Synthesis, Characterisation and Biological Activity of some Heterocyclic Scaffold's.

Bhil Batuk M[1], Kahan I Pandya[2] and Sharma S[1*].

Department of Chemistry Hemchandracharya North Gujarat University Patan3 84265,Gujarat, India

ABSTRACT :

Pyrazole-1-carbothioamide and pyrido[2,3-d]pyrimidinethione, Triazolopyridopyrimidinines and Thiazoles, Synthesis of some compound and characterisation it by spectral analysis and elemental analysis, biological activity of compound.

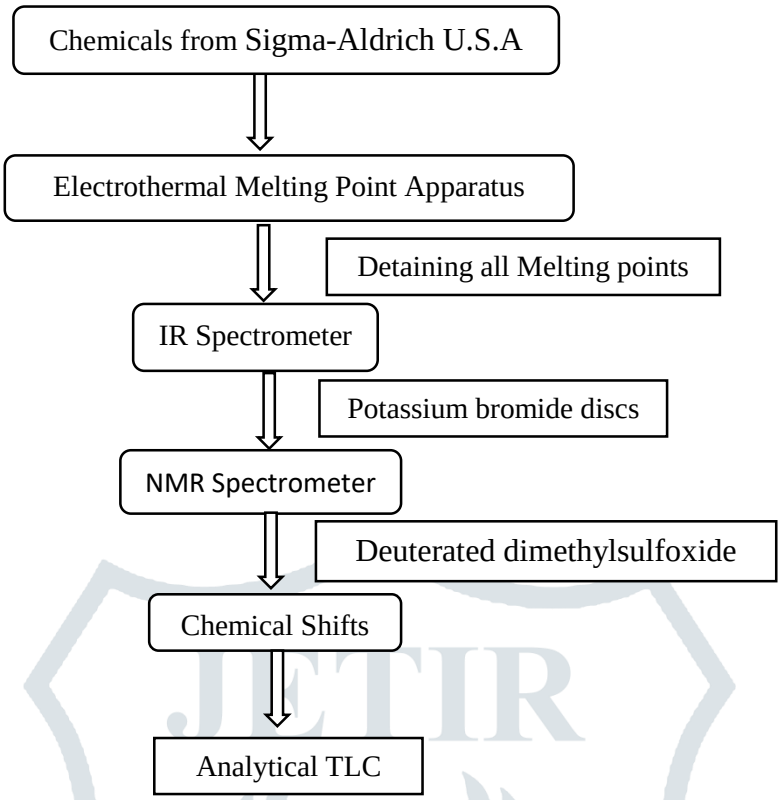
KEY WORDS : Pyrazole, Thione, Analysis and Biological activity.

Introduction :

The extensive biological activity linked with indole derivatives, together certainly happening and synthetic, confirms that the synthesis of indole by-products remains a subject of existing attention[1]. Monoindole and bisindole have been intensively deliberate and the consequences exposed that greatest of them have biological happenings; for example, indole-3-carbinol, originate in Brassica plants, is a possible cancer defensive agent [2]. Thiazoles can be create in medicine growth for the action of allergies, hypertension, inflammation, schizophrenia, microbial infections, HIV, sleep complaints, and for the action of pain [3], as fibrinogen receptor opponents with antithrombotic action and as new inhibitors of microbial DNA gyrase B. The 1,2,4-triazolopyrimidines have also involved rising attention owing to their significant pharmacological actions, such as antitumor, antimalarial, antimicrobial, anti-inflammatory, and antifungal activity, as well as macrophage initiation[4]. 1,3,4-Thiadiazole by-products have involved substantial attention due to their wide reaching biological doings such as antifungal, antihepatitis B viral, antibacterial, antileishmanial, anti-inflammatory, antituberculosis, analgesic, CNS depressant, anticancer, antioxidant, antidiabetic, molluscicidal, antihypertensive, diuretic, analgesic, antimicrobial, antitubercular, and anticonvulsant actions[5]. In light of these evidences, we have created some new thiazole, dihydropyrido[3,2-e][1,2,4]triazolo[4,3-a]pyrimidine and 1,3,4-thiadiazole derivatives using 3-acetylindole as a common precursor and separated these mixtures for their anticancer actions.

The unique structural features and multipurpose biological actions of attached indoles have completed them an attractive target for the growth of new pharmacological lead mixtures. Indole alkaloids display diverse kind of biological properties such as cytotoxic, antitumor, antiviral, antimicrobial, antiparasitics, antiserotonin and anti-inflammatory activities. The dimeric indole alkaloids with a fused six, seven, or eight-membered ring between two indole rings are known and some of them possessed anticancer activity. The indolo[3,2-*a*]carbazole with a bis-annelated six-membered ring, displayed antitumor properties. The other examples of bioactive indole fused alkaloids include ellipticine and olivacine. Ellipticine and olivacine which contain a pyrido[4,3-*b*]carbazole nucleus, have remarkable antitumour activities. The tetracyclic indoles and were found to exhibit *in vitro* activity against human nasopharyngeal carcinoma (HONE-1) and gastricadeno carcinoma (NUGC-3) cell lines.5 The pyrrolo[2,3-*e*]indole derivative showed *in vitro* cytotoxic activity in PC-3(prostate) cell line .

Flow sheet diagram for synthesis method :



Synthesis and Methodology

Reaction between 3-aryl-1-(1H-indol-3-yl) prop-2-en-1-ones 3a,b with thiosemicarbazide (4) afforded 3-(1H-indol-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbthioamide (5). Similar treatment with 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (6) generated 5-aryl-7-(1H-indol3-yl)-2-thioxo2,3-dihydropyrido[2,3-d] pyrimidin-4(1H)-ones7a,b, respectively. Structures 5 and 7 were expounded by chemical transformation, elemental analysis, and spectral data.

