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Cytidine derivatives as inhibitors of methyltransferase enzyme

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Abstract

In this work, cytidine and fifteen of its derivatives have been examined to detect their Methyltransferase (MTF) enzyme inhibitory activity. 3D models of the ligands and MTF were extracted from PubChem and Protein Data Bank (PDB), respectively. All ligand structures were first optimized to obtain their minimum energy structures. Molecular features were obtained for the optimized structures. The molecular docking process was performed for all of the ligands versus MTF enzyme to obtain the interacting ligand-receptor complexes. The results indicated that, the derivatives of cytidine revealed better enzyme inhibitory activity compared with that of the original structures. Moreover, chemical modifications showed different impacts on the molecular features and enzyme activities. Therefore, it is important to choose the type of modifications to the desired chemical structure. Among the investigated derivatives, D4: Galocitabine showed the best properties to be proposed as the best inhibitors, and it is a great candidate for further investigations.

Keywords: Cytidine; methyltransferase; cancer; docking.

Introduction

Since the early days of DNA recognition by Watson and Crick, numerous attempts have been dedicated to findthe properties of this biological building block [1-5]. Moreover, structural characterization of the nucleobases, adenine, guanine, cytosine, thymine, and uracil have attracted researchers to explore synthetic analogous for the novel materials [6-8]. Contribution to intermolecular integrations (such as hydrogen bonds) is another important factor for nucleobases, in which the 3D shapes of DNA are constructed by the hydrogen bonds [9-14]. In addition to the initial biological importance of the nucleobases in living systems, their therapeutic behaviors have also been followed by researchers to evaluate synthetic medicinal compounds [15]. In this case, cytidine, which is the nucleotide of cytosine, revealed significant activities in cancer therapy [16-19]. It is well-known that cancer is the most important health disorder of the centuries, in which numbers of people in all around the world have serious problems with cancer [20-23]. Herein, by the importance of cancer therapy of nowadays research, exploring

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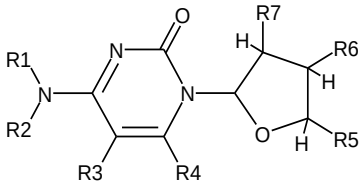
potent inhibitors of cancer growth has been become almost the first choice of studies for the researchers in the life sciences fields [24].

Enzymes have important roles in initiating bio-reactions in living systems; however, their activities are sometimes out of order, leading to serious problems [25]. Methyltransferase (MTF), which is responsible for methylation of DNA and proteins, is one of those enzymes with serious problems in hyperactivity [26]. Although methylation is very much important for several tasks of genetic engineering in the human body, it could cause cancer [26]. Therefore, MTF inhibition is one of the proposed treatments for cancer therapy by avoiding the hyper-methylation [26].

In this this work, we have investigated cytidine and its available derivatives (Table 1) to assess their inhibitory activity for MTF enzyme. So, we have employed *in silico* methodologies to perform the molecular docking for the virtual screening processes [27-29]. Available cytidine derivatives have been screened and the potent ones for MTF inhibition were proposed. All the cytidine structures were optimized to reach a minimum energy level. It is worth to note that the *in silico* methodologies are such versatile techniques to evaluate information at the atomic/molecular scales for complicated living systems besides *in vitro* and *in vivo* environments[30].

Table 1. The models of original cytidine and derivatives



No.	PubChem ID	Name	R1	R2	R3	R4	R5	R6	R7
0	6175	Cytidine	H	H	H	H	CH_3O	OH	OH
D1	60953	Capecitabine	$C_6H_{11}O_2$	H	F	H	CH_3	OH	OH
D2	65091	Deoxycytidine Triphosphate	H	H	H	H	$CH_6P_3O_{10}$	OH	H
D3	65177	Cytarabine 5'-Monophosphate	H	H	H	H	CH_4PO_4	OH	OH
D4	65950	Galocitabine	$C_{10}H_{11}O_4$	H	F	H	CH_3	OH	OH
D5	101544	Cytidine 2'-Monophosphate	H	H	H	H	CH_3O	OH	H_2PO_4
D6	107461	N4-Acetylcytidine	H	C_2H_3O	H	H	CH_3O	OH	OH
D7	114682	Aracytidine	H	H	H	H	CH_3O	OH	OH
D8	169016	2'-Deoxy-5-(Hydroxymethyl)Cytidine	H	H	CH_3O	H	CH_3O	OH	H
D9	688503	N-Isobutyryl-2'-Deoxycytidine	H	C_4H_6O	H	H	CH_3O	OH	H
D10	6435808	Tezacitabine	H	H	H	H	CH_3O	OH	CHF
D11	6918726	Valopicitabine	H	H	H	H	CH_3O	$C_5H_{10}NO_2$	OH
D12	10037499	5'-Deoxy-5-Fluorocytidine;	H	H	F	H	CH_3	OH	OH
D13	11346228	N4-Acetyl-2'-deoxycytidine	H	C_2H_3O	H	H	CH_3O	OH	H
D14	14308791	5-Chlorocytidine	H	H	Cl	H	CH_3O	OH	OH
D15	21136976	5-(1-Propynyl)-cytidine	H	H	C_3H_3	H	CH_3O	OH	OH

