SYNTHESIS AND EVALUATION OF 2-AMINO-5-(PYRIDIN-3YL)-1, 3,4 THIADIAZOLE DERIVATIVES

[1]Rajitha K
Associate professor, Department of Pharmaceutical Chemistry, National College of Pharmacy, Manassery, Calicut
Email id - raji.divya1986@gmail.com
[2]Athira B
Assistant professor, National College of Pharmacy, Manassery, Calicut
Email id - athirasubhash422@gmail.com
[3]Akash Marathakam
Professor, National College of Pharmacy, Manassery, Calicut
Email id - akashmarathakam@gmail.com
[*]**Sreejith M**
Professor, Nazreth College of Pharmacy, Thiruvalla
[*]**Corresponding Author Email id**- sreejith.madhavankutty@gmail.com

ABSTRACT

Thiadiazole molecule exhibit broad spectrum of biological activities like antibacterial, anti-inflammatory, anticonvulsant, antifungal, antitubercular, anticancer activities. The aim and objective of the work was to develop novel 1,3,4 thiadiazole derivatives, docking studies and to evaluate their cytotoxicity and antibacterial activity. In this present study 6 novel 1,3,4 thiadiazole derivatives were synthesized by Schiff and Mannich reaction of 2-amino-5-(pyridine-3yl)-1,3,4 thiadiazole with substituted aromatic aldehydes and secondary amines respectively. The synthesized compounds were then established on the basis of IR, ^{1}H NMR and MASS spectral datas and screened for invitro anticancer activity on Lymphoma Cancer Cell line DLA and antibacterial activity against E.coli and bacillus subtilis. The derivatives showed moderate cytotoxic activity on DLA cell lines and good antibacterial activity.

KEYWORDS: - *1, 3, 4 Thiadiazole, Docking, Cytotoxicity, Antibacterial activity, DLA.*
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INTRODUCTION

Drug discovery and development is an intense, lengthy and an interdisciplinary endeavor. Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and lead discovery followed by lead optimization and preclinical *in vitro* and *in vivo* studies to determine if such compounds satisfy a number of preset criteria for initiating clinical development [1, 2]. Heterocycles are common structural units in marketed drugs and in medicinal chemistry targets in the drug discovery process. Heterocycles with a variety of shapes and electronics and physicochemical properties provide fertile grounds. [3]

Docking is a technique which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. The strength of association or binding affinity between two molecules can be predicted from this orientation [4]

Thiadiazole nucleus have wide spectrum of pharmacological activities such as antimicrobial, ant tubercular, antileshmanial,

anti- inflammatory, analgesic, CNS depressant, anti-convulsant, anticancer, antioxidant, anti-diabetic, molluscidal, antihypertensive, diuretic, analgesic properties. For instance, 1, 3, 4 thiadiazole derivatives have demonstrated a broad spectrum of biological properties in both pharmaceutical and agrochemical fields. They have known to exhibit diverse biological activities such as *in-vitro* inhibition of cycloxygenase and 5-lipoxygenase activities [5, 6]

Synthesis and characterization

All the chemicals and reagents used in this research work were of analytical or synthetic grade. Melting point of the synthesized compounds was determined by open capillary method and is uncorrected. IR spectra of the synthesized compounds were recorded using SHIMADZU FT-IR Spectrophotometer in the range of 4000-500 cm^{-1}, ^{1}H NMR of the synthesized compounds was recorded in DMSO on Bruker 500 MHz FT NMR. Chemical shifts were reported in δ (ppm) relative to Tetra Methyl Silane (TMS) as internal standard. MASS spectra of the synthesized compounds were recorded by using JEOL-JMS 600H Mass spectrometer. Purity of the compound was ascertained by TLC over precoated, preactivated glass plates with solvent system n-Hexane and ethyl acetate (50:50). Purity of the compounds was ascertained by TLC and consistency of the R_f value.