Reaction between 5-aryl-7-(1H-indol-3-yl)-2-thioxo-2,3-dihydropyrido[2,3-d] pyrimidin-4(1H)-ones 7a or 7b with the appropriate hydrazonoyl halides 8a-j in dioxane containing TEA under reflux produced 2-(1H-indol-3-yl)-9-substituted-4,7-disubstituted pyrido[3,2- e][1,2,4]triazolo[4,3-a]pyrimidin-5(7H)-ones 19a-1, respectively. Structure, 19 was expounded by elemental analysis, spectral data analysis, and alternative synthesis routes. Thus, the treatment of 7-amino-3-substituted-1-phenyl-[1,2,4] triazolo[4,3-a]pyrimidin-5(1H)-ones 20a,e,i 49 with chalcone3a in boiling acetic acid produced the products that were alike in all respects (mp., mixed mp., and spectra) with the equivalent 19a,e,i. The mechanism in outline seems to be the possible lane for the formation of 19 from the reaction of thione7 with 8 via two pathways. In the first pathway, 1,3- the addition of the thiol tautomer 7 to the nitrilimine 9 would give the thiohydrazonate ester 16 which could go through nucleophilic cyclization in order to produce spiro compounds 17. Ring opening to accomplish 18 is followed by cyclization with loss of hydrogen sulphide would then produce 19.

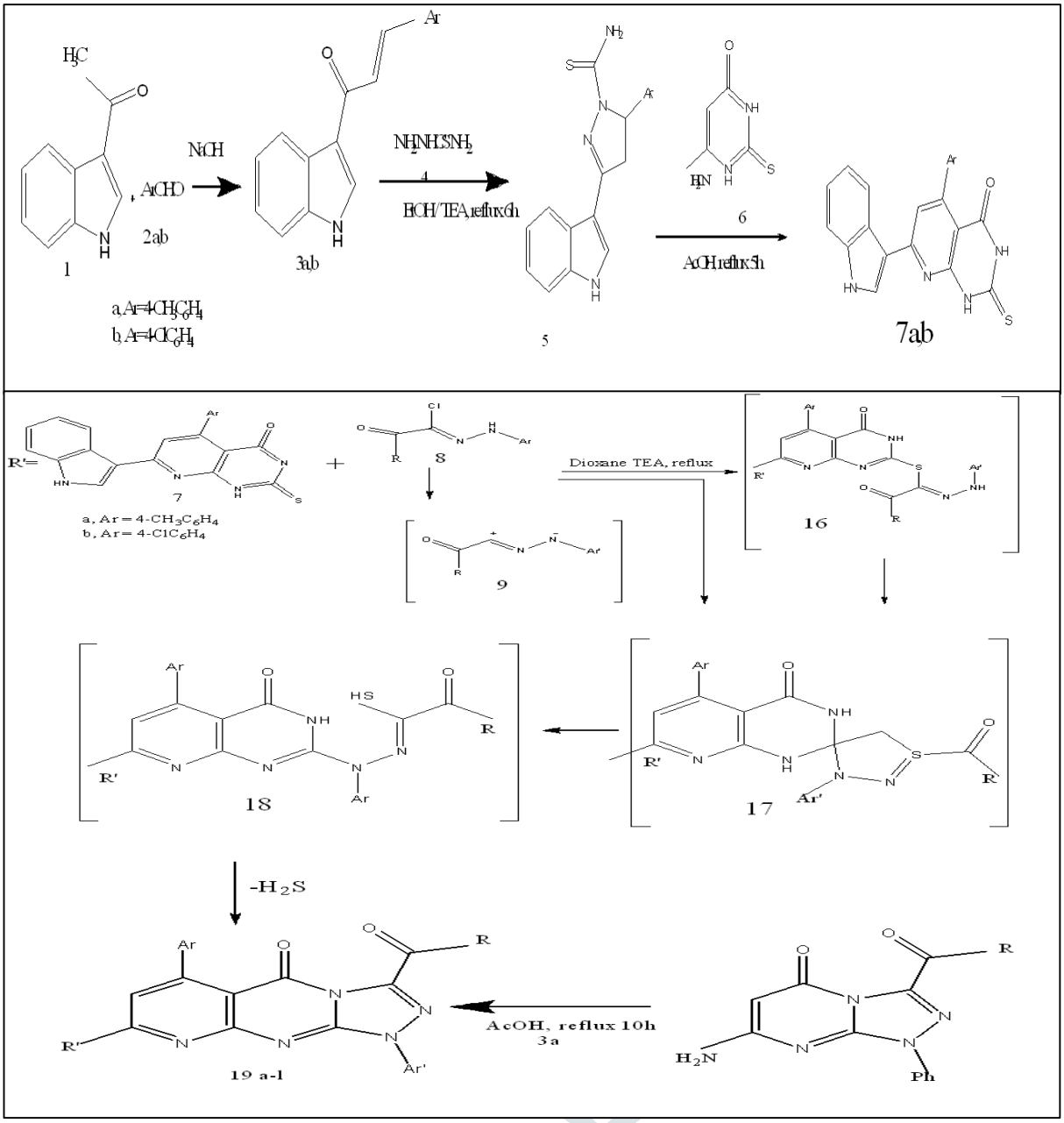
In the second pathway, an initial 1,3-cycloaddition of nitrilimine 9 to the C=S double bond of 7 would give 17 directly. Attempts that made for the isolation of thiohydrazonate ester 16, spiro intermediate 17 and thiohydrazide 18 did not succeed, even under mild conditions, as they readily undergo in situ cyclization, which is then followed by the elimination of hydrogen sulphide to give the final product 19.

