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Anti-proliferation and molecular docking study of s-triazinyl uracil hybrids

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ABSTRACT

A series of uracil substituted s-triazinyl derivatives (U1T, U2T & U3T) were synthesized. All the hybrids were in vitro evaluated for their anti-proliferative activity in human cancer cell lines, namely HepG2 hepatocellular carcinoma and normal hepatocytes. Docking studies have been performed with suitable enzyme to study the mode of action. 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. Finally, a theoretical kinetic study was established to predict the ADME of the active hybrids. These compounds are worthy of further evaluation as anticancer agents.

Keywords: nucleobasemimitic, Pharmacokinetic properties, HepG2 cell lines, docking studies, anti-cancer drugs. Cyanuric chloride derivatives, nucleoside analogues.

INTRODUCTION

Identification of cytotoxic compounds led the development of anticancer therapeutics for several decades. Advances in cancer treatment, however, continued to be limited by the identification of unique biochemical aspects of malignancies that could be exploited to selectively target tumor cells. Schwartsmann *et al*., noted in 1988 that of over 600,000compounds screened, less than40 agents were routinely used in the clinic [1]. Conventional screening models for anticancer agents are geared toward the selection of cytotoxic drugs. The history of cancer chemotherapy has been widely described [2]. Most An understanding of toxicities, adverse effects, and special dosing considerations of existing anticancer compounds is important to the design of effective drug combinations and to the interpretation of the toxicological profile of new chemical entities. Most cytotoxic anticancer agents are dosed to maximum tolerated levels to achieve maximum cell kill. Cytotoxic anticancer compounds were discovered by serendipity or as inhibitors of metabolic pathways involved in cell division. There is clearly an important role for nucleosides in the treatment of cancer, and the design of new agents within his class of compounds is still warranted. However, design, synthesis, and evaluation of new analogues as potential anticancer agents is not currently a major emphasis in the drug development community. Although toxicity is still a problem and is an issue that is hard to circumvent with antimetabolites (or other classical cytotoxic agents), the information provided in the preceding pages clearly indicates that small structural changes can have profound effects on the biological activity of nucleoside analogues and suggests that new agents with useful activities can still be identified.

The main mechanism of action of these classic cytotoxic drugs is inhibition of the increased rate of DNA synthesis and replication, or to destroy DNA in tumor cells. Cytotoxic drugs can interact with cells via different mechanisms and are divided into groups accordingly; alkylating agents (e.g. melphalan), antimetabolites (e.g. cytarabine, fluorouracil), topoisomerase inhibitors (e.g. etoposide, doxorubicin) and microtubule interacting agents (e.g. vincristine, paclitaxel) (Nygren 2001).In contrast to the traditional DNA-targeting cytotoxic agents, these drugs were designed to specifically act on their targets and thereby to be less toxic to normal cells. In drug development, it is therefore important to find novel anticancer drugs with both good clinical effects and low toxicity (Parent-Massin *et al.* 2010; Valeri *et al.* 2010). Currently more investigations are going on with the xenobiotic in the hope of reducing the toxicity [3]

So far researchers have designed nucleoside analogue cytotoxic drugs with deoxyribose or ribose moiety which was connected to any one of the five nucleobase moiety, but we have chosen 1,3,5-Triazine. s-triazine derivatives are considerable interest among the chemist because of their anti-tumor activity[4-9]. In our earlier work we designed and synthesized s-triazine nucleobase derivatives which are all found to anticancer activity with low toxicity [10].First time we are synthesized a drug that will easily catabolized by the enzyme and minimize the toxicity. In this present work we have reported the anticancer activity, ADMET and docking studies of s-triazinyl uracil derivatives.

MATERIALS AND METHODS

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 37^{o}C. 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[11]

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.

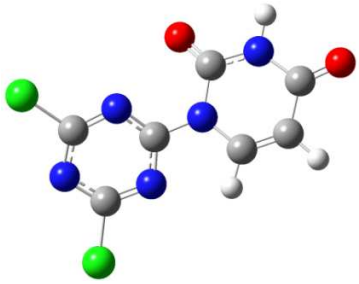
2.2. Computational Studies

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: 1ZXM, 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 using OPLS-2005 force field [12]. Receptor Grid Generation programs were used to prepare all the protein Grid and all ligands were optimized by LigPrep program by using OPLS-2005 force field to generate lowest energy state of ligands[13]. 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. 2D structures of compounds were subjected to a computational program using Qikprop module of Schrodinger software for the *in silico* determination of pharmacokinetic properties [14].

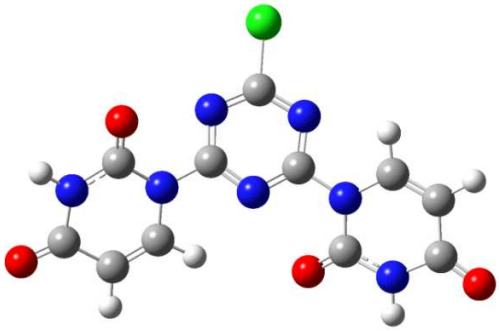
RESULTS AND DISCUSSION

A series of triazinyl derivatives with uracil nucleobase by mono, di, and tri-substitution in cyanuric chloride at the 2, 4 and/or 6positions was taken for antitumor evaluation. The compounds was synthesised using the procedure as per the

