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QSAR Studies and Molecular Shape Analysis of Azonafide Derivatives as Topoisomerase II Inhibitors

R. SARAVANA KUMAR, N. S. H. N. MOORTHY AND P. TRIVEDI*
Department of Pharmacy, S.G. S. I. T. S.,
23, Park Road, Indore-452003.

Topoisomerase II inhibitor (2-[2'-(dimethylamino) ethyl]-1,2 dihydro-3H-dibenz (d, e, h) isoquinoline-1,3-dione) derivatives as potential anticancer molecule were subjected to quantitative structure activity relationship analysis. Transition melts temperatures (ΔTm) of DNA binding strength and other physicochemical descriptors were correlated with the biological activity (melanoma, ovarian cancer and leukemia). The result shows that transition melt temperatures, superdelocalizability, heat of formation, hydrophobic character and C10-charge density are important for the biological activity. From the quantitative structure activity relationship analysis studies, it was noticed that the hydrophobic character and C10-charge play an important role in biological activity of 8-substituted and 9 and 10-substituted azonafides. It indicates that a particular charge density distribution is required for better biological activity of the compound. Molecular shape analysis suggests that there is less difference in non-common overlap steric volume while comparing biological activity with reference compound. The higher carbon chain at 8th and 10th position will result in increased hydrophobicity and in turn will have better biological activity.

The effectiveness of cancer chemotherapy is mostly limited due to two major problems, which are still to be overcome, the lack of selectivity of anticancer agents and the occurrence of intrinsic or acquired resistance leading to significant side effects and some times failure of treatments[1]. Research on enzyme inhibitors specifically on topoisomerase II inhibitors is one of the attractive targets for cancer chemotherapy[2]. This enzyme is present in large amount in all cancer cells, but in negligible amounts in normal cells[3]. The cytotoxic effects of DNA topoisomerase II inhibitors are presumably due to trapping of inhibitors in to the DNA cleavable complex. DNA and a topoisomerase II enzyme form a reversible and cleavable complex, which is stabilized by the ternary complex of drugs. Stabilization of the complex leads to interruption of DNA strand scission, passing and resealing[4].

*For correspondence
E-mail: drpiyushtrivedi@hotmail.com

The series of azonafides (parent structure in fig. 1) have shown good activity against melanoma, ovarian cancer and leukemia. The azonafide derivatives were subjected to quantitative structure activity relationship (QSAR) studies. The transition melts temperatures (ΔTm), is measured by increase in drug and DNA binding strength and is statistically significant[5]. The molecular shape analysis is a formal approach for incorporating conformational flexibility and shape data into a QSAR. The outcome of the MSA is an optimized QSAR that can be used for activity estimation and ligand evaluation.

MATERIALS AND METHODS

The biological activity data was acquired from Salah *et al*[5]. The biological activity data were found out by cell culture method (Table 1). In the present study Silicon graphics Indigo XY[2] workstation was used. Structures of all compounds were sketched using 3D-sketcher module of the Cerius[2]. The energy calculations were done using universal

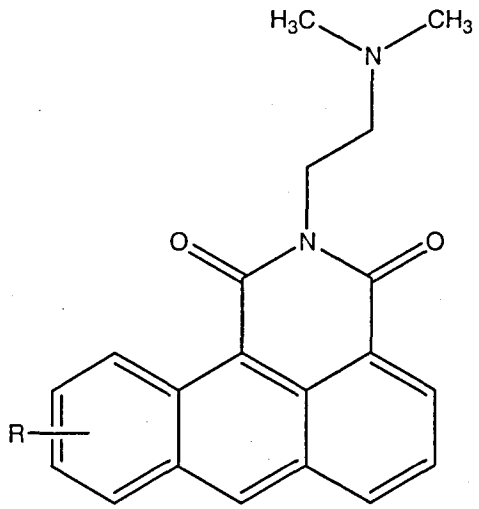

Fig. 1:Parent structure of azonafide derivatives.

force field. All the structures were energy minimized by standard minimizer algorithms. In the minimization process, first steepest descent (SD) method was used to eliminate the bad contacts, after which more accurate minimizing methods like conjugate gradient (CG) and truncated Newton-Raphson (N-R) methods were used[6].

