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Antibacterial bioassay and computational studies of various novel naphthyridine series

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ABSTRACT

*The biological evaluation, QSAR and docking studies ofNaphthyridine derivatives are carried out to optimize their inhibitory activity against DNA topoisomerase. The biological activities of these derivatives are correlated to different molecular properties. The AM1and PM3 semi empirical methods are used to estimate vertical ionization potentials (IPv's), electron affinity (EA) , electro negativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP),hydration energy(HE), ionization potential(IP) and charges. The different modeled equations are proposed by regression analysis. The leave-one-out cross-validation method is used to estimate the predictive power of final QSAR equations. The hardness (η), was found to be indicative molecular property by regression analysis. Docking studies of naphthyridines with DNA topoisomerase were made to support the finding of QSAR studies. Analysis of results of both QSAR and Docking studies suggested that remarkable inhibitory activity is exhibited by molecule **5, 7, and8**.The hydrogen bond interactions along with hydrophobic and electrostatic interactions are mapped to confirm their potencies.*

Keywords: semi empirical Methods, Naphthyridine derivatives, QSAR, Regression analysis, Docking.

INTRODUCTION

Amino-substituted 2-pyridines have attracted attention due to their promising features as an important core structure for the development of biologically active molecules [1]. Pharmaceuticals with the 2- pyridone skeleton have emerged as antitumor [2], antifungal [3], antibacterial [4], antiviral, antithrombotic [5] agents. Meanwhile it is well known that the 2-pyridone-ring system is a valuable building block in natural product synthesis.

It is well known that the introduction of fluorine atoms or a fluoroalkyl group can greatly modify the physico-chemical features and thus the biological properties of a molecule [6, 7]. Moreover the presence of pyridyl ring into a parent compound may improve its properties and biological activities in the pharmaceutical and agrochemical compounds. And many pyridyl containing compounds are also known to possess a wide range of biological and pharmaceutical activities [8, 9].

Naphthyridines, as antibacterial agents are found to inhibit topoisomerase (Top) [10] .Human topoisomerase type I (Top1) is a member of the topoisomerase family of enzymes that resolve the topological problems associated with DNA super coiling during various essential cellular processes [11]. It forms a covalent link with the 3′-end of the cut DNA strand in the Top1-DNA cleavage complex at its catalytic tyrosine 723 residue, relieving torsional strain in DNA via reversible single-strand nicks [12]. Top1 is important for the successful replication, transcription and recombination of DNA, as well as chromatin remodeling, making it an attractive drug target for anticancer therapy.
The goal of our research was (i) to gain further insight into the structural features related to the antibacterial activity of the compounds from the quinolone series and (ii) to suggest new substituents or structures with potentially enhanced antibacterial activity.

EXPERIMENTAL SECTION

BIOASSAY

Bioassay or biological evaluation is done by Macro broth dilution test and expressed as MIC value. Serial dilutions of antibacterial agents (Naphthyridine derivatives) are dispensed into appropriately labeled tubes. Each tube is then inoculated with a standardized nutrient broth suspension of the bacteria being tested. The primary advantage of the broth dilution test is that it permits a quantitative estimate of both the inhibitory and bactericidal activities of the antimicrobial agent.

The methods described here are those currently being used in multicenter collaborative studies by the Subcommittee on Antibacterial Susceptibility Testing of the NCCLS[13].

Procedure

Preparation of antibacterial drugs and dilution schemes: Because antibacterial drug preparation is critical step in the performance of reproducible assays, commercially prepared macro broth dilution tubes are unavailable, and techniques for preparation of the tubes vary from those generally employed with antibacterial - antimicrobial agents.

(a) Formulation

10mg of unknown Naphthyridine
1 ml dimethylsulfoxide (DMSO)
$C_1 \times V_1 = C_2 \times V_2$
10,000 µg/ml x 1 ml = 2.5 µg/ml x V_2
V = (10,000 µg/ml x 1 ml)/2.5µg/ml

(b) Preparation

Weighed out 10mg of given sample powder in a small eppendoff. Dissolved the powder with 1 ml of DMSO.Diluted 1 ml (10,000 µg/ml) sample in 14.63 ml of Sabauraud's broth (SAB) to make an initial concentration of 2.5 µg/ml. Prepared 11 racks of test tubes labeled 25-0.0781 µg/ml (i.e., 2.2, 1.25, 0.625, 0.312, 0.156,and 0.0781). Labeled 10-50 ml centrifuge tubes starting with tube 1 labeled as 2.5 µg/ml. Placed 2 ml of SAB in tube 2 through tube 10. Placed 4 ml of 2.5µg/ml drug concentration in tube 1. Performed a serial dilution of 2 ml from tube1 to tube 2, continued the serial dilution to tube 10(pour off 2ml from the last tube).

Starting with the test tube containing the 0.0781µg/ml concentration of sample, taken 0.5ml of the diluted sample into a properly labeled culture tube. Repeated this procedure with all the dilutions. Arranged drug concentration tube for each antibacterial agent in ascending order, with the highest concentration on the left. Added 4.5 ml of diluted fungal culture to each tube containing 0.5 ml of diluted drug sample tubes (Naphthyridine derivatives). (Culture concentration: 1x 10^5cfu/ml. QC strain Bacillus Subtilis[MTCC 96]) this dilutes the drug concentration to 1:10 to obtain the concentration indicated on the tube. After setting up the each set of sample tubes, vortex the inoculum suspension to resuspend the bacteria. For positive growth control, 4.5ml of final inoculum to 0.5ml of broth. For negative growth control, add 5ml of broth to tube. Incubate MIC tubes at 30°C for 24-48hrs.