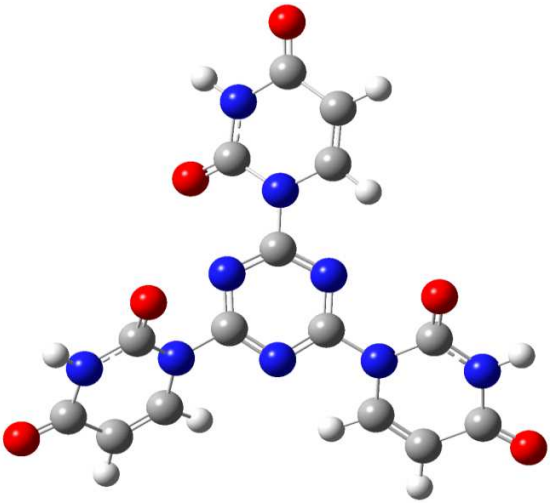
literature and optimized by DFT method[10]. The probe compound structures are given in **figure 1.**We assumed that the toxicity would be minimised by connecting s-triazine and uracil moieties and thus it has been proved by the *in-vitro* studies. On the basis of two major factors regarding cancer drug candidate we designed these hybrids accordingly. 1. The xenobiotic molecule should already present in the cell 2. Enzymes pathway was taken in to account of cancer and normal cell. The IC_{50} values of the probe compounds are listed in the **table 1**



1-(4, 6-dichloro-1,3,5-triazine-2-yl)pyrimidine-2,4(1H, 3H)-dione) (U1T)



1, 1'-(6-chloro-1,3,5-triazine-2,4-diyl)bis(pyrimidine-2,4(1H, 3H)-dione) (U2T)



1, 1', 1"-(1,3,5-triazine-2,4,6-triyl)tris(pyrimidine-2,4(1H, 3H)-dione) (U3T)

Figure 1. Optimized structures of s-triazine uracil derivatives

Table 1.IC_{50}values of the probe compounds obtained by *in vitro* method

Compounds	IC_{50} Value of HepG2 cancer cell (µg/ml)	IC_{50}ValueofNormalhepatocytes (µg/ml)
U1T	16	82
U2T	22	85
U3T	20	65
Doxorubicin	4	12

All the values obtained in ±5

Table 1 showed the IC_{50} values of the probe compounds. It revealed that the title compounds are very much active against the HepG2 cancer cell lines. The IC_{50}values suggested that compound U1T, U2T exhibited 7.6 fold, 5.3 fold activity respectively while U3T has 4.5 fold when compared to doxorubicin which has 3.2 fold activity against cancer cell. It suggested that our hybrids exhibited best activity with low toxicity compare to standard doxorubicin. The cytotoxic effect was cell type specific targeting preferentially cancer cells. From the IC_{50} values one can conclude that these drugs are not inhibiting any of the enzymes implied that their specificity is in some other way. Fluorescence images of treated cell lines showed the viability of cell in the presence of s-triazinyl uracil hybrids.

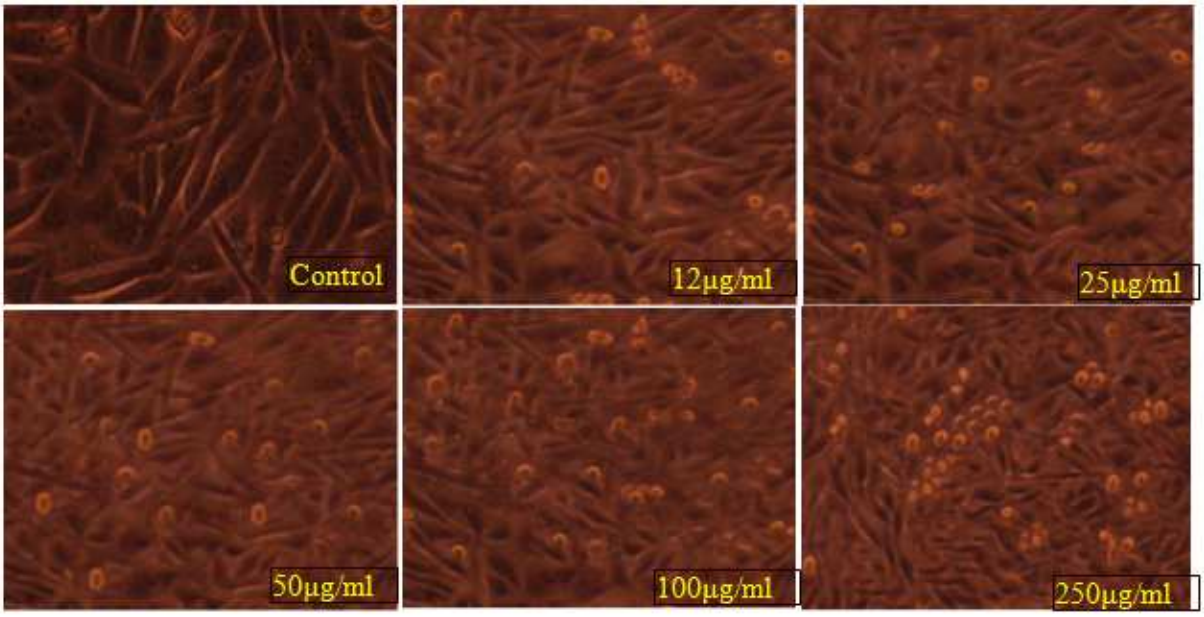


Figure 2.Fluorescence microscopic images of normal hepatocytes by MTT assay method in the presence of compound U1T

