Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(12):1263-1271

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Microwave assisted synthesis of novel heterocyclic compounds and evaluation of their biological activities

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ABSTRACT

*The synthesis of novel heterocyclic compounds**2a-d** have been carried out by the [2+2]cyloaddition of chloroketene to 3,4-dihydropyrimidone, **1a-d**. **3a & 3b** are synthesised by the [2+2] cycloaddition of dichloroketene to the dihydropyrimidone**1a&1c** respectively. The structures of these compounds have been confirmed on the basis of their elemental analysis and spectral data. The synthesised compounds **2a-d** and **3a&3b** were screened for their antimicrobial activity. They have been tested for antibacterial activity against Escherichia coli, Staphylococcus aureus, Shigella boydii, Aeromonas spp and Bacillus. subtilis and for antifungal activity against Aspergillus flavus, Aspergillus niger, Fusarium spp , Trichophyton mentagrophytes* and *Penicillium spp. The compounds **2b & 3b** were screened for their cytotoxic activity on Hep2 Cell line*

Keywords: Antimicrobial activity, antibacterial activity, antifungal activity, cytotoxic activity, Hep 2 Cell line.

INTRODUCTION

In 1986 the first chemists took advantage of microwaves for heating chemical reactions, in the same way they had been heating food for decades [1]. In 1986 Gedye, who worked at Laurentian University, published that reaction rates increased significantly when heated by a domestic Toshiba microwave (on power 7).The reaction presented included acid hydrolysis of an amide, KMnO4 oxidation, esterification and SN2 substitution[2]. Months later Giguere and Majetich reported the use of microwave ovens to perform pericyclic reactions[3]. Microwave heating has led to cleaner, faster, and often higher yielding reactions. During the last two decades, the activity in this new technique has experienced exponential growth and has been extensively reviewed [4]. Kappe and Dallinger have reported the impact of microwaves on drug discovery [5]. Even microwave-assisted reactions under solvent-free conditions promoted the synthesis of Zincke's salt and its conversion to chiral pyridinium salts in water [6]. Moreover, Varma and co-workers have reported the drug discovery by using aqueous microwave chemistry [7]. Microwave- assisted organic transformations using benign reaction media have also been reported [8, 9]. A survey of the literature shows that there is no report on the microwave assisted [2+ 2] cycloaddition reactions of ketenes to >C=C< of the 3,4-dihydropyrimidone. Hence In continuation of our work [10,11] on the reactions of 3,4-dihydropyrimidones, we report the synthesis of the novel heterocyclic compounds, **2(a-d)** by the microwave assisted [2+2] cycloaddition of chloroketene to the >C=C< of the 3,4-dihydropyrimidinones **1(a-d)**.The novel heterocyclic compounds **3(a-b)** were synthesised by the microwave assisted [2+2] cycloaddition of dichloroketene to the >C=C< of the 3,4-dihydropyrimidinones, **1a** and**1c.** We also report on the antimicrobial activity of the novel heterocyclic compounds, **2(a-d)** and **3(a-b)** and the cytotoxic activity of the novel heterocyclic compounds **2(b)& 3(b)**.

EXPERIMENTAL SECTION

All m.ps are uncorrected. The homogeneity of the compounds was checked by Thin Layer Chromatography (TLC) over silica gel. ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as an internal standard on a Bruker 300

spectrometer at 500 MHz.^{13}C NMR were recorded on a Bruker 300 spectrometer at 75 MHz and mass spectra on Jeol DX 303 spectrometer.

General procedure for the synthesis of 3,4-dihydropyrimidones , 1(a-d), by Grind Stone Method[10]
A mixture of urea, aromatic aldehyde and ethyl acetoacetae / acetyl acetone in the mole ratio 1.5: 1: 1 was ground in a mortar and pestle in the presence of catalytic amount of phosphoric acid (5 drops) for 30 minutes. The reaction mixture was kept aside for 30 minutes and then poured into a beaker containing ice cold water (50 ml). The product, **1(a-d)**,obtained was filtered, washed with water and dried. It was then recrystalised from ethanol.

