NOVEL 1, 1-DIMETHYL-3-PHENYL-3-(5-PHENYL-1, 3, 4- THIADIAZOL-2-YL) UREA DERIVATIVE HAS POTENTIAL ANTIPROLIFERATIVE ACTIVITY AGAINST HUMAN LEUKEMIA CELL LINES - K562

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Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins. Based on the DNA changes in cells, proliferating cycle of tumor cells can be divided into 4 phases. Pre-synthetic phase (Gap 1 phase or G1 phase). Cells chiefly make preparations for the synthesis of DNA. Synthetic phase (S phase). Cells are synthesizing their DNA. Post-synthetic phase (Gap 2 phases or G2 phase). DNA duplication has been finished and they are equally divided to the two of future sub-cells. Mitosis phase (M Phase). Each cell is divided into two sub-cells. Some of these new cells enter the new proliferating cycle, the others become non-proliferating cells. G0 phase cells have proliferation ability but do not divide temporally. When proliferating cells are suffered heavy casualties, G_0 phase cells will get into proliferating cycle and become the reasons of tumor recurrence.G_0 phase cells are usually not sensitive to antineoplastic drugs, which is the important obstacle to tumor.chemotherapy. The antiproliferative activities of these compounds wee evaluated against a Cytotoxicity analysis of compounds against leukemia cell line - K562 organism homo sapiens(human) organ bone – marrow, tissue - lymphoblast, disease – chronic myelogenous leukemia(CML) one human tumor cell lines(K562) by applying the MTT colorimetric assay. The 1, 3-disubstituted urea derivatives show good antiproliferative activity against human cancer cell lines (K562). The hydroxyl groups on the phenyl ring reduced the antiproliferative activities.

Key words: Cancer, urea derivative**,** antiproliferative activities, malignant behavior

INTRODUCTION

In the present study 1, 3-disubstituted urea derivatives (compounds A) were synthesized. The antiproliferative activities of this compound were evaluated against a panel of one human tumor cell lines (K562) by applying the MTT colorimetric assay. The series of 1,3-disubstituted urea derivatives show good antiproliferative activity against human cancer cell lines (KB and K562) .The potent in vitro antiproliferative activity of these derivatives and their selectivity for quite important points for an anticancer drug candidate with fewer side effects. Structure activity relationships were also discussed based on the obtained experimental data. 2-amino-5-phenyl1, 3, 4-thiadiazolebearing different substituent were synthesized and evaluate their antiproliferative activities. The hydroxyl groups on the phenyl ring reduced the antiproliferative activities of 1, 3-disubstituted urea derivatives. The OH groups could be responsible for a reduction in the permeability of the cell membrane. Generally, an aromatic ring on N-3 seems to be in favor of enhancing the inhibitory activity, compounds introduced a nitro group substituent at C-3 position on the aromatic ring approved to generally decrease activity. The compound has been characterized by elemental analysis IR, 1H NMR, Mass spectral data.

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METHOD

Thionyl chloride, Dimethylformamide, Ethyl acetate, Dry pyridine, Ethanol, Benzaldehyde Chloro benzaldehyde, Nitrobenzaldehyde, Methoxy benzaldehyde. Human tumor cell lines (K562).

Synthesis Compound

(A) Aldehyde was dissolved in 25 ml of ethanol, and amine was added to the solution. The reaction mixture was refluxed for1 h.

