DESIGN, SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW 1,3-OXAZOLE DERIVATIVES AS ANTICANCER AGENTS AGAINTS LIVER CANCER CELL LINE

Corresponding (AUTHOR)

Seeba Anjum

Student

Shri Ram Group of Institutions, Faculty of Pharmacy, Jabalpur

EMAIL: s483105a@gmail.com

CONTACT NO: 8889747863

Dr. Arun Patel

Principal (Shri Ram Group of Institutions,Faculty of Pharmacy, Jabalpur)

Mr. Shailendra Patel

Associate Professor (Shri Ram Group of Institutions, Faculty of Pharmacy, Jabalpur)

ABSTRACT

The purpose of this work is to design, synthesized and pharmacological evaluation of new 1,3-oxazole derivative for the treatment of cancer cells, specially against liver cancer cells. One of the main causes of sickness and mortality worldwide is cancer, which has long been associated with humankind's curse. Chemotherapy continues to be considered the preferred treatment for cancer despite the variety of therapeutic options available. Unfortunately, a number of anticancer drugs that are now on the market have caused catastrophic side effects in addition to multidrug resistance. As a result, significant efforts have been made to find novel, active medications with enhanced anticancer activity and fewer side effects. Medicinal chemists have been paying close attention to the advent of heterocyclic ring-containing anticancer drugs in recent years. As a versatile heterocyclic molecule, 1,3-oxazole and its derivatives have broad-spectrum pharmacological capabilities, including anticancer activity through numerous mechanisms against cancer cell lines that are drug-susceptible, drug-resistant, and even multidrug-resistant.

The 1,3-oxazole moiety is therefore a helpful template for the creation of brand-new anticancer drugs. The current state of research on 1,3-oxazole derivatives with prospective therapeutic uses as anticancer drugs will be thoroughly reviewed in this study, with an emphasis on the compounds' chemical structures, anticancer activity, and mechanisms of action. Using a 1,3-oxazole derivative, 12 compounds were designed, produced, and evaluated according to different chemical properties.

Keywords:

1,3 – Oxazole, Anticancer Drug, Heterocyclic, Pharmacological Evaluation, Drug Resistant

INTRODUCTION

Liver cancer

Liver cancer is the growth and spread of unhealthy cells in the liver. Cancer that starts in the liver is called primary liver cancer. Cancer that spreads to the liver from another organ is called metastatic liver cancer. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer.

Hepatocellular carcinoma: Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults, and is the most common cause of death in people with cirrhosis. It occurs in the setting of chronic liver inflammation, and is most closely linked to chronic viral hepatitis infection (hepatitis B or C) or exposure to toxins such as alcohol or Aflatoxin.

Types of liver cancer

Liver cancer is divided into two types

A. Primary liver cancer

B. Secondary liver cancer

A) Primary liver cancer: In primary liver cancer, the cancer originates in the liver.

- Hepatocellular carcinoma (HCC)
- Cholangiocarcinoma (bile duct cancer)
- Gallbladder cancer
- Fibrolamellar
- Angiosarcoma

B) Secondary liver cancer: A secondary cancer is when cancer cells break away from where the cancer started and grow elsewhere in the body.

Stage I: The tumor is in the liver and has not spread to another organ or location

Stage II: Either there are several small tumors that all remain in the liver, or one tumor that has reached a blood vessel

Stage III: There are various large tumors or one tumor that has reached the main blood vessels. Cancer may have also reached the gallbladder

Stage IV: The cancer has metastasized. This means that it has spread to other parts of the body.

A sufficiently large number of chemical compounds – tubulin polymerization inhibitors that bind to tubulin using the colchicine binding site were synthesized and tested recently. These compounds include oxazole, thiazole, and benzimidazole derivatives.

Heterocyclic systems are a part of large number of drugs and biologically relevant molecules. Often the presence of hetero atoms or groupings imparts preferential specificities in their biological responses. The chemistry and biological study of heterocyclic compounds has been interesting field for a long time and oxazole is one such moiety which has gained attention in recent times due to its increasing importance in the field of medicinal chemistry.

Oxazole is a doubly unsaturated 5-membered ring having one oxygen atom at position 1 and a nitrogen at position 3 separated by a carbon in-between. It was first prepared in 1947, has a boiling point of 69°C and is a stable liquid at room temperature.

Substitution pattern in oxazole derivatives play a pivotal role in delineating the biological activities like antimicrobial, anticancer, antitubercular, anti-inflammatory, antidiabetic, antiobesity and antioxidant etc. Oxazole and its derivatives are a part of number of medicinal compounds which includes aleglitazar (antidiabetic), ditazole (platelets aggregation inhibitor), mubritinib (tyrosine kinase inhibitor), and oxaprozin (COX-2 inhibitor).