Reading MIC

Starting with lowest concentration and working towards the highest concentration for each drug grasped the drug-free control plus one or two drug-inoculum tubes by the caps, and held them up to view by transmitted light. Gently shaken the tubes and noted down the tubes showing the turbidity as positive. One tube before the tube showing turbidity is taken as the MIC value.

Results are recorded and tabulated.

Computational Calculations

Molecular Structure Building

A series of compounds whose inhibitory activity was determined were selected for the present study and the program of window Hyperchem software Inc [14] was used in modeling studies. The molecules were generated and the energy was minimized using molecular modeling pro. The window version software SPSS10] [44] was used in the regression analysis.

Table 1. Structural skeleton and inhibition effect of Naphthyridine derivatives (Fig-1)

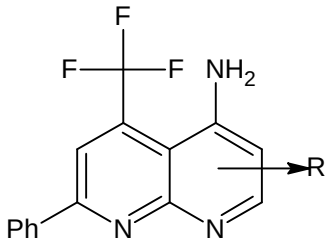
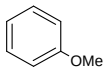
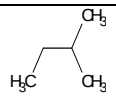
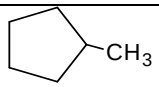
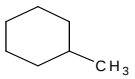
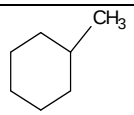
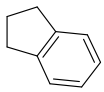
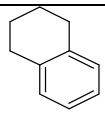
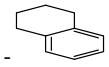


Fig-1. Structural skeleton Naphthyridine

Comp	derivatives	R Structure	MIC(µg/ml)	Activity= Log1/MIC
1	Naphthyridine		0.25	0.6020
2	Naphthyridine		12	-1.0791
3	Naphthyridine		0.20	0.6989
4	Naphthyridine		3.8	-0.5797
5	Naphthyridine		0.5	0.3010
6	Naphthyridine		4	-0.6020
7	Naphthyridine		0.45	0.3467
8	Naphthyridine		0.18	0.7447
9	Naphthyridine		14	-1.1461
10	Naphthyridine		0.5	0.3010
11	Naphthyridine		0.48	0.3187

Data Set and validation

The physicochemical parameters, such as vertical ionization potentials (IPv's) electron affinity (EA) , electronegativity (χ), hardness (η), softness (S), electrophilic index (ω), partition coefficient (LogP), charges,

hydration energy(HE) and polarisability (Pol) were obtained for Naphthyridine derivatives compounds. QSAR technique was applied to those Naphthyridine derivatives that were varied at the (R) position. The appropriate descriptors were used as independent variables for deciding in DNA Topoisomerase inhibitory activity.

CHEMICAL DESCRIPTORS

***Calculated Properties* – Semi empirical methods**

Quantum chemical calculations AM1 [15] and PM3 [16] semi empirical theory levels are employed for full optimization of the selected neutral compounds. The geometrical structures of the radicals studied are optimized independently from the neutral molecules prior to the calculation of energies, treated as open shell systems. Using the program of window Hyperchem software Inc performs all calculations. The AM1 and PM3-based reactivity descriptors for title compounds were computed [17].

Correlation Analysis

We obtained the correlation matrix between inhibitory activity and respective calculated properties for **Naphthyridine derivatives**. The more relevant regression models were selected following criteria: The correlation coefficient (R), the Fisher ratio values (F) and the standard deviations(s), standard error estimate (SEE), percentage of effective variable(%EV) and R^2adjusted(R^2_{adj}).

Docking Studies and Validation

GOLD[18] and Argus lab 4.0.1[19] are Molecular modeling and Drug docking softwares which helps in computational virtual screening to find the lead compounds. Argus lab, which provides a user-friendly graphical interface and uses Shape Dock algorithm, was used to carry out docking studies of the DNATopoisomerase.

The 3D structure of Topoisomerase was retrieved from Protein Data Bank (PDB ID 1SC7) with an X-ray resolution of $2A^O$ [20]. Docking poses were obtained by applying Chem score and Gold score, fitness functions available for scoring. As easily interpretable results were obtained based on a recently published work [21] all the results reported in the present paper are referred to the Chem score fitness functions. These complexes were prepared for docking studies by adding hydrogen atoms, removing water molecules and co-crystallized inhibitors and refined by using the Deep View/SwissPdbViewer3.7 (SP5)[22]. Enzyme-inhibitor interactions within a radius equal to 15 Å centered on reported bound inhibitors were taken into account. As a conclusive part of docking we expect, generated results should yield RMSD values below 1.5 Å. Successful docking has been performed for the selected set of **Naphthyridine derivatives** and their corresponding Chem score with their respective RMSD have been produced in the **Table 5.**

RESULTS AND DISCUSSION

Simple linear regression model

The biological activity data and the physicochemical properties IPv, IP, EA, EI, EN, Hard, Soft, LogP, HE and Pol of Naphthyridine derivatives are given in **Tables 1-3.** The data from these tables were subjected to regression analysis. The Correlation matrices were generated with **11** analogs. The term close to 1 indicates high co-linearity, while the value below 0.5 indicates that no co-linearity exist between more than the two parameters.