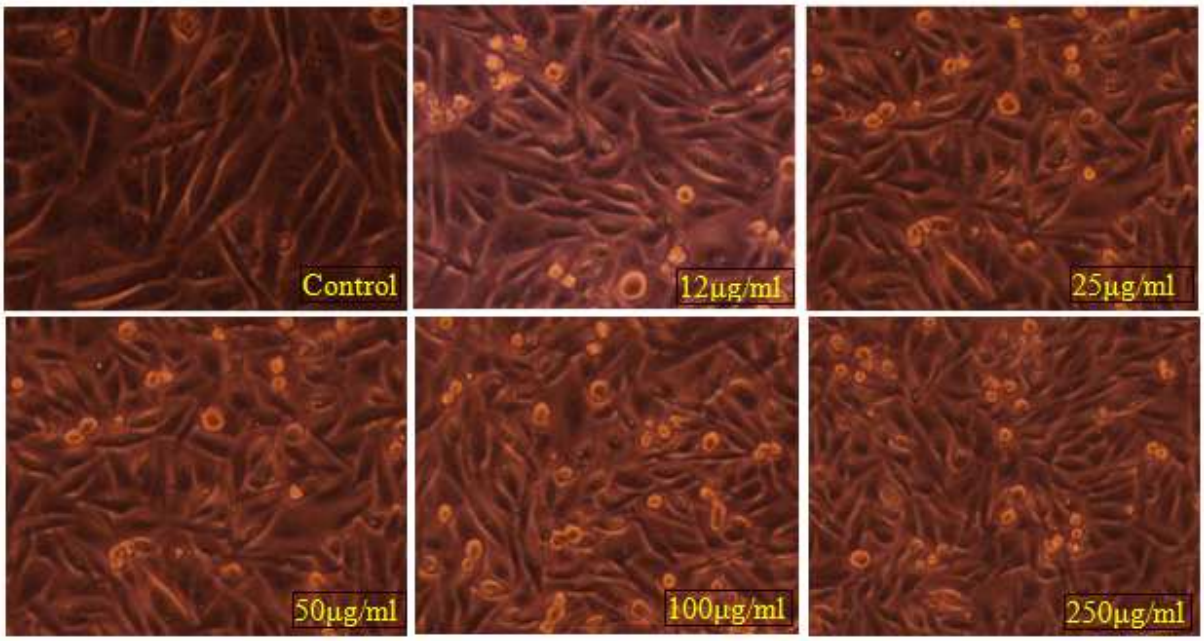


Figure 3.Fluorescence microscopic images of HepG2 cancer cells by MTT assay method in the presence of compound U1T

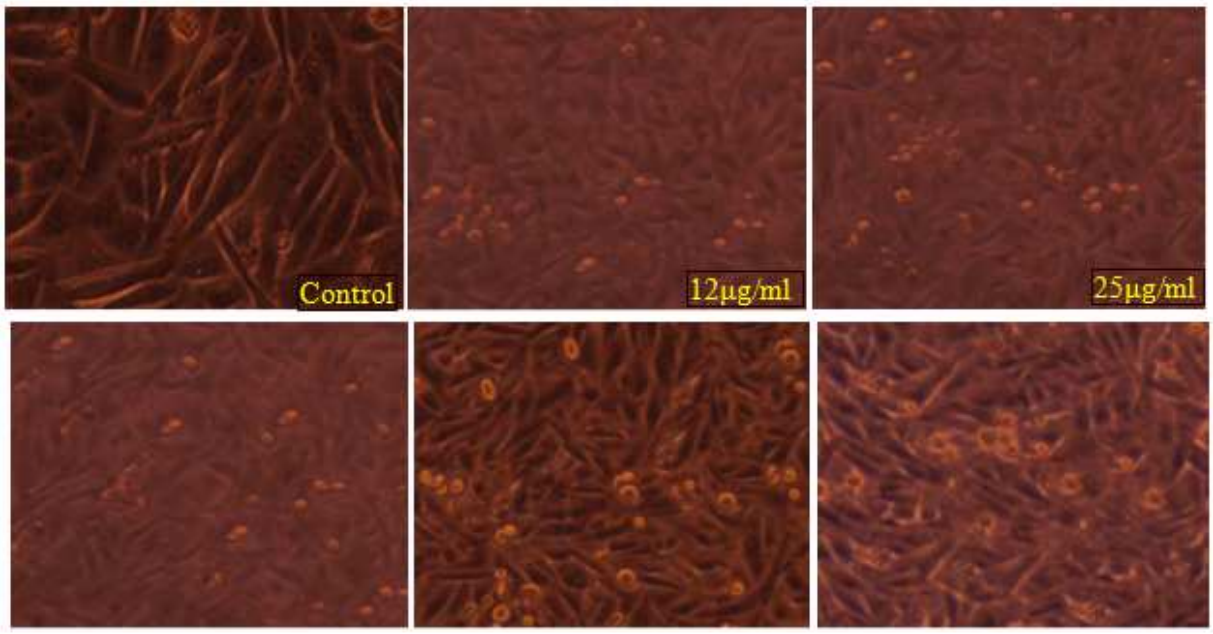


Figure 4.Fluorescence microscopic images of normal hepatocytes by MTT assay method in the presence of compound U2T