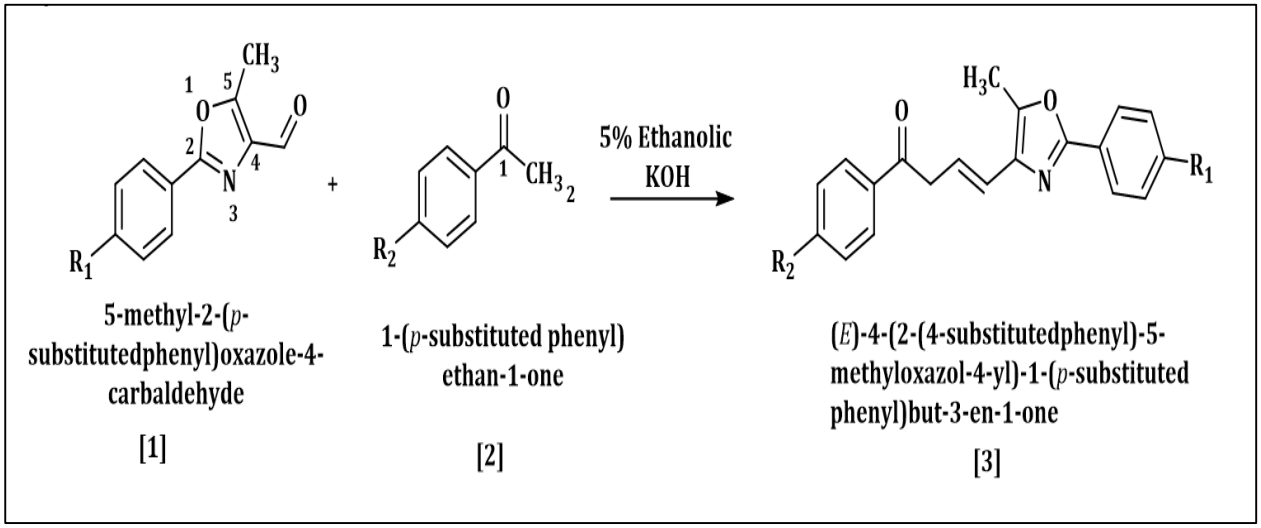
MATERIAL AND METHOD

The 5-methyl-2-(p-tolyl)oxazole-4-carbaldehyde, 2-(4-ethylphenyl)-5-methyl oxazole-4-carbaldehyde, 1-(4-nitrophenyl)ethan-1-one, 1-(4-chlorophenyl) ethan-1-one, 1-(4-bromophenyl)ethan-1-one, 1-(4-aminophenyl)ethan-1-one, 1-(4-ethylphenyl)ethan-1-one, 1-(p-tolyl)ethan-1-one purchased from sigma Aldrich. All the chemicals were purchased from Merck India. Commercial grade solvents used for the reactions were distilled before use.

Synthesis scheme

Synthesis of new imidazole derivatives involves the following step.

Synthesis of (E)-4-(2-(4-substituted phenyl)-5-methyloxazol-4-yl)-1-(p-substituted phenyl)but-3-en-1-one



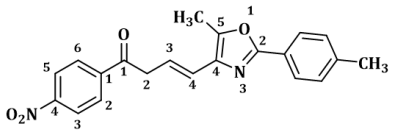
Procedure:

To a magnetically stirred mixture of 1-(4-substitutedphenyl)ethan-1-one (0.01M) in ethanolic sodium hydroxide in a 250 ml round bottom flask, a solution of 5-substituted-2-(p-substituted phenyl)oxazole-4-carbaldehyde (0.01M) in 20 ml ethanol was added drop wise using addition funnel during 20-30 min at room temperature. The reaction mixture was heated in water bath at 45–50°C and the reaction was continued for further 2 h to complete the reaction (TLC: 10%, ethyl acetate and petroleum ether). The reaction mixture was then poured into ice cold water to precipitate the product as yellow solid, which was then filtered, dried and crystallized from ethanol. Yield: 76–81%. Table 1 shows the quantity of chemicals taken.

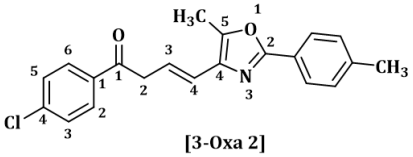
Table 1: Quantity of chemicals taken

S No	Name of Chemicals	Mol. formula	Mol. Weight	Quant(gm)
1	5-methyl-2-(p-tolyl)oxazole-4-carbaldehyde	$C_{12}H_{11}NO_2$	201.23	2.01
2	2-(4-ethylphenyl)-5-methyloxazole-4-carbaldehyde	$C_{13}H_{13}NO_2$	215.25	2.15
3	1-(4-nitrophenyl)ethan-1-one	$C_8H_7NO_3$	165.15	1.65
4	1-(4-chlorophenyl)ethan-1-one	C_8H_7ClO	154.59	1.54
5	1-(4-bromophenyl)ethan-1-one	C_8H_7BrO	199.05	1.99
6	1-(4-aminophenyl)ethan-1-one	C_8H_9NO	135.17	1.35
7	1-(4-ethylphenyl)ethan-1-one	$C_{10}H_{12}O$	148.21	1.48
8	1-(p-tolyl)ethan-1-one	$C_9H_{10}O$	134.18	1.34

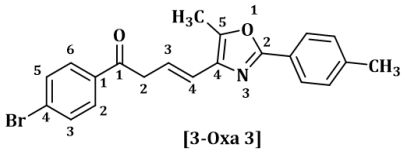
Structure Of Synthesized Compounds



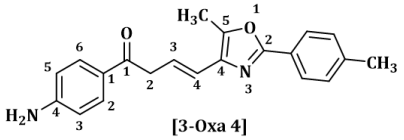
[3-Oxa 1]



