RESEARCH ARTICLE

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Green synthesis, characterization, *in silico* molecular docking and *in vitro* anticancer activity of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids

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ABSTRACT: Background: The dihydropyrimidines has structural resemblance with clinically important pyridines that the pyrimidines, which exhibit wide range of biological activities such, are antibacterial, anticancer, antiviral, antitumor and anti-inflammatory. **Aim:** Primarily, the study was aimed to synthesize and characterize the hybrid bioactive skeletons that is 1,2,3-triazole linked pyrimidine-2-thione derivatives. The secondary objective of the study to evaluate the *in-vitro* anticancer activity of synthesized thione derivatives. **Methods:** The 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids was synthesized by Green protocol. The *in vitro* anticancer activity of these synthesized new thione derivatives was evaluated by MTT assay on human cancer cell line K562. **Results:** The synthesized thione Compounds showed inhibition constant of 7.84 mM. The compound hybrids 5a and 5c (7.84mM) showed the lowest inhibition constant. The compounds hybrid 5a and 5c showed lesser intermolecular energy (-3.37kcal/mol) with better and stronger DNA Topoisomerase inhibitory activity. **Conclusion:** The synthesized compounds hybrid- 5a and 5c showed good anti-cancer activity. The hybrid- 5b and 5d showed very poor anti-cancer activity.

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KEYWORDS: Molecular docking, *in silico*, anticancer, 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids, MTT assay, QSAR.

INTRODUCTIONS:

Computerized conformational analysis used to predict the 3D structure of drug with the receptor. QSAR used to elucidate the mechanism of action of drugs at the molecular level and physicochemical property like hydrophobicity. 1,2,3-triazole nucleus with three nitrogen atoms and electron rich property has been paid special attention in the development of new drugs due to large medicinal potentiality of triazole-based derivatives. These exciting achievements encourage continuous efforts to develop of 1, 2, 3- triazole compounds for the treatment of infective diseases. Considering the importance of chalcone and triazole compounds, and as

an extension of our researches on bioactive heterocyclic compounds. Triazole moiety (Fig 1) is able to easily bind with various enzymes and receptors in organisms through coordination bonds, hydrogen bonds, ion-dipole, cation-p, pep stacking, hydrophobic effect and van der Waals force etc., which helpfully modulate the physicochemical and pharmacokinetics properties [1,2].

Fig 1. Structure of 1,2,3-triazole and dihydropyrimidine-2-thione nucleus.

On the other hand, dihydropyrimidines (Fig 1) has structural resemblance with clinically important Hantzsch pyridines. Also, literature study reveals that the pyrimidines exhibit wide range of biological activities viz. antibacterial, anticancer, antiviral, antitumor, anti-inflammatory etc. Moreover, Pyrimidine-2-thiones were found to inhibit the synthesis of t-RNA under certain conditions and thus act as anti-tumour and anti-thyroid agents.

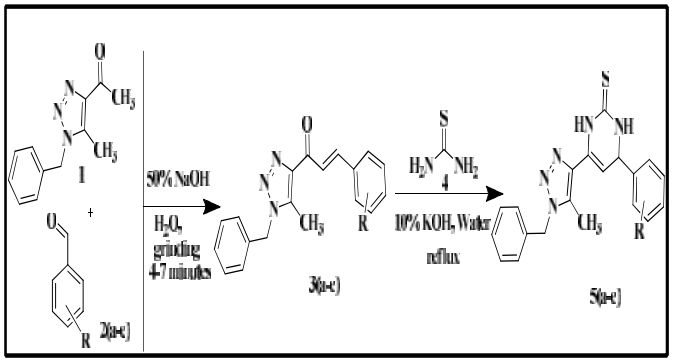

Fig 2. Scheme for synthesis of 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids.

The utilization of simple molecules that is 1,2,3-triazole-pyrimidine-2-thione hybrids with different functionalities is a valuable contribution in the chemistry of heterocycles. Particularly, heterocyclic hybrids are single frameworks wherein more than one heterocyclic moieties are being grafted with a view of designing more effective and enhance the bioactive entities [3,4].