The perusal of correlation matrix indicates that Hard, is the predicted parameters from AM1 method. From regression methods (backward, forward, removed and stepwise) Hard was found to be explainable variable. The regression technique was applied through the origin using these explainable parameters.

Activity = 0.526 x Hard (0.055) ------ (1)

N = 11; R = 0.950; R^2 = 0.902; R^2adj=0.892; %EV = 90.20; SEE = 0.6895; F=92.236; Q =1.37781;

In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. **Eq.1** shows that the values of %EV are less and to improve its value, outliers were sought and eliminated.

After the elimination of the outlier (1, 2, 3 and 9), a second model was developed. Overall, there is an increase in R and %EV (90.20 –95.50) values, and a decrease in SEE (0.6895 -0.49).

Activity = 0.558 x Hard (0.049) ----- (2)
N =7; R= 0.977; R^2= 0.955; R^2adj =0.948; %EV = 95.50; SEE = 0.49; F =127.350; Q =1.99388;

Eq.2 is an improved model since it explains the biological activity to the extent of (95.50%). In this way, the predictive molecular descriptor Hard was considered as variables.

From the correlation matrix table, it reveals Hard was found to be explainable variables. A uni parametric QSAR equation with Hard was generated in PM3 method also.

Activity = 0.511 x Hard (0.054) ----- (3)
N = 11; R = 0.949; R^2= 0.900; R^2adj =0.890; %EV = 90.00; SEE = 0.6959; F = 90.357; Q = 1.3637;

Eq.3 shows that the values of %EV is less and to improve its value, outliers were sought and eliminated, In addition, the plot of observed activity versus predicted activity was not found to be satisfactory. Hence, the predictive ability of the model is not good. After the elimination of the outlier (**1, 2, 3 and 9**), a second model was developed.

Activity = 0.563 x Hard (0.041) ----- (4)
N =11; R = 0.982; R^2 = 0.964; R^2adj=0.959; %EV = 96.40; SEE = 0.4502; F =189.058; Q =2.18125;

In an attempt to investigate the predictive potential of proposed models, the cross-validation parameters (q^2_{cv} and PRESS) were calculated and used. The predictive power of the equations was confirmed by leave-one-out (LOO) cross-validation method. The cross-validation evaluates the validity of a model by how well it predicts the data rather than how well it fits the data. The cross-validation parameter**,** $q^2_{cv,}$ is mentioned in the respective equations **(Table 4).**

Eq.2 and 4 of AM1 and PM3 methods respectively give good q2cv values, which should be always smaller than %EV. A model is considered to be significant when q^2_{cv}>0.3.

Another cross-validation parameter, PRESS which is the sum of the squared differences between the actual and that predicted when the compound is omitted from the fitting process, also supports the predictive ability of **Eqs.2 and 4**. Its value decreases from **Eq.1 to Eq.3.**

The quality factor Q = R/SEE that indicates the higher the value of R, and the lower the value of SEE, the higher is the magnitude of Q and the better will be the correlation. In present case, Q increases from 1.37781 to 1.99388 and 1.3637 to 2.18125 **(Eq. 1 to 4).**

Table 2. Values obtained for the AM1 computational method

Compound	IP_V(AM1)	IP	EA	EN	η	S	ω	LogP	HE	Pol
1	-9.0094	-1.4712	-8.9947	-5.2329	3.7617	0.1329	3.6398	2.04	-7.42	37.43
2	-8.9262	-1.4448	-9.0032	-5.224	3.7792	0.1323	3.6106	2.04	-7.19	37.43
3	-9.0067	-1.3609	-9.089	-5.225	3.8641	0.1294	3.5326	1.81	-6.84	39.36
4	-8.767	-1.2511	-8.9355	-5.0933	3.8422	0.1301	3.3759	1.04	-8.86	39.9
5	-9.0031	-1.668	-9.2459	-5.457	3.789	0.132	3.9297	2.24	-3.86	35.11
6	-8.8116	-1.5315	-8.9493	-5.2404	3.7089	0.1348	3.7022	3.23	-4.06	34.34
7	-8.9211	-1.4347	-9.1574	-5.296	3.8614	0.1295	3.6319	1.62	-3.78	34.34
8	-8.8928	-1.4254	-9.1193	-5.2724	3.847	0.13	3.613	2.02	-3.54	36.17
9	-8.798	-1.521	-8.9303	-5.2256	3.7047	0.135	3.6855	2.02	-3.57	36.17
10	-8.9339	-1.3183	-8.9928	-5.1556	3.8372	0.1303	3.4634	1.57	-6.04	38.49
11	-8.8387	-1.3521	-8.8289	-5.0905	3.7384	0.1337	3.4658	1.96	-5.61	40.33