Computational details

First, 3D models of cytidine and fifteen of its derivatives (Table 1) were downloaded from the PubChem databank [31]. They were optimized at the B3LYP/6-31G* density functional theory (DFT) level employing the Gaussian package to achieve the global minimum structures [32]. Molecular features including values of the highest occupied and the lowest unoccupied molecular orbitals (HOMO and LUMO), differences of HOMO and LUMO levels as energy gaps (E_g), dipole moments (D_m) and partition coefficient (*Log* P) have been also evaluated by the results of optimization processes (Table 2). Till now, the ligand structures have become ready for further investigation versus the MTF enzyme. The 3D structure of the MTF enzyme was downloaded from the Protein Data Bank (PDB) with ID: 1XVA, and its chain-A was extracted and prepared using the discovery studio package for incorporating in molecular docking processes [33,34]. AutoDock-tool was employed to prepare required files of molecular docking processes by assigning 70*70*70 grid box and 300 runs of genetic algorithm [35]. Molecular docking processes were performed using the AutoDock4 package for all of the sixteen ligands with MTF receptor [35]. The results of binding energies (E_b) and interacting amino acids are summarized in Table 3 and their corresponding graphical schemes are summarized in Figures 1 and 2 (supplementary file).

Table 2. Structural features of the original cytidine and derivatives

No.	Formula	HOMO /ev	LUMO /ev	E_g /eV	D_m Debye)	LogP
0	$C_9H_{13}N_3O_5$	-6.20	-0.72	5.48	7.79	-1.73
D1	$C_{15}H_{22}FN_3O_6$	-6.50	-1.65	4.85	6.54	0.74
D2	$C_9H_{16}N_3O_{13}P_3$	-6.23	-0.81	5.42	7.89	0.46
D3	$C_9H_{14}N_3O_8P$	-6.57	-1.15	5.42	5.10	-0.98
D4	$C_{19}H_{22}FN_3O_8$	-6.19	-1.85	4.34	9.57	0.24
D5	$C_9H_{14}N_3O_8P$	-6.03	-0.62	5.41	8.78	-0.97
D6	$C_{11}H_{15}N_3O_6$	-6.41	-1.34	5.07	9.36	-1.31
D7	$C_9H_{13}N_3O_5$	-5.80	-0.70	5.10	4.76	-1.75
D8	$C_{10}H_{15}N_3O_5$	-5.66	-0.27	5.39	6.89	-1.63
D9	$C_{13}H_{19}N_3O_5$	-5.89	-1.03	4.86	4.34	0.23
D10	$C_{10}H_{12}FN_3O_4$	-6.09	-0.65	5.44	6.20	-1.52
D11	$C_{15}H_{24}N_4O_6$	-5.93	-1.05	4.88	6.65	-1.02
D12	$C_9H1_2FN_3O_4$	-6.28	-1.13	5.15	5.79	-0.81
D13	$C_{11}H_{15}N_3O_5$	-5.93	-0.89	5.04	8.34	-0.7
D14	$C_9H_{12}ClN_3O_5$	-6.30	-1.05	5.25	6.10	-1.21
D15	$C_{12}H_{15}N_3O_5$	-5.71	-0.71	5.00	8.58	-0.55

Results and discussion

The models of this work include ligands of cytidine and fifteen of its derivatives as described in Table 1. The obtained results of optimization processes (Table 2) revealed different molecular properties of the ligand structures. The values of E_g, the difference of HOMO and LUMO levels indicated different grades of reactivates for the ligand structures. The lower value of E_g was found to be more favorable for the reactivity incorporation with other molecular systems. The results indicated that, the value of E_b for D4 was the best one among the interacting ligands with receptor, in which its E_g was also the smallest one among the ligands. It is very significant to relate the structural features to the corresponding activities (SAR), in which the HOMO and LUMO quantum descriptors are among the most

important structural features. These values that are characteristic for each structure could be mentioned as personalized for each structure; therefore, they could reveal insightful information at the molecular scales especially for the complicated biological systems.

