

## ORIGINAL RESEARCH

# Design and synthesis of some new heterocyclic benzylidene hydrazide derivatives for their antileishmanial activity

Ritesh Bhole<sup>1</sup>, Prashant Patil<sup>2</sup>, Pritam Agale<sup>2</sup>, Sanjay Wate<sup>2</sup>

**ABSTRACT:** Benzylidene hydrazide is a highly active moiety against *Leishmania donovani*, and has become a core structure for the design of new antileishmanial agents. The 5-nitrothiophen-2-yl-benzylidene hydrazide derivative presents a potent IC<sub>50</sub> value. With this background, it is attempted to discern the structural and physicochemical requirements for the inhibition of *Leishmania donovani*. The techniques of quantitative structure activity relationship and docking are valuable molecular modeling tools for drug design. In the present study, 3-DQSAR of some *Leishmania donovani* inhibitors was carried out using VLife MDS, while interaction studies were carried using Schrodinger molecular modeling interface. The developed QSAR models showed  $q^2 = 0.9849$ ,  $pred\_r^2 = 0.6770$  with kNN analysis by stepwise forward and backward method. Docking study revealed important interactions of designed compounds with the active binding site of *Leishmania donovani*. Designed compounds were synthesis and screened against *Leishmania donovani*. Three compounds exhibited IC<sub>50</sub> values lower than standard drugs. Brief SAR analysis revealed that substitution is important to the activity.

**KEY WORDS:** benzylidene hydrazide derivatives, antileishmanial activity, 3D-QSAR, docking

## INTRODUCTION

Diseases caused by protozoan parasites are responsible for considerable morbidity and mortality, especially in developing countries. The most prevalent parasitic disease is malaria, but leishmaniasis is also considered to be a genuine emerging disease, afflicting worldwide over 12 million people in 88 countries with an annual incidence of about 2 million (1). Leishmaniasis is defined as a cluster of vector-borne diseases with diverse clinical manifestations, caused by the obligate intracellular protozoan parasite of the genus *Leishmania* (2). Its manifestations include three broad groups of disorders: visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis (3).

The treatment of leishmaniasis is far from satisfactory. Since the 1940s, the pentavalent antimony

compounds sodium stibogluconate (Pentostam, Glaxo Wellcome, UK) and meglumine antimoniate (Glucantime, Rhone-Poulenc Rorer, France) have been the mainstays of antileishmanial therapy (4,5). These drugs present high toxicity besides requiring parenteral administration for extended periods, especially in cases of visceral leishmaniasis. Moreover, in recent years, widespread resistance to pentavalent antimonial agents has been observed, especially in cases of *Leishmania*/HIV co-infection (6). These agents have been improved with the advent of new formulations or dosage regimens but there is an obvious need for new drugs with structures and mechanisms of action different from those of drugs in use to date with better potency and toxicity profiles (7,8).

The techniques of quantitative structure activity relationship and docking are valuable molecular

## AFFILIATIONS

<sup>1</sup>Department of Pharm Chemistry and Drug Discovery, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune (MS), India

<sup>2</sup>Department of Pharm Chemistry and Drug Discovery, Sharad Pawar College of Pharmacy, Wanadongri, Nagpur (MS), India

## CORRESPONDENCE

Ritesh Bhole

E-mail:  
ritesh.edu@gmail.com

Received:  
06.12.2012

Revision:  
26.12.2012

Accepted:  
10.01.2013

modeling tools for drug design. In the present manuscript, we report 3D QSAR model developed along with docking studies of *Leishmania donovani* inhibitors. Quantitative structure activity relationship (QSAR) searches information relating chemical structure to biological and other activities by developing a QSAR model (9,10). Molecular docking describes the generation, manipulation or representation of three-dimensional structures of molecules and associated physicochemical properties. It is the process by which the two molecules are fit together in complementary fashions in 3D space and design the molecules rationally. QSAR studies were done on VLife MDS, while docking calculations were done using Schrodinger GLIDE.

Though both of these softwares Viz, V-Life Science and Schrodinger can perform QSAR and Molecular Docking Studies we use V-Life Sci for QSAR and Schrodinger for Molecular Docking Studies based on its accuracy and precision based on reported studies (11).

## RESULTS AND DISCUSSION

### 3D-QSAR

3-DQSAR study was performed on a series of 23 compounds of *Leishmania donovani* inhibitors using V-Life MDS software Version 3.5.15

Statistical results 3-DQSAR analysis showed that QSAR model has good internal as well as external predictability (Table 1). For 3D QSAR a kNN-MFA with stepwise forward backward variable selection method was used resulted in several statistically significant models, of which the corresponding best model is reported herein. The model selection criterion is the value of  $q^2$ , the internal predictive ability of the model, and that of  $\text{pred}_r^2$ , the ability of the model to predict the activity of external test set. For activity against *Leishmania donovani*, model was found to be statistically most significant, especially with respect to the internal predictive ability ( $q^2 = 0.9849$ ) of the model. As the cross-validated correlation coefficient ( $q$ ) is used as a measure of reliability of prediction, the correlation coefficient suggests that our model is reliable and accurate. The predicted versus the experimental selectivity values for the training and test sets are depicted in (Figure 1). The value of  $\text{pred}_r^2$  was obtained for the test set and gave better results, with a value of 0.6770. Thus, the developed model displays good predictivity in regular cross validation.

3D QSAR studies helped to find out the importance of electronegative with bulkier groups at these positions. The electro-

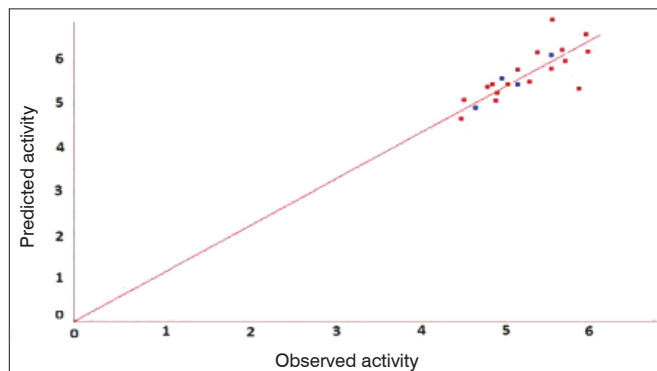
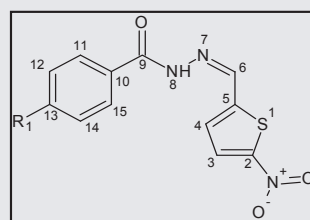


FIGURE 1.

static data point generated was E\_203 (-1.3257 to -1.1251), E\_864 (-0.7001 to -0.0473) and H\_104 (0.1069,0.1220) (Figure 2). It was found that the electronegative groups like alkoxy groups with increase in bulk were essential for potent *Leishmania donovani* inhibition activity and accordingly the substitutions were carried out for designing of NCEs.