General Procedure for the Synthesis of 2(a-d), under microwave Irradiation
To chloroacetyl chloride (0.01mole) in ice cold condition, added drops of triethylamine (0.01mole). The 3,4 - dihydropyrimidone **1(a-d),** (0.01mole) is then added and stirred well for10mins. To the mixture, ethanol (20ml) is added and then irradiated with microwave irradiation (300W) for 30 seconds. On evaporation of the solvent, the corresponding solid product, **2(a-d),** is obtained. This showed a new single spot on TLC. It was recrystalized from ethanol. The obtained products were characterized by means of spectral (^{1}H-NMR, ^{13}C-NMR & Mass spectrum) data and their melting points.

General Procedure for the Synthesis of 3(a-b), under microwave Irradiation
Thionyl chloride (0.01mole) was added to dichloroacetic acid (0.01 mole) in ice cold condition and stirred well for 10 mins. To this ice cold mixture, triethylamine (0.01mole) was added and stirred well for 30 mins. Then 3,4 - dihydropyrimidone **1(a** &**c)** (0.01mole) is added. The mixture is dissolved in dichloromethane and adsorbed on silica gel. The adsorbed mixture is then irradiated with microwave irradiation (180W) for 1 minute. The product formed, as evidenced by TLC, was extracted with dichloromethane. On evaporation of the solvent, the corresponding solid product, **3(a-b)**, is obtained. This showed a new single spot on TLC. It was recrystalized from ethanol. The obtained products were characterized by means of spectral (^{1}H-NMR, ^{13}C-NMR & Mass spectrum) data and their melting points.

Antimicrobial activity
In this work, we report the *in vitro* study of antimicrobial activity of the novel heterocyclic compounds, **2(a-d), 3a** and **3b** against Gram +ve bacteria (Staphylococcus aureus & Bacillus subtilis), Gram –ve bacteria (Escherichia coli, *Shigella boydii & Aeromonas spp*) and fungi (*Aspergillus flavus, Aspergillus niger, Fusarium spp* , *Trichophyton mentagrophytes* and *Penicillium spp.*).

Antimicrobial assay
Screening of antimicrobial activity was carried out in the following sequence:
(i) Preparation of nutrient agar (ii) Preparation of Mc Ferland standards (iii) Inoculums preparation. (iv) *In vitro* Antimicrobial Sensitivity Determination by Agar well diffusion method.

(i) Preparation of nutrient agar
The nutrient agar was prepared by dissolving beef extract(1.5g), peptone(0.5g), yeast extract (1.5g), sodium chloride (0.5g) and agar (1.5g) 100ml of distilled water. The pH was adjusted to 7.2 followed by sterilization in an autoclave at 121^{0}C / 15LB for 15 minutes. The sterile molten agar media was then cooled to 50^{0}C. About 15 ml of the media was poured on a sterile petri-plate and allowed to cool to room temperature.

(ii) Preparation of 0.5 Mc Ferland standards
0.5ml of solution A (1.175g of barium chloride in 100 ml of distilled water) was added to 99.5 ml of solution B (1 ml of 0.36 N sulphuric acid in 100 ml of distilled water) and mixed well with magnetic stirrer, then distributed in test tubes with a screw cap of the same size as those containing the bacterial/ fungal culture.

(iii) Preparation of bacterial and fungal inoculums.
The cooled sterile broth medium was poured into sterile petri-plates having a uniform depth of 4mm; this is equivalent to approximately 25 ml in a 90mm plate. Once the medium had solidified then the culture was inoculated on the medium. The turbidity of the culture was adjusted with sterile broth so as to correspond to 0.5 Mc Ferland standards. Immediately after standardisation, a sterile cotton swab was immersed in the bacterial/ fungal suspension and then rotated and compressed against the wall of the test tube, so as to remove the excess fluid.
(iv) *In vitro* Antimicrobial Sensitivity Determination by Agar well diffusion method.

In vitro antimicrobial sensitivity of the antibiotics and the test compounds synthesised were determined by well diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [12]. The well diffusion test was performed using medium, as per the procedure described by Magaldi *et al* [13] .A sterilized

10 mm cork borer was used to make agar wells on the sterile nutrient agar plates. 24 hours sub cultured bacteria/ fungi were inoculated in the petri-plates, with a sterile cotton swab. Compounds were dissolved in DMSO solvent separately and poured in the wells with varying concentrations ranging from from 50µL, 100µL, 150µL and 200µL, using a micropipette. 100% DMSO was used as a control. The plates were incubated for 24 hours at 37^0C. Antibiotic amphicillin /chloramphenicol was used as a reference antibacterial agent and Amphotericin-B were used as a reference antifungal agent.The tests were carried out in triplicates.