Table 3. Values obtained for the PM3 computational method

Compound	IP_V(PM3)	IP	EA	EN	η	S	ω	LogP	HE	Pol
1	-8.9811	-1.3095	-8.8379	-5.0737	3.7642	0.1328	3.4194	2.04	-7.42	37.43
2	-9.0544	-1.212	-8.9754	-5.0937	3.8817	0.1288	3.3421	2.04	-7.12	37.43
3	-9.0722	-1.1209	-8.9069	-5.0139	3.893	0.1284	3.2287	1.81	-6.78	39.36
4	-8.8759	-1.1778	-8.797	-4.9874	3.8096	0.1312	3.2647	1.04	-8.74	39.9
5	-8.9737	-1.4616	-8.9477	-5.2047	3.7431	0.1336	3.6185	2.24	-3.85	35.11
6	-8.8524	-1.4182	-8.8738	-5.146	3.7278	0.1341	3.5518	3.23	-4.27	34.34
7	-8.8771	-1.4097	-8.8881	-5.1489	3.7392	0.1337	3.5450	1.62	-3.79	34.34
8	-8.8576	-1.4129	-8.879	-5.146	3.733	0.1339	3.5468	2.02	-3.49	36.17
9	-8.8597	-1.3892	-8.8656	-5.1274	3.7382	0.1338	3.5164	2.02	-3.76	36.17
10	-8.9798	-1.2049	-8.9045	-5.0547	3.8498	0.1299	3.3183	1.57	-6.01	38.49
11	-8.8811	-1.3076	-8.7902	-5.0489	3.7413	0.1336	3.4068	1.96	-5.84	40.33

Table 4. Observed activity and predicted activity values of Naphthyridine derivatives by usingAM1and PM3Eqs

Compound	EQ.(2)Am1			EQ(4)PM3	
	Observed	Predicted	Residual	Predicted	Residual
1	2.602	-	-	-	-
2	0.9209	-	-	-	-
3	2.6989	-	-	-	-
4	1.4203	2.2983	-0.8780	1.9455	-.5252
5	2.301	1.9640	0.3370	2.0281	.2729
6	1.398	1.4526	-0.0546	1.9060	-.5080
7	2.3467	2.4177	-0.0710	1.9710	.3757
8	2.7447	2.3280	0.4167	2.1664	.5783
9	0.8539	-	-	-	-
10	2.301	2.2673	0.0337	1.9652	.3358
11	2.3187	1.6422	0.6765	1.9092	.4095

Docking Analysis

The compounds were then docked using each of the two docking software's. The Chemscore data and Gold fitness and the energy values are given in Table 8. The binding energies obtained in Argus lab ranged from -7.2427 to -5.4545 kJ/mol. The results of Gold are analyzed in terms of Chem score (ranging from 21.09 to 21.97) and Gold score (77.82 to -382.08).

Docking results revealed that cyclo hexyl and methyl substituent group (R) on the compounds play a key role in ligand binding interactions with protein. The predicting scoring functions of these compounds have shown good correlation with Argus binding energy values.

Molecules **5** and **7** have best Chem score and Gold fitness with minimum binding energy values compared to all other molecules and has best vanderwaals interactions and Hydrogen bonding interactions with optimum clash penalty and showing best lipophilic character (**Table 5**) . The best binding modes of molecules (chosen as best in docking studies) and its interactions in the active pocket of poly [ADP-ribose] polymerase have been illustrated in **figure4 and 5**.

The docking simulation of the most active compound 5, 7 and 8 toward DNA Topoisomerase (PDB ID 1SC7) showed that the most enzyme–inhibitor complex was stabilized by hydrophobic interactions occurring between the aromatic moieties of the ligand and lipophilic residues of the binding site. In particular the compound 5, 7 and 8 groups were oriented towards the hydrophobic region lined by Arg364, Leu530, and His511. The molecule 5, 7and 8 has been reported with appreciable biological activity values 0.5µg/ml, 0.45 µg/ml and 4.0 µg/ml.

Table 5. Energy and Chemscore values of the docked ligands

Comp	Chem Score	DG	S(hbond)	S(metal)	S(lipo)	DE(clash)	DE(int)	Gold fitness	Argus binding energy
5	21.56	-23.71	0.90	0.00	130.01	0.51	1.64	73.12	-6.3117
7	21.09	-22.88	0.86	0.00	124.06	0.04	1.74	77.82	-6.7639
8	21.97	-23.80	0.87	0.00	131.81	0.02	1.82	-382.08	-5.7949

CONCLUSION

In conclusion, our present studies have established predictive QSAR models that are quite reliable to efficiently guide further modification in the molecules for obtaining better drugs. AM1 and PM3 are the semi empirical methods employed in QSAR studies. Both of them provided good statistical results in terms of R^2,R^2_{adj} and standard error of estimate (SEE), suggesting the significant correlations of molecular structures with its biological activities. GOLD and Argus lab were employed to support QSAR studies. The GOLD and Argus lab were employed to dock the inhibitors into the active site of Topoisomerase and these docking studies revealed the vital interactions and binding conformation of the inhibitors. Docking studies reveal higher values of Chemscore, Gold fitness and good Argus binding energy values, which support QSAR studies. And the best molecules from this study were found to be molecules **5, 7 and 8** (Table -5). Therefore, QSAR studies and the docking approach of Naphthyridine derivatives as Topoisomerase inhibitors can be successfully modeled using mono parametric equations. (The **Eq.2** and **4** from AM1 and PM3).The linear dependence of inhibitory nature on Hardness was evident from **Figure 2 and 3.**

Subsequently, it is concluded the following from Quantitative structure activity relationships and molecular modeling studies. 1) The presence of basic skeleton (Phenyl trifluoromethyl Pyridine, and Dihydropyridine 4-Amine moiety) is necessary for the broad spectrum of antibacterial activity. 2) Introducing Fluorine group (Fig-1)

(electron withdrawing group, -ve inductive effect) increases the antibacterial activity. 3) The presence of cyclohexane substituents shows good antibacterial activity due to their stability compared to other substituents (Bayer's strain theory) and methyl group on them minimizes the strain which enhances the antibacterial activity.