Most stable conformations for each compound were generated and its analysis was performed using GRID method. These conformers were used for calculating other physicochemical parameters. Semi-empirical quantum mechanical calculations were performed using modified neglected differential overlap (MNDO) method. The Austin model 1(AM1) Hamiltonian of molecular orbital package (MOPAC) module was used for calculating atomic charges and electron density on various atoms. The Connolly surface (solvent accessible area) of the lowest energy conformers of each molecule was computed and viewed on a SGI IRIS Indigo using the programme Cerius[2] with probe radius (1.04A°), Dot density (8.0A°) and VDW scale factor (1.0)[7]. The other parameters for all the compounds were calculated by standard procedure in Cerius[2].

The correlation between the biological activity and physicochemical parameters was found through stepwise multiple regression analysis using the methods of least squares also called SPA (stepwise predicted activity). The cross correlation matrix for the –log (BA) and various physicochemical descriptors were calculated (Table 2). The statistical measures used in this study are correlation coefficient (r), squared correlation coefficient (r^2), Fischer's value and standard deviation[8].

The compound AZA 44 is highly effective against all the three types of cancer and it has less cardiotoxicity. So that compound was taken as a reference compound for the Molecular shape analysis study. The correlation between biological activity and MSA descriptor was obtained only in 10-substituted azonafides for activity against leukemia.

RESULTS

Among several 3D QSAR equations, some equations were selected based on the criterion correlation coefficient (r), number of compounds and parameters those having least inter-correlation with each other (Table 2). The results of linear stepwise multiple regression analysis are as follows. Considering 8-substituted azonafide having ΔTm, for melanoma cancer the following equation was obtained, -Log IC_{50} (Mel)=7.4515+1.23409x π -1.80264 x π^2, where, n is 8, r is 0.962, r^2 is 0.926, cvr^2 is 0.838, F_{-test} is 31.320 and SD is 6.03715, while in ovarian cancer, -LogIC_{50} (Ova)=9.38474+0.1651 x Fh_2O–0.0317234 x H_F_MOPAC, Where, n is 8, r is 0.875, r^2 is 0.766, cvr^2 is 0.482, F_{-test} is 8.176 and SD is 6.14455 and in leukemia the equation is, -LogIC_{50} (Leu)=7.67428–2.63452 x π–6.51807 x π^2, Where, n is 8, r is 0.974, r^2 is 0.948, cvr^2 is -0.375, F_{-test} is 36.34 and SD is 47.647886, Where, Mel is melanoma, Ova is ovarian cancer and Leu is leukemia.

The study of 8-substituted azonafide suggests that the π^2 negatively contribute in the melanoma and leukemia but hydrophobic parameter (π) positively contributes in melanoma and negatively in leukemia. Heat of formation (H_F_MOPAC) negatively and desolvation free energy for water (Fh_2O) positively contributed against ovarian cancer.

Considering 9 and10 substituted azonafides, for melanoma cancer the following equation was obtained, -LogIC_{50} (Mel)= 4.36367–2.71223 x C10-Charge+0.174093 x ΔTm+0.320378 x Sr, where, n is 13, r is 0.895, r^2 is 0.801, cvr^2 is 0.343, F_{-test} is 12.055 and SD=6.017877 and the equation obtained for ovarian cancer is, -LogIC_{50} (Ova)=5.9035+0.0920215 x Δ Tm, Where, n is 13, r is 0.558, r^2 is 0.311, cvr^2 is -0.776, F_{-test} is 4.9739 and SD=5.908108, while in leukemia is -LogIC_{50} (Leu)=7.67428–2.63452 x π - 6.51807 x π^2. Where, n is 13, r is 0.750, r^2 is 0.563, cvr^2 is 0.468 and SD=7.186511.