Alternate Synthesis of 19a,e,i

Equimolar amounts of 1-(1*H*-indol-3-yl)-3-(*p*-tolyl)prop-2-en-1-one (**3a**) (0.261 g, 1 mmol) and 7-amino-1-phenyl-[1,2,4]triazolo[4,3-*a*]pyrimidin-5(1*H*)-one derivatives **20a**,**e**,**i** (1 mmol) in acetic acid (15 mL), was refluxed for 10 hours and then cooled to room temperature. The solid which was precipitated is collected, washed with water, dried, and recrystallized from DMF to give the corresponding products,

19a,**e**,**i** which were identical in all respects (mp, mixed mp, and IR spectra) with those obtained from the reaction of thione **7a** with hydrazonoyl chlorides **8a**,**e**,**i** but the % yields are 69%, 67%, and 70%, respectively.

Reaction scheme :



Physical data :

Sr. No.	Code Name	Substitution R	Substitution Ar	Substitution Ar`	Molecular Formula	Molecular weigh	% of Yield	M.P ^{0}C	% C		% H		% N	
									Cal.	Found.	Cal.	Found.	Cal.	Found.
1	19a	CH_3CO	Ph	Ph	$C_{31}H_{22}N_6O_2$	510.55	80	253-254	72.92	72.76	4.34	4.32	16.46	16.28
2	19b	CH_3CO	4-MeC_6H_4	4-MeC6H4	$C_{32}H_{24}N_6O_2$	524.57	76	242-244	73.27	73.12	4.61	4.48	16.02	15.93
3	19c	CH_3CO	4-ClC_6H_4	4-ClC6H4	$C_{31}H_{21}ClN_6O_2$	544.99	83	264-265	68.32	68.20	3.88	3.69	15.42	15.31
4	19d	CH_3CH_2CO	4-BrC_6H_4	4-BrC6H4	$C_{31}H_{21}BrN_6O_2$	589.44	78	254-256	63.17	63.09	3.59	3.42	14.26	14.17
5	19e	CH_3CH_2CO	Ph	Ph	$C_{32}H_{24}N_6O_3$	540.57	75	212-214	71.10	71.07	4.47	4.39	15.55	5.37
6	19f	CH_3CH_2CO	4-MeC_6H_4	4-MeC6H4	$C_{33}H_{26}N_6O_3$	554.60	77	238-240	71.47	71.38	4.73	4.70	15.15	15.04
7	19g	CH_3CH_2CO	4-$MeOC_6H_4$	4-MeOC6H4	$C_{33}H_{26}N_6O_4$	570.60	70	193-195	69.46	69.39	4.59	4.48	14.73	14.59
8	19h	PhNHCO	4-ClC_6H_4	4-ClC6H4	$C_{32}H_{23}ClN_6O_3$	575.02	78	243-245	66.84	66.69	4.03	4.01	14.62	14.47
9	19i	NHPh	Ph	Ph	$C_{36}H_{25}N_7O_2$	587.63	82	268-270	73.58	73.52	4.29	4.15	16.69	16.57
10	19j	NHPh	4-ClC_6H_4	4-ClC6H4	$C_{36}H_{24}ClN_7O_2$	622.07	80	279-281	69.51	69.42	3.89	3.81	15.76	15.59
11	19k	CH_3	4-ClC_6H_4	Ph	$C_{30}H_{19}ClN_6O_2$	530.96	78	271-273	67.86	67.69	3.61	3.54	15.83	15.71
12	19l	CH_3	4-ClC_6H_4	4-MeC6H4	$C_{31}H_{21}ClN_6O_2$	544.99	78	252-254	68.32	68.25	3.88	3.69	15.42	15.31

Table -1 physical data

Spectral discussion :

1H NMR SPECTRA:

1H NMR spectra of the synthesized compounds were recorded on Bruker Advance II 400 spectrometer. Sample solutions were made in DMSO solvent using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned. Numbers of protons identified from H NMR spectrum and their chemical shift (δ ppm) were in the agreement of the structure of the molecule.

MASS SPECTRA :

Mass spectra of the synthesized compounds were recorded on Shimadzu mass Spectrophotometer. The molecular ion peak was found in agreement with molecular weight of the respective compound.

IR SPECTRA :

IR spectra of the synthesized compounds were recorded on Shimadzu FT IR spectrophotometer using Diffused Reflectance Attachment (DRA) System using Potassium Bromide.

ELEMENTAL ANALYSIS :

Elemental analysis of the synthesized compounds was carried out on PerkinElmer 2400 CHN Analyzer which showed calculated and found percentage values of Carbon, Hydrogen and Nitrogen in support of the structure of synthesized compounds. The spectral and elemental analysis data are given for individual compounds.

Spectral data :

9-Acetyl-2-(1*H*-indol-3-yl)-7-phenyl-4-(*p*-tolyl) pyrido[3,2-e] [1,2,4] triazolo[4,3-a] pyrimidin-5 (7*H*)-one (19a).

Physical appearance	Yellow Solid
Yield	80%
Molecular Formula	$C_{31}H_{22}N_6O_2$
Molar Mass	510.55 g/mol.
Melting Point	253-255°C
IR (KBr)	v 3435 (NH), 1706, 1633 (2C=O), 1590 (C=N)cm1 ;
1H-NMR (DMSO-d6)	δ 2.35 (s, 3H, CH3), 2.49 (s, 3H, CH3), 7.30–8.38 (m, 14H, Ar-H and pyridine-H),8.72 (s, 1H, indole-H2), 12.02 (br s, 1H, NH); 13C-NMR (DMSO-d6) d: 21.0, 25.5, 109.4, 113.6, 115.8,119.0, 120.9, 121.0, 123.9, 126.2, 127.1, 129.2, 129.6, 131.4, 131.8, 133.8, 136.4, 140.9, 146.2, 148.4, 161.0,163.9, 167.1, 180.6;
MS	m/z (%) 510 (M+ , 36), 407 (23), 334 (22), 233 (27), 105 (100), 77 (22).