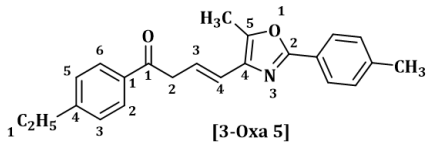
[3-Oxa 2]



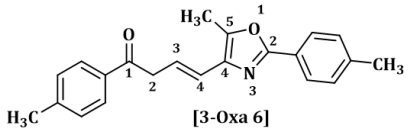
[3-Oxa 3]



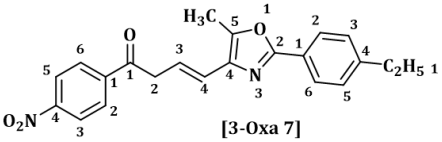
[3-Oxa 4]



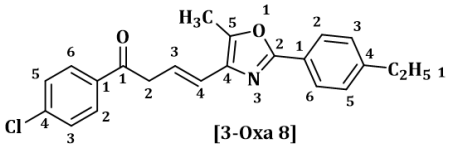
[3-Oxa 5]



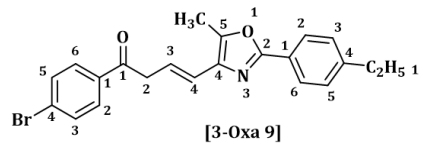
[3-Oxa 6]



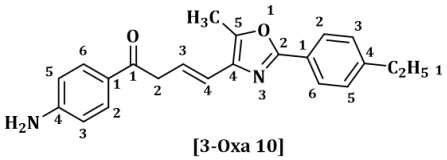
[3-Oxa 7]



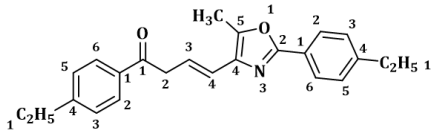
[3-Oxa 8]



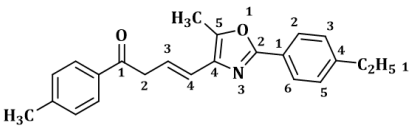
[3-Oxa 9]



[3-Oxa 10]



[3-Oxa 11]



[3-Oxa 12]

Table 2: Characterization of the synthesized compounds

S. No.	Code	Chemical formula	Mol. Weight	Percent Yield	Melting point
1	3-Oxa1	$C_{21}H_{18}N_2O_4$	362.39	78	142-144°C
2	3-Oxa2	$C_{21}H_{18}ClNO_2$	351.83	82	136-138°C
3	3-Oxa3	$C_{21}H_{18}BrNO_2$	396.28	76	155-157°C
4	3-Oxa4	$C_{21}H_{20}N_2O_2$	332.40	75	133-135°C
5	3-Oxa5	$C_{23}H_{23}NO_2$	345.44	73	114-116°C
6	3-Oxa6	$C_{22}H_{21}NO_2$	331.42	68	120-122°C
7	3-Oxa7	$C_{22}H_{20}N_2O_4$	376.41	62	115-117°C
8	3-Oxa8	$C_{22}H_{20}ClNO_2$	365.86	80	132-134°C
9	3-Oxa9	$C_{22}H_{20}BrNO_2$	410.31	85	142-144°C
10	3-Oxa10	$C_{22}H_{22}N_2O_2$	363.43	80	162-164°C
11	3-Oxa11	$C_{24}H_{25}NO_2$	359.47	75	124-126°C
12	3-Oxa12	$C_{23}H_{23}NO_2$	345.44	78	118-120°C

List of Synthesized compounds: A total of twelve compounds were synthesized and purified using column chromatography. The IR, 1HNMR, 13CNMR spectroscopy, mass, and elemental analyses were used to analyse all compounds (Table 3).

Table 3: List of Final synthesized compounds

S. No.	Code	Chemical name
1	3-Oxa1	(E)-4-(5-methyl-2-(p-tolyl)oxazol-4-yl)-1-(4-nitrophenyl)but-3-en-1-one
2	3-Oxa2	(E)-1-(4-chlorophenyl)-4-(5-methyl-2-(p-tolyl)oxazol-4-yl)but-3-en-1-one
3	3-Oxa3	(E)-1-(4-bromophenyl)-4-(5-methyl-2-(p-tolyl)oxazol-4-yl)but-3-en-1-one
4	3-Oxa4	(E)-1-(4-aminophenyl)-4-(5-methyl-2-(p-tolyl)oxazol-4-yl)but-3-en-1-one
5	3-Oxa5	(E)-1-(4-ethylphenyl)-4-(5-methyl-2-(p-tolyl)oxazol-4-yl)but-3-en-1-one