Synthetic procedure

Preparation of 5-(pyridin-3-yl)-1, 3, 4 thiadiazol-2-amine **(I)**

A mixture of 0.1 mole of nicotinic acid and 0.1 mole of thiosemicarbazide was taken in a 250 ml round bottom flask and added $POCl_3$ drop wise with constant stirring. It was refluxed gently for 3hrs. Then the reaction mixture was cooled to 5^0C, to this crushed ice was added very carefully and reaction mixture was further refluxed for 4hrs.cooled and filtered. The filtrate was neutralized with saturated solution of KOH; the precipitated solid was filtered, washed with cold water. Dried and recrystallised from absolute ethanol. Melting point 241^0C.Percentage yield was found to be 81%. [7]

Schiff base derivatives

Preparation of N-[(E)-substituted methylidine]-5-(pyridin-3yl)-1, 3, 4 thiadiazol-2-amine. **(II)**

An equimolar mixture of 2-amino-5-pyridin (3-yl) 1, 3, 4 thiadiazole (0.01mol) and different aromatic aldehydes (0.01mole) in 35ml of absolute ethanol was refluxed on water bath for about 5hrs. After refluxing the mixture was poured into the ice-cold water. The precipitate formed was filtered and recrystallised from ethanol. [APTS (a-d)] [8]

Synthesis of 2-substituted-3-[5-(pyridin-3yl) -1, 3, 4 thiadiazol 2-yl] 1, 3 thiazolidin-4 one. **(II-a)**

A mixture of N-[(E)-substituted methylidine]-5-(pyridin-3yl)-1, 3, 4 thiadiazol-2-amine (0.01mol) and thioglycollic acid (0.01mol) in ethanol (25ml) were refluxed on a water bath for about 6hrs.After refluxing the mixture was poured into the ice-cold water. The precipitate formed was filtered and recrystallized from methanol. [APTS (1a-4d)] [8]

Mannich base derivatives

Synthesis of *N*, *N*-disubstituted-*N*'-[5-(pyridin-3-yl)-1, 3, 4-thiadiazol-2-yl] methanediamine. **(III)**

An equimolar mixture of 2-amino-5-(pyridine-3yl) 1, 3, 4 thiadiazole (0.01mol) and different secondary amines (0.01mol) in methanol (35ml) and add HCl (2ml) then formaldehyde (2ml) add drop wise. This mixture was refluxed on a water bath for about 6hrs. After refluxing a mixture was poured into the ice-cold water. The precipitate formed was filtered and recrystallized from ethanol. [APTM (1-3)] [9]