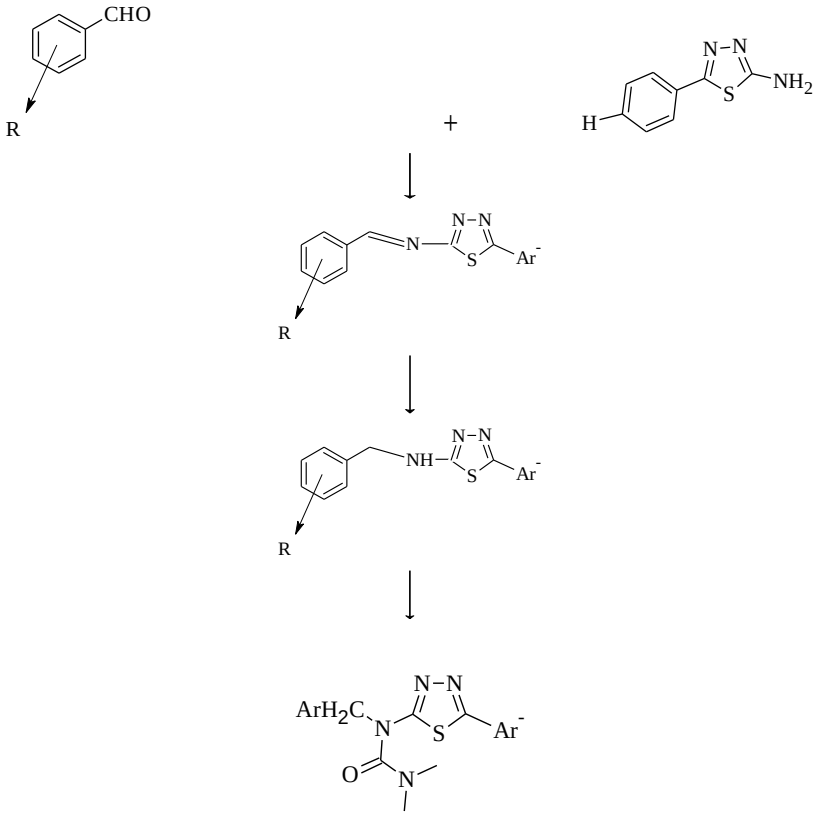
B) 0.05 mmol of $NaBH_4$ was then added to the reaction solution slowly, and stirred under 50°C for 23 h. The

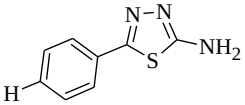
mixture was evaporated under vacuum, and dissolved in EtOAc (30 ml). The solution was washed with 20 ml water twice, dried over anhydrous sodium sulfate, and evaporated. Purification by silica gel afforded pure products.

(C) The mixture of CH_2Cl_2 (15 ml) dry DMF (3 ml, 40 mmol) and $SOCl_2$ (7 ml, 0.10 mol) was stirred to reflux at 70°C for4 h and cooled. The solvents and excess $SOCl_2$ were then removed under reduced pressure. The residue dissolved inCH_2Cl_2 (15 ml) was added to dry pyridine (4 ml)

(D) and various amines (40 mmol). The reaction mixture was stirred at 50 to 60°c for 5 to 6 h, and then added to 20 ml ice-water, organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2 to 10 ml). The organic layer was combined and washed with saturated NaHCO3, dried with anhydrous Na_2SO4 for 0.5 h and concentrated under vacuum; ultimately the residue was purified by silica gel column (eluent EtOAc / petroleum ether, 1:2 - 2:1).

Scheme for synthesis compound:-

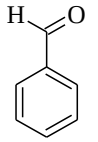


Compound	Structure	Mol. Formula	Mol. wt.	Nature
I	 5-phenyl-1,3,4-thiadiazol-2-amine	$C_8H_7N_3S$	177.2	Pink color crystal

Tab-1 Synthesized amine compound

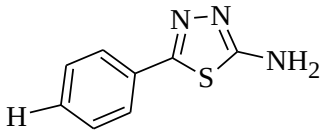
SYNTHESIS OF COMPOUND:

STEP-1

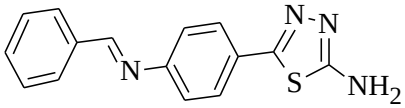


Benzaldehyde

+

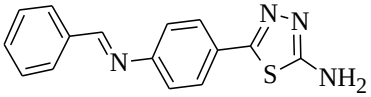


5-phenyl-1, 3, 4-thiadiazol-2-amine Ethanol-reflux (1-hr.)