Table 3. Molecular docking results

No.	E_b kcal/mol	Interacting Amino Acids	
		Hydrogen Bonds	Non-Hydrogen Bonds
0	-5.38	HIS142 – TYR242 – PRO187 – ARG175	LEU240 - TYR33 – ILE202 – PRO188 – GLY189 – TYR177 – ASN138 – GLY137 – TYR283 – LEU218 – ALA186
D1	-6.32	HIS142 - SER139 - THR67 - THR37	ASN116 - VAL84 - ALA115 - ASP85 - VAL63 - ALA64 - CYS65 - GLY66 - GLY68 - ARG38 - VAL69 - ILE34 - TRP117
D2	-5.78	GLY137 - ALA64 - THR67 - THR37 - ASP70 - GLY68 - VAL69 - ILE34 - ASN191	ASN138 - TYR33 - SER139 - LEU136 - ASP62 - CYS65 - GLY66 - MET90 - ARG38 - TYR283 - TYR194
D3	-5.58	ILE34 - THR67 - TYR33 - TYR37 - ASN191 - ASP70	ARG38 - TYR193 - GLY137 - TYR44 - IEU136 - VAL69 - MET90 - GLY66 - TYR194
D4	-8.43	HIS142 - THR67 - ASP85 - TRP117	ALA115 - VAL85 - SER87 - GLY68 - CYS65 - VAL69 - ILE34 - LEU143 - LEU136
D5	-5.98	SER139 - ALA64 - CYS65 - GLY68 - VAL69 - TYR33 - ILE34 - THR67	HIS142 - ASN138 - GLY137 - LEU136 - TYR194 - SER71 - THR37 - GLY35 - ARG38 - MET90 - GLY66 - ASP70 - ASP62
D6	-5.94	THR67 - ASP70 - VAL69 - THR37	MET90 - GLY66 - LEU136 - TYR44 - GLY137 - CYS65 - GLY68 - ILE34 - ARG38 - TYR194
D7	-5.69	HIS142 - ALA115 - ASP85 - VAL63 - TRP117	LEU143 - ASN116 - VAL84 - LEU120 - ALA64 - SER87
D8	-5.86	THR67 - ASP70 - VAL69 - THR37 - ALA64 - SER139 - ILE34 - GLY68	GLY66 - CYS65 - LEU136 - GLY137 - HIS142 - MET90 - GLY35 - ARG38 - TYR194
D9	-6.24	HIS142 - THR67 - ASP70 - VAL69 - ASP85 - CYS65	GLY68 - ALA84 - SER87 - SER71 - ARG38 - TRP117 - ALA64 - MET90
D10	-5.67	ASN116 - SER139 - ASP85	PHE140 - ALA64 - ALA115 - ALA156 - LEU118 - HIS142 - TRP117 - LEU143 - ALA86
D11	-6.78	THR37 - ASP70 - ALA64 - ASP62	TYR194 - VAL69 - ARG38 - TYR33 - PHE238 - TRP30 - LEU240 - HIS142 - GLY66 - SER139 - GLY68 - GLY137 - CYS65 - LEU136 - ILE34 - MET90
D12	-6.07	THR67 - VAL69 - ALA64 - ASP70 - GLY68	SER139 - GLY66 - MET90 - ILE34 - THR37 - CYS65 - LEU136
D13	-6.14	HIS142 - TRP117 - ASN116 - ALA86	LEU143 - SER87 - ALA115 - ASP85 - ALA64 - LEU118 - GLY66
D14	-5.62	THR37 - ASP70 - ALA64 - ILE34 - GLY68 - THR67	SER139 - MET90 - CYS65 - LEU136 - GLY66 - GLY137 - ARG38 - GLY35 - TYR194 - VAL69
D15	-6.3	TYR33 - THR37 - THR67 - CYS65 - VAL69 - ALA64 - ASP64 - ASP70	ARG38 - ASN191 - GLY137 - TYR44 - IEU136 - MET90 - GLY66 - TYR194 - GLY68 - SER139

The values for HOMO, LUMO and E_g of other structures showed that, the tendency of ligands to receptor could be different from the lower and higher strengths. Dm as another molecular descriptor, could very well describe the electronic sphere of molecular systems, in which the higher values could reveal deviation of electronic distribution from spherical form. D4, as the characteristic ligand revealed relatively the highest value of Dm among the considered ligands. Log P is the other descriptor showing the tendency of ligand solubility in non-polar versus polar solvents, in which the values upper than

zero indicate the favorability of non-polar solubility whereas the values below zero indicated the favorability of polar solubility. Based on the functional groups, the values of *Log* P varied among the ligands from very much non-polar solubility of D1 to very much polar solubility of D7. As a conclusion of the molecular descriptors, it could be concluded that the structural features are very much important for the ligands for their further features description and clarification.