TABLE 2. Structure, Experimental data and Predicted Activity of Benzohydrazides used in Training and Test Set using (SA-kNN method) model 1



No.	Substituents		IC50 ( $\mu\text{M}$ )		Dataset	Residual
	R <sub>1</sub>	R <sub>2</sub>	Exp.	Pred.		
1.	H	H	6.54	5.45	Train	-0.0792
2.	Cl	H	1.26	0.41	Train	-0.4876
3.	OCH <sub>3</sub>	H	7.21	4.69	Train	-0.1868
4.	NH <sub>2</sub>	H	2.24	4.49	Train	0.302
5.	OH	H	0.89	1.29	Train	0.151
6.	CH <sub>3</sub>	H	4.49	4.84	Train	0.0326
7.	CF <sub>3</sub>	H	0.82	0.41	Train	-0.3011
8.	NO <sub>2</sub>	H	2.81	6.54	Train	0.3668
9.	H	OCH <sub>3</sub>	8.52	4.84	Train	-0.2456
10.	H	CH <sub>3</sub>	4.84	4.49	Train	-0.0326
11.	H	CF <sub>3</sub>	3.79	2.78	Train	-0.1299
12.	H	NO <sub>2</sub>	2.78	0.89	Train	-0.4947
13.	Cl	Cl	0.41	1.29	Train	0.4876
14.	F	F	2.15	1.92	Train	-0.0491
15.	F	Cl	1.92	2.15	Train	0.0491
16.	NO <sub>2</sub>	OCH <sub>3</sub>	2.00	2.78	Test	0.143
17.	NO <sub>2</sub>	OH	5.45	1.29	Train	-0.636
18.	OCH <sub>3</sub>	NH <sub>2</sub>	4.69	7.29	Train	0.1868
19.	CH <sub>3</sub>	NH <sub>2</sub>	2.224	2.30	Test	0.0115
20.	CH <sub>3</sub>	OH	2.30	4.49	Train	0.2905
21.	CH <sub>3</sub>	OCH <sub>3</sub>	2.19	8.52	Test	0.5900
22.	Cl	SO <sub>2</sub> NH <sub>2</sub>	8.06	8.52	Test	0.0241

TABLE 1. Statistical results of 3D QSAR studies by kNN method

S.N.	Statistical parameter	Simulated annealing	Genetic algorithm	Forward backward
1	N	20	20	20
2	k	2	2	2
3	df	15	16	17
4	$q^2$	0.9706	0.9304	0.9849
5	$q^2\text{SE}$	0.2999	0.4613	0.2150
6	$\text{Pred}_r^2$	0.7153	0.6974	0.6770
7	$\text{Pred}_r^2\text{SE}$	0.3508	0.3617	0.3737
8	Contributing descriptor	E_864 E_712 H_104 E_203	H_163 E_49 E_70	E_864 (-0.7001,-0.0473) E_712 (5.0506,5.4668) E_203 (-1.3257,-1.1251) H_104 (0.1069,0.1220)

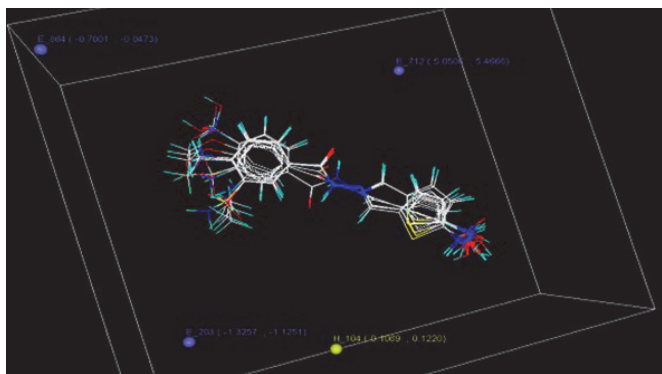


FIGURE 2.

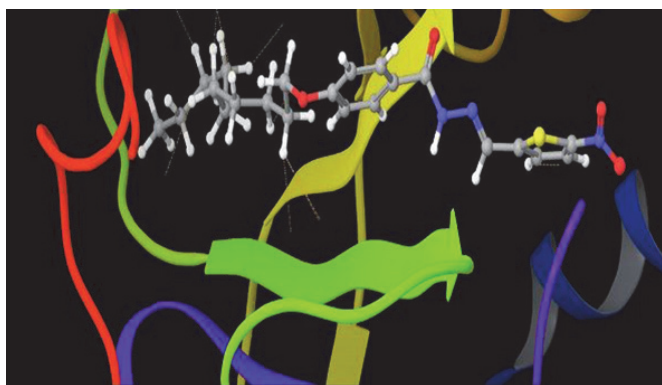


FIGURE 3.