Cytotoxicity assay:

(a)Cell line and culture:

Hep2 cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at37 °C.

(b) Reagents:

MEM was purchased from Hi Media Laboratories. Fetal Bovine Serum (FBS) was purchased from Cistron laboratories. Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT) and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In order to study the antitumor activity of a new drug, it is important to determine the cytotoxicity concentration of the drug. Cytotoxicity tests define the upper limit of the extract concentration, which is non-toxic to the cell line. The concentration nontoxic to the cells is chosen for antiviral assay.

After the addition of the drug, cell death and cell viability was estimated. The result is confirmed by additional metabolic intervention experiment such as MTT assay.

(c) Procedure:

1. 48 hrs monolayer culture of Hep2 cells at a concentration of one lakh/ml/well (10cells/ml/well) seeded in 24 well titer plate.
2. The plates were microscopically examined for confluent monolayer, turbidity and toxicity if the cells become confluent.
3. The growth medium (MEM) was removed using micropipette. Care was taken so that the tip of the pipette did not touch the cell sheet.
4. The monolayer of cells was washed twice with MEM without FCS to remove the dead cells and excess FCS.
5. To the washed cell sheet, added 1 ml of medium (without FCS) containing defined concentration of the sample in respective wells.
6. Each dilution of the drug ranges from 1:1 to 1:64 and they were added to the respective wells of the 24 wells titer plates.
7. To the cell control wells added 1 ml MEM (w/o) FCS.
8. The plates were incubated at 37 °C in 5 % CO_2 environment and observed for cytotoxicity using inverted microscope

MTT Assay:

MTT is 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide.

(a)Principle:

MTT is cleaved by mitochondrial dehydrogenase in viable cells, yielding a measurable purple product formazan. This formazan production is proportionate to the viable cell number and inversely proportional to the degree of Cytotoxicity.

(b)Procedure:

i) After incubation for cytotoxicity, removed the medium from the wells carefully for MTT assay.
ii) Each well washed with MEM (w/o) FCS for 2-3 times. And added 200 µl of MTT conc. of (5 mg / ml).
iii) And incubated for 6-7 hrs in 5 % CO_2 incubator.
iv) After incubation added 1 ml of DMSO in each well and mixed by pipette and left aside for 45 sec.
v) If any viable cells are present,then formazan crystals are formed which after adding solublizing reagent (DMSO) shows a purple colour formation.
vi) The suspension is transferred in to the cuvette of spectrophotometer and an O.D values is read at570nm by taking DMSO as a blank.
vii) Graphs are plotted by taking conc. of the drug on X-axis and relative cell viability on Y-axis.

viii) % cell viability = A570 of treated cells / A570 of control cells × 100

RESULTS AND DISCUSSION

The reaction for different title compounds is outlined in **Scheme 1.** Physical data of the compounds are shown in **Table-1.** The structures of all the compounds (**2a – 2d**) and **3a & 3b** were supported by spectral data. The IR, ^{1}H NMR, ^{13}CNMR and mass spectra are in agreement with the proposed structures.