6	3-Oxa6	(E)-4-(5-methyl-2-(p-tolyl)oxazol-4-yl)-1-(p-tolyl)but-3-en-1-one
7	3-Oxa7	(E)-4-(2-(4-ethylphenyl)-5-methyloxazol-4-yl)-1-(4-nitrophenyl)but-3-en-1-one
8	3-Oxa8	(E)-1-(4-chlorophenyl)-4-(2-(4-ethylphenyl)-5-methyloxazol-4-yl)but-3-en-1-one
9	3-Oxa9	(E)-1-(4-bromophenyl)-4-(2-(4-ethylphenyl)-5-methyloxazol-4-yl)but-3-en-1-one
10	3-Oxa10	(E)-1-(4-aminophenyl)-4-(2-(4-ethylphenyl)-5-methyloxazol-4-yl)but-3-en-1-one
11	3-Oxa11	(E)-1-(4-ethylphenyl)-4-(2-(4-ethylphenyl)-5-methyloxazol-4-yl)but-3-en-1-one
12	3-Oxa12	(E)-4-(2-(4-ethylphenyl)-5-methyloxazol-4-yl)-1-(p-tolyl)but-3-en-1-one

Qualitative analysis

a) Melting point: Open capillary tubes were used to determine the melting point of the produced compounds, as stated in Table 4.

b) Solubility: At room temperature (18-300°C), the solubility of the produced product was tested in various solvents. Table 5 shows the solubility of the produced chemical.

c) Bromine test for un-saturation: With continual shaking, dissolve the synthesized final chemical in a suitable solvent and add 4-5 drops of bromine water. The presence of a brown color discharge indicates that the chemical was unsaturated and that a double bond was present. Wherever possible, the synthesized compounds were submitted to qualitative analyses for nitrogen, sulphur, and halogen. Elemental vario EL III Carlo-Erba 1108 was used for quantitative examination of nitrogen, oxygen, and sulphur.

d) IR spectra were recorded on Bruker alpha-II software.

e) NMR spectra were recorded on C13 Advance Brucker DRX 400 MHz spectrometer and for 13C NMR at 100 MHz.

f) Mass spectra were recorded on Jeol-sx 102/DA-6000 mass spectrometer using fast moving bombardment (FAB) technique.

Table 4: Physicochemical properties of the synthesized compounds

S No	Code	Chemical formula	Mol. Weight	Percent Yield	Melting point
1	3-Oxa1	$C_{21}H_{18}N_2O_4$	362.39	78	142-144°C
2	3-Oxa2	$C_{21}H_{18}ClNO_2$	351.83	82	136-138°C
3	3-Oxa3	$C_{21}H_{18}BrNO_2$	396.28	76	155-157°C
4	3-Oxa4	$C_{21}H_{20}N_2O_2$	332.40	75	133-135°C
5	3-Oxa5	$C_{23}H_{23}NO_2$	345.44	73	114-116°C
6	3-Oxa6	$C_{22}H_{21}NO_2$	331.42	68	120-122°C
7	3-Oxa7	$C_{22}H_{20}N_2O_4$	376.41	62	115-117°C
8	3-Oxa8	$C_{22}H_{20}ClNO_2$	365.86	80	132-134°C
9	3-Oxa9	$C_{22}H_{20}BrNO_2$	410.31	85	142-144°C
10	3-Oxa10	$C_{22}H_{22}N_2O_2$	346.43	80	162-164°C
11	3-Oxa11	$C_{24}H_{25}NO_2$	359.47	75	124-126°C
12	3-Oxa12	$C_{23}H_{23}NO_2$	345.44	78	118-120°C

Table 5: Solubility Data of Synthesized Compounds

S No	Code No.	Cool Water	Hot Water	Ethyl Alcohol	Ether	Acetone	Chloroform	DMSO
1	3-Oxa1	-	-	+++	++	-	-	+++
2	3-Oxa2	-	-	+++	++	+	-	+++
3	3-Oxa3	-	-	+++	++	+	+	+++
4	3-Oxa4	-	-	+++	++	++	-	+++
5	3-Oxa5	-	-	+++	++	++	-	+++
6	3-Oxa6	-	-	+++	++	+	-	+++
7	3-Oxa7	-	-	+++	++	+	+	+++
8	3-Oxa8	-	-	+++	++	-	-	+++
9	3-Oxa9	-	-	+++	++	+	-	+++
10	3-Oxa10	-	-	+++	++	++	-	+++
11	3-Oxa11	-	-	+++	++	-	-	+++
12	3-Oxa12	-	-	+++	++	+	-	+++

- Characterization of the synthesized compounds by IR, NMR, mass and elementary analysis

1. **Compound code: 3-Oxa1**

IR (cm^{-1}): 3052, 1656, 1612, 1580, 1125, 735

^{1}HNMR (400 MHz, δ ppm): 2.56 (3H, s, -CH_3), 2.52 (3H, s, -CH_2), 7.45 (2H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.97 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 119.8, 121.5, 140.7, 127.5, 125.2, 129.9, 131.0, 132.2, 133.1, 135.7, 150.8, 157.6, 186.7, 10.8 (CH_3); 10.6 (CH_3), 37.8 (CH_2), 53.2 (CH)

FAB Mass (m/z): 362.30

2. **Compound code: 3-Oxa2**

IR (cm^{-1}): 3052, 1658, 1615, 1578, 1125, 735

^{1}HNMR (400 MHz, δ ppm): 2.53 (3H, s, -CH_3), 2.52 (3H, s, -CH_2), 7.52 (2H, m, Ar-H), 7.60 (2H, m, Ar-H), 7.69 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.95 (2H, m, Ar-H), 8.00 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 10.8 (-CH_3); 10.6 (-CH_3), 37.8 (-CH_2-), 53.2 (-CH-), 21.7 (-CH_3), 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7

