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Research Article

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Pharmacokinetic properties, docking study and anti-cancer evaluation of s-triazine-cytosine hybrids

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ABSTRACT

A series of cytosine substituted s-triazinyl derivatives (C1T, C2T &C3T) were synthesized. Pharmacokinetic parameters such as absorption, distribution, metabolism, excretion of these hybrids were determined by in silico method. Docking studies have been performed with their catabolic enzyme. The viability of HepG2 liver cancer cells and normal hepatocyte in the presence of these hybrids were assessed by MTT assay. The IC_{50} values of these compounds showed less viability exhibited in tumor cells compare to normal cells. The hybrid molecules distinguish between cancer cell from normal cell and reducing the toxicity.

Keywords: s-triazine nucleoside analogue, Pharmacokinetic properties, HepG2 cell lines, docking studies, anti-cancer drugs.

INTRODUCTION

Cancer is one of the most widespread and feared diseases in the Western world today - feared largely because it is known to be difficult to cure. The main reason for this difficulty is that cancer results from the uncontrolled multiplication of subtly modified normal human cells. One of the main methods of modern cancer treatment is drug therapy (chemotherapy). The majority of drugs used for the treatment of cancer today are cytotoxic (cell-killing) drugs that work by interfering in some way with the operation of the cell's DNA.A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects.

Cytotoxic drugs work by interfering with DNA replication because cancer cells are rapidly synthesized new DNA. No qualitative differences has been discovered so far between the DNA and associated enzymes of cancer cells and normal cells. Currently more investigations are going on with the chemicals of natural origin in the hope of reducing the toxicity [1]

1,3,5-Triazine possessing threefold symmetry allows versatile modifications uncomplicated by regiochemical concerns to provide useful biologically active compounds.[2-4]This strategy was an important development in drug discovery in the context of rapid identification and optimization of biological active lead compounds.[5]1,3,5-triazines and its derivatives are of considerable interest among the chemist because of their anti-tumor activity. [6-10] In our earlier work we designed and synthesized s-triazine nucleobase derivatives which are all found to anticancer activity with low toxicity [11]. Several pyrimidine derivatives have been reported for its potent anti-cancer activity [12-17]. So far researchers designed a xenobiotic molecule that has been inhibiting the protein

activity, first time we are synthesized a drug that will easily be catabolized by the enzyme and minimize the toxicity. In this present work we have reported the anticancer activity, pharmacokinetic properties and docking studies of s-triazinecytosine derivatives.

EXPERIMENTAL SECTION

2.1. MTT assay

The compound was dissolved in different concentration (10 to 250µg/ml) in 10% Dimethyl Sulfoxide (DMSO) to give a final concentration of DMSO not more than 0.5% and did not affect cell survival.

Cell viability test

The viability of cells was assessed by MTT assay (Mosmann, 1983) using HepG2 Liver cancer cell lines. The cancer cells were plated separately in 96 well plates at a concentration of 1×10^5 cells/well. After 24 h, cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37ºC. After starvation, cells were treated with different concentrations of test compound (10-100µg/ml) for 24 h. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37ºC in a CO_2 incubator. The 50% inhibitory concentration value (IC_{50}) of the test compound was identified for untreated cell line[18]

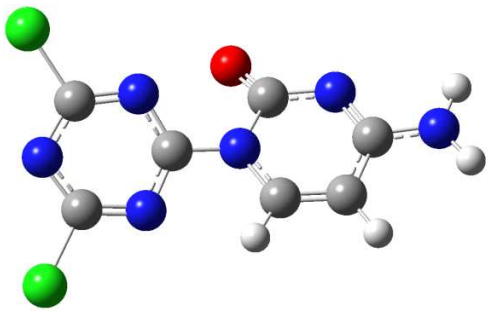
The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipetting up and down. Spectrophotometrical absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graph pad prism5 software.