Scheme I

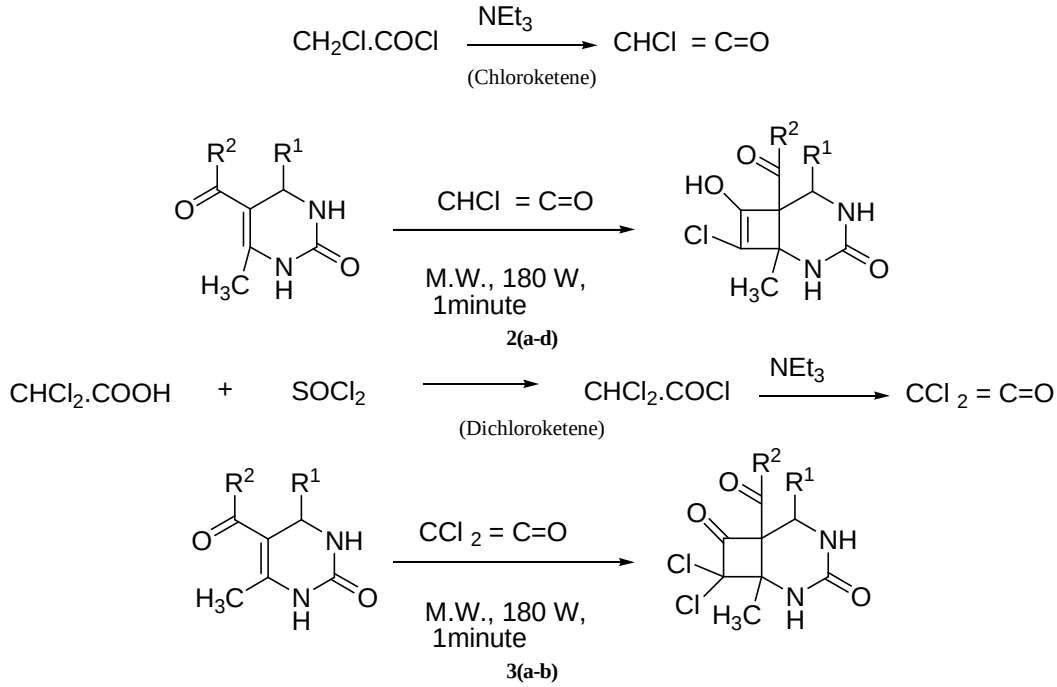
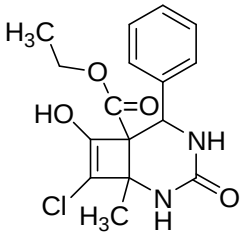


Table I: Physical data of compounds 2(a-d) & 3(a-b)

Entry	Product	R^1	R^2	Molecular Formula	Melting Point (^{0}C)	Yield (%)
1	**(2a)**	$-C_6H_5$	$-OC_2H_5$	$C_{16}H_{17}N_2O_4Cl$	195	81
2	**(2b)**	$-4CH_3.C6H5$	$-OC_2H_5$	$C_{17}H_{19}N_2O_4Cl$	212	80
3	**(2c)**	$-4OCH_3$. C6H5	$-OC_2H_5$	$C_{17}H_{19}N_2O_5Cl$	208	82
4	**(2d)**	-3OH. C6H5	$-OC_2H_5$	$C_{16}H_{17}N_2O_5Cl$	199	88
5	**(3a)**	-C6H5	$-OC_2H_5$	$C_{16}H_{16}N_2O_4Cl_2$	186	90
6	**(3b)**	$-4OCH_3$. C6H5	$-OC_2H_5$	$C_{17}H_{18}N_2O_5Cl_2$	175	91

Spectroscopic data 2a.



2a

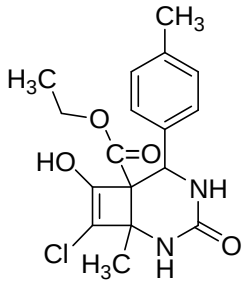
IR (KBr, cm^{-1}) : 2700-3700 (broad, -OH str, enolic), 3244 & 3116 (-NH str.), 1724 (-C=O str. of – COOEt), 1700(C=O str. of –CONH).

^{1}H NMR (400 MHz, $CDCl_3$ / TMS) δ (ppm) : 1.2 (t, 3H,J=7.2 Hz, methyl protons of –COOEt),1.6(bs, 1H, D_2O exchangeable NH proton) 2.3 (s, 3H $-CH_3$), 4.0(quartet, 2H, J= 3Hz,$-OCH_2$Protons of COOEt), 5.4 (s, 1H, methine Proton), 5.7(bs, 1H, D_2O exchangeable NH proton) 7.2 (m, 5H, aromatic Protons), 8.0 (bs, 1H, -OH, enolic)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) : 14, 19, 56(2C), 60(2C), 101, 127,128(2C), 129(2C), 144, 146, 166(2C).

Mass spectrum: m/z ($M^{+\cdot}$) = 336.