TABLE 3. XP docking of compounds (4.a-4.e) with 2WUU receptor

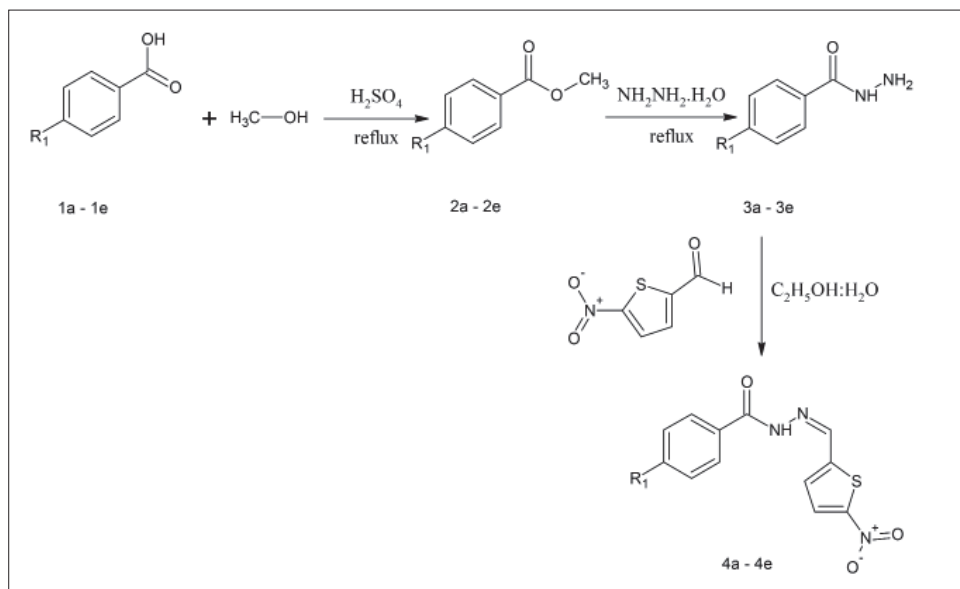
Sr. No.	Compd	Glide Score	Emodel Score	Glide Energy	Pose No.	H-bond	Good vdw	Bad vdw	Ugly vdw
1	4.a	-10.2	-99.4	-61.6	2	2	444	10	0
2	4.b	-9.95	-87.1	-55.9	19	2	373	13	0
3	4.c	-9.89	-80.4	-53.6	66	2	324	8	0
4	4.d	-9.61	-91.2	-58.1	3	2	387	14	0
5	4.e	-9.44	-81.5	-55.4	53	2	323	6	0
Std	Pentamidine	-10.18	-56.0	-41.1	1	2	331	15	0

### Docking Study

The docking study was performed of the compounds predicted from 3D QSAR in the active site of the protein with side chain flexibility. The docking study revealed hydrogen bond interactions of molecules with different active site residues present in catalytic pocket and specific pocket. Docking pose of compound 4a showed hydrogen bond interactions of aliphatic chain with receptor active site (Figure 3). All selected interacted with *leishmania donovani* receptor out of which compound 4a showed highest Docking score (GLIDE Score), H Bond energy and affinity towards receptors (Table 3).

### Chemistry

The synthesis of the intermediate and target compounds were performed by the reaction illustrated in Scheme 1. Compound 2a-2e namely substituted methylbenzoate was synthesized in



COMPOUNDS				R <sub>1</sub>
1a	2a	3a	4a	-O(CH <sub>2</sub> ) <sub>9</sub> CH <sub>3</sub>
1b	2b	3b	4b	-(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>
1c	2c	3c	4c	-(CH <sub>2</sub> ) <sub>6</sub> CH <sub>3</sub>
1d	2d	3d	4d	-O(CH <sub>2</sub> ) <sub>7</sub> CH <sub>3</sub>
1e	2e	3e	4e	-(CH <sub>2</sub> ) <sub>5</sub> CH <sub>3</sub>

SCHEME 1. Synthetic approach to obtain the library of compounds.

**TABLE 4.** Predicted and actual activity of compounds 4a-4e

Sr. No.	Compd	IC <sub>50</sub> (μM)	
		Leishmania donovani	
		Predicted activity	Actual activity
1.	4.a	0.822	0.578
2.	4.b	0.971	0.627
3.	4.c	0.749	0.778
4.	4.d	1.23	1.304
5.	4.e	1.392	1.421
6.	Pentamidine (Standard)	-	1.249

excellent yield by esterification of substituted benzoic acid with methanol. The structures of the compounds **3a-3e** were confirmed on the basis of IR spectra which showed the presence of characteristic absorption peaks at 1520-1500 (C=C vibrations), 1610-1500 (C-O stretching), 890-850 (benzene 1,4-disubstituted), which confirms esterification. The intermediate (2a-2e) undergoes nucleophilic substitution reaction in presence of hydrazine hydrate to form an intermediate substituted benzohydrazide (3a-3e). The structures of the reaction products were confirmed by IR, which showed characteristic peak at 1620-1600 (C-O stretching), 3180-3160 (N-H stretching), 1650-1620 (C=N stretching) confirms amination. The final step was carried out by condensing intermediate (3a-3e) with 5-nitrothiophene aldehyde resulting in the formation of substituted N-[(5-nitrothiophen-2-yl)methylidene]-benzohydrazide (4a-4e). The IR spectra showed bands at 3215 - 3230 (N-H stretching) and 1309 - 1348 (C-S stretching).

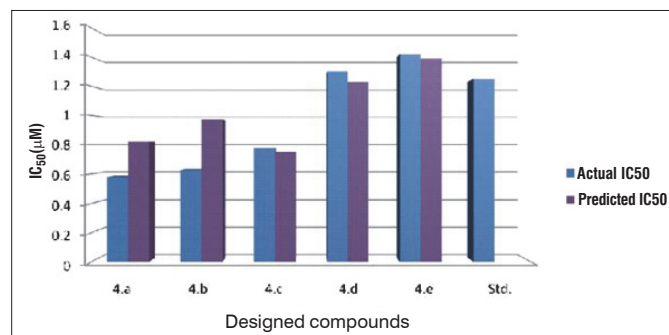
#### Biological evaluation

The five predicted compounds from 3D QSAR and confirmed from docking were tested, in vitro, against *L. donovani* promastigote forms at concentrations ranging from 10 to 0.01 μg/mL using well plates and RPMI 1640 medium supplemented with 10% fetal calf serum at 26°C, as described in section 5. The observed IC<sub>50</sub> values are summarized in (Table 4). The results obtained show that synthesized and tested compounds (4a), (4b) and (4c) exhibited very promising activities when compared with the standard drug pentamidine, while (4d) and (4e) showed moderate antileishmanial activity (Figure 4).

The predicted IC<sub>50</sub> values from QSAR studies were compared with the actual IC<sub>50</sub> values the results showed that compounds (4c), (4d) and (4e) exhibited comparable IC<sub>50</sub> values to that of predicted IC<sub>50</sub> values, while (4a) and (4b) showed variation from predicted values (Figure 4).