9-Acetyl-2-(1*H*-indol-3-yl)-4,7-di-*p*-tolylpyrido[3,2-e] [1,2,4] triazolo[4,3-*a*] pyrimidin-5(7*H*)-one (19b).

Physical appearance	Yellow Solid
Yield	76%
Molecular Formula	$C_{32}H_{24}N_6O_2$
Molar Mass	524.57 g/mol.
Melting Point	242-244°C
IR (KBr)	v 3421 (NH), 1707, 1642 (2C=O), 1589 (C=N) cm-1 ;
1H-NMR (DMSO-d6)	δ 2.23 (s, 3H, CH3), 2.34 (s, 3H, CH3), 2.48 (s, 3H, CH3), 7.07-8.28 (m, 13H, Ar-Hand pyridine-H), 8.70 (s, 1H, indole-H2), 12.03 (br s, 1H, NH);
MS	m/z (%) 524 (M+, 22), 509 (100),381 (32), 231 (96), 173 (79), 55 (63). C, 73.12; H, 4.48; N, 15.93.

9-Acetyl-7-(4-chlorophenyl)-2-(1*H*-indol-3-yl)-4-(*p*-tolyl) pyrido[3,2-*e*] [1,2,4] triazolo[4,3-*a*] pyrimidin-5(7*H*)-one (19c).

Physical appearance	Yellow Solid
Yield	83%
Molecular Formula	$C_{31}H_{21}ClN_6O_2$
Molar Mass	544.99 g/mol.
Melting Point	264-265°C
IR (KBr)	v 3386 (NH), 1710, 1644 (2C=O), 1589 (C=N)cm-1 ;
1H-NMR (DMSO-d6)	δ 2.35 (s, 3H, CH3), 2.44 (s, 3H, CH3), 7.09–8.34 (m, 13H, Ar-H and pyridine-H), 8.71 (s, 1H, indole-H2), 12.09 (br s, 1H, NH);
MS	m/z (%) 545 (M+, 3), 490 (24), 258 (30),152 (47), 29 (100). C, 68.20;H, 3.69; N, 15.31.

Ethyl2-(1*H*-Indol-3-yl)-5-oxo-7-phenyl-4-(*p*-tolyl)-5,7-dihydropyrido[3,2-*e*] [1,2,4]triazolo[4,3-a]-pyrimidine-9-carboxylate (19e).

Physical appearance	Yellow Solid
Yield	75%
Molecular Formula	$C_{32}H_{24}N_6O_3$
Molar Mass	540.57 g/mol.
Melting Point	212-214°C
IR (KBr)	v 3342 (NH), 1718, 1644 (2C=O),1579 (C=N) cm-1 ;
1H-NMR (DMSO-d6)	δ 1.29 (t, J = 7.1 Hz, 3H, CH3), 2.34 (s, 3H, CH3), 4.29 (q, J = 7.1Hz, 2H, CH2), 7.13–8.30 (m, 14H, Ar-H and pyridine-H), 8.73 (s, 1H, indole-H2), 12.12 (br s, 1H, NH);
MS	m/z (%) 540 (M+, 2), 521 (16), 361 (13), 270 (69),167 (38), 91 (100). C, 71.07; H, 4.39; N, 15.37.

Ethyl2-(1*H*-Indol-3-yl)-5-oxo-4,7-di-*p*-tolyl-5,7-dihydropyrido[3,2-*e*] [1,2,4] triazolo[4,3-*a*]pyrimidine-9-carboxylate (19f).

Physical appearance	Yellow Solid
Yield	77%
Molecular Formula	$C_{33}H_{26}N_6O_3$
Molar Mass	554.60 g/mol.
Melting Point	238-240°C
IR (KBr)	v 3348 (NH), 1746, 1673 (2C=O), 1576 (C=N)cm-1 ;
1H-NMR (DMSO-d6)	δ 1.30 (t, J = 7.1 Hz, 3H, CH3), 2.23 (s, 3H, CH3), 2.35 (s, 3H, CH3), 4.26(q, J = 7.1 Hz, 2H, CH2), 7.08–8.32 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H2), 12.09 (brs, 1H, NH);
MS	m/z (%) 554 (M+ , 4), 522 (24), 431 (100), 326 (88), 282 (25), 91 (10).

Ethyl2-(1*H*-Indol-3-yl)-7-(4-methoxyphenyl)-5-oxo-4-(*p*-tolyl)-5,7 dihydropyrido[3,2-*e*] [1,2,4] triazolo-[4,3-*a*] pyrimidine-9-carboxylate (19g).

Physical appearance	Yellow Solid
Yield	70%
Molecular Formula	$C_{33}H_{26}N_6O_4$
Molar Mass	570.60 g/mol.
Melting Point	193-195°C
IR (KBr)	v 3390 (NH), 1740,1674 (2C=O), 1578 (C=N) cm-1 ;
1H-NMR (DMSO-d6)	δ 1.32 (t, J = 7.1 Hz, 3H, CH3), 2.35 (s, 3H, CH3),3.82 (s, 3H, OCH3), 4.34 (q, J = 7.1 Hz, 2H, CH2), 7.03–8.34 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H,indole-H2), 12.10 (br s, 1H, NH);
MS	m/z (%) 570 (M+, 10), 354 (52), 311 (62), 267 (85), 72 (54), 59 (100). C, 69.39; H, 4.48; N, 14.59.

Ethyl7-(4-Chlorophenyl)-2-(1*H*-indol-3-yl)-5-oxo-4-(*p*-tolyl)-5,7 dihydropyrido[3,2-*e*][1,2,4]triazolo-[4,3-*a*]pyrimidine-9-carboxylate (19h).