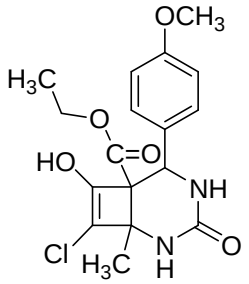
2b.



2b

IR (KBr, cm^{-1}): 2500-3600 (broad, -OH str, enolic), 3244 & 3116 (-NH str.), 1720 (-C=O str. of – COOEt), 1706(C=O str. of –CONH).
^{1}H NMR (400 MHz, $CDCl_3$ / TMS) δ (ppm) : 1.2 (t, 3H, J=7.0 Hz, methyl protons of –COOEt), 1.8(bs, 1H, D_2O exchangeable NH proton) 2.3 (s, 3H $–CH_3$), 3.8(s, 3H, $-CH_3$), 4.1(quartet, 2H, J= 7Hz, $-OCH_2$ protons of – COOEt), 5.4(s, 1H, methine proton), 6.0 (bs, 1H, D_2O exchangeable NH proton) 6.8(d, 2H, J=8Hz, aromatic proton), 7.2 (m, 2H, aromatic protons), 8.5 (bs, 1H, -OH, enolic)
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14, 19, 21, 55, 60, 77, 101, 114, 126, 128, 129,136, 138, 141, 146, 159, 166.
Mass spectrum: m/z ($M^{+\cdot}$ + 1) = 351.

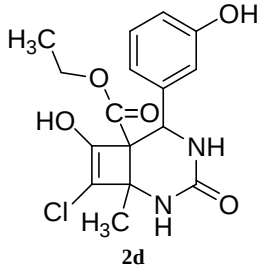
2c.



2c

IR (KB r, cm^{-1}): 2500-3600 (broad, -OH str, enolic), 3242 & 3111 (-NH str.), 1723 (-C=O str. of –COOEt), 1705(C=O str. of –CONH).
^{1}H NMR (400 MHz, $CDCl_3$ / TMS) δ (ppm) : 1.2 (t, 3H, J=7.0 Hz, methyl protons of –COOEt), 1.7(bs, 1H, D_2O exchangeable NH proton), 2.3 (s, 3H, $–CH_3$), 3.8 (s, 3H, $-OCH_3$), 4.1.(quartet, 2H, J=2.5Hz, OCH_2 protons of COOEt), 5.3 (s, 1H, methine proton), 5.9(bs, 1H, D_2O exchangeable NH proton), 6.8 (d, 2H, J = 8.5Hz, aromatic protons), 7.2(d, 1H, J = 8.5Hz, aromatic proton) 7.3 (s, 1H, aromatic proton), 8.4 (bs, 1H, enolic OH proton).
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14, 19, 55(2C), 60, 77, 102, 114(2C), 128(2C), 136(2C), 146, 154, 159, 166.
Mass spectrum: m/z ($M^{+\cdot}$) = 366.

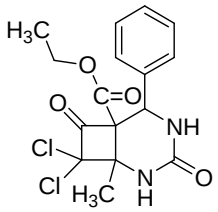
2d.



2d

IR (KBr, cm^{-1}): 2500-3600 (broad, -OH str, enolic), 3514 & 3351 (-NH str.), 1727 (-C=O str. of –COOEt), 1708(C=O str. of –CONH).
^{1}H NMR (400 MHz, $CDCl_3$ / TMS) δ (ppm): 1.2 (t, 3H, J=7.0 Hz, methyl protons of COOEt), 1.6(bs, 2H, D_2O exchangeable NH protons), 2.3(s, 3H $–CH_3$), 3.1(quartet, 2H, J=7.5Hz, OCH_2 protons of COOEt), 5.4 (s, 1H, methine proton), 6.6(bs, 1H, enolic OH proton), 7.2(m, 4H, aromatic protons), 7.5 (bs,1H, phenolic OH proton).
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) : 14, 18, 21, 55, 60, 102, 126, 129, 130, 137, 140, 145, 154, 165, 168.
Mass spectrum: m/z ($M^{+\cdot}$) = 352.34.

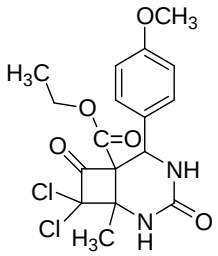
3a.



