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Introduction of Modified Fused Coumarin and Screening of Biological Activity against Breast Cancer Cell Lines

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Abstract

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. A cancer that forms in the cells of the breasts is known as Breast Cancer. Breast cancer is the most common invasive cancer in women and the second leading cause of cancer death in women after lung cancer. The coumarin is well known naturally occurring heterocyclic compound having wide range of medicinal properties. The furo-fused coumarin is one of fused derivative of coumarin having various activities along with anti-tumor. Various furo-fused derivatives of coumarin have been found active against breast cancer. The synthesis of nitroethenes and their condensation with 4-hydroxy coumarins results in furofused coumarins. These synthesized compounds were screened for anti-cancer activity by cell viability study against Breast Cancer Cell Line (Michigan Cancer Foundation-7).

Keywords: Cancer; Breast cancer; Coumarin; Nitroethene; Fused-coumarin and cell viability: MCF-7 study

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Introduction

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place. When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors. Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemia, generally do not form solid tumors. Cancerous tumors are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor. Unlike malignant tumors, benign tumors do not spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening **(Figure 1)** [1].

Literature Review

Cancer cells differ from normal cells in many ways that allow them to grow out of control and become invasive. One important difference is that cancer cells are less specialized than normal cells. That is, whereas normal cells mature into very distinct cell types with specific functions, cancer cells do not. This is one reason that, unlike normal cells, cancer cells continue to divide without stopping. In addition, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis, which the body uses to get rid of unneeded cells. Cancer cells may be able to influence the normal cells, molecules, and blood vessels that surround and feed a tumor, an area known as the microenvironment. For instance, cancer cells can induce nearby normal cells to form blood vessels that supply tumors with oxygen and nutrients, which they need to grow. These blood vessels also remove waste products from

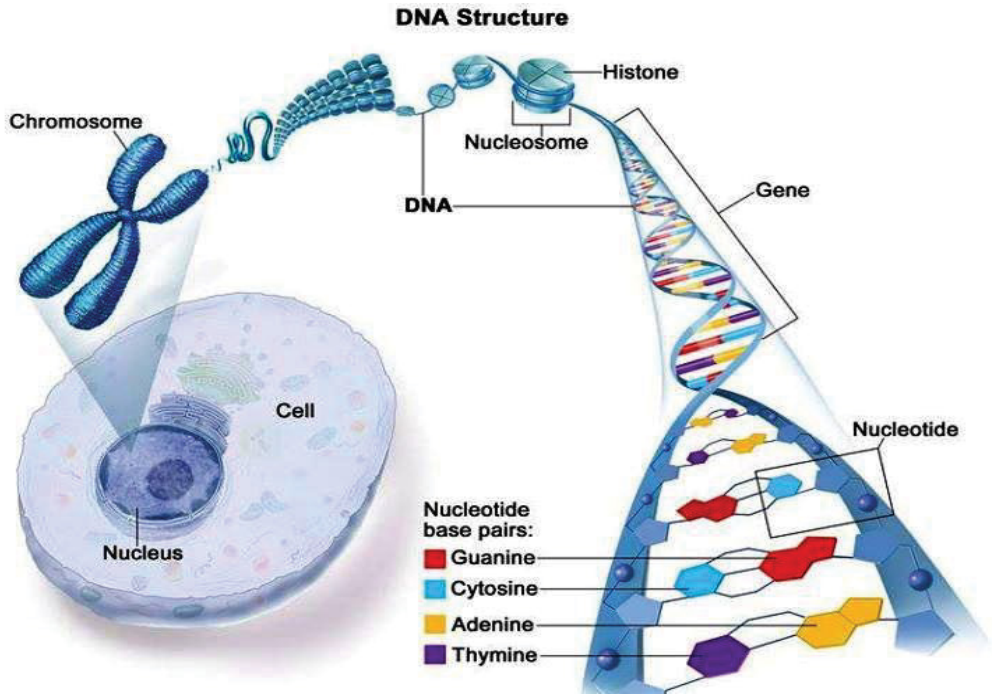


Figure 1 Cancer is caused by certain changes to genes, the basic physical units of inheritance. Genes are arranged in long strands of tightly packed DNA called chromosomes (**Credits:** Terese Winslow).

tumors. Cancer cells are also often able to evade the immune system, a network of organs, tissues, and specialized cells that protects the body from infections and other conditions. Although the immune system normally removes damaged or abnormal cells from the body, some cancer cells are able to "hide" from the immune system. Tumors can also use the immune system to stay alive and grow. For example, with the help of certain immune system cells that normally prevent a runaway immune response, cancer cells can actually keep the immune system from killing cancer cells [1].