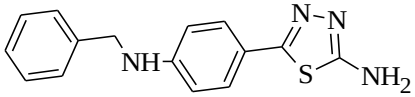
5-(4-{[(*E*)-phenylmethylidene] amino} phenyl)-1, 3, 4-thiadiazol-2-amine

STEP-2



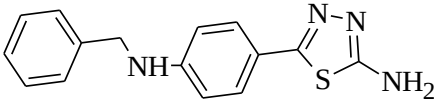
+ $NaBH_4$

5-(4-{[(*E*)-phenylmethylidene] amino} phenyl)-1, 3, 4-thiadiazol-2-amine



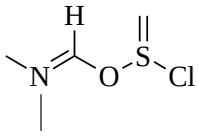
5-[4-(benzyl amino) phenyl]-1, 3, 4-thiadiazol-2-amine

STEP-3

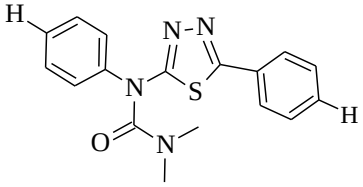


5-[4-(benzyl amino) phenyl]-1, 3, 4-thiadiazol-2-amine

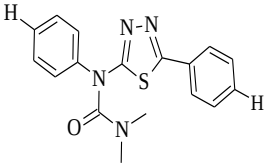
+



N-[(*E*)-{[chloro (methylidene)-□[4]-sulfanyl] oxy} methylidene] methanamine

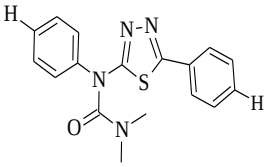


1, 1-dimethyl-3-phenyl-3-(5-phenyl-1, 3, 4-thiadiazol-2-yl) urea.

Code	Compounds	Mol. Formula	Mol.Wt.	Melting Point °C	Crystals Nature	% Yield
S001	 1,1-dimethyl-3-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)urea	$C_{17}H_{16}N_4OS$	324.40014	215-217	Brown color	80%

Tab 2 Synthesized Compound

Physicochemical parameters: Compound:



1,1-dimethyl-3-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)urea

Color – Yellowish Brown color; Odour - odourless; Nature- Yellowish Brown crystal; MeltingPoing – 215 -217 °C

S. No.	Name of Solvents	Solubility					
		Normal Tem.			Hot.Tem.		
		+	±	-	+	±	-
1.	Water			✓			✓
2	Ethanol		✓		✓		
3.	Methanol		✓		✓		
4.	Chloroform		✓		✓		
5.	Benzene			✓	✓		
6.	Carbon Tetrachloride	✓				✓	
7.	Ethyl acetate	✓				✓	
8.	Pyridine	✓				✓	
9.	Dimethyl formamide	✓				✓	
10.	Dimethyl sulfoxide	✓				✓	

Tab 3. Solubility Profile

TLC:

Preparation of the plate: Chromatography a variety of coating materials is available, but silica gel is most frequently used. Slurry of the adsorbent (silica gel, cellulose powder, etc.) is spread uniformly over the plate by means of one of the commercial forms of spreader, the recommended thickness of adsorbent layer being 150-250 J.lm.After air-drying overnight, or oven-drying at 80-90 °C for about 30 minutes, it is ready for use.

Sample application: The origin line, to which the sample solution is applied, is usually located 2-2.5 cm from the bottom of the plate.

Development of plates: Development is allowed to proceed until the solvent front has travelled the required distance (usually 10-15 cm), the plate is then removed from the chamber and the solvent front immediately marked with a pointed object.

Compound	**Solvent system**	**Ratio**	**Rf Value**
SOO1	EtOAc:Petrolium ether	1:2	0.7

Tab 4. Rf value of synthesized compound

Elemental Detection of synthesized compound:

Test	Observation	Result
(1) 2ml sodium extract+3 drops of freshly prepared $FeSo_4$+2-drops of NAOH and boil it then cool it +add 1 ml. dil.HCL+ $FeCl_3$ solution.	After some time green – blue ppt. obtained.	Nitrogen present.
Lassiagens Test-Sulphur Test		
(1)2ml.sodium extract+2ml.freshly prepared sodium nitrate.	Violet color obtained then after some times it disappears in purple color.	Sulphur present.

Tab 5. Lassiagens Test-Nitrogen Test:

Test	Observation	Result
(1)Take a small amount of sample+2ml. of NAOH solution and heat it then attach red litmas paper over the mouth of the test tube.	Ammonia gas is evolved and Red litmus paper gets blue color	Amide present.