Physical appearance	Yellow Solid
Yield	78%
Molecular Formula	$C_{32}H_{23}ClN_6O_3$
Molar Mass	575.02 g/mol.
Melting Point	243-245°C
IR (KBr)	v 3383 (NH), 1742,1675 (2C=O), 1577 (C=N) cm1 ;
1H-NMR (DMSO-d6)	δ 1.31 (t, J = 7.1 Hz, 3H, CH3), 2.35 (s, 3H, CH3),4.37 (q, J = 7.1 Hz, 2H, CH2), 7.07-8.34 (m, 13H, Ar-H and pyridine-H), 8.72 (s, 1H, indole-H2), 12.09 (brs, 1H, NH);
MS	m/z (%) 575 (M+, 17), 352 (12), 293 (29), 126 (23), 72 (59), 59 (100). C, 66.69; H, 4.01; N, 14.47.

2-(1*H*-Indol-3-yl)-5-oxo-N,7-diphenyl-4-(*p*-tolyl)-5,7-dihydropyrido [3,2 *e*] [1,2,4] triazolo[4,3-*a*]-pyrimidine-9-carboxamide (19i).

Physical appearance	Yellow Solid
Yield	82%
Molecular Formula	$C_{36}H_{25}N_7O_2$
Molar Mass	587.63 g/mol.
Melting Point	268-270°C
IR (KBr)	v 3385, 3213 (2NH), 1679, 1641(2C=O), 1598 (C=N) cm-1 ;
1H-NMR (DMSO-d6)	δ 2.35 (s, 3H, CH3), 7.02–8.34 (m, 19H, Ar-H andpyridine-H), 8.72 (s, 1H, indole-H2), 10.92 (br s, 1H, NH), 12.10 (br s, 1H, NH);
MS	m/z (%) 587 (M+ ,2), 441 (30), 271 (43), 158 (100), 128 (43), 91 (31). C, 73.52; H, 4.15; N, 16.57.

All of the synthesized compounds (19a-l) were tested for their antibacterial and antifungal activity (MIC) *in vitro* by broth dilution method with two Gram-positive bacteria *Staphylococcus aureus* ATTCC 25923 and *Enterococus faecalis* ATCC 29212, two Gram-negative bacteria *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and two fungal strains *Candida albicans* ATCC 10231 and *Aspergillus Niger* ATCC 1015 taking chloramphenicol, and ketoconazole as standard drugs. The standard strains were procured from the Microbial Type Culture Collection (MTCC), Microbiology lab, Sterling Hospital, Gujarat, India.

The minimal inhibitory concentration (MIC) values for all the newly synthesized compounds, defined as the lowest concentration of the compound preventing the visible growth, were determined by using micro dilution broth method according to NCCLS standards.

Minimal Inhibition Concentration [MIC]

The main advantage of the **Broth Dilution Method** for MIC determination lies in fact that it can readily be converted to determine the MIC as well.

1. Serial dilutions were prepared in primary and secondary screening.

2. The control tube containing no antibiotic is immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 0C overnight.

3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.

5. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

Antitumor Activity Assay

The human carcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). These cells were grown on RPMI-1640 medium which is supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, and 50 µg/mL gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 incubator and were sub-cultured for two to three times a week. For anti-tumor assays, the tumour cell lines were suspended in a medium at concentration 5 x 10^4 cell/well in Corning® 96-well tissue culture plates, then incubated for 24 hours. The tested compounds were then added to 96-well plates (six replicates) to achieve eight concentrations of each compound (started from 200 to 1.56 µg/mL). Six vehicle controls with media or 0.1% DMSO were run for each 96-well plate as a control. After 24 hours of incubation, the number of viable cells was determined by MTT assay. Therefore, the media were removed from the 96-well plate and replaced with 100 µL of fresh culture RPMI 1640 medium without phenol red and 10 µL of the 12 mM MTT (3-[4,5-dimethylthiazol- 2-yl]-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO, USA) stock solution (5 mg of MTT in 1 mL of PBS) to each well including the untreated controls. The 96-well plates were then incubated at 37 ºC with 5% of CO_2 for 4 hours. 85 µL aliquots of the media were removed from the wells, and 50 µL of DMSO was added to each well and mixed thoroughly using the pipette and incubated at 37 ºC for 10 minutes. The relation between surviving cells and drug concentration was plotted to get the survival curve of each tumour cell line after treatment with the specified compounds. The 50% Inhibitory Concentration (IC_{50}) and the concentration required to cause toxic effects in 50% of intact cells were estimated from graphic plots of the dose-response curve for each concentration [47,48].

Biological screening of compound :

Compounds	R	Ar	Ar'	Gram positive bacteria		Gram positive bacteria		Fungi		IC_{50}
				s.Aureus	S.Pyogenes	E.coli	P.Aeruginosa	C. Albicans	A. Niger	
19a	Me	4-MeC_6H_4	Ph	250	250	500	500	200	100	6.88
19b	Me	4-MeC_6H_4	4-MeC_6H_4	50	50	50	100	100	500	4.68
19c	Me	4-MeC_6H_4	4-ClC_6H_4	25	25	25	25	50	100	69.85
19d	Me	4-MeC_6H_4	4-BrC_6H_4	500	500	500	500	500	500	4.83
19e	OEt	4-MeC_6H_4	Ph	500	500	500	250	500	500	5.49
19f	OEt	4-MeC_6H_4	4-MeC_6H_4	500	500	500	500	1000	500	3.05
19g	OEt	4-MeC_6H_4	4-$MeOC_6H_4$	25	100	50	100	25	50	18.61
19h	OEt	4-MeC_6H_4	4-ClC_6H_4	100	100	100	250	50	100	6.07
19i	NHPh	4-MeC_6H_4	Ph	200	200	200	250	200	200	2.04
19j	NHPh	4-MeC_6H_4	4-ClC_6H_4	500	500	500	500	500	500	1.01
19k	Me	4-ClC_6H_4	Ph	200	500	500	500	500	1000	1.27
19l	Me	4-ClC_6H_4	4-MeC_6H_4	200	500	250	200	500	1000	14.01
Chlora-mphrnicols	-	-	-	50	50	50	50	-	-	-
kitaconazole	-	-	-					50	50	-
doxorubicin	-	-	-							0.75

Table -2 : Biological screening of compound

Methods used for primary and secondary screening

Each synthesized compounds was diluted obtaining 2000 µg mL-1 concentration, as a stock solution. Inoculum size for check strain was adjusted to 108 cfu (colony forming unit) per cubic centimeter by comparing the turbidness.