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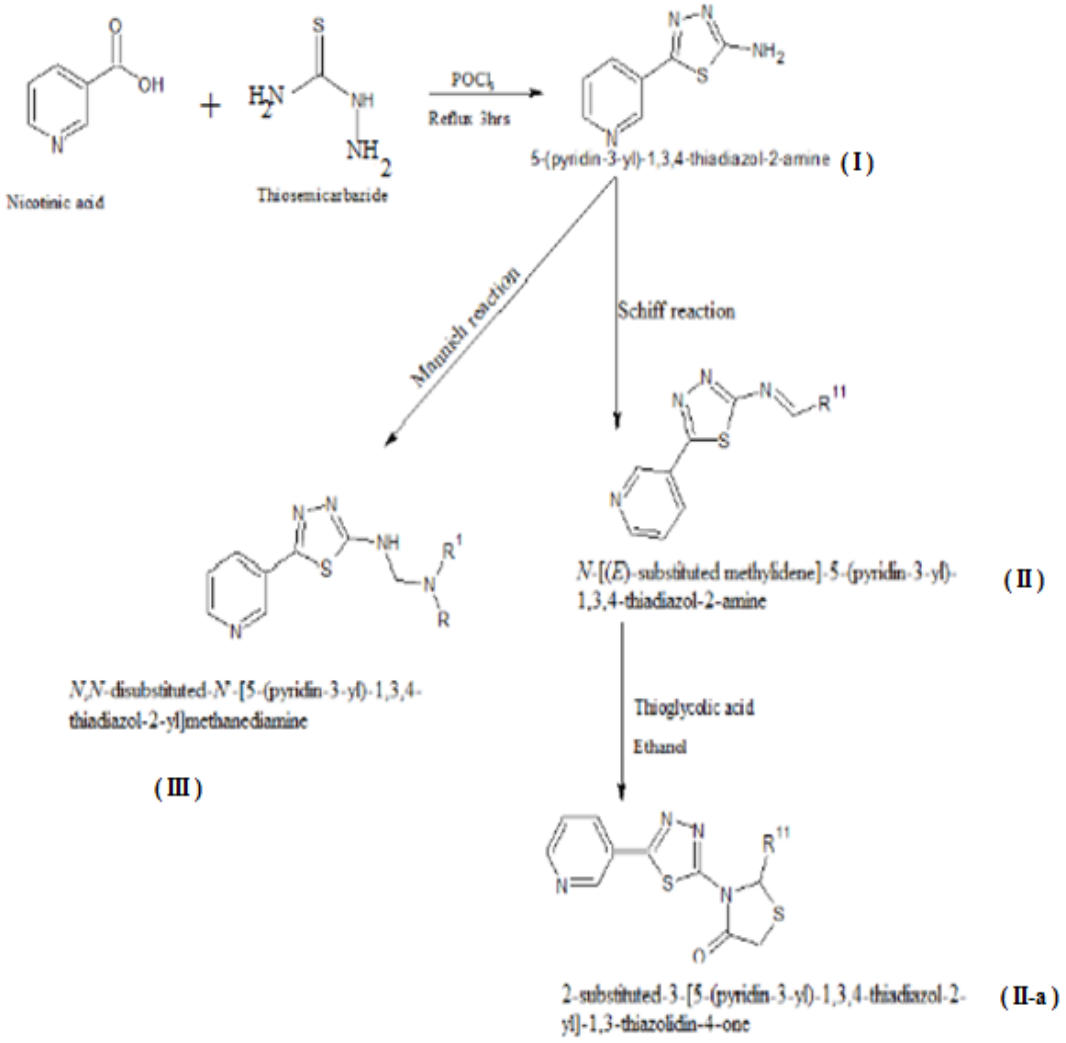
In-vitro cytotoxicity study

The anticancer activity of the selected analogous were screened using Tryphan blue expansion method on DLA (Dalton's Lymphoma Ascites) cell lines from Amala cancer Research institute, Thrissur.

Antibacterial screening

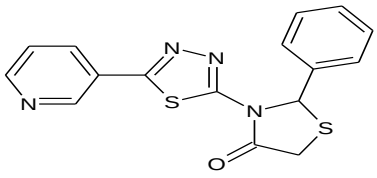
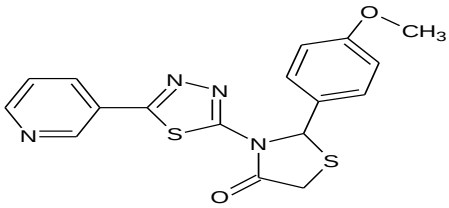
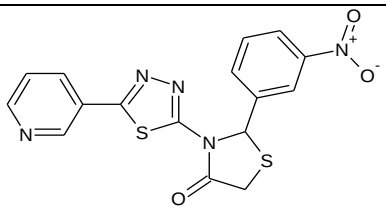
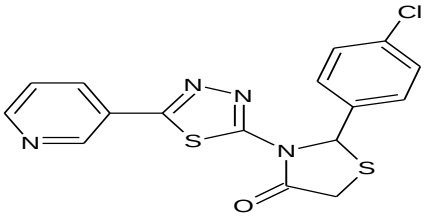
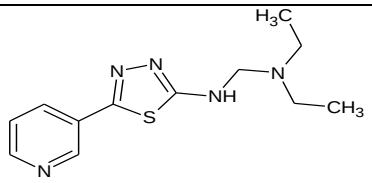
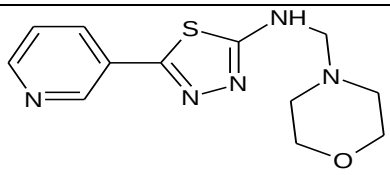
The synthesized analogous were screened for antibacterial activity against gram positive (+) bacteria (Bacillus subtilis) and gram negative bacteria (Escherichia coli) using cup plate agar diffusion method at Department of Pharmaceutical Microbiology and Biotechnology lab, National College of Pharmacy, Manassery.