2.2. Computational Studies

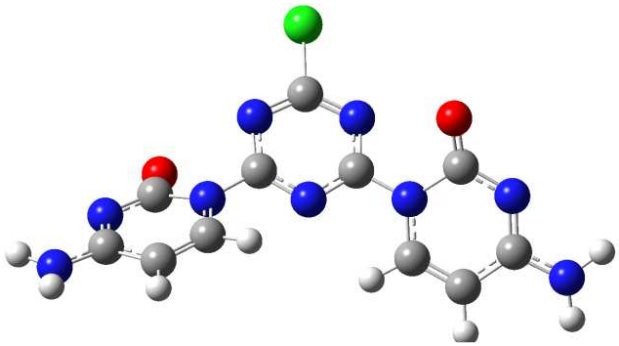
Molecular docking experiment was carried out to study the exact binding location of ligand on protein. Molecular docking simulation was performed with the aid of Maestro 9.3.5 version. Three dimensional (3D) structure of all protein was retrieved from the Protein Data Bank (PDB) using PDB ID:1MQ0, 1HVY (http://www.rcsb.org/pdb) and was optimized by removing water molecules and hetero, docking was subsequently performed using the Flexible docking algorithm considering the default parameters. All groups were deleted from receptor beyond the radius of 4Å of reference ligand and the resulting protein structure refined and minimized by protein preparation Wizard[19]using OPLS-2005 force field. Receptor Grid Generation programs were used to prepare all the protein Grid and all ligands were optimized by LigPrep[20]program by using OPLS-2005 force field to generate lowest energy state of ligands. A binding sphere covering all the active site residues was generated using the Define and Edit Binding Site module, of the total poses identified, the compounds were synthesised and optimized by Gaussion 09 package with DFT method 6-311G(d.p) as basis set. Pharmacokinetic properties were determined using Qikprop[21] module of Schrodinger software.

RESULTS AND DISCUSSION

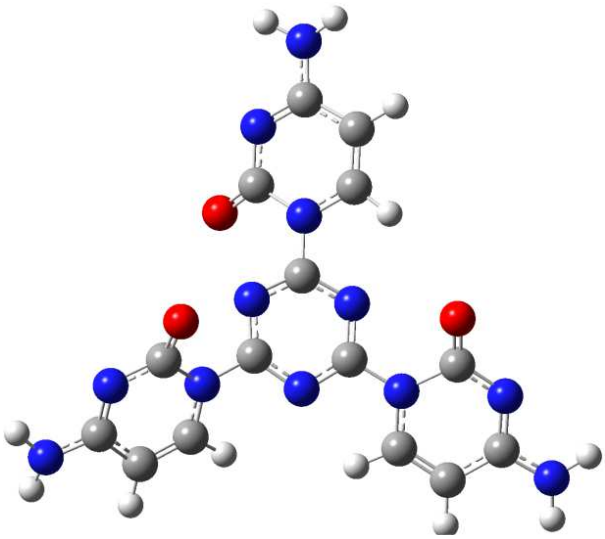
A series of triazinyl derivatives with cytosine nucleobase by mono, di, and tri-substitution in cyanuric chloride at the 2, 4 and 6positions was taken for antitumor evaluation. The compounds was synthesised using the procedure as per the literature [11] and optimized by DFT method. The probe compound structures are given in **figure 1.**The selection of nucleobase moiety is playing an important role because it was present in the DNA. So we assumed that the toxicity would be minimised and thus it has been proved by *in-vitro* studies. The IC_{50} values of the probe compounds are listed in the **table 1**



4-amino-1-(4,6-dichloro-1,3,5-triazine-2-yl)pyrimidine-2(1*H*)-one **(C1T**)



1, 1'-(6-chloro-1,3,5-triazine-2,4-diyl)bis(4-aminopyrimidine-2(1*H*)-one **(C2T)**



1, 1',1"-(1,3,5-triazine-2,4,6-triyl)tris(4-aminopyrimidine-2(1H-one **(C3T)**

Figure 1. Optimized structures of s-triazinecytosine derivatives

Table 1.IC_{50} values of the title compounds

Compounds	IC_{50} Value of HepG2 cancer cell (µg/ml)	IC_{50}Value of Normal hepatocytes (µg/ml)
C1T	40	68
C2T	18	83
C3T	10	85
Doxorubicin	4	12

All the values obtained in ±5

Table 1 shows the IC_{50} values of the probe compounds. It revealed that the title compounds are very much active against the HepG2 cancer cell lines. When comparing to cancer cell in normal cell doxorubicin has 3 fold cytotoxicity while C1T exhibits 2 fold cytotoxicity, C2T and C3T having 6 fold, 8 fold cytotoxicity respectively. It revealed that our hybrids act as better anti-cancer drugs. From the IC_{50} values one can conclude that these drugs are not inhibiting the enzymes which are responsible for DNA replication implied that their specificity is in some other way. All the drugs may interact with the DNA replication associated enzymes or by their corresponding catabolic enzyme cytidinedeaminaseor inhibit thymidylate synthase or it may directly go to the DNA and interact with it. In our drug nucleobase moiety is available so it may be catabolized by its corresponding enzyme due to availability of natural nucleotide already present in the cell for replication or the enzyme may allow our candidate drugs for replication due to imbalance of natural nucleotide for replication.