FAB Mass (m/z): 351.80

3. **Compound code: 3-Oxa3**

IR (cm^{-1}): 3048, 1662, 1615, 1585, 1125, 735

^{1}HNMR (400 MHz, δ ppm): 2.52 (3H, s, -CH_3), 2.50 (3H, s, -CH_2), 7.45 (2H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.65 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.90 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 120.8, 125.3, 145.2, 127.6, 128.5, 130.5, 132.5, 130.5, 132.5, 134.6, 136.2, 150.3, 157.5, 188.5, 37.5 (CH_2), 53.2 (CH), 21.7 (CH_3),

FAB Mass (m/z): 396.20

4. **Compound code: 3-Oxa4**
   IR (cm^{-1}): 3045, 1658, 1612, 1585, 1125, 735
   ^{1}HNMR (400 MHz, δ ppm): 2.52 (3H, s, -CH_3), 2.48 (3H, s, -CH_2), 7.42 (2H, m, Ar-H), 7.60 (2H, m, Ar-H), 7.65 (1H, d, -CH=CH-), 7.70 (1H, d, -CH=CH-), 7.92 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)
   ^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 120.5, 126.1, 142.6, 127.5, 128.0, 130.0, 131.5, 132.1, 133.9, 135.6, 135.7, 150.8, 159.2, 187.8, 37.5 (CH_2), 53.0 (CH), 21.5 (CH_3),
   FAB Mass (m/z): 332.30
5. **Compound code: 3-Oxa5**
   IR (cm^{-1}): 3049, 1658, 1612, 1578, 1125, 737
   ^{1}HNMR (400 MHz, δ ppm): 2.56 (3H, s, -CH_3), 2.50 (3H, s, -CH_3), 7.42 (2H, m, Ar-H), 7.60 (2H, m, Ar-H), 7.68 (1H, d, -CH=CH-), 7.72 (1H, d, -CH=CH-), 7.95 (2H, m, Ar-H), 8.01 (2H, m, Ar-H)
   ^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7, 10.8 (-CH_3); 10.6 (-CH_3), 37.8 (-CH_2-), 53.2 (-CH-), 21.7 (-CH_3),
   FAB Mass (m/z): 345.00
6. **Compound code: 3-Oxa6**
   IR (cm^{-1}): 3050, 1660, 1610, 1580, 1127, 739
   ^{1}HNMR (400 MHz, δ ppm): 2.55 (3H, s, -CH_3), 2.50 (3H, s, -CH_3), 7.45 (2H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.78 (1H, d, -CH=CH-), 7.95 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)
   ^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7, 10.7 (CH_3), 10.8 (CH_3); 37.8 (CH_2), 53.2 (CH), 21.7 (CH_3)
   FAB Mass (m/z): 331.40
7. **Compound code: 3-Oxa7**
   IR (cm^{-1}): 3050, 1660, 1610, 1580, 1127, 739
   ^{1}HNMR (400 MHz, δ ppm): 2.55 (3H, s, -CH_3), 2.52 (3H, s, -CH_2), 7.45 (2H, m, Ar-H), 7.63 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.95 (2H, m, Ar-H), 8.04 (2H, m, Ar-H)
   ^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7, 10.6 (CH_3), 37.8 (CH_2), 53.2 (CH), 21.7 (CH_3)
   FAB Mass (m/z): 376.20
8. **Compound code: 3-Oxa8**
   IR (cm^{-1}): 3050, 1660, 1610, 1580, 1127, 739
   ^{1}HNMR (400 MHz, δ ppm): 2.55 (3H, s, -CH_3), 2.52 (3H, s, -CH_3), 7.45 (2H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.97 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)
   ^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7, 10.8 (-CH_3); 37.8 (-CH_2-), 53.2 (-CH-), 21.7 (-CH_3)
   FAB Mass (m/z): 365.80

9. Compound code: 3-Oxa9

IR (cm^{-1}): 3050, 1660, 1610, 1580, 1127, 739

^{1}HNMR (400 MHz, δ ppm): 2.57 (3H, s, -CH_3), 2.55 (3H, s, -CH_2), 7.47 (2H, m, Ar-H), 7.67 (2H, m, Ar-H), 7.72 (1H, d, -CH=CH-), 7.78 (1H, d, -CH=CH-), 7.99 (2H, m, Ar-H), 8.04 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 10.8 (-CH_3); 10.6 (-CH_3), 37.8 (-CH_2-), 53.2 (-CH-), 21.7 (-CH_3), 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7

FAB Mass (m/z): 410.28

10. Compound code: 3-Oxa10

IR (cm^{-1}): 3050, 1660, 1610, 1580, 1127, 739

^{1}HNMR (400 MHz, δ ppm): 2.55 (3H, s, -CH_3), 2.52 (3H, s, -CH_3), 7.45 (2H, m, Ar-H), 7.65 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.97 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 121.1, 125.3, 145.7, 128.0, 128.2, 130.1, 131.9, 132.3, 134.1, 136.7, 136.8, 151.8, 159.6, 188.7, 10.6 (-CH_3), 37.8 (-CH_2-), 53.2 (-CH-), 21.7 (-CH_3)