Tab 6. Amide Test

Test	Observation	Result
(1)small amount of sample in test tube +melt it then ammonia is evolved after some times when it resolidify dissolve it in 1ml. of dil.NAOH solution+2 drops of dil. Copper sulphate solution	Violet colors are developed then finally change in blue color.	Urea present.

Tab-7. Urea Test (Biuret Test)

Test	Observation	Result
Take a small amount of sample in conc.HCl+2 ml. water then cool in ice cold water + 2 ml.of dil. Sodium nitrate solution.	Yellow ppt.obtained.	Primary amines present.

Tab-8. Amines Test

Peaks cm-1	Due to	Probable Group
1700.0 (Strong peak)	C=O , stretching	Amide
1250.2 (Strong peak)	C-C , stretching	Benzene
768.00 (Strong peak)	C-S ,stretching	Thiourea
3216.0 (Medium peak)	N-H str.(asymmetric stretching)	Primary Amide
690.6(Weak peak)	C-H , stretching	(Aromatic ring)
1635.7(Weak peak)	N=C, stretching	Nitrate

Tab 9. Infra Red / (KBr) (cm-1) spectral study of the synthesized compounds; SOO1- 1, 1-dimethyl-3-phenyl-3-(5-phenyl-1, 3, 4-thiadiazol-2-yl) urea