#### CONCLUSION

In conclusion, a series of novel 5-nitroheterocyclic benzohydrazide derivatives were designed and synthesized. 3D QSAR models have good statistical significance and high predictivity. The developed 3D QSAR models revealed the importance of different physicochemical properties of compounds in the *Leishmania donovani* inhibition. It was also found that descriptors like electrostatic and hydrophobic contribute significantly in the activity while, steric descriptor contributes negatively in the activity. Docking study revealed important interactions of compounds in the active binding site. The aliphatic chain present in the compounds showed good affinity towards

**FIGURE 4.**

the active site residues. Designed compounds showed good predictive activity and GLIDE score. The compounds, (4c), (4d) and (4e), exhibit the antileishmanial activity as expected from the QSAR studies. The compound (4a), (4b) and (4c) had shown highest antileishmanial activity while other compounds (4d) and (4e) showed moderate antimicrobial activity. The activity was compared with pentamidine as standard drug.

#### EXPERIMENTAL WORK

##### Hardware and software

All molecular modeling studies (3D) were performed using the Molecular Design Suite (VLife MDS software package, version 3.5; from VLife Sciences, Pune, India), conformational analysis was carried out using Schrodinger molecular modeling user interface implemented Dell Desktop Computers with a Dual core processor of Intel and Windows operating system (12).

##### Data set

A data set comprising 23 compounds belonging to 5-nitrothiophen-2-yl-benzylidene hydrazide derivatives as *Leishmania donovani* inhibitors were taken from the literature (13). While preparing the data set, compounds whose pharmacological screening was performed by same experimental protocol and conditions were considered. The chemical structures and pIC<sub>50</sub> values for the complete set of compounds are listed in (Table 2).

##### Structure conformation generation

Structures of compounds were sketched using the 2D structure draw application and converted to 3D structures. All the structures were minimized and optimized with the Merck Molecular Force Field (MMFF) method taking the root mean square gradient (RMS) of 0.01 kcal/mol Å° and the iteration limit to 10,000. All the structures were ionized at neutral pH 7. Conformers for each structure were generated using ConfGen by applying OPLS-2005 force field method and least energy conformer was selected for further study and all the compounds were aligned by template based method.

##### 3D QSAR

In the present study, (7.48650 to 31.8668) × (-16.7361 to 0.3877) × (-8.4230 to 7.30490) Å° grid at the interval of 2.00 was generated around the aligned compounds. The steric, electrostatic and hydrophobic interaction energies are computed at the lattice points of the grid using a methyl probe of charge +1 of gasteiger-marsili type. These interaction energy values are

considered for relationship generation and utilized as descriptors to decide nearness between molecules. The QSAR models were developed using Stepwise (SW) Forward - Backward, Simulated (SA) Annealing and Genetic Algorithm (GA) variable selection method with  $pIC_{50}$  activity field as dependent variable and physico-chemical descriptors as independent variable having cross-correlation limit of 0.9, 0.7 and 1.0 for model 1, model 2 and model 3 respectively. Selection of test and training set was done by sphere exclusion method having dissimilarity value of 4.2, 5.3 and 4.9 for model 1, model 2 and model 3 respectively. Variance cut off point was 0.0. Numbers of maximum and minimum neighbors were 5 and 2 respectively.

### Flexible Docking

For docking study, co-crystallized structure of *Leishmania donovani* (PDB id 2WUU) (14) was taken from Brookhaven Protein Data Bank (www.rcsb.org) and prepared by using the Protein preparation wizard removing water and cofactors from the protein, optimizing hydrogen bonding and deleting the ligand present in crystal structure (15). The binding site shows a high degree of flexibility, which poses a big challenge to investigate possible binding modes of a given ligand. So these side chain residues which are close enough to the active ligand and interacting with it were considered as flexible during the docking. Because of the stochastic nature of the docking search algorithm, we have employed multiple runs (10 runs) for each ligand protein setup to ensure convergence to the lowest-energy solution and reranking the poses found afterwards. The most promising poses returned when the docking run terminates was further analyzed in the pose organizer.

### Synthesis of Designed Compounds

The synthesis of the intermediate and target compounds were performed by the reaction illustrated in Scheme 1. Compound **2a - 2e** namely, substituted methyl benzoate was synthesized in excellent yield by esterification of compound **1a - 1e**. Reaction of **2a - 2e** with hydrazine hydrate gives compounds **3a - 3e**. Condensation of product **3a - 3e** with nitrothiophene aldehyde affords compounds from **4a-4e**.

Chemicals were obtained from Alfa Aesar (UK), Loba Chemie/ S.D. Fine-Chem. /E. Merck. Melting points (m.p.) were detected with open capillaries using Thermo Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). HNMR was obtained using a BRUKER AVANCE II 400 Spectrophotometer using  $CDCl_3$ . All chemical shift values were recorded as  $\delta$  (ppm). The purity of compounds was checked by thin layer chromatography (Merck, silica gel, HF, type 60, 0.25 mm). The elemental analysis was performed at RTM Nagpur University, India. Elemental analyses on 4a-4e for C, H, N were within 0.4% of theoretical values.

### Synthesis procedure

#### Synthesis of Compound 2a - 2e

The mixture of substituted benzoic acid (1a-1e) (1 mol) and methanol (30mol) in presence of sulphuric acid was refluxed for 4-6 hr; excess solvent was removed under vacuum. The solid crystals separated were filtered, dried and recrystallized from ethanol.

**2a:** Yield: 83%, mp 195–197°C,  $R_f$ : 0.44 [ethanol: benzene (1:1)], IR (KBr):  $cm^{-1}$  1506 (C=C vibrations), 1609 (C-O stretching), 884 (benzene 1, 4 -disubstituted),  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.4 (s, 3H, decoxy,  $CH_3$ ), 1.39 (m, 2H, decoxy,  $OCH_2$ ), 1.53 (m, 2H, decoxy,  $CH_2$ ), 3.66 (m, 2H, decoxy,  $CH_2$ ), 2.48 (m, 2H, decoxy,  $CH_2$ ), 3.28 (m, 2H, decoxy,  $CH_2$ ), 1.57 (m, 2H, decoxy,  $CH_2$ ), 3.41 (m, 2H, decoxy,  $CH_2$ ), 1.2 (m, 2H, decoxy,  $CH_2$ ), 1.32 (m, 2H, decoxy,  $CH_2$ ), 7.31-7.32 (d,  $H_{12}$  and  $H_{14}$ ), 7.63 (d,  $H_{11}$  and  $H_{15}$ ), EIMS (m/z): 292 ( $M^+$ ).