FAB Mass (m/z): 346.40

11. Compound code: 3-Oxa11

IR (cm^{-1}): 3052, 1662, 1615, 1585, 1125, 730

^{1}HNMR (400 MHz, δ ppm): 2.55 (3H, s, -CH_3), 2.52 (3H, s, -CH_2), 7.45 (2H, m, Ar-H), 7.60 (2H, m, Ar-H), 7.75 (1H, d, -CH=CH-), 7.72 (1H, d, -CH=CH-), 7.92 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 120.1, 124.3, 142.7, 125.0, 128.0, 132.5, 134.5, 136.2, 151.0, 159.0, 188.7, 10.7 (CH_3); 21.6 (CH_3); 10.5 (CH_3); 37.2 (CH_2), 53.8 (CH),

FAB Mass (m/z): 359.40

12. Compound code: 3-Oxa12

IR (cm^{-1}): 3045, 1658, 1607, 1575, 1120, 735

^{1}HNMR (400 MHz, δ ppm): 2.55 (3H, s, -CH_3), 2.52 (3H, s, -CH_2), 7.45 (2H, m, Ar-H), 7.62 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.97 (2H, m, Ar-H), 8.02 (2H, m, Ar-H)

^{13}CNMR (100 MHz, $CDCl_3$, δ ppm): 121.3, 145, 127.5, 132.0, 132.5, 134.2, 136.5, 151.0, 159.2, 188.7, 10.8 (-CH_3), 37.6 (-CH_2-), 53.2 (-CH-), 21.8 (-CH_3)

FAB Mass (m/z): 345.42

RESULT AND DISCUSSION

The synthesis of 1,3-oxazole carried by the reaction of 1-(4-substituted phenyl)ethan-1-one with 5-substituted-2-(p-substituted phenyl) oxazole-4-carbaldehyde. The yellow color solid was obtained after the reaction and completion of the reaction was monitored by the TLC method. The twelve oxazole derivatives were synthesized and analyzed using IR, 1HNMR, mass spectral, and elemental analysis. The compound's elemental analysis and melting point were also recorded and evaluated, and both were within acceptable limits. The IR spectra of synthesized imidazole compounds have revealed the following features spectrum at 3045, 1658, 1607, 1575, 1120, 735 cm^{-1}.

The 1H NMR spectrum of compound revealed the following features: 2.55 (3H, s, -CH_3), 2.52 (3H, s, -CH_2), 7.45 (2H, m, Ar-H), 7.62 (2H, m, Ar-H), 7.70 (1H, d, -CH=CH-), 7.75 (1H, d, -CH=CH-), 7.97 (2H, m, Ar-H), 8.02 (2H, m, Ar-H); The ^{13}CNMR spectrum of compound revealed the following features: 121.3, 145., 127.5, 132.0, 132.5, 134.2, 136.5, 151.0, 159.2, 188.7, 10.8 (-CH_3), 37.6 (-CH_2-), 53.2 (-CH-), 21.8 (-CH_3). The mass spectrum of all twelve compounds was recorded, and it was virtually identical to the molecular weight of the compounds.

Pharmacological Evaluation

***Ex-vivo* study of HeLa cell**

Cell culture

HeLa human cervical carcinoma cells obtained from the American Type Culture Collection were cultured in RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin and 100 mg/ml streptomycin. The cells were incubated at 37°C in a 5% CO_2 humidified atmosphere.

Anticancer screening of the compounds of Oxazole derivatives (3-Oxa1 to 3-Oxa12) was carried out by the standard procedure followed by NCI for screening. The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fatal bovine serum and 2 mM l-glutamine. For a typical screening experiment, cells are inoculated into 96 well µl plates in 100 µl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed ***in situ*** with TCA, to represent a measurement of the cell population for each cell line at the time of drug-addition (Tz).

Experimental drugs are solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is thawed and diluted to twice. Aliquots of 100µl of these different drug dilutions are added to the appropriate microtiter wells already containing 100µl of medium, resulting in the required final drug concentrations.

Following the drug addition, the plates are incubated for an additional 48 h at 37°C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant is discarded, and the plates are washed five times with tap water and air dried.

SRB ASSAY

Sulforhodamine B (SRB) solution (100 µl) at 0.4% (w/v) in 1% acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing six times with 1% acetic acid and the plates are air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, ©, and test growth in the presence of drug at the six concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels.

Percentage growth inhibition is calculated as: [(Ti – Tz)/(C – Tz)] x 100 for concentrations for which Ti>/=T [(Ti - Tz) / Tz] x 100 for concentrations for which Ti<Tz

***In-vitro* anticancer activity**

SRB ASSAY:

Anti-cancer activity of Oxazole derivatives (3-Oxa1 to 3-oxa12):- The Twelve compounds were synthesized and evaluated for the anticancer activity. The cells are grown in supplemented RPMI-1640 medium for 24 h. The test compounds were dissolved in DMSO and incubated with cells at six concentrations with 10-fold dilutions, the highest being 10^{-4}M and the others being 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} M. The assay is terminated by addition of cold trichloroacetic acid, and the cells are fixed and stained with Sulforhodamine B (SRB assay). Bound stain is solubilized, and the absorbance is read on an automated plate reader. The cytostatic parameter 50% growth inhibition (GI50) was calculated from time zero, control growth and the Six-concentration level absorbance. The cytotoxic parameter that is, inhibitory concentrations (LC50) represent the average of two independent experiments. Compound 3-Oxa1 inhibits proliferation of liver cancer cells and has a high inhibitory effect on selected cell lines (HeLa). The data of percentage of growth inhibition was represented in Table 6.