Synthetic Scheme



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Table No- 1 List of synthesized compounds

Code	Structure of the compound	IUPAC name of the compound
APTS-1a		2-phenyl-3-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-thiazolidin-4-one
APTS-2b		2-(4-methoxy phenyl)-3-[5-(pyridin-3-yl)1,3,4-thiadiazol-2-yl]-1,3-thiazolidin-4-one
APTS-3c		2-(3-nitrophenyl)-3-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-thiazolidin-4-one
APTS-4d		2-(4-chlorophenyl)-3-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-thiazolidin-4-one
APTM-1		N,N-diethyl-N'-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]methanediamine
APTM-2		N-(morpholin-4-yl-methyl)-5-(pyridin-3-yl)-1,3,4-thiadiazol-2-amine

RESULT AND DISCUSSION

INSILICO MOLECULAR MODELING

10 different analogues of 1,3,4 thiadiazole has been done, all the compounds obeyed Lipinski rule of five by Molinspiration.

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Table No: 2 Molecular properties of drugs available in the markets

Standard drugs	MolarVolume (cm^3)	Parachor (cm^3)	Polarizability ($10^{-24}cm^3$)
Anastrazole	270.2±7.0	688.7±8.0	35.68±0.5
Fluorouracil	84.5±5.0	220.4±6.0	10.24±0.5
Lapatinib	420.6±3.0	1159.2±4.0	60.44±0.5
Imatinib	393.0±3.0	1110±6.0	58.31±0.5

Table No: 3 Molecular properties of proposed derivatives.

Compound code	Molar volume (cm^3)	Parachor (cm^3)	Polarizability($10^{-24}cm^3$)
APTS-1a	238.5 ± 3.0	693.6 ± 6.0	36.40 ± 0.5
APTS-2b	262.5 ± 3.0	752.2 ± 6.0	39.05 ± 0.5
APTS-3c	250 ± 3.0	750.6 ± 6.0	39 ± 0.5
APTS-4d	250.5 ± 3.0	730.7 ± 6.0	38.34 ± 0.5
APTM-1	216.6±3.0	589.9±4.0	29.87±0.5
APTM-2	207.9±3.0	592.9±6.0	29.63±0.5

Table No: 4 Lipinski rule analysis of standard drugs Molinspiration.

Drug	milogP	MW	nON	nOHNH	nrotb	Nviolation
Anastrazole	2.854	293.374	5	0	4	0
Flurouracil	-0.587	130.078	4	2	0	0
Imatinib	3.889	493.615	8	2	7	0
Lapatinib	6.162	581.069	8	2	11	2

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Table No: 5 Lipinski rule analysis of proposed derivatives by Molinspiration

Compound Code	milogP	MW	nON	nOHNH	nrotb	Nviolation
APTS-1a	2.881	340.433	5	0	3	0
APTS-2b	2.938	370.459	6	0	4	0
APTS-3c	2.816	385.43	8	0	4	0
APTS-4d	3.559	374.878	5	0	3	0
APTM-1	2.128	263.37	5	1	6	0
APTM-2	1.222	277.353	6	1	4	0

Molecular docking

Table No: 6 Docking scores of drugs available in market

Receptor	Standard Inhibitor	Energy	Best Pose
Aromatase	Anastrazole	-7.7650	2
ErbB-2	Lapatinib	-11.1057	19
Thymidylate synthase	Fluoro uracil	-6.15116	122
Tyrosine kinase	Imatinib	-8.23542	57

Table No-7 Docking Scores for proposed derivatives against Aromatase and ErbB-2 receptors