In view of the above and continuation of our earlier reported work [5,6], the present research was planned to synthesize and explore the hybrid bioactive skeletons that is 1,2,3-triazole linked pyrimidine-2-thione derivatives via green protocol. Furthermore, all the synthesized hybrids were evaluated for docking and *in-vitro* anticancer studies.

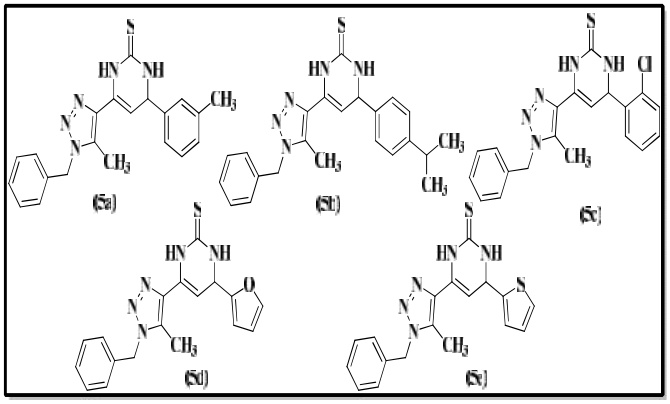

Fig 3. The synthesized hybrids of 1,2,3-triazolyl dihydropyrimidine-2-thione (5a-e).

MATERIALS:

The chemicals [Dimethyl sulphoxide, thiourea and 3-(4,5-dimethylthiazo1-2-y1)-2,5-diphenyltetrazolium bromide (MTT)] were of analytical grade and purchased from Merck, India. The thiourea was distilled properly prior to use. The human cancer cell line K562 was procured from Sigma-Aldrich.

METHODOLOGY:

Synthesis of 1,2,3-triazole linked pyrimidine-2-thione derivatives:

The compounds were synthesized by reaction between 1,2,3-triazolyl chalcone and aromatic aldehydes in the presence of 50 % aqueous sodium hydroxide grinding for 4 to 7 min. Then the above product was treated with thiourea, 10 % potassium hydroxide and water reflux to form 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids [7,8]. The scheme of synthesis is given in Fig 1 and 2.

Characterization of synthesized compounds:

The synthesized compounds derivatives were characterized by using C^1 and C^{14} NMR (Bruker, Germany) [9,10].

Anticancer (Cytotoxic) assay:

The *in vitro* Anticancer of the synthesized thione hybrid compounds was measured by the MTT assay on human cancer cell line K562 as per standard protocol [11-14]. Doxorubicin was used as a positive control. Initially the cancer cells were harvested and plated in 96 well plates at a concentration of 1×10^4 cells/well. The cells were incubated at 37 °C for 24 h under condition of humidity and 5 % CO_2, which facilitate cell attachment. A

solution of 3-(4,5-dimethylthiazo1-2-y1)-2,5-diphenyltetrazolium bromide (MTT) of concentration 5 mg/ml was prepared in the phosphate buffered saline (PBS). About 20 µl of MTT solution was added to each well. Each well was incubated at 37 °C for 4 h. The absorbance of the wells was read with a microplate reader (Elisa-reader) at 570 nm. Cell survival was calculated using the formula.

Survival (%) = [(At−Am)/(Au−Am)]×100 ……..(1)

Where, At is absorbance of treated cells, Am is absorbance of culture medium and Au is absorbance of untreated cells. The inhibitory concentration (IC_{50}), which was the concentration required for 50 % inhibition of cell growth, as compared to that of control values, was calculated from a dose response curve.

Table 1. Calculation of bioactivity score for newly designed heterocyclic compounds.