Primary screen: In primary screening 1000 µg mL-1, 500 µg mL-1 and 250 µg mL-1 concentrations of the synthesized compounds were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms.

Secondary screen: The compounds found active in primary screening were similarly diluted to obtain 200 µg mL-1, 100 µg mL-1, 50 µg mL-1, 25 µg mL-1 and 12.5 µg mL-1 concentrations.

Reading Result: The best dilution showing a minimum of nintynine behaviour midification zone is taken as MIC. The result of this is much affected by the size of the inoculums. The test mixture should contain 108 organism/mL.

Result :

Antibacterial and Antifungal activity of synthesised compound found good some moderate and some poor as compare to chloramphenicol and kitaconazole.

For substituent at position 3: the organic compound cluster (CO_2Et) provides higher activity than the organic compound cluster (CONHPh) or the acetyl radical (Ac) (**19e** > **19i** > **19a**).Generally, on fixing the substituents at position 3, the electron-donating group (methyl or methoxy) at C4 of the phenyl ring enhances the antitumor activity while the electron-withdrawing group (chlorine) decreases the antitumor activity (**19b** > **19a** > **19c** and **19g** > **19f** > **19e** > **19h** as compare to doxorubicin.

Conclusion :

Heterocyclic scaffolds play an important role in the field of medical science special in antimicrobial, antifungal, and anticancer. The current work is helpful to prevent disease which happened because of fungal, microbial as well as cancer.

References :

1. Parmentier, J.G.; Poissonnet, G.; Goldstein, S. Practical Syntheses of 5-Trifluoromethyl-1H-indoles. Heterocycles **2002**, 56, 465–476.
2. Black, D.S.; Craig, D.C.; Rezaie, R. Synthesis of mixed heterocalixarenes from benzofuranyl methanols and activated indoles. Chem. Commun. **2002**, 8, 810–811.
3. Gaifa, L.; Wayne, K.N. Synthesis of novel indole analogues of mycophenolic acid as potential antineoplastic agents. Tetrahedron **2000**, 56, 2583–2590.
4. Aiello, F.; Valacchi, G. Synthesis and Evaluation of Indole Based Molecules for Treatment of Oxidative Stress-Related Diseases. Curr. Top. Med. Chem. **2014**, 14, 2576–2589.
5. Broadbent, T.A.; Broadbent, H.S. The chemistry and pharmacology of indole-3-carbinol (indole-3-methanol) and 3-(methoxymethyl)indole. Part I. Curr. Med. Chem. **1998**, 5, 337–352. [PubMed]
6. Riby, J.E.; Chang, G.H.; Firestone, G.L.; Bjeldanes, L.F. Ligand-independent activation of estrogen receptor function by 3,3'-diindolylmethane in human breast cancer cells. Biochem. Pharmacol. **2000**, 60, 167–177.
7. Hargrave, K.D.; Hess, F.K.; Oliver, J.T. N-(4-Substituted-thiazolyl)oxamic acid derivatives, new series of potent, orally active antiallergy agents. J. Med. Chem. **1983**, 26, 1158–1163.
8. Rudolph, J.; Theis, H.; Hanke, R.; Endermann, R.; Johannsen, L.; Geschke, F.U. Seco-Cyclothialidines: New concise synthesis, inhibitory activity toward bacterial and human DNA topoisomerases, and antibacterial properties. J. Med. Chem. **2001**, 44, 619–626.
9. Ergenc, N.; Capan, G.; Gunay, N.S.; Ozkirimli, S.; Gungor, M.; Ozbey, S.; Kendi, E. Synthesis and hypnotic activity of new 4-thiazolidinone and 2-thioxo-4,5-imidazolidinedione derivatives. Arch. Pharm. Pharm. Med. Chem. **1999**, 332, 343–347.
10. Jaen, J.C.;Wise, L.D.; Caprathe, B.W.; Tecle, H.; Bergmeier, S.; Humblet, C.C.; Heffner, T.G.; Meltzner, L.T.; Pugsley, T.A. 4-(1,2,5,6-Tetrahydro-1-alkyl-3-pyridinyl)-2-thiazolamines: A novel class of compounds with central dopamine agonist properties. J. Med. Chem. **1990**, 33, 311–317.
11. Sharma, R.N.; Xavier, F.P.; Vasu, K.K.; Chaturvedi, S.C.; Pancholi, S.S. Synthesis of 4-benzyl-1,3-thiazole derivatives as potential anti-inflammatory agents: An analogue-based drug design approach. J. Enzym. Inhib. Med. Chem. **2009**, 24, 890–897.
12. Patt, W.C.; Hamilton, H.W.; Taylor, M.D.; Ryan, M.J.; Taylor, D.G., Jr.; Connolly, C.J.C.; Doherty, A.M.; Klutchko, S.R.; Sircar, I.; Steinbaugh, B.A.; et al. Structure-activity relationships of a series of 2-amino-4-thiazole containing renin inhibitors. J. Med. Chem. **1992**, 35, 2562–2572.
13. Bell, F.W.; Cantrell, A.S.; Hoegberg, M.; Jaskunas, S.R.; Johansson, N.G.; Jordon, C.L.; Kinnick, M.D.; Lind, P.; Morin, J.M.; Noreen, R.; et al. Phenethylthiazolethiourea (PETT) compounds, a new class of HIV-1 reverse transcriptase inhibitors. 1. Synthesis and necessary structure-activity relationship studies of PETT analogs. J. Med. Chem. **1995**, 38, 4929–4936.
14. Badorc, A.; Bordes, M.F.; de Cointet, P.; Savi, P.; Bernat, A.; Lale, A.; Petitou, M.; Maffrand, J.P.; Herbert, J.M. New orally active non-peptide fibrinogen receptor (GpIIb-IIIa) antagonists: Identification of ethyl 3-[N-[4-[4-amino[(ethoxycarbonyl) imino]methyl]phenyl]-1,3-thiazol-2-yl]-N-[1-(ethoxycarbonyl)methyl] piperid-4-yl]amino]propionate (SR 121787) as a potent and long-acting antithrombotic agent. J. Med. Chem. **1997**, 40, 3393–3401.
15. Carter, J.S.; Kramer, S.; Talley, J.J.; Penning, T.; Collins, P.; Graneto, M.J.; Seibert, K.; Koboldt, C.; Masferrer, J.; Zweifel, B. Synthesis and activity of sulfonamide-substituted 4,5-diaryl thiazoles as selective cyclooxygenase-2 inhibitors. Bioorg. Med. Chem. Lett. **1999**, 9, 1171–1174.
16. Tsuji, K.; Ishikawa, H. Synthesis and anti-pseudomonal activity of new 2-isocephems with a dihydroxypyridone moiety at C-7. Bioorg. Med. Chem. Lett. **1994**, 4, 1601–1606.