Figure 2. Plot of Observed Verses Predicted activity (AM1 Method).

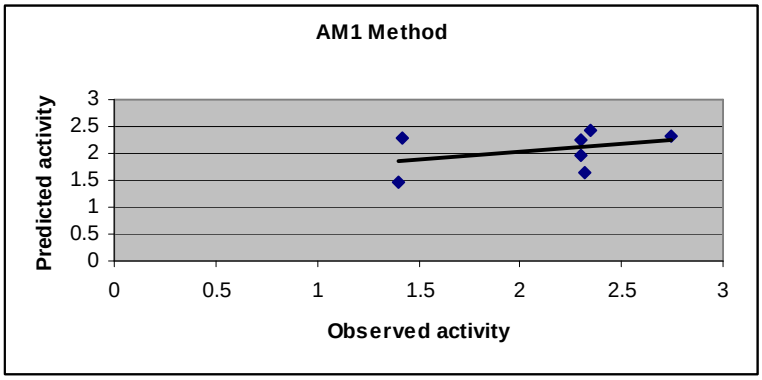


Figure 3. Plot of Observed Verses Predicted activity (PM3 Method)

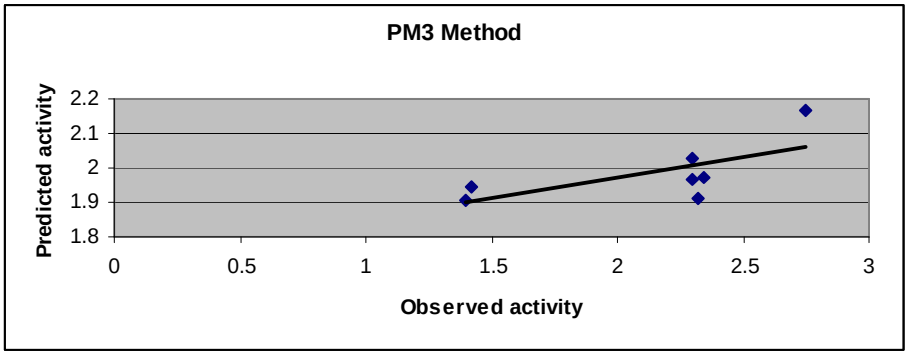


Figure 4. Best pose of molecule 5 and secondary structure of Topoisomerase (PDB ID 1SC7)

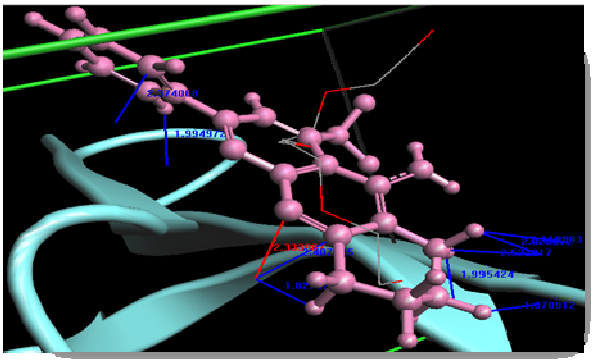
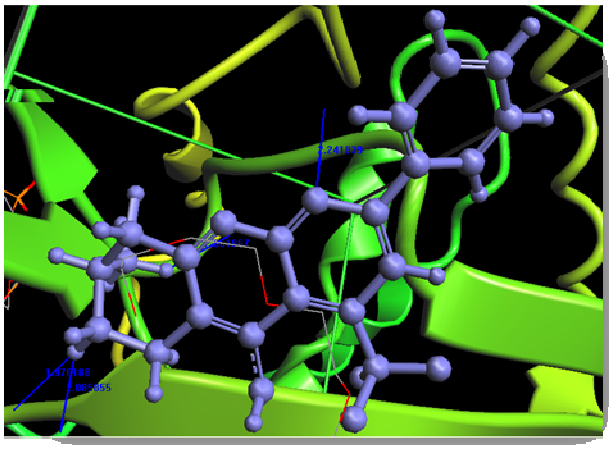


Figure 5. Best pose of molecule 7 and secondary structure of Topoisomerase (PDB ID 1SC7)