Table 6: Percentage growth inhibition of NCI-H226 cell line

S. No.	Concentration (µM)	0.1	1	10	25	50	100
	Compound	Percentage of Growth Inhibition					
1	3-Oxa1	0.32	1.63	9.17	38.99	60.83	76.03
2	3-Oxa2	0.26	1.33	7.45	31.68	49.42	61.78
3	3-Oxa3	0.18	0.92	5.16	21.93	34.21	42.77
4	3-Oxa4	0.28	1.43	8.02	34.11	53.22	66.53
5	3-Oxa5	0.20	1.02	5.73	24.37	38.01	47.52
6	3-Oxa6	0.22	1.12	6.30	26.80	41.82	52.27
7	3-Oxa7	0.30	1.53	8.60	36.55	57.02	71.28
8	3-Oxa8	0.24	1.22	6.88	29.24	45.62	57.02
9	3-Oxa9	0.16	0.81	4.58	19.49	30.41	38.01
10	3-Oxa10	0.22	1.12	6.30	26.80	41.82	52.27
11	3-Oxa11	0.18	0.92	5.16	21.93	34.21	42.77
12	3-Oxa12	0.28	1.43	8.02	34.11	53.22	66.53

The SRB assay indicates that the Compound 3-Oxa1 has shown the 76.03% of inhibition, compound 3-Oxa2 has shown 61.78% of inhibition, compound 3-Oxa3 has shown 42.77% of inhibition, compound 3-Oxa2 has shown 66.53% of inhibition, compound 3-Oxa2 has shown 47.52% of inhibition, compound 3-Oxa2 has shown 52.27% of inhibition, compound 3-Oxa2 has shown 71.28% of inhibition, compound 3-Oxa2 has shown 57.02% of inhibition, compound 3-Oxa2 has shown 38.01% of inhibition, compound 3-Oxa2 has shown 52.27% of inhibition, compound 3-Oxa2 has shown 42.77% of inhibition and compound 3-Oxa2 has shown 66.53% of inhibition. The compounds 3-Oxa1, 3-Oxa4, 3-Oxa7 and 3-Oxa12 has shown the percentage of inhibition more than 60 percentage. The compound 3-Oxa1 and 3-

Oxa7 has the most potent compounds in the series having the 76.03 and 71.28 percentage of inhibition respectively.

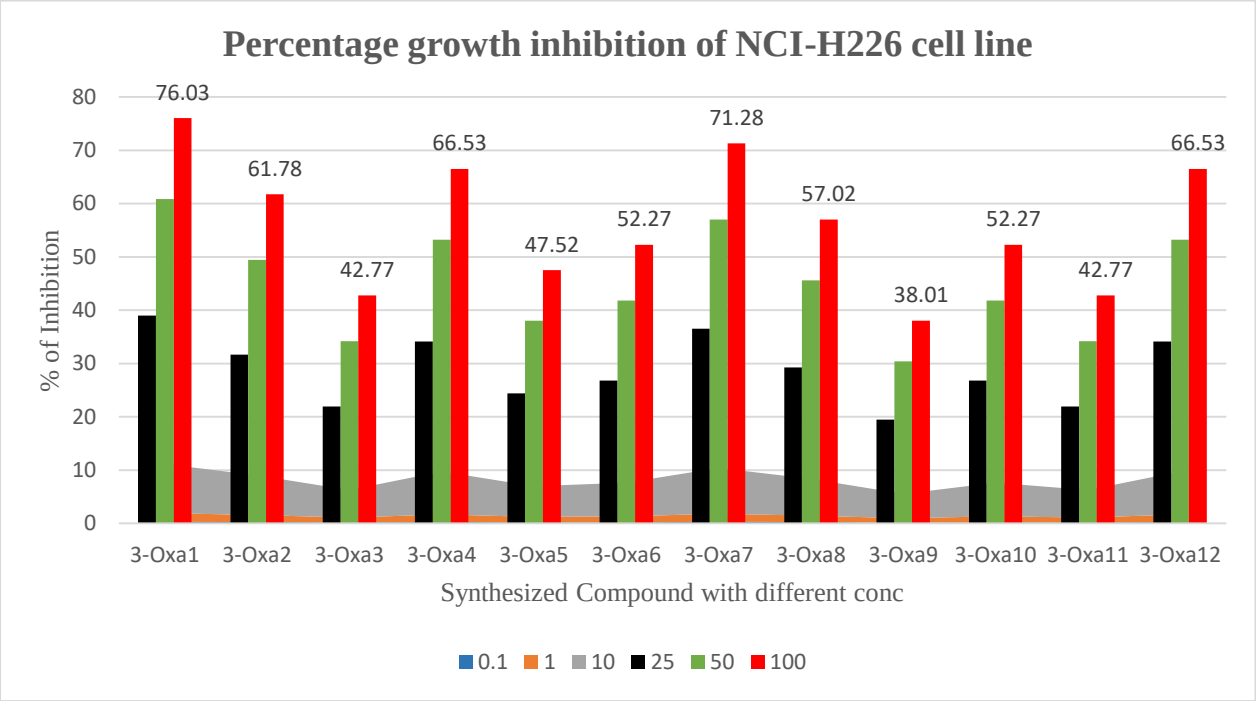


Figure 1: Concentration dependent inhibition of NCI-H226 cell proliferation by synthesized compounds