The cause of cancer

Cancer is a genetic disease that is caused by changes to genes that control the way our cells function, especially how they grow and divide. Genetic changes that cause cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by certain environmental exposures. Cancer-causing environmental exposures include substances, such as the chemicals in tobacco smoke, and radiation, such as ultraviolet rays from the sun. Each person's cancer has a unique combination of genetic changes. As the cancer continues to grow, additional changes will occur. Even within the same tumor, different cells may have different genetic changes. In general, cancer cells have more genetic changes, such as mutations in DNA, than normal cells. Some of these changes may have nothing to do with the cancer; they may be the result of the cancer, rather than its cause. Some fundamental explanations are shown in **Figure 2**.

The drivers of cancer

The genetic changes that contribute to cancer tend to affect three main types of genes Proto-oncogenes, tumor suppressor genes, and DNA repair genes. These changes are sometimes called "drivers" of cancer. Proto-oncogenes are involved in normal cell growth and division. However, when these genes are altered in certain ways or are more active than normal, they may become cancer-causing genes (or oncogenes), allowing cells to grow and survive when they should not. Tumor suppressor genes are also involved in controlling cell growth and division. Cells with certain alterations in tumor suppressor genes may divide in an uncontrolled manner. DNA repair genes are involved in fixing damaged DNA. Cells with mutations in these genes tend to develop additional mutations in other genes. Together, these mutations may cause the cells to become cancerous. As scientists have learned more about the molecular changes that lead to cancer, they have found that certain mutations commonly occur in many types of cancer. Because of this, cancers are sometimes characterized by the types of genetic alterations that are believed to be driving them, not just by where they develop in the body and how the cancer cells look under the microscope.

The spreading of cancer

A cancer that has spread from the place where it first started to another place in the body is called metastatic cancer. The process by which cancer cells spread to other parts of the body is called metastasis. Metastatic cancer has the same name and the same type of cancer cells as the original, or primary, cancer. For example, breast cancer that spreads to and forms a metastatic tumor in the lung is metastatic breast cancer, not lung cancer. Under a microscope, metastatic cancer cells generally look the same as cells of the original cancer. Moreover, metastatic cancer

cells and cells of the original cancer usually have some molecular features in common, such as the presence of specific chromosome changes. Treatment may help prolong the lives of some people with metastatic cancer. In general, though, the primary goal of treatments for metastatic cancer is to control the growth of the cancer or to relieve symptoms caused by it. Metastatic tumors can cause severe damage to how the body functions, and most people who die of cancer die of metastatic disease **(Figure 3)**.

Types of cancer

There are more than 100 types of cancer. Types of cancer are usually named for the organs or tissues where the cancers form. For example, lung cancer starts in cells of the lung, and brain cancer starts in cells of the brain. Cancers also may be described by the type of cell that formed them, such as an epithelial cell or a squamous cell. The detailed types are described in following list **(Table 1)**.