**2b:** Yield: 69%, mp 44 - 46°C,  $R_f$ : 0.52 [ethanol: benzene (1:1)], IR (KBr):  $cm^{-1}$  1514 (C=C vibrations), 1502 (C-O stretching), 884 (benzene 1, 4 -disubstituted),  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.78 (s, 3H, octyl,  $CH_3$ ), 2.47 (m, 2H, octyl,  $CH_2$ ), 1.55 (m, 2H, octyl,  $CH_2$ ), 1.21 (m, 2H, octyl,  $CH_2$ ), 2.45 (m, 2H, octyl,  $CH_2$ ), 1.34 (m, 2H, octyl,  $CH_2$ ), 3.24 (m, 2H, octyl,  $CH_2$ ), 2.44 (m, 2H, octyl,  $CH_2$ ), 6.97-6.64 (d,  $H_{12}$  and  $H_{14}$ ), 7.77-7.0 (d,  $H_{11}$  and  $H_{15}$ ), EIMS (m/z): 248 ( $M^+$ ).

**2c:** Yield: 83%, mp 122–124°C,  $R_f$ : 0.57 [ethanol: benzene (1:1)], IR (KBr):  $cm^{-1}$  1506 (C=C vibrations), 1609 (C-O stretching), 884 (benzene 1, 4 -disubstituted),  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.71 (s, 3H, heptyl,  $CH_3$ ), 3.01 (m, 2H, heptyl,  $CH_2$ ), 2.12 (m, 2H, heptyl,  $CH_2$ ), 1.32 (m, 2H, heptyl,  $CH_2$ ), 1.53 (m, 2H, heptyl,  $CH_2$ ), 1.35 (m, 2H, heptyl,  $CH_2$ ), 2.74 (m, 2H, heptyl,  $CH_2$ ), 6.90-6.68 (d,  $H_{12}$  and  $H_{14}$ ), 7.8–7.15 (d,  $H_{11}$  and  $H_{15}$ ), EIMS (m/z): 234 ( $M^+$ ).

**2d:** Yield: 71%, mp 44 - 46°C,  $R_f$ : 0.6 [ethanol: benzene (1:1)], IR (KBr):  $cm^{-1}$  1514 (C=C vibrations), 1502 (C-O stretching), 880 (benzene 1,4 -disubstituted),  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  3.78 (s, 3H, octoxy,  $OCH_2$ ), 2.47 (m, 2H, octoxy,  $CH_2$ ), 1.55 (m, 2H, octoxy,  $CH_2$ ), 1.21 (m, 2H, octoxy,  $CH_2$ ), 2.45 (m, 2H, octoxy,  $CH_2$ ), 1.34 (m, 2H, octoxy,  $CH_2$ ), 3.24 (m, 2H, octoxy,  $CH_2$ ), 2.44 (m, 2H, octoxy,  $CH_2$ ), 6.99-6.78 (d,  $H_{12}$  and  $H_{14}$ ), 7.7-7.10 (d,  $H_{11}$  and  $H_{15}$ ), EIMS (m/z): 264 ( $M^+$ ).

**2e:** Yield: 57%, mp: 52 - 54°C,  $R_f$ : 0.63 [ethanol: benzene (1:1)], IR (KBr):  $cm^{-1}$  1610 (C-O stretching), 1516 (C=C vibrations), 875 (benzene 1,4 -disubstituted),  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.68 (s, 3H, hexyl,  $CH_3$ ), 3.28 (m, 2H, hexyl,  $CH_2$ ), 1.94 (m, 2H, hexyl,  $CH_2$ ), 2.47 (m, 2H, hexyl,  $CH_2$ ), 3.87 (m, 2H, hexyl,  $CH_2$ ), 1.34 (m, 2H, hexyl,  $CH_2$ ), 6.7-6.58 (d,  $H_{12}$  and  $H_{14}$ ), 7.54-7.23 (d,  $H_{11}$  and  $H_{15}$ ), EIMS (m/z): 220 ( $M^+$ ).

#### Synthesis of Compound 3a - 3e

The mixture of (2a-2e) (0.02mol) and hydrazine hydrate (0.6 mol) was refluxed for 12 hr. The excess solvent was removed under vacuum and the reaction mixture was cooled at 4-5°C. The solid crystals separated were filtered, washed with cold water, dried and recrystallized from ethanol.

**3a:** Yield: 74.91%, mp 198 - 200°C,  $R_f$ : 0.48 (ethyl acetate), IR (KBr):  $cm^{-1}$  3178 (N-H stretching), 1648 (C=N stretching), 1506 (C=C vibrations), 1609 (C-O stretching), 1328 (aromatic -CH stretching),  $^1H$ -NMR (DMSO- $d_6$ ):  $\delta$  2.4 (s, 3H, decoxy,  $CH_3$ ), 1.39 (m, 2H, decoxy,  $CH_2$ ), 1.53 (m, 2H, decoxy,  $CH_2$ ), 3.66 (m, 2H, decoxy,  $CH_2$ ), 2.48 (m, 2H, decoxy,  $CH_2$ ), 3.28 (m, 2H, decoxy,  $CH_2$ ), 1.57 (m, 2H, decoxy,  $CH_2$ ), 3.41 (m, 2H, decoxy,  $CH_2$ ), 1.2 (m, 2H, decoxy,  $CH_2$ ), 1.32 (m, 2H, decoxy,  $CH_2$ ), 7.31-7.32 (d,  $H_{12}$  and  $H_{14}$ ), 7.63 (d,  $H_{11}$  and  $H_{15}$ ), 9.6 (d,  $H_8$ ), EIMS (m/z): 292 ( $M^+$ ).