Among the three title compounds only C1T having high IC_{50} values suggested that those drugs might have catabolized by their corresponding enzyme and excreted so high concentration is needed to attack DNA. Compounds which are having lesser IC_{50} values may attack the DNA which leads to apoptosis, while compound C3T has low IC_{50}value (10µg/ml)The mechanism can be explained by another way, all drug molecules having nucleobase moiety (nucleobase mimetic) so their catabolic enzyme may allow these drugs for replication because it needs nucleotides that leads to obtained low IC_{50} values or some of them go to inhibit some other protein which is also leads to apoptosis. C3T was not docked with cytidinedeaminase suggests that there is some other mechanism also possible which will be investigated in future. Further the cytotoxicity can be explored by the following cell viability obtained from the normal cell lines which is showed in the **Table 2.**

Table 2. Percentage viability of Normal cells and tumor cells on compounds C1T, C2T and C3T

Concentration (µg/ml)	HepG2 cancer cells (%)			Normal hepatocytes (%)		
	Compounds			Compounds		
	C1T	**C2T**	**C3T**	**C1T**	**C2T**	**C3T**
0	100	100	100	100	100	100
12	80	47	47	87	88	89
25	57	46	43	81	84	82
50	45	43	37	83	78	74
100	21	20	29	40	43	63
250	6	10	12	11	19	49

All the values obtained in ±5

The viability table clearly revealed that our hybrids target only cancer cells and thus minimized the toxicity suggested that they acted against cell proliferation. At lower concentration (40-50µM) the normal cells are more viable when compared to tumor cell. The toxicity can be explained by enzymology aspects.

3.1. Enzymology

Researchers have been reported that in the cancerous cell some important enzymes are less or more active [22-25]. We have designed a drug in a novel way that we are target a missing enzyme so that we have achieved minimum toxicity which was proved by MTT assay. Cytidinedeaminase is the catabolic enzyme of cytidine nucleotides. Thymidylate synthase is an important enzyme for natural pyrimidine nucleotide balance in the cell. So considering the TS activity is very essential for anti-cancer activity of our drug candidate. Under expression of TS lead to thymine less death [26-27]. This may be the reason for distinguish the normal cell from cancer cell and minimize the toxicity. It can be explained in docking studies. Similarly the other enzyme activity also assessed by the researchers and found variations among them between the cancerous cell and normal cell, as a chemist we have considered this enzymology aspect and designed the xenobiotic molecule that minimized toxicity. Further studies may be carried out and developed by biochemist, biotechnologist, pharmacologist, and physicians.

Table 3. Docking results of the probe compounds

Compounds	**Cytidinedeaminase (1MQ0)**					
	Docking Score	Binding energy kcal/mol	Glide evdw	Glide ecoul	Hydrogen bond energy	Interacting residues
C1T	-3.29	-29.77	-24.90	-4.88	-0.91	Glu 67 Ser 97
C2T	-4.37	-38.28	-30.79	-7.45	-1.46	Glu 67 Ser 97 Tyr 60
C3T	No Dock	-	-	-	-	-
Thymidylate Synthase (1HVY)						
C1T	-4.68	-44.67	-37.85	-6.83	-0.7	-
C2T	-7.11	-56.42	-48.89	-7.54	-1.20	-
C3T	-4.83	-45.19	-27.93	-17.26	-2.89	Leu 221

Evdw-van der waals energy, ecoul-coulomb energy

3.2. Molecular Docking

In silico docking studies are the powerful tool for designing a drug candidate molecule. So far chemists have been designing drug molecules that target the protein to inhibit its action by strongly binds with protein. The same idea

has been employed in the case of anti-cancer drugs, but the main problem in the cancer drug design, xenobiotic molecule will also kill the normal cell and causing side effects. Our motive is the drug would be killed the cell or be catabolized by suitable enzyme. We have chosen cytidinedeaminase and thymidylate synthase for docking studies which are maintaining the nucleoside balance in the cell. Docking studies were performed with nucleoside catabolic enzymes and the parameters are shown in **Table 3.**