In 9 and 10 substituted azonafides, ΔTm positively contributed against melanoma and ovarian cancer. C10-charge negatively and superdelocalizability (Sr) positively contributed only in melanoma. The results of the QSAR equations indicate that mostly ΔTm, electronic, quantum mechanical,

TABLE 1: STRUCTURE AND BIOLOGICAL ACTIVITY OF AZONAFIDE DERIVATIVES.

Structure code	Substituents	Log (1/ IC_{50})		
		Melanoma	Ovarian cancer	Leukemia
AZA	H	7.15	7.24	8.16
AZA15	10-Cl	7.19	7.11	8.29
AZA16	10-I	6.20	6.75	6.51
AZA17	10-C_6H_5	5.73	6.46	7.16
AZA18	10-F	7.80	8.17	8.10
AZA19	4-CH_3	6.75	6.91	7.27
AZA20	10-CH_3	7.09	7.17	7.39
AZA21	4-OH	5.12	4.83	5.17
AZA22	4-OCH_3	6.74	6.30	7.28
AZA23	4-$NH(CH_2)_2N(CH_3)_2$	6.53	6.24	6.90
AZA26	10-Cl, DM*	7.36	6.99	8.24
AZA31	4-Cl	6.89	6.75	8.00
AZA31a	4-NH_2	8.02	7.92	8.27
AZA31b	4-$NHCOCH_3$	6.01	6.21	6.22
AZA31c	4-$NHCOC(CH_3)_3$	5.06	6.18	5.66
AZA34	10-NH_2	7.20	7.70	8.60
AZA35	8-NH_2	4.69	5.02	5.17
AZA36	11-NH_2	6.02	5.76	6.17
AZA37	9-Cl	7.05	6.64	8.11
AZA38	9-OH	8.34	7.66	8.27
AZA39	9-OCH_3	6.79	6.27	7.27
AZA39a	9-$NHCOCH_3$	7.22	7.89	6.84
AZA40	10-OH	5.92	5.72	6.77
AZA41	10-OCH_3	7.28	7.11	7.68
AZA42	10-C_2H_5	7.12	7.20	7.60
AZA43	10-NO_2	7.44	7.64	7.60
AZA44	10-CN	7.96	7.92	7.84
AZA44a	10-$NHCOCH_3$	7.01	7.31	7.31
AZA44b	10-$NHCOC(CH_3)_2$	6.26	6.48	6.36
AZA45	8-Cl	7.11	7.41	0.00
AZA46	8-OH	6.57	6.67	6.97
AZA47	8-OCH_3	7.28	7.28	7.89
AZA47a	8-$NHCOCH_3$	5.92	5.70	6.31
AZA47b	8-NO_2	7.42	7.30	7.30
AZA47c	8-I	6.08	5.68	5.68
AZA48	11-Cl	5.69	5.05	5.75
AZA49	11-OH	6.27	5.72	6.72
AZA49a	11-NO_2	7.13	6.65	7.64
AZA49b	11-$NHCOCH_3$	5.61	5.61	6.61

From the table DM is demethyl, AZA is azonafide and Log 1/IC_{50} is negative logarithm of 50% inhibition concentration.

TABLE 2: CROSS CORRELATION MATRIX FOR ACTIVITY AGAINST MELANOMA, OVARIAN CANCER AND LEUKAEMIA.