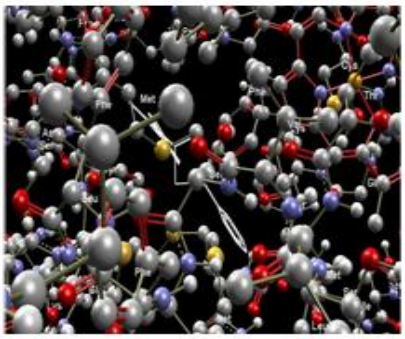
Compound code	Aromatase		ErbB-2	
	Energy score(Kcal/mol)	Best pose	Energy score(Kcal/mol)	Best pose
APTS-1a	-10.17	32	-8.43	62
APTS-2b	-9.40	29	-6.29	48
APTS-3c	-9.54	20	-6.43	31
APTS-4d	**-11.9692**	**37**	**-6.22**	**6**
APTM-1	-6.91	75	-7.06	71
APTM-2	-8.43	38	-8.61	35

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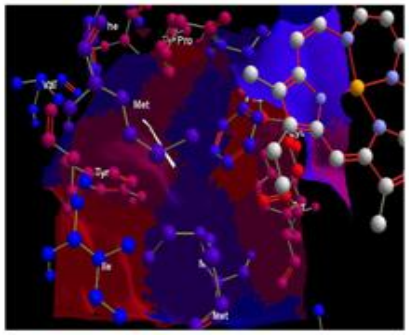
Table No: 8 Docking scores of proposed derivatives against Thymidylate synthase and Tyrosine Kinase receptors.

ssss Compound code	Thymidylate synthase		Tyrosine kinase	
	Energy score (kcal/mol)	Best pose	Energy score (kcal/mol)	Best pose
APTS-1a	-7.75	60	-9.675	68
APTS-2b	-8.34	52	-8.62	43
APTS-3c	-7.60	31	-8.87	39
APTS-4d	**-8.96**	**62**	**-9.62**	**65**
APTM-1	-6.10	78	-7.14	63
APTM-2	-6.76	33	-7.52	48

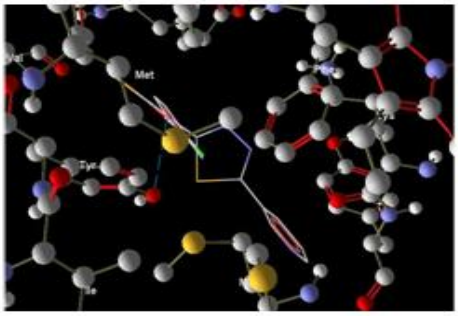
Fig No: 1 Docking images of APTS-4d binding to Aromatase



Docking View



Hydrophobic Interaction



Hydrogen Bonding Interaction

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Table No-9 Preliminary characterizations of newly synthesized compounds

Compound code	Molecular Formula	Molecular weight	Melting point	Percentage yield	R_f value
APTS-1a	$C_{16}H_{12}N_4OS_2$	340.42	178	65	0.78
APTS-2b	$C_{17}H_{14}N_4O_2S_2$	370.44866	135	60	0.69
APTS-3c	$C_{16}H_{11}N_5O_3S_2$	385.420	65	61	0.73
APTS-4d	$C_{16}H_{11}ClN_4OS_2$	302.78194	51	53	0.74
APTM-1	$C_{12}H_{17}N_5S$	263.36188	130	75	0.78
APTM-2	$C_{12}H_{15}N_5OS$	277.34	105	79	0.66

Spectral data of synthesized compounds

The synthesized compounds were confirmed with IR and 1H NMR and MASS spectra

Table No 10 IR spectral analysis

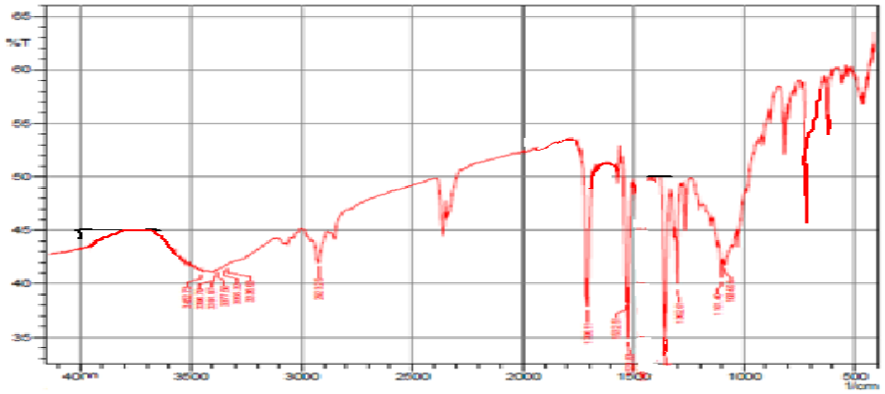
Compound code	IR peaks(cm^{-1})
APTS-3c	C=C Str (1523), C=O Str (1706), N=O Str (1357), C-S-C str (652)
APTM-1	C=C Str (1531), CH_2 Str (2948), NH Str (3102)