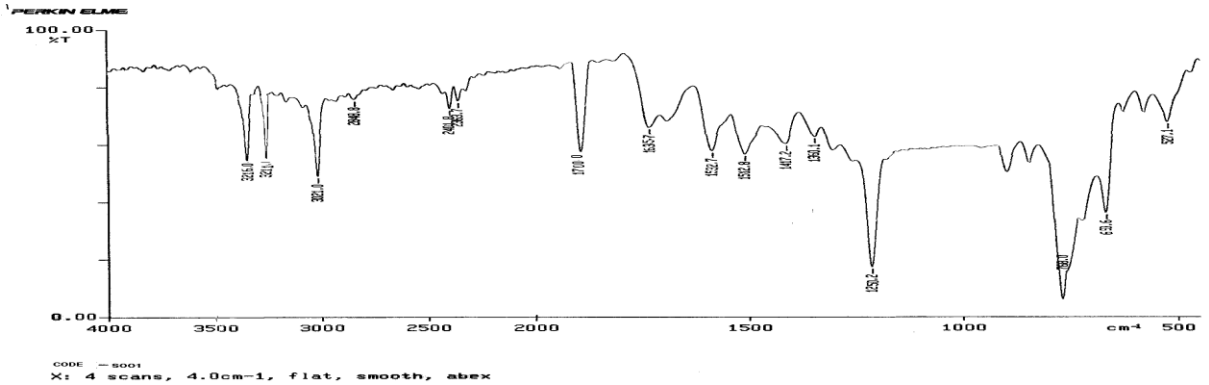


Fig.1 spectral study of the synthesized compounds

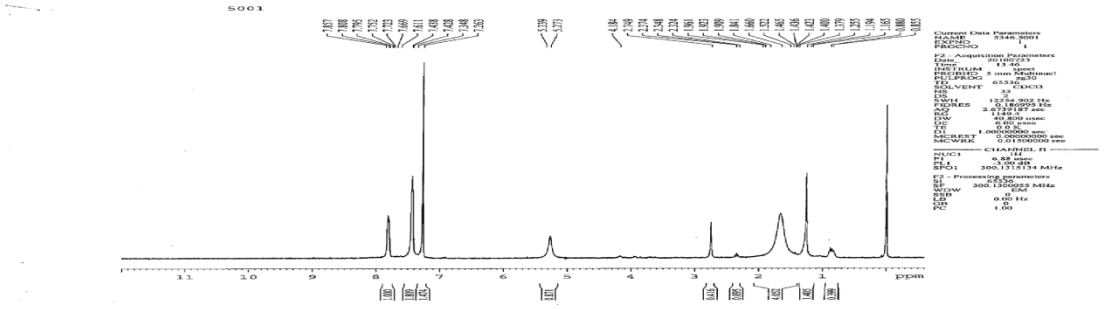


Fig.2. NMR Proton- H^1 Spectroscopy

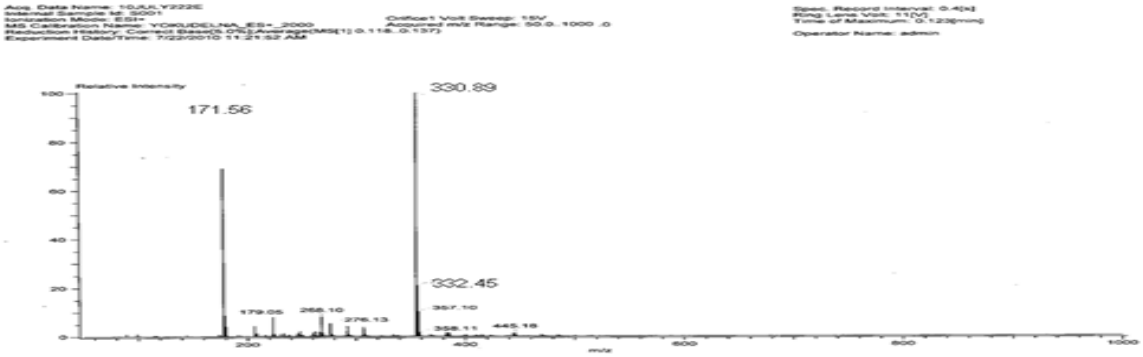


Fig.3 Mass spectroscopy

Determination of IC50 value:

Assay	Percent Inhibition IC50
Cell Line	K- 562
Organism	Homo sapiens (human)
Organ	bone marrow
Tissue	lymphoblast
Disease	chronic myelogenous leukemia (CML)
Growth	Properties suspension
Age	53 years
Gender	Female.

Table:-10. Cytotoxicity analysis of compounds against leukemia (K-562)

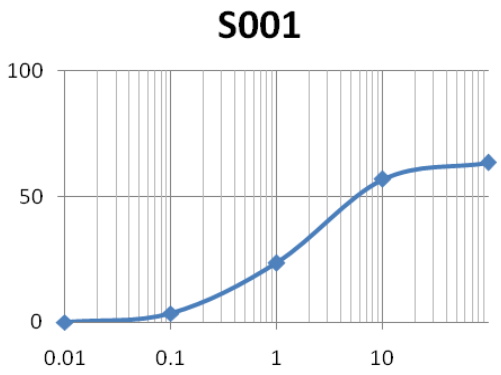


Fig 4 X axis – concentration in micromole; Y axis- % growth inhibition

RESULT & DISCUSSION

Cells were incubated with different concentrations of the extract for 5 days in a 96 well plate, after which the live cells which did not take in stain and dead cells which took in stain were counted. For counting the cell suspension was mixed with an equal volume of trypan blue and was counted. Concentration that inhibited the growth of cells at 50% (IC50) was computed. Substances with low IC50 indicate potential for cytotoxicity. The synthesized compounds was confirmed by physic-chemical properties (melting point,TLC) and by IR spectral analysis. Sample-S001 was found most potent compound for cytotoxic activity.(a) 1,1-dimethyl-3-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)urea.

CONCLUSION

The continuous cell line K-562 was established by Lozzio andLozzio from the pleural effusion of a 53-year-old female with chronic myelogenous leukemia in terminal blast crises. [22609]The cell population has been characterized as highly undifferentiated and of the granulocytic series. [26059]Studies conducted by Anderson, et al., on the surface membrane properties led to the conclusion that the K-562 was a human erythroleukemia line. [26060]The K-562 cell line has attained widespread use as a highly sensitive in vitro target for the natural killer assay. Cells were incubated with different concentrations of the extract for 5 days in a 96 well plate, after which the live cells which did not take instain and dead cells which took in stain were counted. For counting, the cell suspension was mixed with an equal volume of trypan blue and counted. Concentration that inhibited the growth of cells at 50% (IC50) was computed. Substances with low IC50 indicate potential for cytotoxicity.

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