3a

IR (KBr, cm^{-1}): 3244 &3114 (-NH str.), 1724 (-C=O str. of – COOEt), 1702(C=O str.), 1649(-C=O str. of – CONH).
^{1}H NMR (400 MHz, $CDCl_3$ / TMS) δ (ppm): 1.2 (t, 3H, J=7.5 Hz, methyl protons of –COOEt), 2.38 (s, 3H $–CH_3$), 4.1(quartet, 2H, J= 3Hz, methylene protons of –COOEt), 5.40 (s, 1H, methine proton), 5.95 (s, 2H, D_2O exchangeable NH protons), 7.3 (m, 5H, aromatic protons).
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14, 18, 46, 55, 60, 65, 102,127, 128, 130, 143, 145, 155, 165, 168, 170.
Mass spectrum: m/z ($M^{+\cdot}$) = 370.7659

3b.



3b

IR (KBr, cm^{-1}): 3364 &3244 (-NH str.), 1724 (-C=O str. of – COOEt), 1705(C=O str.), 1650(-C=O str. of - CONH).
^{1}H NMR (400 MHz, $CDCl_3$ / TMS) δ (ppm): 1.2 (t, 3H, J=7.0 Hz, methyl protons of –COOEt), 2.35 (s, 3H $–CH_3$), 3.79 (s, 3H, $-OCH_3$), 4.1 (quartet, 2H, J= 3Hz, methylene protons of –COOEt), 5.375 (s, 1H, methine proton), 5.96 (s, 2H, D_2O exchangeable NH protons), 6.86 (quartet, 2H, J = 2Hz, aromatic protons), 7.24 (m, 2H, aromatic protons).
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 14, 18, 46, 55, 60, 65, 77, 102, 114 (2C), 128, 136, 145, 155, 159, 165, 168.
Mass spectrum: m/z ($M^{+\cdot}$) = 400

Biological activities
The results of *in vitro* study of antimicrobial activity of **2(a-d) & 3(a** & **b)** against each of the five bacterial species (*E.coli, Staphylococcus aureus, Shigella boydii, Aeromonas spp* and *B.subtilis*) are reported in the **Table- 2**

Table-2

S. No.	Organisms (Bacteria)	Zone of Inhibition (mm)					
		2a (Std.)[a]	**2b (Std.)[a]**	**2c (Std.)[a]**	**2d (Std.)[a]**	**3a (Std.)[b]**	**3b (Std.)[b]**
1	*E.coli*	0.6 (1.9)	0.5 (0.9)	0.8 (1.2)	0.4 (0.9)	10 (21)	10 (21)
2	*Staphylococcus aureus*	1.1 (2.1)	0.8 (1.4)	0.7 (1.8)	0.9 (1.9)	7 (21)	- (21)
3	*Shigella boydii*	0.9 (1.8)	0.6 (1.2)	0.4 (1.0)	0.4 (0.9)	- (20)	8 (20)
4	*Aeromonas spp*	1 (3)	- (3)	2 (5)	3 (5)	8 (23)	8 (23)
5	*Bacillus subtillus*	- (4)	- (1)	- (5)	- (2)	7 (19)	7 (19)

***(Std)[a]** = Amphicillin. **(Std)[b]** = Chloromphenicol. - = No zone of Inhibition.*

The foregoing data shows that **2(a, c** &**d)**, are moderately active against,*E.coli, Staphylococcus aureus, Shigella boydii* and *Aeromonas spp.* .They are resistant to *B.subtilis.* **2b** is moderately active against *E.coli, Staphylococcus aureus, Shigella boydii* .It is resistant against *Aeromonas spp* and *B.subtilis.* **3a** is moderately active against *Aeromonas spp, E.coli, B.subtilis* and *Staphylococcus aureus* .It is resistant to *Shigella boidii.* **3b** is moderately active against *Aeromonas spp, Shigella boidii, E.coli, B.subtilis* and it is resistant to *Staphylococcus aureus*

The results of the antifungal activity of the tested novel compounds against five fungi (*Aspergillus flavus, Aspergillus niger, Fusarium spp* , *Trichophyton mentagrophytes* and *Penicillium spp.*).are summerised in the **Table -3**.