Hybrid	GPCR ligand	ICM	KI	NRL	PI	EI
5a	-0.42	-0.43	0.66	-0.76	-0.66	-0.45
5b	-0.37	-0.36	-0.62	-0.65	-0.60	-0.38
5c	-0.42	-0.42	-0.74	-0.74	-0.73	-0.48
5d	-0.51	-0.62	-0.89	-0.90	-0.89	-0.53
5e	-0.51	-0.60	-0.81	-0.90	-0.78	-0.47

ICM – Ion Channel Modulator, KI – Kinase Inhibitor, NRL – Nuclear Receptor Ligand, PI- Protease Inhibitor and EI – Enzyme Inhibitor.

Table 2. Calculation of physiochemical properties for newly designed heterocyclic compounds.

Hybrid	nOHNH	nviola	nrot	Volu
5a	3.97	54.77	375.5	5
5b	5.06	54.77	403.5	5
5c	4.18	54.77	395.9	5
5d	2.80	67.91	351.4	6

RESULTS AND DISCUSSION:

About five hybrid derivative compounds were synthesized that are 1,2,3-triazolyl dihydropyrimidine-2-thione hybrids of 5a-e. The synthesized compounds NMR data obtained as mentioned below. The NMR data of five thione hybrid derivatives (Fig 3) are given in Fig 4 to 13.

Characterization data of synthesized compound 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydropyrimidine-2(1H)-thione (5a) was White solid in nature; m.p.176 °C; Yield: 89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H), 7.48 – 7.01 (m, 9H), 6.73 (s, 1H), 5.50 (s, 2H), 5.22 (d, *J* = 3.3 Hz, 1H), 5.07 (d, *J* = 1.9 Hz, 1H), 2.33 (s, 3H), 2.20 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.33, 139.53, 138.62, 137.43, 134.16, 130.37, 129.83, 129.62, 129.14, 129.05, 128.98, 128.58, 127.32, 127.10, 126.81, 126.11, 99.63, 56.66, 52.18, 21.10, 9.52.

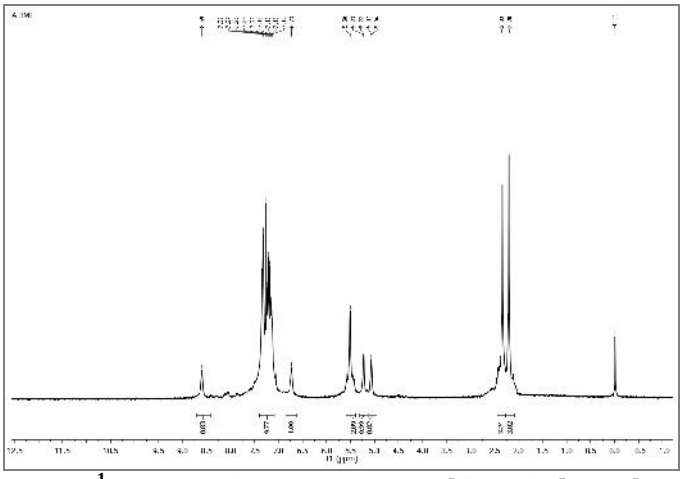

Fig 4. ^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydro pyrimidine-2(1H)-thione (5a).

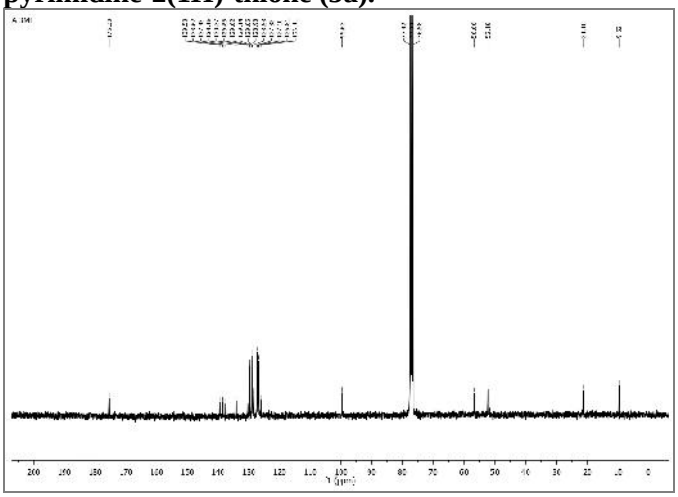

Fig 5. ^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(m-tolyl)-3,4-dihydro pyrimidine-2(1H)-thione (5a).