17. Astakhov, A.V.; Chernyshev, V.M. Molecular structure of 3-amino[1,2,4]triazolo-[4,3-a] pyrimidin-5-one in various tautomeric forms: Investigation by DFT and QTAIM methods. Chem. Heterocycl. Compd. **2014**, 50, 319–326.

18. Gomha, S.M. A facile one-pot synthesis of 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]-1,2,4-triazolo [4,5-a]pyrimidin-5-ones. Monatshefte Chem. **2009**, 140, 213–220.

19. Fares, M.; Abou-Seri, S.M.; Abdel-Aziz, H.A.; Abbas, S.E.S.; Youssef, M.M.; Eladwy, R.A. Synthesis and antitumor activity of pyrido[2,3-d]pyrimidine and pyrido[2,3-d] [1,2,4]triazolo[4,3-a]pyrimidine derivatives that induce apoptosis through G(1) cell-cycle arrest. Eur. J. Med. Chem. **2014**, 83, 155–166.

20. Gomha, S.M.; Ahmed, S.A.; Abdelhamid, A.O. Synthesis and cytotoxicity evaluation of some novel thiazoles, thiadiazoles, and pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one incorporating triazole moiety. Molecules **2015**, 20, 1357–1376.

21. Liu, X.H.; Sun, Z.H.; Yang, M.Y.; Tan, C.X.; Weng, J.Q.; Zhang, Y.G.; Ma, Y. Microwave assistant one-pot synthesis, crystal structure, antifungal activities and 3D-QSAR of novel 1,2,4-triazolo[4,3-a]pyridines. Chem. Biol. Drug Des. **2014**, 84, 342–347.

22. Gomha, S.M.; Badrey, M.G. Eco-friendly regioselective one-pot synthesis of chromeno[4,3-d] [1,2,4]triazolo [4,3-a]pyrimidine. Eur. J. Chem. **2013**, 4, 180–184.

23. Zhang, L.J.; Yang, M.Y.; Sun, Z.H.; Tan, C.X.;Weng, J.Q.;Wu, H.K.; Liu, X.H. Synthesis and antifungal activity of 1,3,4-thiadiazole derivatives containing pyridine group. Lett. Drug Des. Discov. **2014**, 11, 1107–1111.

24. Gomha, S.M.; Khalil, K.D.; El-Zanate, A.M.; Riyadh, S.M. A facile green synthesis and anti-cancer activity of bis-arylhydrazononitriles, triazolo[5,1-c][1,2,4]triazine, and 1,3,4-thiadiazoline. Heterocycles **2013**, 87, 1109–1120.

25. Yan, S.L.; Yang, M.Y.; Sun, Z.H.; Min, L.J.; Tan, C.X.;Weng, J.Q.;Wu, H.K.; Liu, X.H. Synthesis and antifungal activity of 1,2,3-thiadiazole derivatives containing 1,3,4-thiadiazole moiety. Lett. Drug Des. Discov. **2014**, 11, 940–943.

26. Tong, J.Y.; Sun, N.B.; Wu, H.K.; Liu, X.H. Synthesis, crystal structure and biological activity of N-(5-(Otolyl)-1,3,4-thiadiazol-2-yl)cyclopropanecarboxamide. J. Chem. Soc. Pak. **2013**, 35, 1349–1353.

27. Gomha, S.M.; Salah, T.A.; Abdelhamid, A.O. Synthesis, characterization and pharmacological evaluation of some novel thiadiazoles and thiazoles incorporating pyrazole moiety as potent anticancer agents. Monatshefte Chem. **2015**, 146, 149–158.

28. Li, Z.;Wang, X.; Da, Y. Synthesis of 2-(5-(2-chlorophenyl)-2-furoylamino)-5-aryloxymethyl-1,3,4-thiadiazoles under microwave irradiation. Synth. Commun. **2001**, 31, 1829–1836.