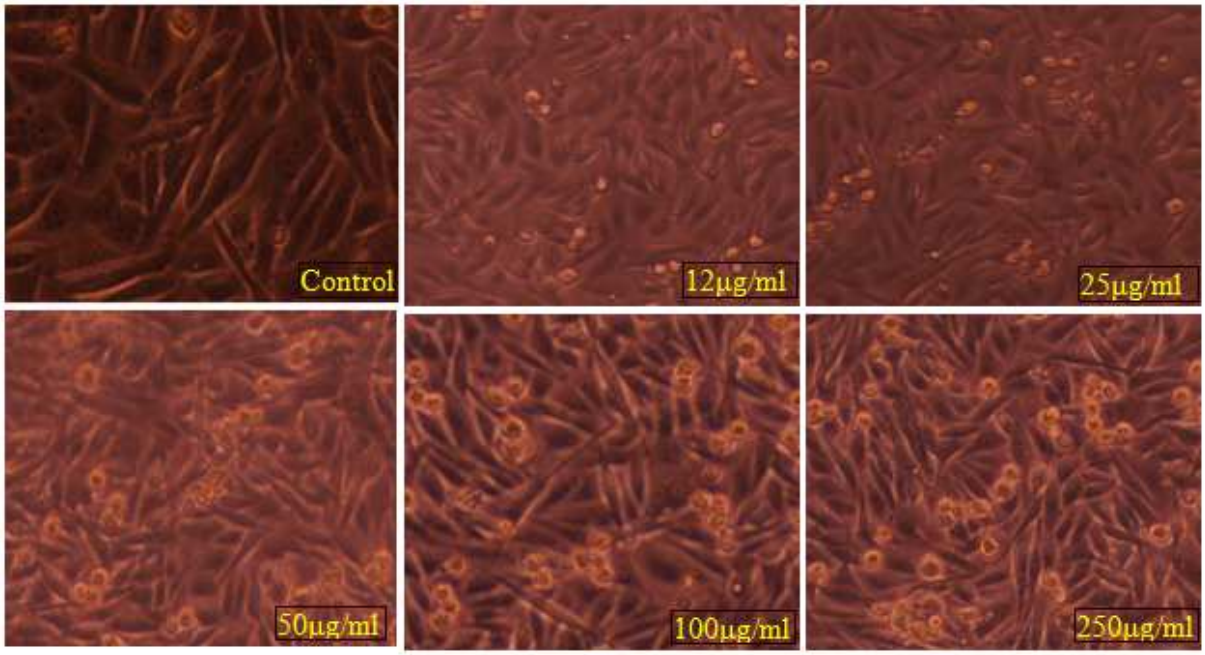


Figure 5.Fluorescence microscopic images of HepG2 cancer cells by MTT assay method in the presence of compound U2T

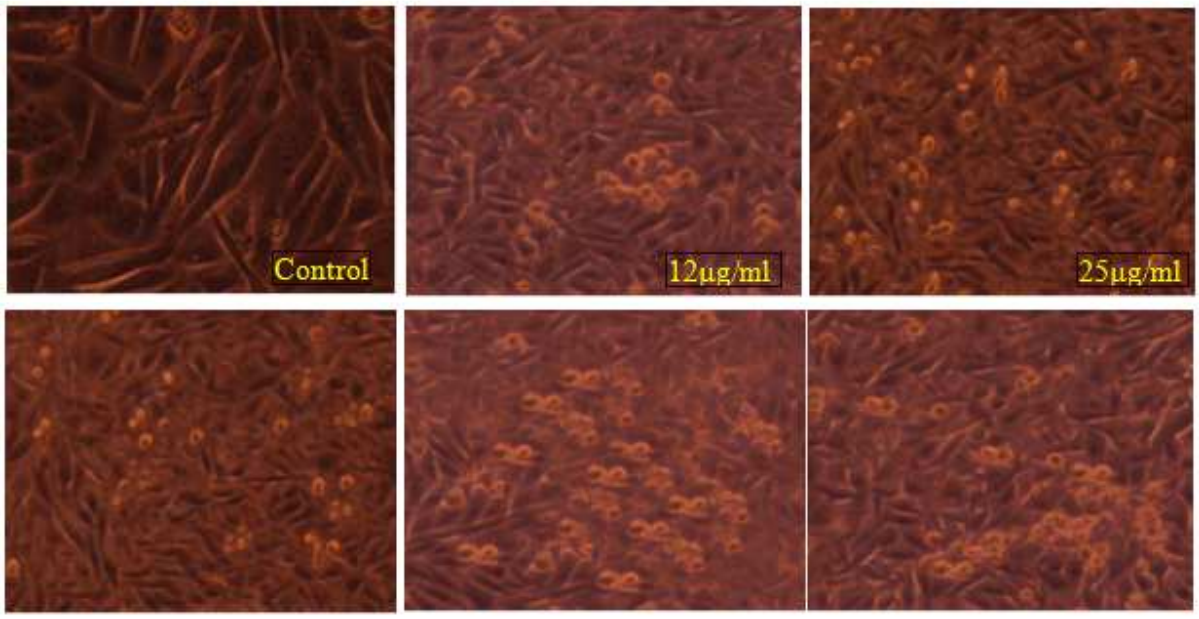


Figure 6.Fluorescence microscopic images of normal hepatocytes by MTT assay method in the presence of compound U3T