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REFERENCES

[1]. CO Kappe.*Angew. Chem. Int. Ed.*, **2004,** 43, 6250–6284.
[2]. RS Varma.*J. Heterocycl. Chem.*, **1999**, 36, 1565–1571.
[3]. JJ Parlow; RG Kurumbail; RA Stegeman; AM Stevens; WC Stallings; MS South. *J.Med. Chem.*, **2003,** 46, 4696–4701.
[4]. LA Hasvold; W Wang; SLGwaltney; TW Rockway; LTJ Nelson; RA Mantei; SA Fakhoury; GM Sullivan; Q Li; NH Lin; L Wang; H Zhang; J Cohen; WZ Gu; K Marsh; JBauch; S Rosenberg; HL Sham. *Bioorg. Med. Chem. Lett.*, **2003**, 13, 4001–4005.
[5]. Q Li; LA Mitscher; LL Shen. *Med. Res. Rev.*, **2000,** 20, 231–293.
[6]. R Filler; Y Kobayashi; LM Yagupolskii. Organofluorine Compounds in MedicinalChemistryand Biomedical Applications"; *Elsevier: Amsterdam*, **1993**.
[7]. MC Liu; TS Lin; JG Cary; AH Cary; AC Santonelli. *J. Med. Chem.*, **1996,** 39,2586.
[8]. GY Li; XH Qian; JN Cui; QC Huang; R Zhang; H Guan. *J. Agric. Food.Chem.*, **2006,** 54,125.
[9]. S Jain; P Mishra. *Indian J. Heterocyclic. Chem.*, **2004**, 13, 307.
[10].Y Zhi; J Yang; T Shengchao; FYuan; YLiu; Y Zhang; P Sun; B Song; Z Chen. *Int. J. Mol Sci*., **2012,** 13(5), 6009–6025.
[11]. Y Pommier. .*Nat. Rev. Cancer*., **2006**, 6, 789–802.
[12]. DA Koster; V Croquette; C Dekker; S Shuman; NH Dekker. .*Nature*., **2005,** 434, 671–674.
[13].NationalCommittee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically. *Tentative standard, M7-T2*.Vol.8.Villanova, PA*:* National Committee for Clinical Laboratory Standards, ***1992.***
[14]. http://www.warezdestiny.com/free-hyp.
[15]. SPSS Software. Consult http://www.spss.com.
[16]. JA Pople; RK Nesbet .*J. Chem. Phys*., **1954**, 22, 571.
[17]. R Mc Weeny; G Dierksen. *J. Chem. Phys*., **1968**, 49, 4852.
[18]. M Reis; B Lobato; J Lameira; AS Santos; CNAlves. *E.J. Med. Chem.*, **2007**, 42, 440-446.
[19]. G Jones; P Willet; RC Glen. *J. mol.boil*., **1995**, 245, 43.
[20]. D Feller; MA Thompson; A Rick; Kendall. *J. Phys. Chem A.*, **1997,** 101, 7292-7298.
[21]. http://www.rcsb.org/pdb
[22]. N Guex; MC Peitsch. *Electrophoresis*, **1997**, 18, 2714-2723.

Table 4. Correlation matrix between the selected variables by using AM1 method

		ACT	IP_V(AM1)	IP	EA	χ	η	S	ω	LOGP	HE	POL
ACT	Pearson Correlation	1.000	-.623	.110	-.434	-.195	.537	-.539	-.026	-.146	.072	.114
	Sig. (2-tailed)	.	.041	.748	.182	.566	.088	.087	.939	.669	.834	.738
	N	11	11	11	11	11	11	11	11	11	11	11
IP_V(AM1)	Pearson Correlation	-.623	1.000	.286	.644	.545	-.367	.371	-.368	-.015	.058	.083
	Sig. (2-tailed)	.041	.	.394	.033	.083	.268	.261	.266	.966	.866	.808
	N	11	11	11	11	11	11	11	11	11	11	11
IP	Pearson Correlation	.110	.286	1.000	.476	.853	.484	-.482	-.988	-.688	-.635	.766
	Sig. (2-tailed)	.748	.394	.	.139	.001	.132	.133	.000	.019	.036	.006
	N	11	11	11	11	11	11	11	11	11	11	11
EA	Pearson Correlation	-.434	.644	.476	1.000	.865	-.539	.540	-.606	-.003	-.371	.542
	Sig. (2-tailed)	.182	.033	.139	.	.001	.087	.086	.048	.993	.262	.085
	N	11	11	11	11	11	11	11	11	11	11	11
X	Pearson Correlation	-.195	.545	.853	.865	1.000	-.044	.045	-.923	-.394	-.582	.758
	Sig. (2-tailed)	.566	.083	.001	.001	.	.898	.895	.000	.230	.060	.007
	N	11	11	11	11	11	11	11	11	11	11	11
η	Pearson Correlation	.537	-.367	.484	-.539	-.044	1.000	-1.000	-.343	-.656	-.239	.194
	Sig. (2-tailed)	.088	.268	.132	.087	.898	.	.000	.302	.029	.478	.567
	N	11	11	11	11	11	11	11	11	11	11	11
S	Pearson Correlation	-.539	.371	-.482	.540	.045	-1.000	1.000	.341	.657	.244	-.197
	Sig. (2-tailed)	.087	.261	.133	.086	.895	.000	.	.304	.028	.470	.562
	N	11	11	11	11	11	11	11	11	11	11	11
Ω	Pearson Correlation	-.026	-.368	-.988	-.606	-.923	-.343	.341	1.000	.619	.636	-.784
	Sig. (2-tailed)	.939	.266	.000	.048	.000	.302	.304	.	.042	.035	.004
	N	11	11	11	11	11	11	11	11	11	11	11
LOGP	Pearson Correlation	-.146	-.015	-.688	-.003	-.394	-.656	.657	.619	1.000	.514	-.578
	Sig. (2-tailed)	.669	.966	.019	.993	.230	.029	.028	.042		.105	.063
	N	11	11	11	11	11	11	11	11	11	11	11
HE	Pearson Correlation	.072	.058	-.635	-.371	-.582	-.239	.244	.636	.514	1.000	-.750
	Sig. (2-tailed)	.834	.866	.036	.262	.060	.478	.470	.035	.105	.	.008
	N	11	11	11	11	11	11	11	11	11	11	11
POL	Pearson Correlation	.114	.083	.766	.542	.758	.194	-.197	-.784	-.578	-.750	1.000
	Sig. (2-tailed)	.738	.808	.006	.085	.007	.567	.562	.004	.063	.008	.
	N	11	11	11	11	11	11	11	11	11	11	11