From the anticancer results by SRB assay, it is observed that compounds with electron withdrawing substituents (*R* = Cl, Br and Nitro) displayed better activity with greater inhibition of cancer cell lines.

CONCLUSION

The heterocyclic moiety being so versatile in nature offers the medicinal chemist to explore more about it in medicinal field and the data mentioned in this article will be a great help to prospective researchers working in this area. Oxazole moiety is an important heterocyclic compound as they are being an essential constituent of large number of marketed drugs and having such diverse spectrum of biological activities, oxazole has immense potential to be investigated for newer therapeutic possibilities and is an important class of lead compounds for development of new chemical entities (NCE) to treat various diseases of clinical importance.

In present research reported the research carried-out indicates that synthesized 1,3-oxazole derivatives will displays anticancer activity. The structures of all the newly prepared compounds were determined using IR, 1H NMR, 13C NMR and mass spectrometry. All the newly prepared compounds were screened for *in vitro* anticancer activity against liver cancer cell lines.

Overall, based on the bio-activity evaluation results, the compound **3-Oxa1** and **3-Oxa7** has showed the best inhibition of liver cancer cell lines and emerged as a potent anticancer agent among all. The synthesized new hybrid of heterocyclic compounds exhibited as promising candidates for the development of some new drug molecules.

REFERENCE:

1. Ang SF, Ng ES, Li H, Ong YH, Choo SP, Ngeow J, Toh HC, Lim KH, Yap HY, Tan CK, Ooi LL. The Singapore Liver Cancer Recurrence (SLICER) Score for relapse prediction in patients with surgically resected hepatocellular carcinoma 2015.
2. Breiner KM, Schaller H, Knolle PA. Endothelial cell–mediated uptake of a hepatitis B virus: a new concept of liver targeting of hepatotropic microorganisms 2001.
3. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. The lancet Oncology 2009
4. Chochi Y, Kawauchi S, Nakao M, Furuya T, Hashimoto K, Oga A, Oka M, Sasaki K. A copy number gain of the 6p arm is linked with advanced hepatocellular carcinoma: an array-based comparative genomic hybridization study. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland 2009
5. Curtin JP, Blessing JA, Webster KD, Rose PG, Mayer AR, Fowler WC, Jr, Malfetano JH, Alvarez RD. Paclitaxel, an active agent in non-squamous carcinomas of the uterine cervix: A Gynecologic Oncology Group study. Journal of Clinical Oncology 2001
6. Fan J, Yang GS, Fu ZR, Peng ZH, Xia Q, Peng CH, Qian JM, Zhou J, Xu Y, Qiu SJ, Zhong L. Liver transplantation outcomes in 1,078 hepatocellular carcinoma patients: a multi-center experience in Shanghai, China. Journal of cancer research and clinical oncology 2009
7. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: From genes to environment. Nature Reviews Cancer 2006
8. Heidelbaugh JJ, Bruderly M. Cirrhosis and chronic liver failure: part I. Diagnosis and evaluation. American family physician 2006
9. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology 2018
10. Kumar SS, Saha AK, Kavitha K, Basu SK. Evaluation of clobazam loaded ionically cross-linked microspheres using chitosan. Der Pharmacia Sinica. 2012
11. Lan M, Zhu L, Wang Y. Multifunctional nanobubbles carrying indocyanine green and paclitaxel for molecular imaging and the treatment of prostate cancer. *J* Nanobiotechnology 2020.
12. Li S, Wei J, Yuan L, et al. RGD-modified endostatin peptide 530 derived from endostatin suppresses invasion and migration of HepG2 cells through the αvβ3 pathway. CancerBiotherRadio-pharmaceutics 2011.
13. Barca A, Pani B, Tamaro M, Russo E. Molecular interactions of ruthenium complexes in isolated mammalian nuclei and cytotoxicity on V-79 cells in culture. Mutat Res 1999.

14. Babii SB, Zyabrev VS, Drach BS. Conversion of N-(1-Arylsulfonyl-2,2-dichloroethenyl) carboxamides into derivatives of 4,5-Dimercaptooxazole. Russ. J. Org. Chem. 2001.
15. Drabczyńska A, Müller CE, Schumacher B, Hinz S, Wojciechowska JK, Michalak B, Pękala E, Kieć-Kononowicz K. Tricyclic oxazolo[2,3-*f*] purinediones: potency as adenosine receptor ligands and anticonvulsants. Bioorg Med Chem 2004.