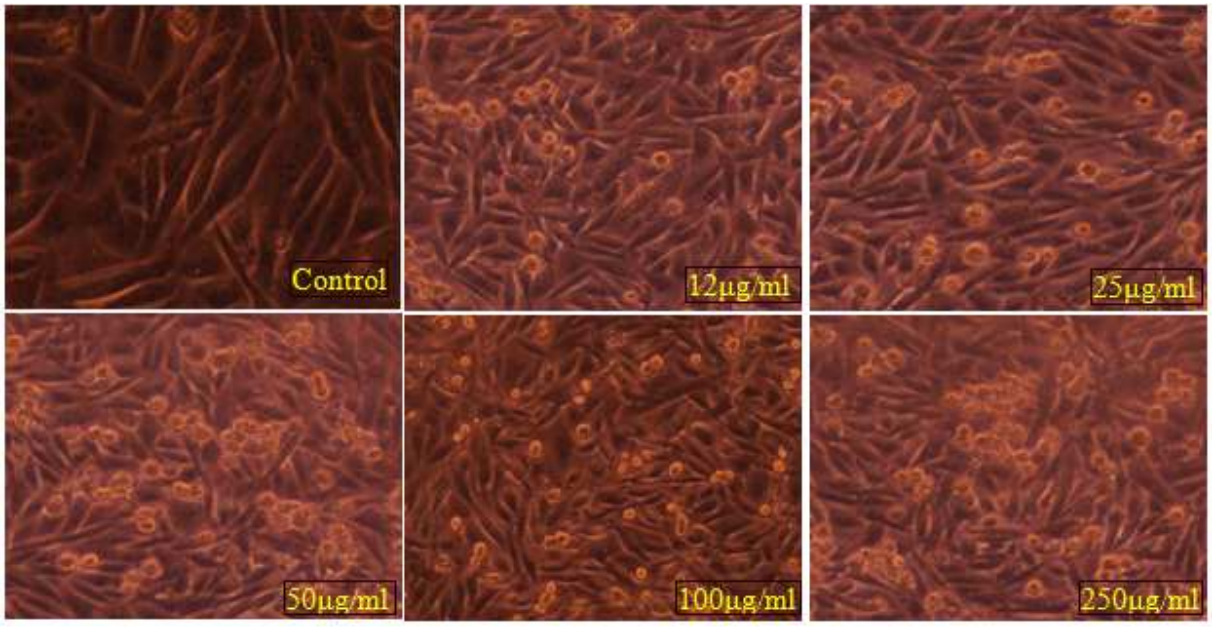


Figure 7.Fluorescence microscopic images of HepG2 cancer cells by MTT assay method in the presence of compound U3T

The cytotoxicity table clearly revealed that normal hepatocytes were less sensitive to our hybrids than tumor cells with dose dependent manner. At higher dosage level 250µM the cytotoxicity was 2 fold in tumor cell, but at the dosage of 50µM viability is more in normal cell when compared to tumor cells. The fluorescence image showed less damage area in normal cell when compared to tumor cells in all the three hybrids.

Table 2. Percentage viability of Normal cells and tumor cells on compounds U1T, U2T and U3T

Concentration (µg/ml)	HepG2 liver cancer cells (%)			Normal Hepatocytes (%)		
	Compounds			Compounds		
	U1T	**U2T**	**U3T**	**U1T**	**U2T**	**U3T**
0	100	100	100	100	100	100
12	54	56	53	85	86	89
25	39	52	48	79	82	70
50	31	41	30	66	74	64
100	18	15	26	49	37	43
250	8	5	8	29	28	18

All the values obtained in ±5

Enzymology

Enzymes played a vital role causing and curing every disease so considering the enzymes leads to discover a novel and active drugs. Enzymes involved drug catabolism process and diminishes the toxicity of xenobiotic molecules in living system. All the anticancer drugs directly or indirectly stop the replication of DNA usually by inhibiting one of the enzymes involved in the replication process since such enzymes are present in both normal cell and cancer cell. Normal cells are also killed by xenobiotic drugs leading to adverse effect including death. Our motive is the drug would kill the cell or catabolized by suitable enzyme [15, 16]. In our hybrid molecule uracil moiety is a natural nucleobase moiety which is well known to the cell, s-triazine moiety also easily catabolized by the enzyme[17], when using our hybrid it is theoretically nontoxic hence it has been proved by in vitro method. But our hybrids mainly target DNA particularly in cancerous cell in a novel way. Doxorubicin and 5-flurouracil inhibited topoisomerase, thymidylate synthase respectively, but uracil substituted triazinyl hybrids acted in a novel way and minimized toxicity, it suggested that predominantly it may attack DNA, still 5-fluoro uracil mechanism is not yet discovered our drug candidates mechanism also should be probed because it acted in a different way[18].

We assumed that disincorporation of hybrid U1T,U2T & U3T into DNA is also possible due to the presence of nucleobase moiety but according to the noble laureate's theory of DNA repairing, the mismatch repair enzyme could not replace our hybrids due to the existence of Watson-crick hydrogen bond with complementary base pair [19, 20],but further replication is not possible due to the rigid stereochemistry of s-triazine moiety because it could not form the phosphodiester back bone formation hence apoptosis triggered, phosphorylate enzyme did not dock with our hybrids further supported our mechanism. Moreover studies should be done for understanding of its mechanism. The sensitivity towards topoisomerase and thymidylate synthase also studied through molecular docking.

Molecular Docking

Docking studies provide remarkable information on binding sites of drugs, estimating the binding energies of each drug conformation with corresponding scores and functions. Docking studies were performed to know the binding mechanism of our hybrid with enzymes. We have chosen topoisomerase and thymidylate synthase for docking studies whether these proteins have been inhibited by our hybrids like doxorubicin and 5-fluro uracil [21,18]. Docking results are shown in **Table 3.**

From the table low docking score indicated non complementarity of our hybrid with protein cavity, less number of hydrogen bonding (2-4) indicated that these hybrids are not inhibiting the activity of protein, in case of thymidylate synthase no hydrogen bond is present between the protein and ligand suggested that this enzyme is not involved in enzymology mechanism, 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, topoisomerase and cytochrome P450 both the normal and tumor cells will damage or otherwise it will inhibit the thymidylate synthase that also will cause cell damage in both cases. In the case of our hybrid molecules, they are allowed by their anabolic enzyme to DNA for replication while the natural nucleoside is deficit; otherwise the hybrid molecule may go to DNA for replication when their catabolic enzyme activity is very low. From the experimental and docking studies it is the indirect evidence that all the hybrids 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 docking images illustrated the various interactions involved between ligand and protein.