Table-3

S. No	Organisms (Fungi)	Zone of Inhibition (mm)					
		2a (Std.)	2b (Std.)	2c (Std.)	2d (Std.)	3a (Std.)	3b (Std.)
1	*Fusarium spp*	- (-)	- (-)	- (-)	- (-)	8 (6)	- (6)
2	*Trichophyton mentazorophytes*	- (-)	- (-)	- (-)	- (-)	7 (17)	8 (17
3	*Aspergillus flavus*	- (-)	- (-)	- (-)	- (-)	8 (12)	7 (12)
4	*Pencillium spp*	- (-)	- (-)	- (-)	- (-)	6 (10)	9 (10)
5	*Aspergillus niger*	- (2)	- (1)	- (2)	- (2)	12 (14)	- (14)

The foregoing data shows that **2(a-d),** are resistant to all the five fungi. **3a,** is highly active against *Fusarium spp* and *Aspergillus niger* and moderately active against *Aspergillus flavus* ,*Trichophyton mentagrophytes* and *Penicillium spp.***3b**, is highly active against *Penicillium spp* and moderately active against *Aspergillus flavus* and *Trichophyton mentagrophytes* It is resistant to *Aspergillus niger* and *Fusarium spp* .

Cytotoxic activity

The data for the anticancer effect of compound **2b** is shown in **Table-4** and for compound **3b** is shown in **Table-5.** The MTT assay for compound **2b** is shown in **Figure-1** and for compound **3b** is shown in **Figure-2.**

Table-4

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.11	20.00
2	500	1:1	0.19	34.54
3	250	1:2	0.24	43.63
4	125	1:4	0.29	52.72
5	62.5	1:8	0.35	63.63
6	31.2	1:16	0.40	72.72
7	15.6	1:32	0.46	83.63
8	7.8	1:64	0.54	98.18
9	Cell control	-	0.55	100

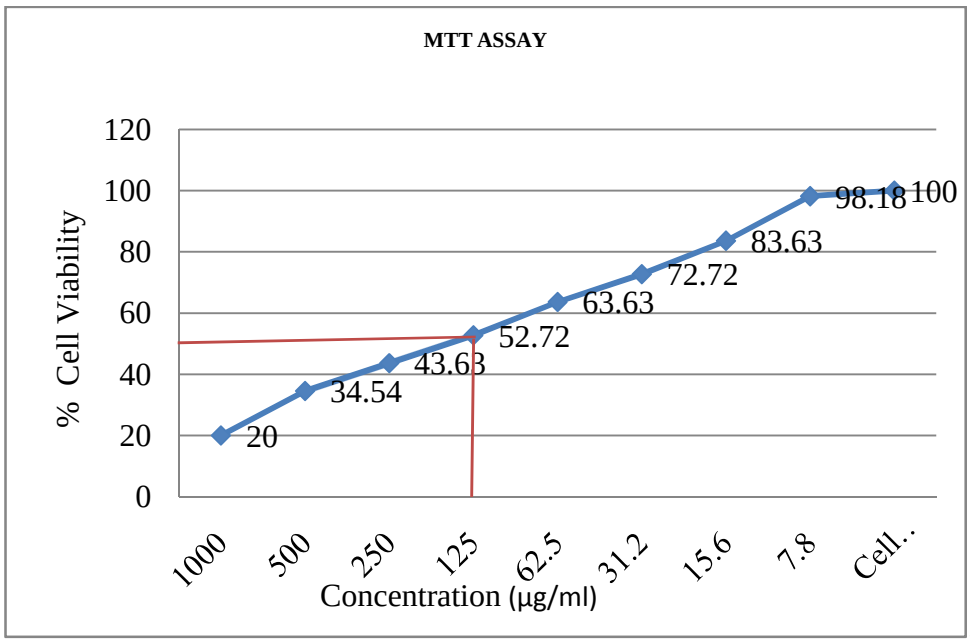


Figure – 1

Table-5

S. No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.07	12.72
2	500	1:1	0.13	23.63
3	250	1:2	0.20	36.36
4	125	1:4	0.27	49.09
5	62.5	1:8	0.36	65.45
6	31.2	1:16	0.42	76.36
7	15.6	1:32	0.47	85.45
8	7.8	1:64	0.53	96.36
9	Cell control	-	0.55	100