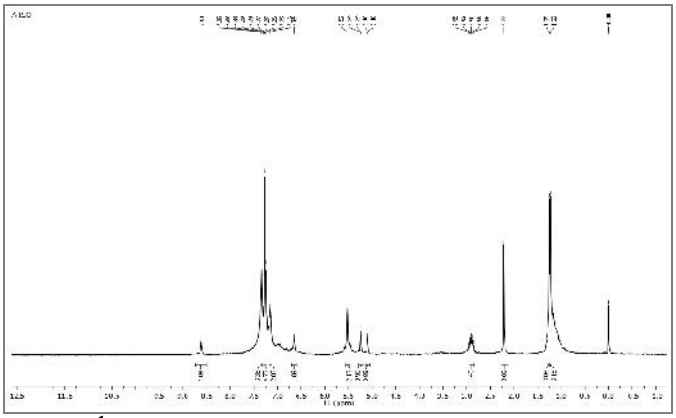

Fig 6. ^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1H)-thione (5b).

Characterization data of synthesized compound 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(4-isopropyl phenyl)-3,4-dihydropyrimidine-2(1H)-thione (5b) was White solid; m.p.173 °C; Yield: 90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.61 (s, 1H), 7.38 – 7.30 (m, 3H), 7.30 – 7.22 (m, 4H), 7.18 – 7.11 (m, 2H), 6.65 (s, 1H), 5.52 (s, 2H), 5.24 (d, *J* = 1.8 Hz, 1H), 5.10 (d, *J* = 1.8 Hz, 1H), 2.96 – 2.79 (m, 1H), 2.22 (s, 3H), 1.25 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.85, 149.42, 139.72, 137.32, 136.80, 134.06, 133.92, 130.41, 129.04, 128.92, 128.48, 127.12, 127.03, 126.87, 125.90, 99.61, 56.42, 52.07, 33.76, 23.85, 9.51.

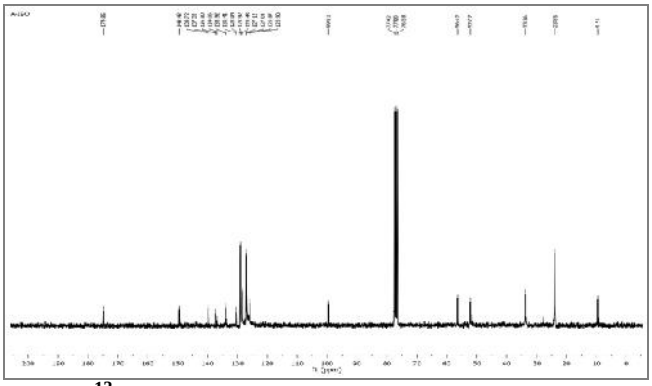

Fig 7. ^{13}C NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(4-isopropylphenyl)-3,4-dihydropyrimidine-2(1H)-thione (5b).

Characterization data of synthesized compound 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(2-chlorophe nyl)-3,4-dihydropyrimidine-2(1H)-thione (5c) was White solid; m.p.198 °C; Yield: 87%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.66 (s, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.41 – 7.23 (m, 6H), 7.21 – 7.10 (m, 2H), 6.88 (s, 1H), 5.72 (d, *J* = 4.0 Hz, 1H), 5.52 (s, 2H), 5.26 (d, *J* = 3.7 Hz, 1H), 2.25 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.58, 139.05, 137.06, 133.96, 133.78, 131.20, 130.56, 129.68, 129.41, 128.96, 128.73, 128.67, 128.43, 127.79, 127.01, 126.70, 97.41, 52.93, 51.99, 9.45.