Table 4a. Correlation matrix between the selected variables, by using AM1 method Correlations

		ACT1	NEUTRAL	IP	EA	χ	η	S	Ω	LOGP	HE	POL
ACT1	Pearson Correlation	1.000	-.688	-.134	-.451	-.326	.396	-.399	.188	-.159	.530	-.081
	Sig. (2-tailed)	.	.088	.774	.310	.476	.380	.376	.687	.733	.221	.863
	N	7	7	7	7	7	7	7	7	7	7	7
NEUTRAL	Pearson Correlation	-.688	1.000	.590	.784	.758	-.269	.273	-.652	-.053	-.594	.444
	Sig. (2-tailed)	.088	.	.163	.037	.049	.560	.553	.113	.911	.160	.319
	N	7	7	7	7	7	7	7	7	7	7	7
IP	Pearson Correlation	-.134	.590	1.000	.654	.905	.372	-.371	-.992	-.699	-.756	.771
	Sig. (2-tailed)	.774	.163	.	.111	.005	.411	.413	.000	.080	.049	.042
	N	7	7	7	7	7	7	7	7	7	7	7
EA	Pearson Correlation	-.451	.784	.654	1.000	.913	-.459	.460	-.746	-.036	-.585	.708
	Sig. (2-tailed)	.310	.037	.111	.	.004	.301	.299	.054	.939	.167	.075
	N	7	7	7	7	7	7	7	7	7	7	7
X	Pearson Correlation	-.326	.758	.905	.913	1.000	-.057	.059	-.952	-.396	-.735	.813
	Sig. (2-tailed)	.476	.049	.005	.004	.	.903	.900	.001	.379	.060	.026
	N	7	7	7	7	7	7	7	7	7	7	7
η	Pearson Correlation	.396	-.269	.372	-.459	-.057	1.000	-1.000	-.250	-.777	-.170	.038
	Sig. (2-tailed)	.380	.560	.411	.301	.903	.	.000	.589	.040	.716	.936
	N	7	7	7	7	7	7	7	7	7	7	7
S	Pearson Correlation	-.399	.273	-.371	.460	.059	-1.000	1.000	.248	.780	.171	-.041
	Sig. (2-tailed)	.376	.553	.413	.299	.900	.000	.	.592	.039	.714	.930
	N	7	7	7	7	7	7	7	7	7	7	7
ω	Pearson Correlation	.188	-.652	-.992	-.746	-.952	-.250	.248	1.000	.618	.756	-.795
	Sig. (2-tailed)	.687	.113	.000	.054	.001	.589	.592	.	.139	.049	.033
	N	7	7	7	7	7	7	7	7	7	7	7
LOGP	Pearson Correlation	-.159	-.053	-.699	-.036	-.396	-.777	.780	.618	1.000	.653	-.594
	Sig. (2-tailed)	.733	.911	.080	.939	.379	.040	.039	.139	.	.112	.160
	N	7	7	7	7	7	7	7	7	7	7	7
HE	Pearson Correlation	.530	-.594	-.756	-.585	-.735	-.170	.171	.756	.653	1.000	-.804
	Sig. (2-tailed)	.221	.160	.049	.167	.060	.716	.714	.049	.112	.	.029
	N	7	7	7	7	7	7	7	7	7	7	7
POL	Pearson Correlation	-.081	.444	.771	.708	.813	.038	-.041	-.795	-.594	-.804	1.000
	Sig. (2-tailed)	.863	.319	.042	.075	.026	.936	.930	.033	.160	.029	.
	N	7	7	7	7	7	7	7	7	7	7	7