From the table low dock scores indicated that these hybrids are not inhibiting any protein, so it may directly attack DNA. If the hybrid molecules inhibit DNA and its associated enzyme such as DNA helicase, single stand binding proteins, primase, DNA polymerase I & III, DNA ligase, gyrase and topoisomerase both the normal and tumor cells will damage or otherwise it will inhibit the cytidinedeaminase and thymidylate synthase that also will case cell damage in both cases. In the case of our hybrids, they are allowed by their catabolic enzyme to DNA for replication while the natural nucleoside is deficit; otherwise the hybrid molecule may go to DNA for replication when the enzyme activity is very low. While the enzyme activity is inhibited there also both cells will be affected that causes toxicity. Due to their bulky size of the C3T molecule, docking will not be taken place so chance is more to go DNA (IC_{50}-10μM). Fig 2 showed 3D diagram of protein-ligand interaction showed both in the C1T, C2T hybrids amino acid residue is interacting with amino group only so it will be deaminased by the enzyme and go to the next metabolite stage, very low docking score of C1T, C3T indicated that this may be catabolized by their enzyme in the case of normal cells, but it will active in tumor cells because of their low enzyme activity. In the case of TS no H-bond is found between any of the drug and the protein showed that this protein is not involved in the enzymology mechanism. From the experimental and docking studies it is the indirect evidence that all the hybrid molecules causing cell damage by attacking DNA molecule which leads to apoptosis. This is the possible way to distinguish tumor cells from normal cells. Two dimensional images of docking diagram showed the amino acid residues and hydrogen bonding and other interactions.

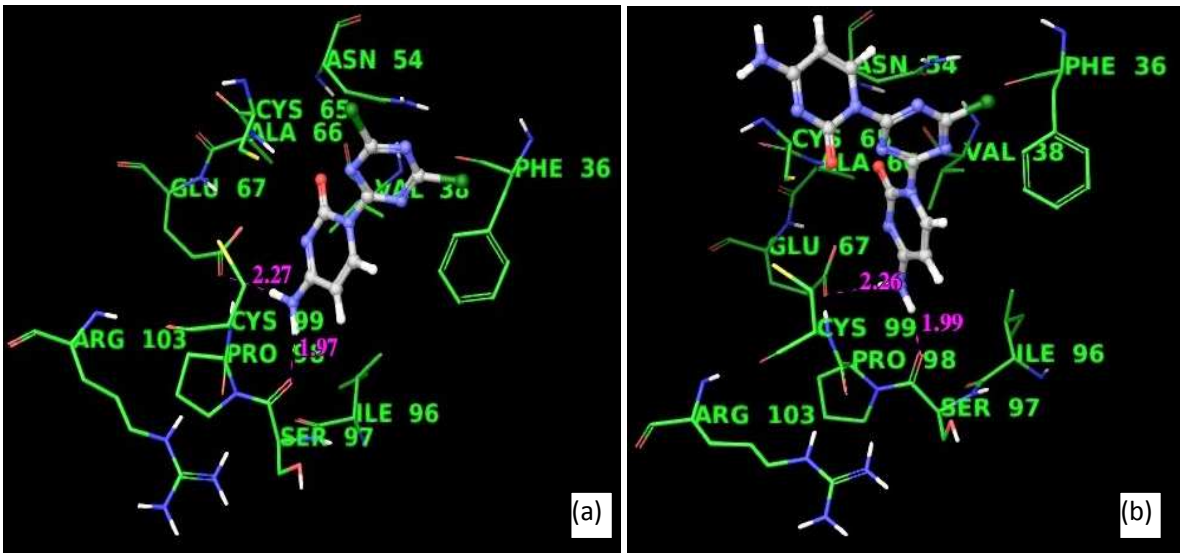


Figure 2.3D docked diagram illustrates the protein-ligand interaction of Cytidinedeaminase (1MQ0) with (a) C1T (b) C2T

3.3. Pharmacokinetic Properties

Lipinski rule of 5filters help in early preclinical development and could avoid costlylate-stage preclinical and clinical failures.C1T abide the Lipinski's rule of five while C2T has one violation and C3T has 2 violations. Among the three hybrids two have possess drug like properties. Lipinski factors are listed in Table 4.

Table 4.Lipinski's properties of probe compounds

Compounds	Factors of Lipinski's rule of five				
	Molecular Weight (<500)	Donor HB (< 5)	Acceptor HB (< 10)	QPlogPo/w[a] (< 5)	Rule of Five
C1T	259.054	2	7	0.264	0
C2T	333.696	4	11	-1.121	1
C3T	408.338	6	15	-2.577	2