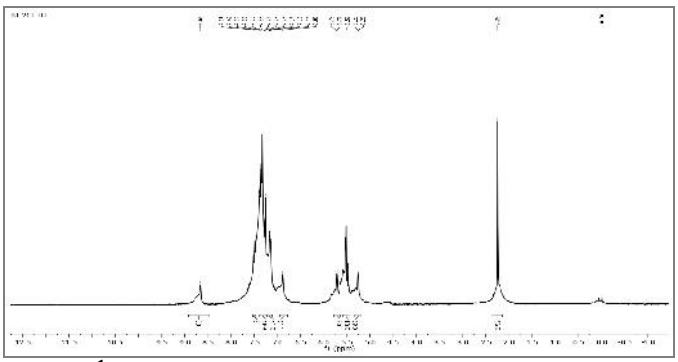

Fig 8. ^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1H)-thione (5c).

Characterization data of synthesized compound 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5d) was White solid; m.p.186 °C; Yield: 88%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.46 – 7.28 (m, 5H), 7.17 (s, 2H), 6.32 (d, *J* = 12.8 Hz, 2H), 5.53 (s, 2H), 5.40 – 5.13 (m, 2H), 2.28 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.65, 155.21, 153.57, 151.62, 145.53, 134.10, 132.96, 129.12, 128.57, 128.13, 127.12, 124.68, 107.25, 106.13, 95.75, 53.75, 52.16, 9.50.

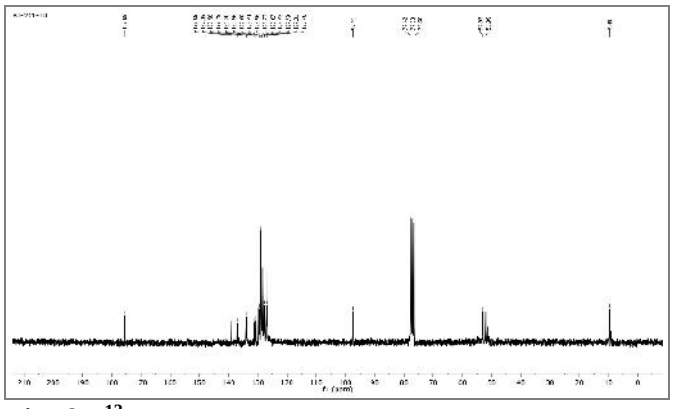

Fig 9. ^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(2-chlorophenyl)-3,4-dihydropyrimidine-2(1H)-thione (5c).

Characterization data of synthesized compound 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5e) was Light yellow; m.p.185 °C; Yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.40 – 6.94 (m, 9H), 5.69 – 5.11 (m, 4H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.13, 146.53, 136.86, 133.77, 133.51, 130.51, 128.52, 127.93, 126.64, 126.53, 126.28, 126.16, 125.38, 124.55, 98.75, 51.44, 50.39, 8.86.

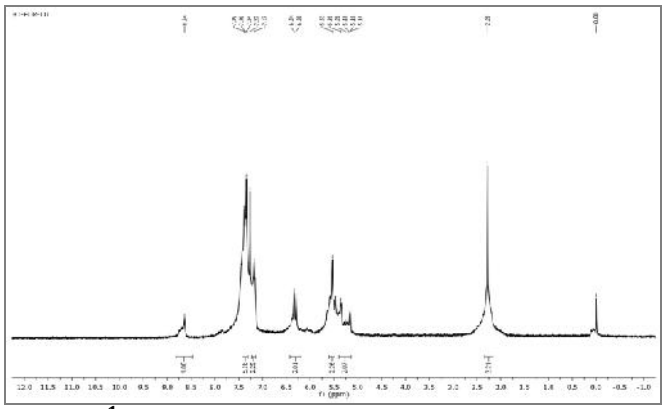

Fig 10. ^{1}H NMR (300 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5d).