Table No-11 ^{1}H NMR spectral analysis

Compound code	^{1}H NMR(DMSO)δ(ppm)
APTS-3c	8.69(s,1H),8.54(d,1H),8.35(d,1H),7.92(t,1H)
APTM-1	8.80(S,1H),8.5(d,1H),7.86(d,1H),2.8(q, N-CH_2-N)

Table No- 12 MASS spectral analysis

Compound code	Molecular ion ($M+H^+$) peak
APTS-3c	385
APTM-1	263



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Fig No-2 IR spectrum of 2-(3-nitrophenyl)-3-[5-(pyridin-3-yl)-1, 3, 4-thiadiazol-2-yl]-1,3-thiazolidin-4-one

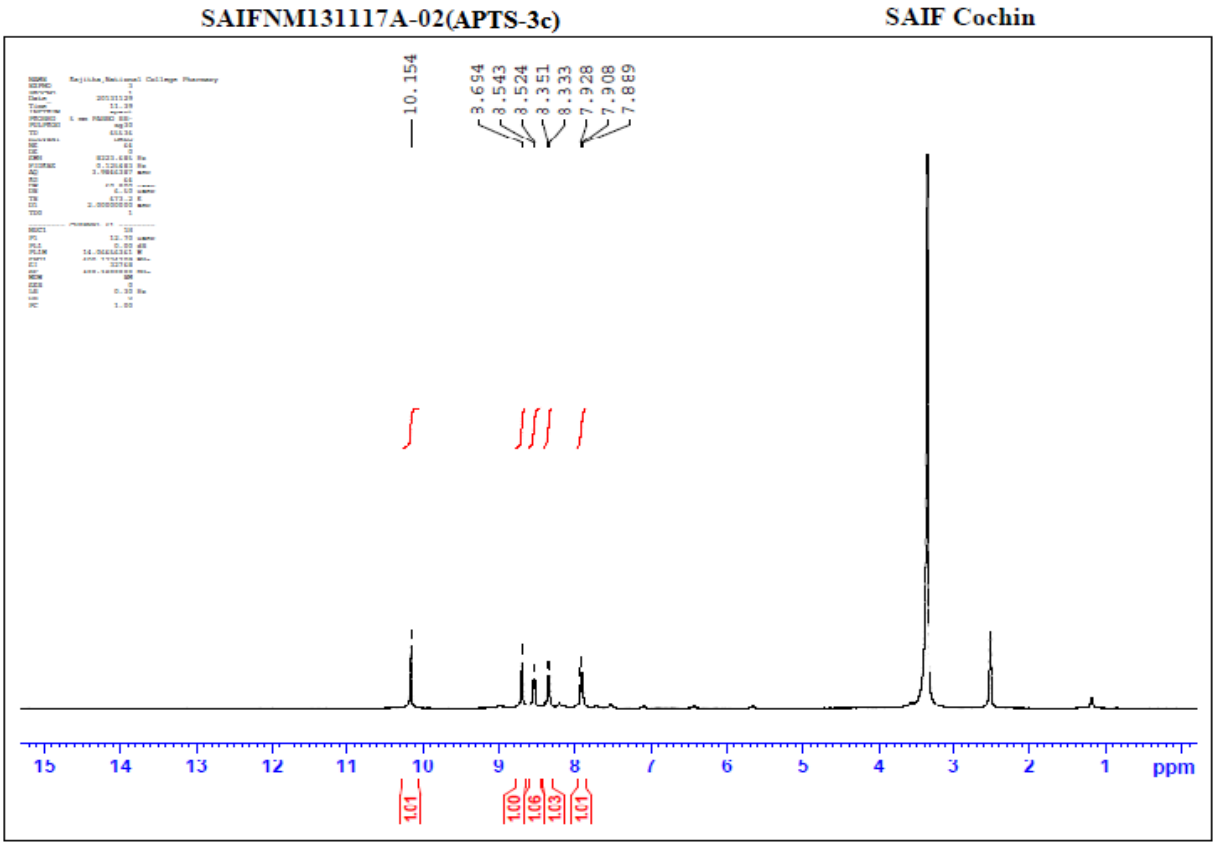


Fig No-3 ^{1}H NMR spectrum of 2-(3-nitrophenyl)-3-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-thiazolidin-4-one (**APTS-3c)**

Pharmacological screening

All the synthesized compounds showed good antibacterial activity against Ecoli and Bacillus subtillis and moderate cytotoxic activity towards the cell line DLA. Among them APTS -4d exhibited significant activity against DLA cell lines.