Table 3. Docking results of the probe compounds

Compounds	Topoisomerase (1ZXM)					
	Docking Score	Binding energy kcal/mol	Glide evdw	Glide ecoul	Hydrogen bond energy	Interacting residues
U1T	-6.62	-41.13	-38.28	-2.85	-1.95	Ser 148 Asn 150
U2T	-6.85	-46.52	-42.58	-3.94	-2.74	Ser 148 Asn 150 Gly166 Tyr 165
U3T	-8.86	-47.24	-31.79	-15.45	-1.03	---
Thymidylate Synthase (1HVY)						
U1T	-5.13	-37.91	-31.98	-5.94	-1.26	---
U2T	-7.76	-55.16	-48.72	-6.44	-1.24	---
U3T	-5.82	-35.95	-30.64	-5.30	-1.52	---

Evdw-van der waals energy, ecoul-coulomb energy

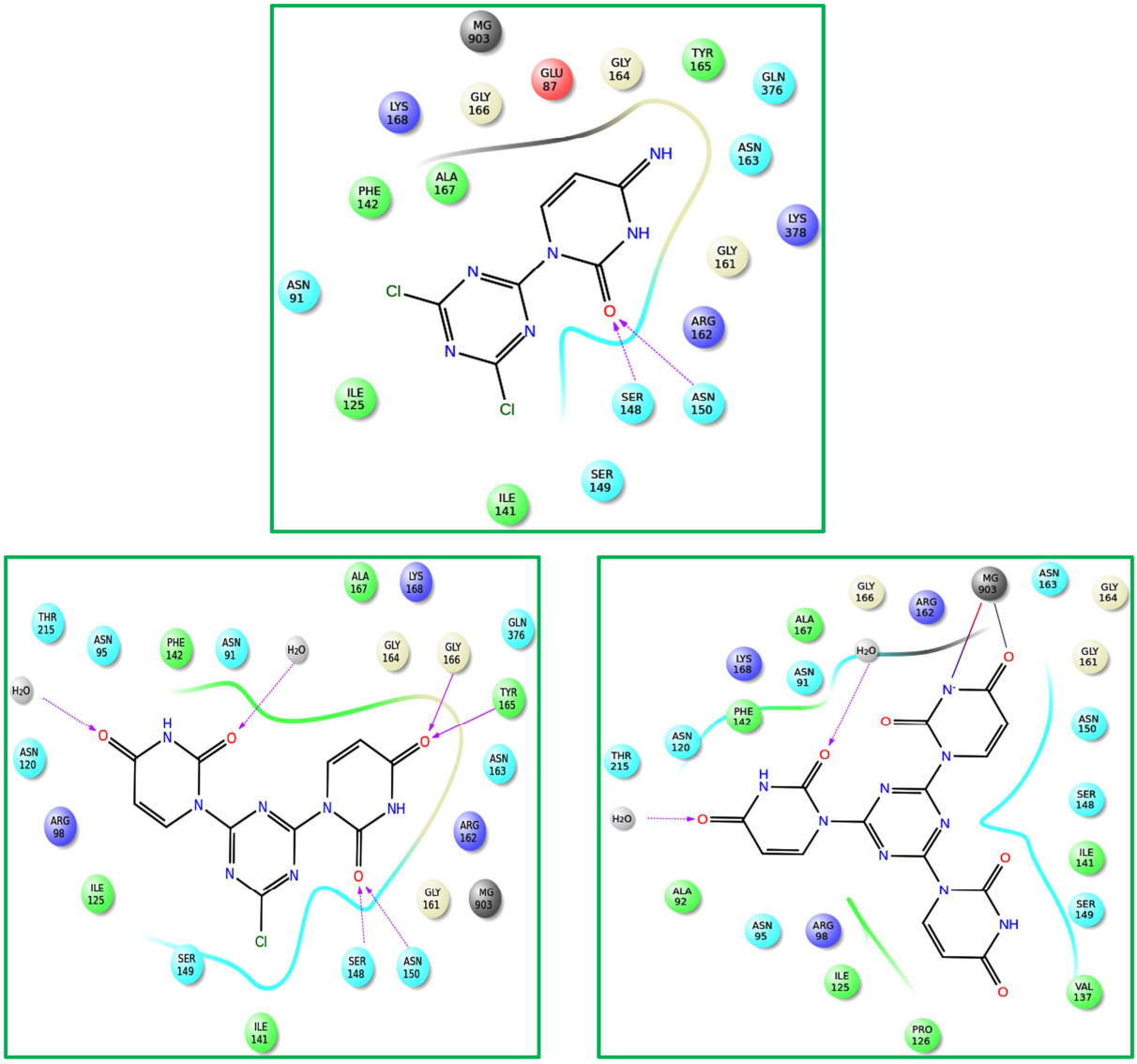


Figure 8. 2D docked diagrams shows the protein-ligand interaction of α-topoisomerase (1ZXM) with (a) U1T (b) U2T (c) U3T