The bioactivity score for newly designed heterocyclic compounds that is thione is given in Table 1.

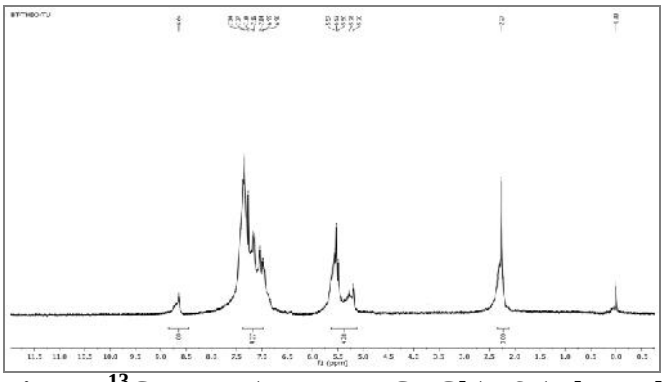

Fig 11. ^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(furan-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5d).

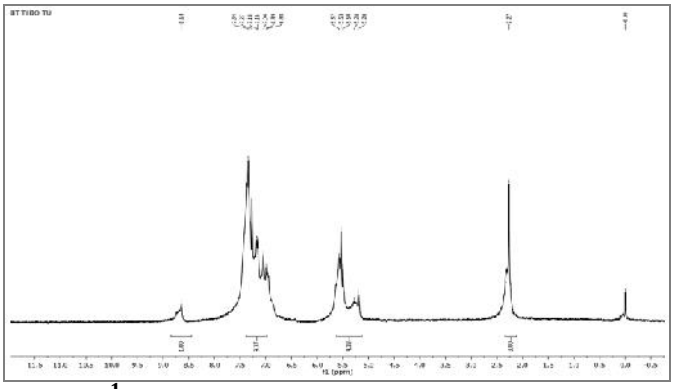

Fig 12. ^{1}H NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5e).

The physiochemical properties for newly designed heterocyclic compounds are given in Table 2 and 3. The protein binding sites of the synthesized compounds is given in Table 4. The binding energy of the compound is given in Table 5. The greater binding energy shown by synthesized hybrid compound 5b, 5d and 5e. The compounds showed inhibition constant of 7.84 mM as shown in Table 6. The compound hybrids 5a and 5c (7.84 mM) showed the lowest inhibition constant which is directly proportional to binding energy.

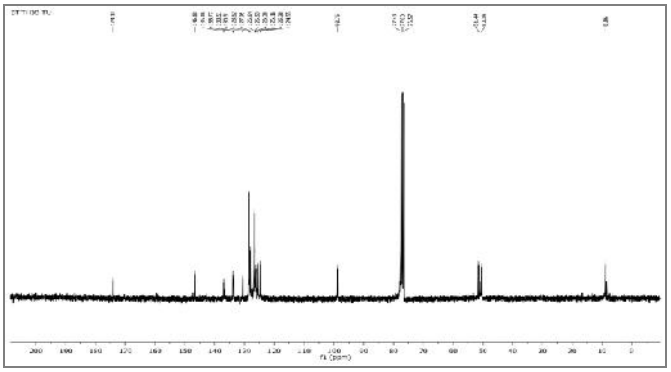

Fig 13. ^{13}C NMR (75 MHz, $CDCl_3$): 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyrimidine-2(1H)-thione (5e).

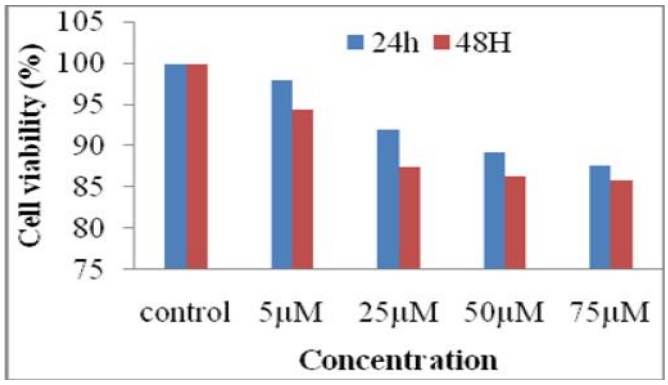

Fig 14. Cytotoxic activity of thione hybrid 5a.