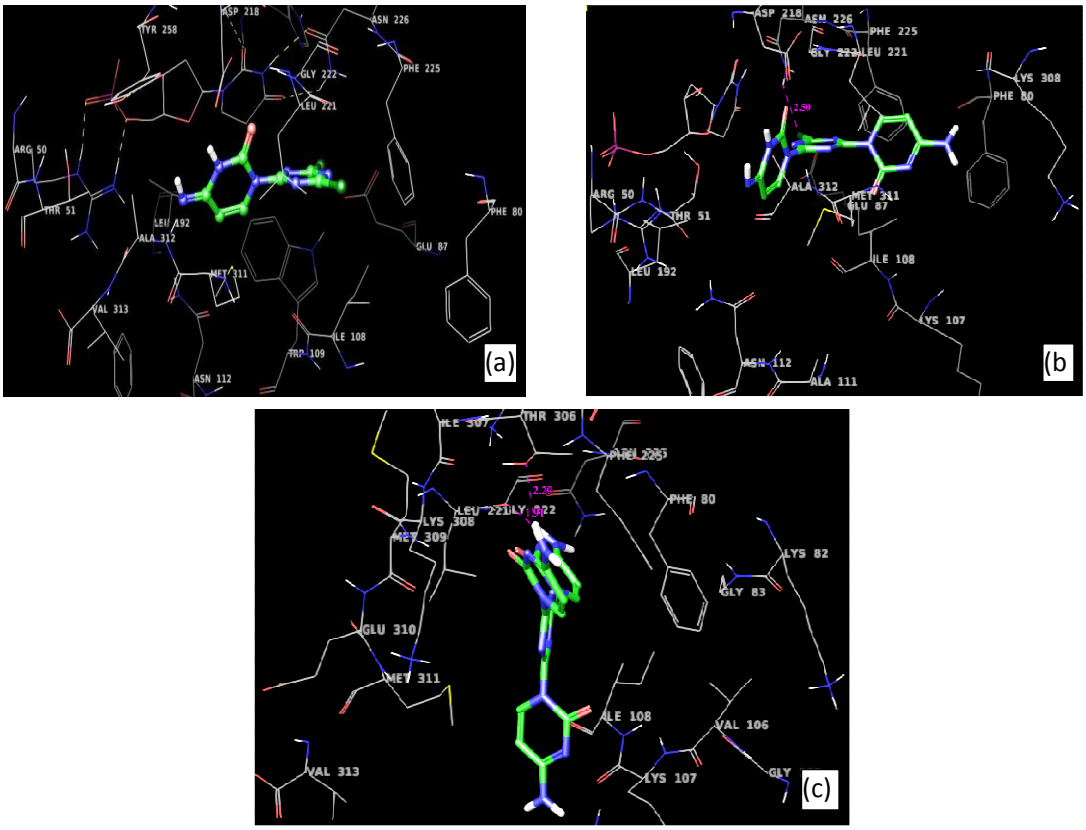


Figure 3.3D docked diagram illustrates the protein-ligand interaction of thymidylate synthase (1HVY) with (a) C1T (b) C2T (c) C3T

The drug-like prediction results by Qikprop are tabulated in table 5. Table showed C1T exhibited acceptable values of human oral absorption, solubility and blood brain barrier penetration except $HERGK^+$ channels. So it can be taken orally. Di substituted compounds have moderate values while tri substituted compounds not give satisfactory results. From the values it can be assumed that these compounds are taken intravenously or applied externally on the surface against cancer.

Table 5 Pharmacokinetic properties of probe compounds

Pharmacokinetic properties	Compounds		
	C1T	C2T	C3T
Percent Human Oral Absorption (> 80 high, < 25 poor)	68.86	27.016	0
QPlogS[b] (–6.5 to 0.5)	-2.81	-3.31	-3.578
QPlogHERG[c] (below –5)	-4.16	-5.133	-5.959
QPlogBB[d] (–3 to 1.2)	-0.749	-2.347	-4.108

[a]Partition Coefficient between octanol and water,
[b]Predicted aqueous solubility; S in mol/L,
[c]Predicted IC_{50} value for blockage of HERG K+ channels,
[d]Predicted blood brain barrier permeability

CONCLUSION

In summary we have evaluated the anti-cancer activity of cytosine substituteds-triazine- hybrids against HepG2 cancer cell lines and normal hepatocellular lines. All the new hybrid molecules selectively target cancer cells and reduced toxicity except. From the cytotoxic study it was found that viability of tumor cells decreased in a dose dependent manner while the normal cells viability remains unchanged up to 40µg/ml. Why and how the molecules are cytotoxic to cancer cells as well as non-toxic to normal cells were explained from docking studies and enzymology aspects. Docking studies indicated that the hybrids may be degraded by catabolic enzymes. The above study suggested that all the hybrid molecules may attack DNA causing apoptosis. For the first time it has been

shown that catabolic enzymes missing in cancer cells may be exploited for highly selective toxicity. Pharmacokinetics screening has been done for future analysis and drug development. It can be utilized as a guide for future studies for designing and screening potent anti-cancer drug development.

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