Table 5.Correlation matrix between the selected variables, by using PM3 method

		ACT	NENT	IP	EA	X	η	S	ω	LOGP	HE	POL
ACT	Pearson Correlation	1.000	-.240	.196	-.150	.003	.187	-.181	-.175	-.146	.090	.114
	Sig. (2-tailed)	.	.477	.564	.660	.992	.583	.594	.606	.669	.791	.738
	N	11	11	11	11	11	11	11	11	11	11	11
NENT	Pearson Correlation	-.240	1.000	-.790	.940	.423	-.960	.964	.622	-.067	-.466	.323
	Sig. (2-tailed)	.477	.	.004	.000	.195	.000	.000	.041	.844	.148	.333
	N	11	11	11	11	11	11	11	11	11	11	11
IP	Pearson Correlation	.196	-.790	1.000	-.654	.164	.884	-.879	-.966	-.300	.113	.132
	Sig. (2-tailed)	.564	.004	.	.029	.630	.000	.000	.000	.370	.740	.699
	N	11	11	11	11	11	11	11	11	11	11	11
EA	Pearson Correlation	-.150	.940	-.654	1.000	.639	-.931	.934	.437	-.130	-.667	.562
	Sig. (2-tailed)	.660	.000	.029	.	.034	.000	.000	.179	.702	.025	.072
	N	11	11	11	11	11	11	11	11	11	11	11
χ	Pearson Correlation	.003	.423	.164	.639	1.000	-.315	.324	-.412	-.475	-.754	.867
	Sig. (2-tailed)	.992	.195	.630	.034	.	.345	.331	.208	.140	.007	.001
	N	11	11	11	11	11	11	11	11	11	11	11
η	Pearson Correlation	.187	-.960	.884	-.931	-.315	1.000	-.999	-.734	-.064	.466	-.283
	Sig. (2-tailed)	.583	.000	.000	.000	.345	.	.000	.010	.852	.148	.398
	N	11	11	11	11	11	11	11	11	11	11	11
S	Pearson Correlation	-.181	.964	-.879	.934	.324	-.999	1.000	.729	.079	-.456	.284
	Sig. (2-tailed)	.594	.000	.000	.000	.331	.000	.	.011	.818	.159	.397
	N	11	11	11	11	11	11	11	11	11	11	11
ω	Pearson Correlation	-.175	.622	-.966	.437	-.412	-.734	.729	1.000	.422	.107	-.358
	Sig. (2-tailed)	.606	.041	.000	.179	.208	.010	.011	.	.197	.754	.280
	N	11	11	11	11	11	11	11	11	11	11	11
LOGP	Pearson Correlation	-.146	-.067	-.300	-.130	-.475	-.064	.079	.422	1.000	.491	-.578
	Sig. (2-tailed)	.669	.844	.370	.702	.140	.852	.818	.197	.	.125	.063
	N	11	11	11	11	11	11	11	11	11	11	11
HE	Pearson Correlation	.090	-.466	.113	-.667	-.754	.466	-.456	.107	.491	1.000	-.761
	Sig. (2-tailed)	.791	.148	.740	.025	.007	.148	.159	.754	.125	.	.007
	N	11	11	11	11	11	11	11	11	11	11	11
POL	Pearson Correlation	.114	.323	.132	.562	.867	-.283	.284	-.358	-.578	-.761	1.000
	Sig. (2-tailed)	.738	.333	.699	.072	.001	.398	.397	.280	.063	.007	.
	N	11	11	11	11	11	11	11	11	11	11	11

Table 5a.Correlation matrix between the selected variables, by using PM3 method

		ACT	NENT	IP	EA	χ	η	SOFT	ω	LOGP	HE	POL
ACT	Pearson Correlation	1.000	-.240	.196	-.150	.003	.187	-.181	-.175	-.146	.090	.114
	Sig. (2-tailed)	.	.477	.564	.660	.992	.583	.594	.606	.669	.791	.738
	N	11	11	11	11	11	11	11	11	11	11	11
NENT	Pearson Correlation	-.240	1.000	-.790	.940	.423	-.960	.964	.622	-.067	-.466	.323
	Sig. (2-tailed)	.477	.	.004	.000	.195	.000	.000	.041	.844	.148	.333
	N	11	11	11	11	11	11	11	11	11	11	11
IP	Pearson Correlation	.196	-.790	1.000	-.654	.164	.884	-.879	-.966	-.300	.113	.132
	Sig. (2-tailed)	.564	.004	.	.029	.630	.000	.000	.000	.370	.740	.699
	N	11	11	11	11	11	11	11	11	11	11	11
EA	Pearson Correlation	-.150	.940	-.654	1.000	.639	-.931	.934	.437	-.130	-.667	.562
	Sig. (2-tailed)	.660	.000	.029	.	.034	.000	.000	.179	.702	.025	.072
	N	11	11	11	11	11	11	11	11	11	11	11
χ	Pearson Correlation	.003	.423	.164	.639	1.000	-.315	.324	-.412	-.475	-.754	.867
	Sig. (2-tailed)	.992	.195	.630	.034	.	.345	.331	.208	.140	.007	.001
	N	11	11	11	11	11	11	11	11	11	11	11
η	Pearson Correlation	.187	-.960	.884	-.931	-.315	1.000	-.999	-.734	-.064	.466	-.283
	Sig. (2-tailed)	.583	.000	.000	.000	.345	.	.000	.010	.852	.148	.398
	N	11	11	11	11	11	11	11	11	11	11	11
S	Pearson Correlation	-.181	.964	-.879	.934	.324	-.999	1.000	.729	.079	-.456	.284
	Sig. (2-tailed)	.594	.000	.000	.000	.331	.000	.	.011	.818	.159	.397
	N	11	11	11	11	11	11	11	11	11	11	11
ω	Pearson Correlation	-.175	.622	-.966	.437	-.412	-.734	.729	1.000	.422	.107	-.358
	Sig. (2-tailed)	.606	.041	.000	.179	.208	.010	.011	.	.197	.754	.280
	N	11	11	11	11	11	11	11	11	11	11	11
LOGP	Pearson Correlation	-.146	-.067	-.300	-.130	-.475	-.064	.079	.422	1.000	.491	-.578
	Sig. (2-tailed)	.669	.844	.370	.702	.140	.852	.818	.197	.	.125	.063
	N	11	11	11	11	11	11	11	11	11	11	11
HE	Pearson Correlation	.090	-.466	.113	-.667	-.754	.466	-.456	.107	.491	1.000	-.761
	Sig. (2-tailed)	.791	.148	.740	.025	.007	.148	.159	.754	.125	.	.007
	N	11	11	11	11	11	11	11	11	11	11	11
POL	Pearson Correlation	.114	.323	.132	.562	.867	-.283	.284	-.358	-.578	-.761	1.000
	Sig. (2-tailed)	.738	.333	.699	.072	.001	.398	.397	.280	.063	.007	.
	N	11	11	11	11	11	11	11	11	11	11	11