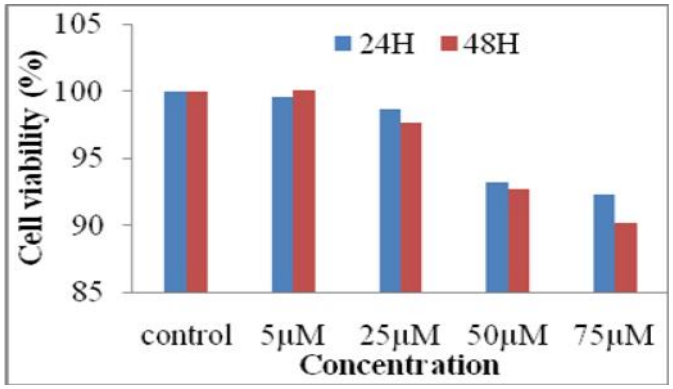

Fig 15. Cytotoxic activity of thione hybrid 5b.

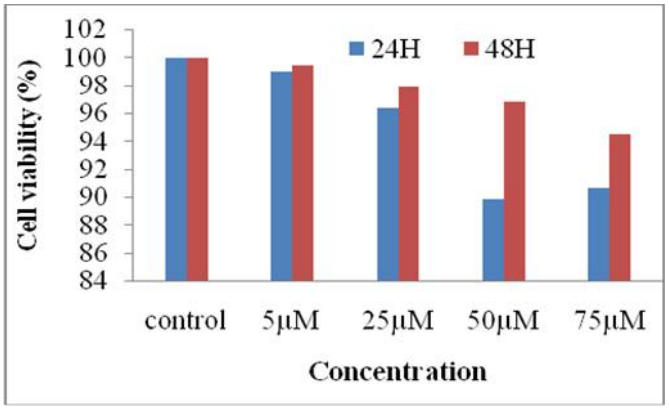

Fig 16. Cytotoxic activity of thione hybrid 5c.

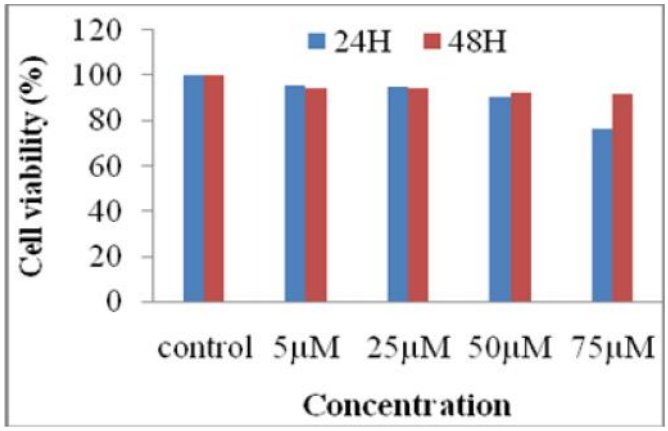

Fig 17. Cytotoxic activity of thione hybrid 5d.

The compounds showed inhibition constant of 7.84 mM as shown in Table 6. The compound hybrids 5a and 5c (7.84 mM) showed the lowest inhibition constant.