29. Gomha, S.M.; Abdel-Aziz, H.A. Synthesis of new heterocycles derived from 3-(3-methyl-1H-indol-2-yl)-3-oxopropanenitrile as potent antifungal agents. Bull. Korean Chem. Soc. **2012**, 33, 2985–2990.

30. Liu, X.; Shi, Y.; Ma, Y.; Zhang, C.; Dong, W.; Pan, L.; Wang, B.; Li, Z. Synthesis, antifungal activities and 3D-QSAR study of N-(5-substituted-1,3,4-thiadiazol-2-yl)cyclopropane carboxamides. Eur. J. Med. Chem. **2009**, 44, 2782–2786.

31. Ahmad, T.; Singh, A.K.; Jaiswal, N.; Singh, D. Synthesis and pharmacological activity of 1,3,4-thiadiazole derivatives: A review. Int. Res. J. Pharm. **2012**, 3, 70–82.

32. Gomha, S.M.; Riyadh, S.M. Synthesis under microwave irradiation of [1,2,4]triazolo[3,4-b] [1,3,4]thiadiazoles and other diazoles bearing indole moieties and their antimicrobial evaluation. Molecules **2011**, 16, 8244–8256.

33. Qiu, X.L.; Li, G.; Wu, G.; Zhu, J.; Zhou, L.; Chen, P.L.; Chamberlin, A.R.; Lee, W.H. Synthesis and biological evaluation of a series of the novel inhibitor of Nek2/Hec1 analogues. J. Med. Chem. **2009**, 52, 1757–1767.

34. Tsoua, H.; MacEwan, G.; Birnberg, G.; Grosu, G.; Bursavich, M.G.; Bard, J.; Brooijmansa, N.; Toral-Barzab, L.; Hollanderb, I.; Mansoura, T.S.; et al. Discovery and optimization of 2-(4-substituted-pyrrolo [2,3-b] pyridin-3-yl)methylene-4-hydroxybenzofuran-3(2H)-ones as potent and selective ATP-competitive inhibitors of the mammalian target of rapamycin (mTOR). Bioorg. Med. Chem. Lett. **2010**, 20, 2321–2325.

35. Mavrova, A.T.; Wesselinova, D.; Tsenov, Y.A.; Denkova, P. Synthesis, cytotoxicity and effects of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives on immunocompetent cells. Eur. J. Med. Chem. **2009**, 44, 63–69.

36. Oleson, J.J.; Slobada, A.; Troy, W.P.; Halliday, S.L.; Landes, M.J.; Angier, R.B.; Semb, J.; Cyr, K.; Williams, J.H. The carcinostatic activity of some 2-amino-1,3,4-thiadiazoles. J. Am. Chem. Soc. **1955**, 77, 6713–6714.

37. Matysiak, J.; Opolski, A. Synthesis and antiproliferative activity of N-substituted 2-amino-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazoles. Bioorg. Med. Chem. **2006**, 14, 4483–4489.

38. Manna, F.; Chimenti, F.; Bolasco, A.; Bizzarri, B.; Filippelli, W.; Filippelli, A.; Gagliardi, L. Anti-inflammatory, analgesic and antipyretic 4,6-disubstituted 3-cyano-2-aminopyridines. Eur. J. Med. Chem. **1999**, 34, 245–254.

39. Ismail, M.M.F.; Khalifa, N.M.; Fahmy, H.H.; Nossier, E.S.; Abdulla, M. Design, docking, and synthesis of some new pyrazoline and pyranopyrazole derivatives as anti-inflammatory agents. J. Heterocycl. Chem. **2014**, 51, 450–458.

40. Butler, R.N. Comprehensive Heterocyclic Chemistry; Katritzky, A.R., Rees, C.W., Scriven, E.F.V., Eds.; Pergamon Press: New York, NY, USA, 1996; Volume 4, pp. 621–678.

41. Huisgen, R.; Grashey, R.; Seidel, M.; Knupfer, H.; Schmidt, R. 1,3-Dipolare additionen, III. Umsetzungen des diphenylnitrilimins mit carbonyl und thiocarbonyl-verbindungen. Justus Liebigs Ann. Chem. **1962**, 658, 169–180.

42. Mosselhi, M.A.N. A convenient synthesis of novel derivatives of pyrido[2,3-d][1,2,4]triazolo[4,3-a] pyrimidine-5,6-dione. Monatshefte Chem. **2002**, 133, 1297–1304.

43. Eweiss, N.F.; Osman, A. Synthesis of heterocycles-2. New routes to acetylthiadiazolines and arylazothiazoles. J. Heterocycl. Chem. **1980**, 17, 1713–1717.

44. Shawali, A.S.; Osman, A. Reaction of dimethylphenacylsulfonium bromide with N-nitrosoacetaryl-amides and reactions of the products with nucleophiles. Bull. Chem. Soc. Jpn. **1976**, 49, 321–324.

45. Shawali, A.S.; Osman, A. Synthesis and reactions of phenylcarbamoyl-arylhydrazidic chlorides. Tetrahedron **1971**, 27, 2517–2528.

46. Asiri, A.M.; Zayed, M.E.M.; Ng, S.W. Ethyl (Z)-2-chloro-2-(2-phenylhydrazin-1-ylidene)acetate. Acta Crystallogr. **2011**, 67, 1962.

47. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods **1983**, 65, 55–63.

48. Fu, L.; Feng, X.; Wang, J.-J.; Xun, Z.; Hu, J.-D.; Zhang, J.-J.; Zhao, Y.-W.; Huang, Z.-B.; Shi, D.-Q. Efficient Synthesis and Evaluation of Antitumor Activities of Novel Functionalized 1,8-Naphthyridine Derivatives. ACS Comb. Sci. **2015**, 17, 24–31.