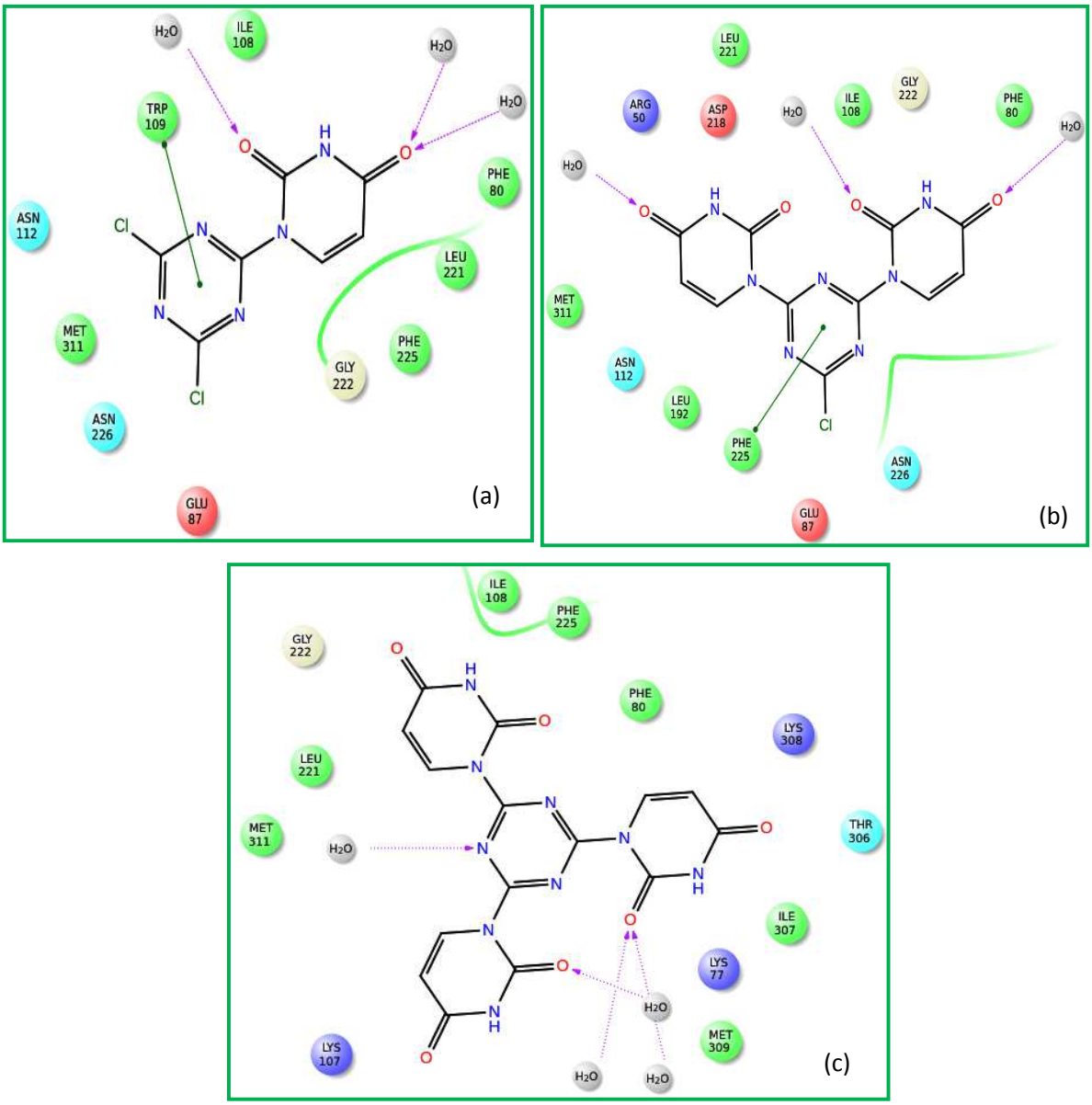


Figure 9. 2D docked diagrams shows the protein-ligand interaction of with Thymidylate synthase (1HVY) (a) U1T (b) U2T (c) U3T

ADME Properties

Lipinski rule of five of all compounds indicated that U1T and U2T compounds obey the rule of five and thus they are having drug-like properties. ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties were predicted using *in silico* methods to know whether the hybrids have potential of adverse effect in human. Among the three hybrids single and di substituted compounds are good consistent with *insilico* pharmacokinetics properties, tri substituted compound (U3T) has more deviations from the Lipinski rule and pharmacokinetics properties suggested that it can be used intravenously but not orally [22-24].The pharmacokinetic properties are listed in table 4

Table 4. Pharmacokinetic properties of probe compounds

Compounds	Factors of Lipinski's rule of five					Pharmacokinetic properties			
	Molecular Weight (<500)	Donor HB (< 5)	Accpt HB (< 10)	QPlogPo/w[a] (< 5)	Rule of Five	Percent Human Oral Absorption (> 80 high, < 25 poor)	QPlogS[b] (–6.5 to 0.5)	QPlogHERG[c] (below –5)	QPlogBB[d] (–3 to 1.2)
U1T	260.039	1	6.5	0.431	0	71.213	-2.68	-3.986	-0.583
U2T	335.666	2	10	-0.763	1	31.505	-3.146	-4.735	-1.954
U3T	411.293	3	13.5	-2.047	1	4.014	-3.398	-5.307	-3.426

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

CONCLUSION

The present study demonstrated the anti-cancer activity of uracil substituted s-triazine hybrids against HepG2cancer cell lines and normal hepatocellular lines. All the new hybrid molecules selectively target cancer cells and reduced toxicity. The fluorescence image and the viability study illustrated the persistence of normal cells at higher concentration when compared to cancer cells. Docking studies revealed that our hybrids may be catabolized by their enzymes, unlike doxorubicin and 5-fluro uracil mechanism our hybrids acted in a novel way which will be further probed. For the first time it has been shown that the catabolic enzymes missing in cancer cells may be exploited for highly selective toxicity. ADMET screening has been done for future analysis and drug development. Future studies are needed to rectify the errors for all the other compounds and finally screen for their in-vitro anti-cancer effect. This study could be utilized for the designing of effective drug for the treatment of cancer.