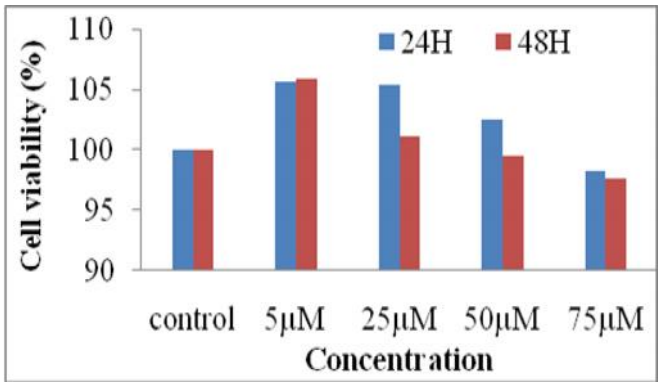

Fig 18. Cytotoxic activity of thione hybrid 5e.

Table 3. Calculation of physiochemical properties for newly designed heterocyclic compounds.

Hybrid	nOHNH	nviola	nrot	Volu
5a	2	0	4	339.9
5b	2	1	5	373.3
4c	2	0	4	336.9
5d	2	0	4	304.9
5e	2	0	4	314.1

Table 4. Potential binding sites of the compound.

Hybrid	Potential Binding Sites
5a	Leu189, Gln201, Asn203, Phe202, His280, Leu269, Leu268, Leu323, Val277, Leu539, Val278, Ile279
5b	Ile185, Leu189, Ile188, Gln201, Phe202, His280, Leu268, Leu323, Phe319, Leu354, Val277, Val278, Ile279
5c	Ile185, Leu189, Ile188, Phe202, Leu268, Tyr326, Leu323, Val277, Leu539, Val278, Ile279, His280.
5d	Gln201, Phe202, Leu268, Leu539, Val278
5e	Pro461, His263, Ala521, Ser518

The compounds showed inhibition constant of 7.84 mM as shown in Table 6. The compound hybrids 5a and 5c (7.84 mM) showed the lowest inhibition constant. Inhibition constant is directly proportional to binding energy. Thus, the DNA Topoisomerase inhibitory activity of the compounds was proved using molecular simulations. The compounds hybrid 5a and 5c showed lesser intermolecular energy (-3.37kcal/mol). This result further indicated that compound hybrid 5a and 5c have better and stronger DNA Topoisomerase inhibitory activity. The anticancer activity (Percentage cell viability) of synthesized hybrid compounds is given in Fig 14 to 18. The synthesized compounds hybrid- 5a and 5c showed good Anticancer activity. Whereas hybrid-5b and 5e showed very poor Anticancer activity.

CONCLUSION:

The result of research study concluded that a series of novel hybrids containing 6-(1-benzyl-5-methyl-1H-1,2,3-triazol-4-yl)-4-(thiophen-2-yl)-3,4-dihydropyri-midine-2(1H)-thione were synthesized. In the cytotoxic assay, compounds 5a and 5c exhibited a significant Anticancer activity with the IC_{50} value of 7.84 mM. The binding mode analysis revealed that the synthesized compounds could easily bind to the Kinase and Protease enzyme active site with good affinity through hydrophobic and H-bond interactions. Finally, the proposed thione hybrid offered the possibility of convenient further modifications, giving rise to lead structures with improved Anticancer activity.

Table 5. Binding energies of the compounds.

Hybrid	Binding Energy (-Ve) (Kcal/Mol)
5a	-2.87
5b	+18.61
5c	-2.87
5d	+18.61
5e	+18.61

Table 6. Inhibition constant of the newly designed compounds.

Para-meters	Hybrid				
	5a	5b	5c	5d	5e
5Rank	1_1	1_1	1_1	1_1	1_1
BE	-2.87	18.61	-2.87	18.6	18.61
IC(Ki) (Mm)	7.84	UA	7.84	UA	UA
IME	-3.37	17.85	-3.37	17.8	17.85
IE	-0.11	-0.43	-0.11	-0.43	-0.43
TE	0.55	0.82	0.55	0.82	0.82
UEE	-0.06	-0.36	-0.06	-0.36	-0.36
Cluster RMS	0.0	0.0	0.0	0.0	0.0
Ref RMS	64.52	79.9	64.52	79.9	79.9

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