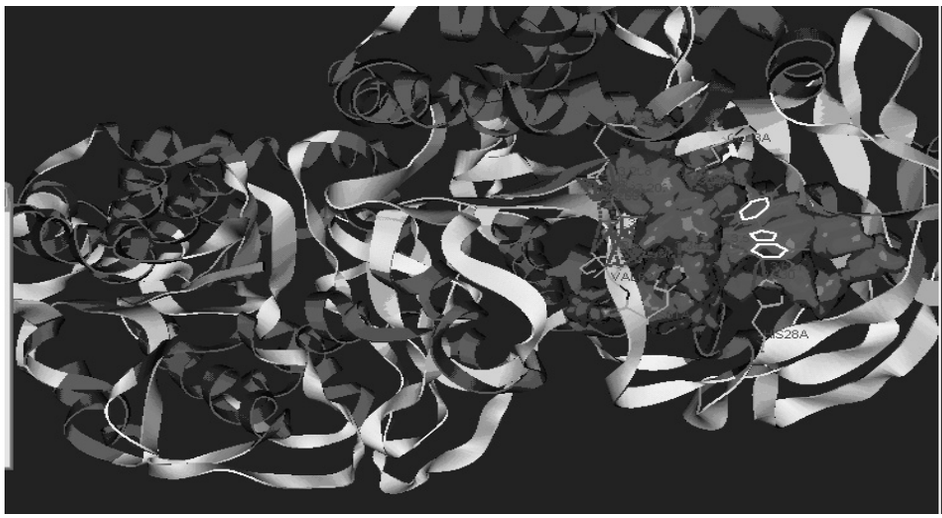


Fig. 9 vdw interaction of compound 3p with β-tubulin.

Conclusion

Molecular modeling study reveals that polar, more electropositive with less bulky substitutions on 2-phenyl benzimidazole-1-acetamides are required to enhance the anthelmintic activity. However observed anthelmintic activity of the benzimidazole analogues may be due to its binding with β-tubulin of intestinal cell of parasite.

References

[1] Lacey E (1988). The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. *Int. J. Parasitol.* **18(7):** 885-936.

[2] Jasmer D.P, Yao C, Rehman A, Johnson S (2000). Multiple lethal effects induced by a benzimidazole anthelmintic in the anterior intestine of the nematode *Haemonchus contortus*. *Mol. Biochem. Parasit.* **105:** 81-90.

[3] MartinR.J, RobertsonA.P, BjornH (1997). Target sites of anthelmintics. *Parasitology* **114:** S111-S124.

[4] Sawant R, Kawade D (2011). Synthesis and biological evaluation of some novel 2-phenyl benzimidazole-1-acetamide derivatives as potential anthelmintic agents. *Acta Pharmaceutica.* **61(3):** 353-361.

[5] Ajmani S, Jadhav K, Kulkarni S.A (2006). Three-dimensional QSAR using the k-nearest neighbor method and its interpretation. *J. Chem. Inf. Model.* **46:** 24-31.

[6] Halgren T.A (1996). Merck molecular force field III, molecular geometries and vibrational frequencies. *J. Comput. Chem.* **17:** 553-586.