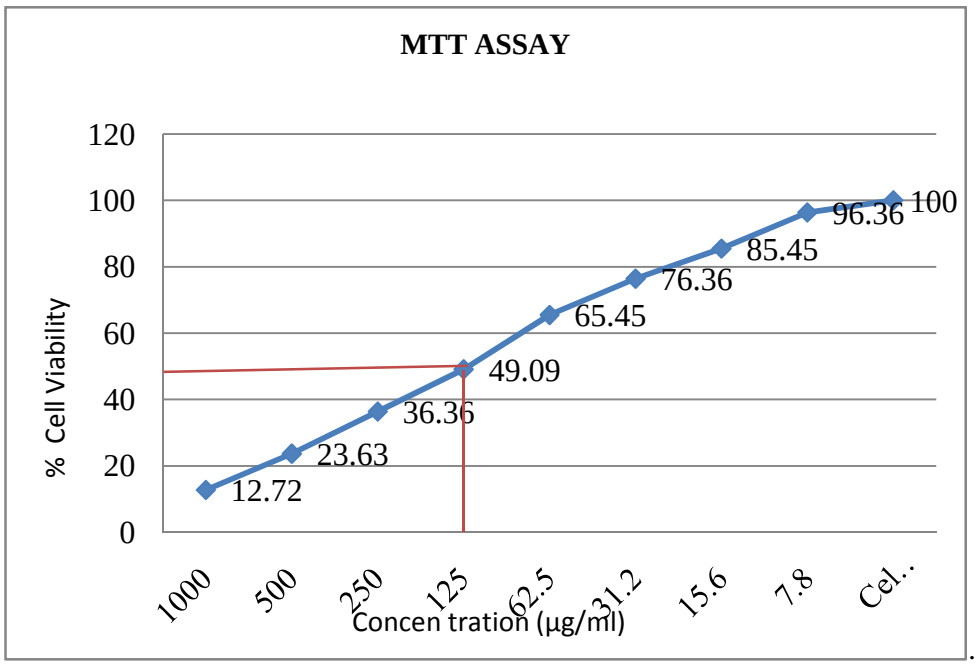


Figure-2

From the foregoing data it is seen that the novel heterocyclic compounds (**2b & 3b),** showed cytotoxic activity positively. Most of the cells were arrested, at the middle concentration taken, during cell and the cell nuclei became condensed and segmented after 48 h incubation which is the indication of apoptosis. The results from light microscope displayed morphological abnormality of the cells after treatment with the heterocyclic organic compounds. On the contrary, the untreated cells (control) did not show these apoptotic characteristics. Most of the condensed and segmented nuclei degraded after 48 h incubation. With these data, one can conclude a heterocyclic ring-dependent activity of the studied heterocyclic organic compounds on the Hep-2 cells. Heterocyclic organic compounds bearing different hetero atoms present different cytotoxic activities.

We observed that the efficacy of heterocyclic organic compounds (**2b & 3b**) at low to medium concentration was quite dependent on the specific cell type. Further experiments to understand the molecular mechanisms underlining the differences would be greatly important to guide the clinical application of these heterocyclic organic compounds. Additional studies are required to understand the molecular basis for this differential response to enhance the effectiveness of heterocyclic organic compounds (**2b** and **3b**) in the treatment of patients with malignant disease.

CONCLUSION

In summary, we have developed a simple, efficient and more eco-friendly method for the synthesis of the novel heterocyclic compounds **2a-d** and **3a & 3b** from3,4-dihydropyrimidinone,**1a-d** using novel [2+2] cycloaddition of ketenes to the >C=C< of the 3,4-dihydropyrimidone. The notable advantages of this method include no usage of solvent (except for recrystallization), simple reaction profile, shorter reaction time and high yields .The antimicrobial searching suggests that all the newly synthesized compounds showed moderate to very good activity against the tested organisms. Among the compounds, **2b** & **3b** showed the most promising cytotoxic activity, suggesting further work with similar analogues.

Acknowledgements
The authors thank the management of The New College, Chennai, India, for providing the necessary facilities and support. Sincere thanks are also due to SAIF, IIT Chennai, India, for recording spectral data.

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