Table No-13 *In-vitro* cytotoxicity of synthesized compounds against DLA cell lines

Compound Code	Percentage cell death (Concentration in µg/ml)				
	10µg/ml	**20µg/ml**	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**
APTS-1a	10	20	31	45	46
APTS-2b	8	15	22	48	50
APTS-3c	11	18	28	52	52
APTS-4d	18	25	35	68	69
APTM-1	5	11	19	40	45

APTM-2	14	20	30	64	68
Doxorubicin (Standard)	20	55	75	100	100

Control tubes contains only one dead cell

Fig no-4 anticancer activities of synthesized Schiff's and Mannich derivatives against DLA cell lines

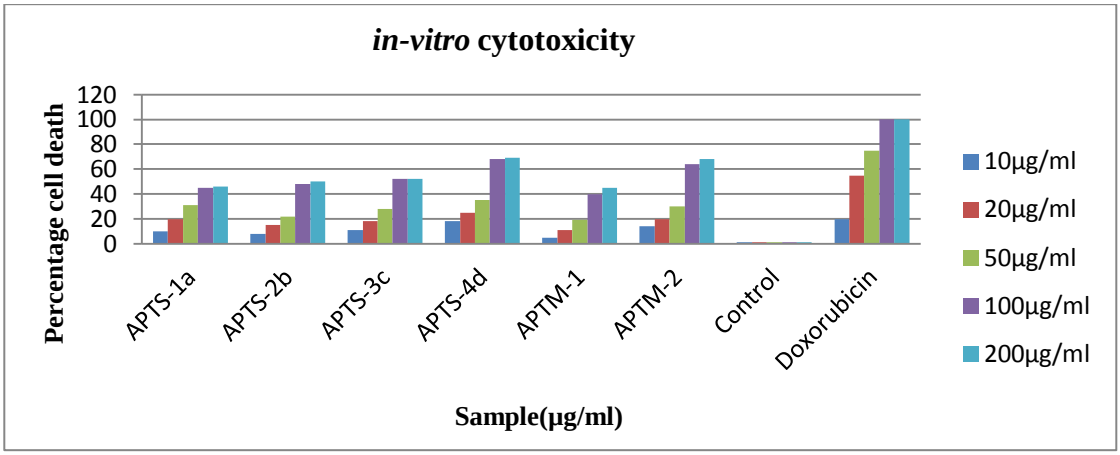


Table No-13 Zone of inhibition for antibacterial activity

Sample (µg/ml)	**Zone of inhibition (mm)**	
	E.coli	***Bacillus subtilis***
Control	0	0
Standard	21	15
APTS-1a	21	20
APTS-2b	15	16
APTS-3c	19	17
APTS-4d	14	10
APTM1	18	11
APTM2	19	17