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Prof. Dr. S. Kabilan, Drug Discovery Lab, Department of Chemistry, Annamalai University. Prof. Dr. SP. Meenakshisundaram, Crystal Growth Lab, Annamalai University, Annamalainagar. University Grant Commission for the Award of Fellowship.

REFERENCES

[1] Schwartsmann G, Winograd B, Pinedo H M, The main steps in the development of anticancer agents.Radiotherapy and Oncology **1988**, 12, 301–313
[2] Wikipedia. History of Cancer Chemotherapy, **2008**. http://en.wikipedia.org/wiki/
[3] William J, Slichenmyer MD, Daniel D, Von Hoff MD,*J. Clin.Pharmacol*,**1990**, 30(9),770-788.
[4]Tirayut Vilaivan, Neungruthai Saesaengseerung, Deanpen Jarprung, Sumalee Kamchonwongpaisan, Worachart Sirawaraporn, YongyuthYuthavong, *Bioorg& Med Chem*, **2003**, 1, 1217–224.
[5] KapilArya,AnshuDandia, *Bioorg& Med Chem*, **2007**, 17, 3298–3304.
[6] MingfangZheng, ChenghuiXu,Jianwei Ma, Yan Sun, Feifei Du, Hong Liu, Liping Lin,Chuan Li, Jian Ding, Kaixian Chen,Hualiang Jiang, *Bioorg& Med Chem*, **2007**,15, 1815–1827.
[7] Sunny Manohar, Shabana I, Khan Diwan S,Rawat, *Bioorg& Med Chem*, **2010**, 20, 322–325.
[8] ArieZask,Jeroen C. Verheijen, David J. Richard, Joshua Kaplan, Kevin Curran, Lourdes Toral-Barza, Judy Lucas, Irwin Hollander, Ker Yu, *Bioorg& Med Chem*, **2010**, 20, 2644–2647
[9] Beibei Liu, Tiemin Sun, Zhixu Zhou, Lei Du, *Med Chem*, **2015,**5(3), 131-148.
[10] M. Shanmugam, K Narayanan, V. Chidambaranathan, S. Kabilan,*SpectrochimActa Part A*, **2013**, 105, 383–390.
[11] T. Mosmann, *J Immunol Methods*, **1983**, 65, 55–63.
[12] Protein Preparation Wizard,*Epik version 2.3*, ***2012****; Impact version 5.7*, Schrodinger, LLC, New York.
[13]*LigPrep, version 2.5*, **2012**, Schrodinger, LLC, New York.
[14] *Qikprop, version 3.5*, **2012**, Schrodinger, LLC, New York.
[15] Viola Tamasi, KatalinMonostory, Russell A. Prough, AndrasFalus, *Cell. Mol. Life Sci*,**2011,**68, 1131–1146.
[16] Marianthi G. Ierapetritou, Panos G. Georgopoulos, Charles M. Roth, Ioannis P,Androulakis,*ClinTransl Sci*.**2009**, 2(3), 228–237.
[17] L. P. Wackett, M.J. Sadowsky, B. Martinez, N. Shapir, *ApplMicrobiolBiotechnol*, **2002**, 58, 39–45.
[18] Ning Zhang, Ying Yin, Sheng-JieXu, Wei-Shan Chen, *Molecules*,**2008,** 13, 1551-1569.

[19] Joshua J. Warren,Timothy J. Pohlhaus,Anita Changela,Ravi R. Iyer,Paul L. Modrich,Lorena S. Beese,*Mole Cell*, **2007**, 26, 579–592
[20] Wilma K. Olson, ManjuBansal, Stephen K. Burley, Richard E. Dickerson, Mark Gerstein, Stephen C. Harvey, Udo Heinemann, Xiang-Jun Lu, Stephen Neidle, ZipporaShakked, Heinz Sklenar, Masashi Suzuki, Chang-Shung Tung, Eric WesthofCynthiaWolberger, Helen M. Berman, *Mol. Biol*, **2001**, 313, 229-237.
[21] Penelope D. Ottewell, Julia K. Woodward, Diane V. Lefley, C. Alyson Evans, Robert E. Coleman, IngunnHolen, *Mol Cancer Ther*,**2009**, 8(10), 2821-2832.
[22] Saba Ferdous, Muhammad UsmanMirza, UsmanSaeed, *Int J Comp App*, **2013**, 67(25), 0975 – 8887
[23] (a) CA Lipinski, F. Lombardo BW Dominy, PJ Feeney, *Adv. Drug Delivery Rev*, **1997**, 23, 3–25; (b) CA Lipinski, F. Lombardo, BW Dominy, PJ Feeney, *Adv. Drug Delivery Rev*, **2001**, 46, 3–26.
[24] SelvamAthavan Alias Anand,ChandrasekaranLoganathan,Nisha Susan Thomas,KuppusamySaravanan, Antony TherasaAlphonsa,SenthamaraikannanKabilan, *New J. Chem*, **2015**,39, 7120-7129.