**3b:** Yield: 81.91%. mp 195 - 197°C,  $R_f$ : 0.4 [ethanol: benzene (1:1)], IR (KBr):  $cm^{-1}$  1506 (C=C vibrations), 1328 (aromatic

-CH stretching), 3180 (N-H stretching), 1609 (C-O stretching), 1635 (C=N stretching), 2856 (CH<sub>3</sub> - O stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 3.78 (s, 3H, octyl,CH<sub>3</sub>), 2.47(m, 2H, octyl,OCH<sub>2</sub>), 1.55 (m, 2H, octyl,CH<sub>2</sub>), 1.21 (m, 2H, octyl,CH<sub>2</sub>), 2.45(m, 2H, octyl,CH<sub>2</sub>), 1.34(m, 2H, octyl,CH<sub>2</sub>), 3.24 (m, 2H, octyl,CH<sub>2</sub>), 2.44 (m, 2H, octyl,CH<sub>2</sub>), 6.97-6.64 (d, H<sub>12</sub> and H<sub>14</sub>), 7.77-7.0 (d, H<sub>11</sub> and H<sub>15</sub>), 9.6 (d, H<sub>8</sub>), EIMS (m/z): 264(M<sup>+</sup>).

**3c:** Yield: 61%. mp 189 – 191°C, R<sub>f</sub>: 0.39 (ethanol: benzene). IR (KBr): cm<sup>-1</sup> 1335 (aromatic -CH stretching), 1655 (C=N stretching), 1611 (C-O stretching), 1524 (C=C vibrations), 3174 (N-H stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 3.71 (s, 3H, heptyl,CH<sub>3</sub>), 3.01 (m, 2H, heptyl,CH<sub>2</sub>), 2.12 (m, 2H, heptyl,CH<sub>2</sub>), 1.32(m, 2H, heptyl,CH<sub>2</sub>), 1.53(m, 2H, heptyl,CH<sub>2</sub>), 1.35(m, 2H, heptyl,CH<sub>2</sub>), 2.74(m, 2H, heptyl,CH<sub>2</sub>), 6.90-6.68 (d, H<sub>12</sub> and H<sub>14</sub>), 7.8-7.15 (d, H<sub>11</sub> and H<sub>15</sub>), 9.3 (d, H<sub>8</sub>), EIMS (m/z): 250 (M<sup>+</sup>).

**3d:** Yield: 59%, mp: 173 – 175°C, R<sub>f</sub>: 0.52 [ethanol: benzene(1:1)], IR (KBr): cm<sup>-1</sup> 1335 (aromatic -CH stretching), 2844 (CH<sub>3</sub>-O stretching), 1639 (C=N stretching), 3178 (N-H stretching), 1524 (C=C vibrations), 1605 (C-O stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 3.78 (s, 3H, octoxy,OCH<sub>2</sub>), 2.47(m, 2H, octoxy,CH<sub>2</sub>), 1.55 (m, 2H, octoxy,CH<sub>2</sub>), 1.21 (m, 2H, octoxy,CH<sub>2</sub>), 2.45(m, 2H, octoxy,CH<sub>2</sub>), 1.34(m, 2H, octoxy,CH<sub>2</sub>), 3.24 (m, 2H, octoxy,CH<sub>2</sub>), 2.44 (m, 2H, octoxy,CH<sub>2</sub>), 6.99-6.78 (d, H<sub>12</sub> and H<sub>14</sub>), 7.7-7.10 (d, H<sub>11</sub> and H<sub>15</sub>), 9.34 (d, H<sub>8</sub>), EIMS (m/z): 280 (M<sup>+</sup>).

**3e:** Yield: 91%, mp: 201 – 203°C, R<sub>f</sub>: 0.68 [ethanol: benzene(1:1)], IR (KBr): cm<sup>-1</sup> 2830 (CH<sub>3</sub> - O stretching), 1520 (C=C vibrations), 3178 (N-H stretching), 1616 (C-O stretching), 1640 (C=N stretching), 1341 (aromatic -CH stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.68 (s, 3H, hexyl,CH<sub>3</sub>), 3.28 (m, 2H, hexyl,CH<sub>2</sub>), 1.94 (m, 2H, hexyl,CH<sub>2</sub>), 2.47 (m, 2H, hexyl,CH<sub>2</sub>), 3.87 (m, 2H, hexyl,CH<sub>2</sub>), 1.34 (m, 2H, hexyl,CH<sub>2</sub>), 6.7-6.58 (d, H<sub>12</sub> and H<sub>14</sub>), 7.54-7.23 (d, H<sub>11</sub> and H<sub>15</sub>), 9.68 (d, H<sub>8</sub>), EIMS (m/z): 236 (M<sup>+</sup>).

### Synthesis of Compound 4a - 4e

The solution of compound (3a-3e) (0.02 mol) and nitrothiophene aldehyde (0.02 mol) was prepared in water: ethanol (2:5) and refluxed with time ranging from 15min to 1hr. The solid crystals separated were filtered, dried and recrystallized from ethanol.

**4a:** Yield: 93%, mp: 176 – 178°C, R<sub>f</sub>: 0.56 [ethanol: benzene (1:1)], IR (KBr): cm<sup>-1</sup> 1555 (aromatic -C-NO<sub>2</sub>), 1647 (C=N stretching), 3165 (N-H stretching), 1482 (C=C vibrations), 1616 (C-O stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.4 (s, 3H, decoxy,CH<sub>3</sub>), 1.39 (m, 2H, decoxy,CH<sub>2</sub>), 1.53 (m, 2H, decoxy,CH<sub>2</sub>), 3.66 (m, 2H, decoxy,CH<sub>2</sub>), 2.48 (m, 2H, decoxy,CH<sub>2</sub>), 3.28 (m, 2H, decoxy,CH<sub>2</sub>), 1.57 (m, 2H, decoxy,CH<sub>2</sub>), 3.41 (m, 2H, decoxy,CH<sub>2</sub>), 1.2 (m, 2H, decoxy,CH<sub>2</sub>), 1.32 (m, 2H, decoxy,CH<sub>2</sub>), 7.4-7.42 (d, 2H, H<sub>12</sub> and H<sub>14</sub>), 7.55-7.56 (d, 1H, H<sub>4</sub>), 7.7 (d, 2H, H<sub>11</sub> and H<sub>15</sub>), 8.11-8.12 (d, 1H, H<sub>3</sub>), 8.66 (s, 1H, H<sub>6</sub>), 12.17 (s, 1H, H<sub>8</sub>); EIMS (m/z): 431 (M<sup>+</sup>).