Fig No-5 Zone of inhibition for antibacterial activity

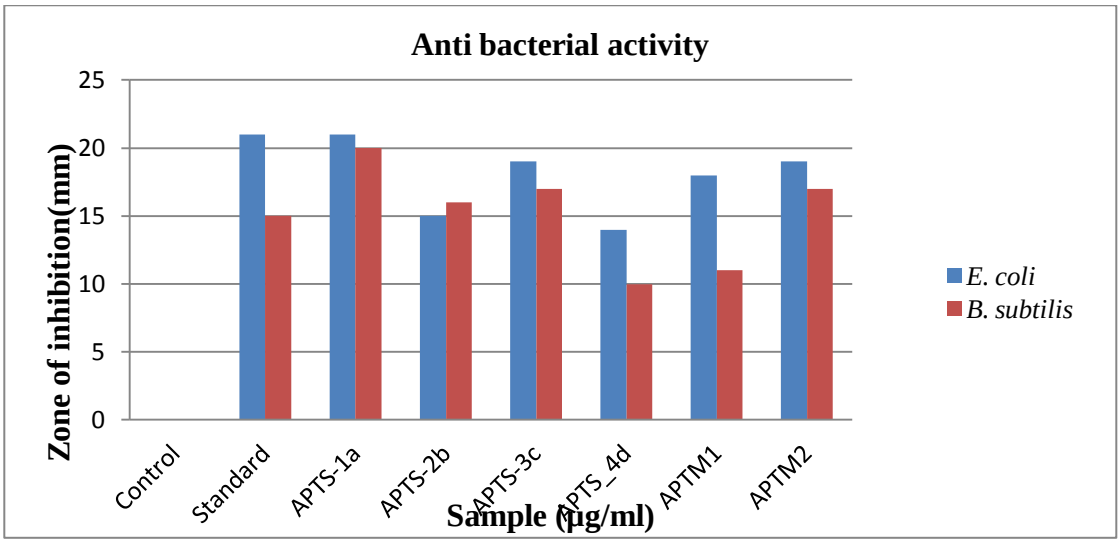


Fig No-6 Antibacterial activity against Gram (+) and Gram (-) bacteria.

Sample(µg/ml	*E. coli*	*B. subtilis*	Series 3
Control	0	0	2
Standard	21	15	2
APTS-1a	21	20	3
APTS-2b	15	16	5
APTS-3c	19	17	
APTS_4d	14	10	
APTM1	18	11	
APTM2	19	17	

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DISCUSSION

Preliminary *insilico* screening performed using Molinspiration. Drug like properties of the proposed analogues were studied and compared with drugs available in the market & all the compounds obeyed Lipinski rule of five.Docking studies of 10 different analogues were carried out against 4 different cancer targets.Docking scores were tabulated and compared with drugs of same category available in the pharmaceutical market.APTS-4d [Chloro benzaldehyde derivatives] obtained better score**(-11.962, -9.62, -8.96** kcal/mol) than that of anastrazole(-7.76504 kcal/mol), imatinib(-8.23 kcal/mol), flourouracil(-6.15 kcal/mol).

10 different analogues were selected for the wet lab synthesis.The synthetic method were then proceed in step wise manner via Schiff and Mannich reaction.*In-vitro* Cytotoxicity studies were carried out by Trypan blue Exclusion method on DLA cell lines.Antibacterial screening were also carried using Cup plate agar diffusion method. Compound APTS-1a-[2-phenyl-3-[5-(pyridin-3-yl)-1,3,4-thiadiazol-2-yl]-1,3-thiazolidin-4-one] exhibit significant activity

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towards gram (–ve) and gram (+ve) organism (100µg/ml) when compared to standard drug Amoxicillin (100µg/ml) and the others shows moderate activity. Compound APTS-2b, APTS-3c and APTM-2 exhibit significant activity towards gram (+ve) organism (100µg/ml) when compared to standard drug amoxicillin (100µg/ml).

CONCLUSION

The present work describes the synthesis and docking studies of novel thiazole-1, 3, 4-thiadiazole derivatives and their *invitro* cytotoxic activity and anti bacterial activity. The purity of the compounds and the completion of reaction thus synthesized were ascertained by consistency in melting point and by TLC and the structures of the synthesized compounds were assigned on the basis of the spectral data.

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