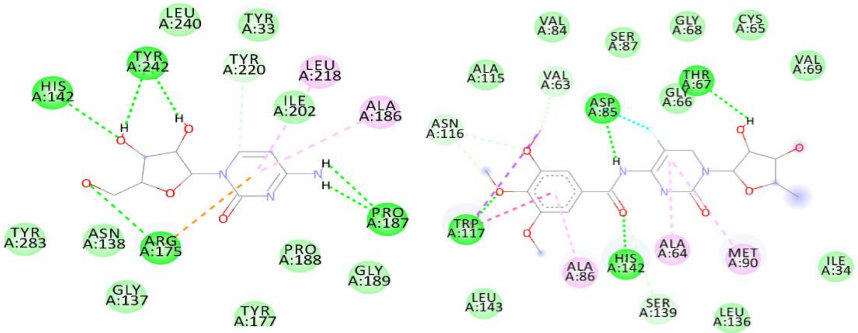


Figure 1. The interacting complexes of original cytidine (left) and D4 (right) with MTF enzyme

The results of molecular docking processes for the original cytidine and fifteen of its derivatives versus MTF enzyme are summarized in Table 3. There are two types of parameters to discuss about docking results, in which one of them is quantitative binding energy (E_b) and the other one is qualitative interacting amino acids (IAA). The schematic model of interaction for the original cytidine and D4 are shown in Figure 1 and all the schematic models are depicted in Figure 2 of supplementary file. Figures 1 and 2 show details of interactions between cytidine and surrounding amino acids, which is very much important for qualitative examination of ligand-receptor complexes. Since the grid box is fixed for all ligands, then the results of docking could be compared to each other. The hydrogen bond and non-hydrogen bond interactions could be seen for all the ligand-receptor complexes. However, the numbers of interactions and also the strength are different for the complexes. The binding strength is defined as an activity for interactions; therefore, the ligands with larger values of bindig energies than the original cytidine are considered here for further discussions. It has been shown earlier that the ligands have different reactivity due to the values of their energy gaps, here it could be seen that the quantity and quality of ligand interactions with receptor are also different. The minimum interaction activity belonged to the original cytidine and all other fifteen ligands were upper than it. The highest activity versus the original cytidine and derivatives belong to D4 (Galocitabine) with binding energy of -8.43 kcal/mol. In this structure, the main functional group was located at the amine group of pyrimidine ring, in which this type of modification was also done for D1, D6, D9 and D13 all with meaningful strong binding energies. Considerable hydrogen and non-hydrogen bonds also took place for these structures. The trend revealed that the structural modification of amine group play a significant role in defining the activity of cytidine derivatives. Addition of halogen atoms, F and Cl to D12 and D14, depicted that the F-addition has higher impact on the activity of cytidine compared with that of the Cl-addition.

Moreover, different functional groups could lead to different activities for the derivatives. The results showed that, the chemical modification of a compound could yield a new structure, in which its molecular features and enzyme inhibition activity could significantly deviate from the original compound. As the concluding remarks of molecular docking results, it could be mentioned that the chemical modification could improve enzyme inhibition activity of cytidine, in which D4 could be proposed as the best derivative for the purpose.

Conclusion

In this research study, we have performed an *in silico* work to investigate the cytidine derivatives as inhibitors of MTF enzyme. Some trends could be evaluated by our results. First, the structural modification of cytidine showed a significant impact on its molecular features, in which its reactivity could be changed. Second, the amine group modification of pyrimidine ring played a significant role in cytidine for enzyme inhibition. Third, halogen addition to the pyrimidine ring had a significant impact, especially by the F-addition. Fourth, although derivatives showed better enzyme inhibition properties than that of the original cytidine, one of derivatives (D4) showed very much significant results, which could be proposed as the best derivative with enzyme inhibitory activity. Finally, the chemical modification of cytidine could yield better ligand for interaction with MTF receptor, in which the type of modification could lead to desired purpose of action.

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