Anal. C<sub>22</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub>S: C (60.23%) H (5.78%) N (8.34%)

**4b:** Yield: 81%, mp : 193-195°C R<sub>f</sub>: 0.68 [ethanol: benzene(1:1)], IR (KBr): cm<sup>-1</sup> 2830 (CH<sub>3</sub> - O stretching), 1520 (C=C vibrations), 1600 (C-O stretching), 1341 (aromatic -CH stretching), 1652 (C=N stretching), 3170 (N-H stretching), 1534 (aromatic -C-NO<sub>2</sub>), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 3.78 (s, 3H, octyl,CH<sub>3</sub>),

2.47(m, 2H, octyl,OCH<sub>2</sub>), 1.55 (m, 2H, octyl,CH<sub>2</sub>), 1.21 (m, 2H, octyl,CH<sub>2</sub>), 2.45(m, 2H, octyl,CH<sub>2</sub>), 1.34(m, 2H, octyl,CH<sub>2</sub>), 3.24 (m, 2H, octyl,CH<sub>2</sub>), 2.44 (m, 2H, octyl,CH<sub>2</sub>), 7.04-7.07 (d, 2H, H<sub>12</sub> and H<sub>14</sub>), 7.52-7.54 (d, 1H, H<sub>4</sub>), 7.87-7.90 (d, 2H, H<sub>11</sub> and H<sub>15</sub>), 8.09-8.10 (d, 1H, H<sub>3</sub>), 8.66 (s, 1H, H<sub>6</sub>), 12.08 (d, 1H, H<sub>8</sub>); EIMS (m/z): 387 (M<sup>+</sup>).

Anal. C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>S: C (61.23%) H (6.50%) N (9.84%)

**4c:** Yield: 64%, mp: 216 – 218°C, R<sub>f</sub>: 0.53 [ethanol: benzene(1:1)], IR (KBr): cm<sup>-1</sup> 1547(aromatic -C-NO<sub>2</sub>), 3175 (N-H stretching), 1615 (C=N stretching), 1531 (C=C vibrations), 1600 (C-O stretching), 1333 (aromatic -CH stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 3.71 (s, 3H, heptyl,CH<sub>3</sub>), 3.01 (m, 2H, heptyl,CH<sub>2</sub>), 2.12 (m, 2H, heptyl,CH<sub>2</sub>), 1.32(m, 2H, heptyl,CH<sub>2</sub>), 1.53(m, 2H, heptyl,CH<sub>2</sub>), 1.35(m, 2H, heptyl,CH<sub>2</sub>), 2.74(m, 2H, heptyl,CH<sub>2</sub>), 6.90-6.68 (d, H<sub>12</sub> and H<sub>14</sub>), 7.8-7.15 (d, H<sub>11</sub> and H<sub>15</sub>), 8.11-8.12 (d, 1H, H<sub>3</sub>), 8.66 (s, 1H, H<sub>6</sub>), 12.17 (s, 1H, H<sub>8</sub>); EIMS (m/z): 373 (M<sup>+</sup>).

Anal. C<sub>19</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>S: C (61.10%) H (6.21%) N (11.25%)

**4d:** Yield: 55%, mp: 182- 184°C, R<sub>f</sub>: 0.6 [ethanol: benzene (1:1)], IR (KBr): cm<sup>-1</sup> 1547 (aromatic -C-NO<sub>2</sub>), 1438 (C=C vibrations), 3180 (N-H stretching), 1334 (C-O stretching, alcohol), 1637 (C=N stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 3.78 (s, 3H, octoxy,OCH<sub>2</sub>), 2.47(m, 2H, octoxy,CH<sub>2</sub>), 1.55 (m, 2H, octoxy,CH<sub>2</sub>), 1.21 (m, 2H, octoxy,CH<sub>2</sub>), 2.45(m, 2H, octoxy,CH<sub>2</sub>), 1.34(m, 2H, octoxy,CH<sub>2</sub>), 3.24 (m, 2H, octoxy,CH<sub>2</sub>), 2.44 (m, 2H, octoxy,CH<sub>2</sub>), 6.99-6.78 (d, H<sub>12</sub> and H<sub>14</sub>), 7.7-7.10 (d, H<sub>11</sub> and H<sub>15</sub>), 8.09-8.10 (d, 1H, H<sub>3</sub>), 8.66 (s, 1H, H<sub>6</sub>), 12.08 (d, 1H, H<sub>8</sub>); EIMS (m/z): 403 (M<sup>+</sup>).

Anal. C<sub>20</sub>H<sub>25</sub>N<sub>3</sub>O<sub>4</sub>S: C (59.53%) H (6.25%) N (10.41%)

**4e:** Yield: 92%, mp: 208 – 210°C, R<sub>f</sub>: 0.59 [ethanol: benzene (1:1)], IR (KBr): cm<sup>-1</sup> 1578(aromatic -C-NO<sub>2</sub>), 2830 (CH<sub>3</sub> - O stretching), 1566 (C=C vibrations), 3178 (N-H stretching), 1648 (C=N stretching), <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>): δ 2.68 (s, 3H, hexyl,CH<sub>3</sub>), 3.28 (m, 2H, hexyl,CH<sub>2</sub>), 1.94 (m, 2H, hexyl,CH<sub>2</sub>), 2.47 (m, 2H, hexyl,CH<sub>2</sub>), 3.87 (m, 2H, hexyl,CH<sub>2</sub>), 1.34 (m, 2H, hexyl,CH<sub>2</sub>), 6.7-6.58 (d, H<sub>12</sub> and H<sub>14</sub>), 7.54-7.23 (d, H<sub>11</sub> and H<sub>15</sub>), 8.11-8.12 (d, 1H, H<sub>3</sub>), 8.66 (s, 1H, H<sub>6</sub>), 12.17 (s, 1H, H<sub>8</sub>); EIMS (m/z): 359 (M<sup>+</sup>).