	Log1/ IC_{50}^{a}	Log1/ IC_{50}^{b}	Log1/ IC_{50}^{c}	ΔTm	Fh_2O	C10	Sr	π	π^2	H_F	NCOSV
Log1/ IC_{50}^{a}	1.000										
Log1/ IC_{50}^{b}		1.000									
Log1/ IC_{50}^{c}			1.000								
ΔTm	0.562		0.190	1.000							
Fh_2O	0.299	0.440	0.061	0.027	1.000						
C10	0.121	0.085	0.251	0.174	0.034	1.000					
Sr	0.075	0.288	0.276	0.069	0.241	0.163	1.000				
π	0.005	0.103	0.158	0.199	0.372	0.026	0.075	1.000			
π^2	0.261	0.155	0.089	0.529	0.053	0.052	0.351	0.443	1.000		
H_F	0.042	0.033	0.062	0.188	0.053	0.156	0.044	0.078	0.392	1.000	
NOSCV	0.283	0.109	0.215	0.263	0.089	0.099	0.069	0.173	0.075	0.155	1.000
		0.170									

In the above table -$LogIC_{50}^{a}$ is logarithm of 50% inhibition concentration of melanoma, $LogIC_{50}^{b}$ is logarithm of 50% inhibition concentration of ovarian cancer, -$LogIC_{50}^{c}$ is logarithm of 50% inhibition concentration of leukaemia, ΔTm is transition melts temperature, Fh_2O is desolvation free energy for water, C10 is charge at C10 substituent position, Sr is superdelocalizability, CAA is Connolly accessible area, π is hydrophobic character, H_F is heat of formation in MOPAC module and NCOSV is non-common steric volume.

spatial and thermodynamic descriptors are responsible for the variation in activity.

From the molecular shape analysis study the equation obtained is, -$LogIC_{50}$ (Leu)= 8.15593–0.0110886 x NCOSV, where, n is 10, r is 0.697, r^2 is 0.486, cvr^2 is 0.290, $F_{.test}$ is 7.551 and SD=4.042610. The molecular shape analysis descriptor NCOSV negatively contributed against leukemia.

In the QSAR study shows all the equations having 99% significance. The significant value is larger than the tabulated values. The out come of the MSA is an optimized QSAR that can be used for activity estimation and ligand evaluation. Although, AZA18 is more active than AZA44, it was not considered as reference compound because of its high cardio toxicity.

DISCUSSION

It is evident from the QSAR studies that C10 - charge, hydrophobic character, molecular super localizability (Sr), transition melt temperature, H_F_MOPAC and non-common overlap steric volume (NCOSV) plays an important role in the biological activity.

The decrease in C10-charge decreases the charge density distribution particularly in the 8, 9 and 10^{th} positions, which forms a complex with the electrophile (tyrosine amino acid) of topoisomerase II enzyme, results in the blockage of cell division and hence, increases the activity. For melanoma, the permeability of drug through the membrane of cancer cells increases when the hydrophobicity is increased.

Molecular superdelocalizability (Sr) is an electronic descriptor, which gives the reactivity of the aromatic ring. Azonafide ring, intercalate with the DNA very freely with increase in the Sr value. Transition melts temperature, measures the influence of substituents size to lipophilicity, cytotoxicity and DNA binding strength of drugs. When the ΔTm value increases the drug intercalate with the DNA strongly and prevent the cell mitosis. The thermal stability of the molecule was found by the quantum mechanical descriptor H_F_MOPAC. It gives the wide range of applicability in conformational analysis and intermolecular modeling. Smaller the H_F, the molecule binds the receptor correctly and is stable.

The molecular shape descriptor non-common overlap steric volume (NCOSV), measures the steric volume of the molecule. If the volume of the molecule is small, the molecule will enter the minor groove of the DNA easily and allow the side chains for free rotation. Based on the above considerations it was assumed that increasing carbon side chain at 8th and 10th substituent position will increase the hydrophobic character, and decrease the charge density. So the substituent in the particular positions forms a complex with the electrophilic group of the topoisomerase II enzyme and reduces the cell proliferation. The larger superdelocalizability and decrease in steric volume will allow the side chain for free rotation to intercalate with the base pairs of DNA. Hence, the position 8th and 10th are important for the anticancer activity.

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