Anal. C<sub>18</sub>H<sub>21</sub>N<sub>3</sub>O<sub>3</sub>S: C (60.15%) H (5.89%) N (11.69%)

### ANTILEISHMANIAL ASSAYS (13)

Antileishmanial activity of the compounds was tested in vitro against a culture of *L. donovani* promastigotes. The parasites were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Chem Co.) at 26°C. A 3-day-old culture was diluted to 5\*10<sup>5</sup> promastigotes/mL. Samples were tested at concentrations from 50 to 3.1µg/mL. Drug dilutions were prepared directly in cell suspension in 96-well plates and were incubated at 26°C for 48 h and growth of Leishmania promastigotes was determined by Alamar Blue assay. Standard fluorescence was measured on a Fluostar Galaxy plate reader at excitation wavelength of 544 nm and emission wavelength of 590 nm. Pentamidine were used as the standard antileishmanial agents. Percentual growth was calculated and plotted versus test concentration for computing the IC<sub>50</sub> values.

## Heterosiklik benziliden hidrazit türevi bazı yeni bileşiklerin sentezi ve antiprotozoal etkileri

**ÖZET:** Benziliden hidrazit artığı taşıyan bileşiklerin *Leishmania donovani*'ye karşı yüksek etkinlik gösterdiği bilindiğinden ilgili yapı *Leishmania donovani*'ye karşı geliştirilen antiprotozoal ilaçların tasarımında önemli bir yere sahiptir. Ayrıca, 5-nitrotiyofen-2-ilbenzilidenhidrazit yapılı bileşiklerin düşük IC50 değerlerine sahip bileşikler olduğu bilinmektedir. Bu bilgiden hareketle *Leishmania donovani*'ye karşı kullanılacak bileşiklerin taşınması gereken yapısal ve fizikokimyasal özellikleri araştırılmış ve ilgili özellikleri taşıyacak olan yeni bileşiklerin tasarımı kantitatif yapı etki ilişkisi (QSAR) yöntemi ve çeşitli moleküler modelleme sistemleri kullanılarak gerçekleştirilmiştir. Yaptığımız çalışma kapsamında *Leishmania donovani*'ye karşı geliştirilen bazı bileşiklerin üç boyutlu kantitatif yapı etki ilişkisi (3-DQSAR) VLife MDS, etkileşme çalışmaları ise Schrodinger moleküler modelleme arayüzü kullanılarak yapılmıştır ( $q_2 = 0.9849$ ,  $pred\_r_2 = 0.6770$  kNN analizi ile). Docking çalışmaları sonucunda, tasarlanan bileşikler ile *Leishmania donovani*'nin aktif bağlanma bölgesi arasında önemli etkileşmeler tespit edilmiştir. Tasarlanan bileşikler sentezlenmiş ve *Leishmania donovani*'ye karşı antiprotozoal etkinlikleri taranmıştır. Sentezlenen seriden üç bileşiğin antiprotozoal etki taraması esnasında standart olarak kullanılan ilaç etken maddelerinden daha etkili olduğu tespit edilmiştir. Elde edilen sonuçlar, yapı etki ilişkisi açısından incelendiğinde süstitüsyonun biyolojik etkiyi etkileyen önemli bir parametre olduğu tespit edilmiştir.

**ANAHTAR KELİMELER:** Benzilidenhidrazit türevleri, antiprotozoal etki, 3D-QSAR, moleküler modelleme

### ACKNOWLEDGMENT

Authors wish to thanks Sharad Pawar College of Pharmacy, University of Nagpur, India for their valuable help in QSAR studies

### REFERENCES

1. Berman J. Chemotherapy of leishmaniasis: recent advances in the treatment of visceral disease. *Curr Opin Infect Dis* 1998; 11:707-10.
2. Sundar S, Rai M. Advances in the treatment of leishmaniasis. *Curr Opin Infect Dis* 2002; 15: 593-98.
3. Desjeux P. Leishmaniasis: Public health aspects and control. *Clin Dermatol* 1996; 14: 417-23.
4. Reithinger R, Dujardin JC, Louzir G, Pirme C, Alexander B, Brooker S. Cutaneous leishmaniasis. *Lancet Infect Dis* 2007; 7:581-96.
5. Mishra J, Saxena A, Singh S. Chemotherapy of leishmaniasis: past, present and future. *Curr Med Chem* 2007; 14:1153-69.
6. Alvar J, Canavate C, Gutierrez-Solar H, Jimenez M, Laguna F, Lopez-Velez V, Molina RJ. *Leishmania* and human immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev* 1997; 10: 298-319.
7. Berman JD, Brewar TG, Roberts RJ. Phase 2 efficacy trial of an oral 8-aminoquinoline (WR6026) for treatment of visceral leishmaniasis. *Clin Infect Dis* 1999; 28: 49-51.
8. Herwaldt BL. Leshminiasis. *Lancet* 1999; 354: 1191-49-9.
9. Jones G, Willett P, Glen RC. A genetic algorithm for flexible molecular overlay and pharmacophore elucidation. *J Comput Aided Mol Design* 1995; 9:532.
10. Zahra GN, Behzad AR. Modeling the antileishmanial activity screening of 5-nitro-2-heterocyclic benzyldiene hydrazides using different chemometrics methods. *Eur J Med Chem* 2010;45:719-26.
11. Guha R, Van Drie JH. Structure--activity landscape index: identifying and quantifying activity cliffs. *J Chem Inf Model* 2008;48:646-58.
12. VLife MDS 3.5, Molecular Design Suite, Vlife Sciences Technologies Pvt. Ltd., Pune, India, 2004. ([www.vlifesciences.com](http://www.vlifesciences.com)) [Accessed 10 January 2011].
13. Rando DG, Avery M, Tekwani BL, Khan S, Ferreira EI. Antileishmanial activity screening of 5-nitro-2-heterocyclic benzyldiene hydrazides. *Bioorg Med Chem* 2008;16: 6724-31.
14. [www.pdb.org/pdb/explore/explore.do?structureId=1PWM](http://www.pdb.org/pdb/explore/explore.do?structureId=1PWM)[Accessed 10 January 2011].
15. GLIDE 2.1, Schrodinger, LLC, New York, NY, 2006 [Accessed 10 January 2011].