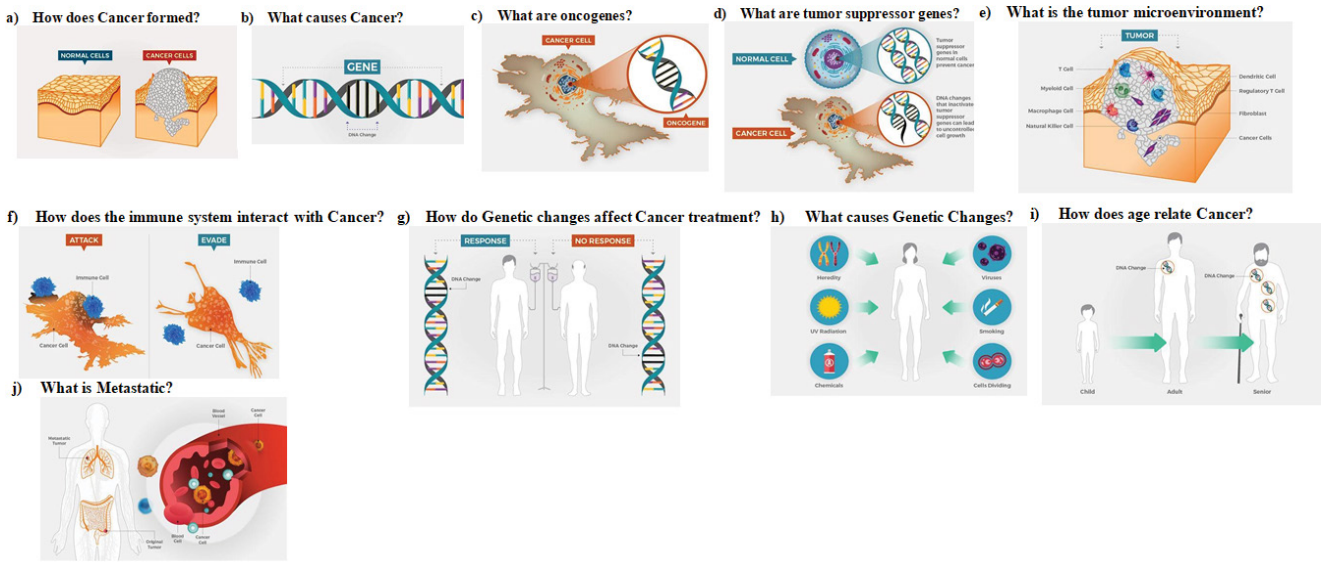


Figure 2 Some fundamentals of Cancer.

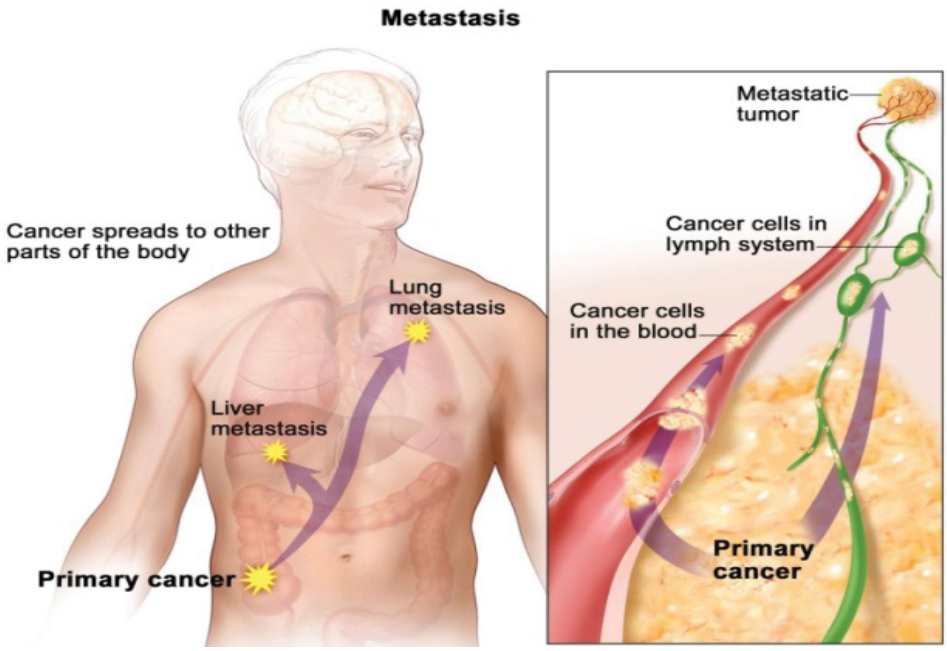


Figure 3 In metastasis, cancer cells break away from where they first formed (primary cancer), travel through the blood or lymph system, and form new tumors (metastatic tumors) in other parts of the body. The metastatic tumor is the same type of cancer as the primary tumor.

Table 1 List of cancer from A to Z (Credits: https://www.cancer.gov/types).

A
Acute Lymphoblastic Leukemia (ALL)
Acute Myeloid Leukemia (AML)
Adolescents, Cancer in
Adrenocortical Carcinoma
AIDS-Related Cancers
Kaposi Sarcoma (Soft Tissue Sarcoma)
AIDS-Related Lymphoma (Lymphoma)
Primary CNS Lymphoma (Lymphoma)
Anal Cancer
Appendix Cancer - see Gastrointestinal Carcinoid Tumors
Astrocytomas, Childhood (Brain Cancer)
Atypical Teratoid/Rhabdoid Tumor, Childhood, Central Nervous System (Brain Cancer)
B
Basal Cell Carcinoma of the Skin - see Skin Cancer
Bile Duct Cancer
Bladder Cancer
Bone Cancer (includes Ewing Sarcoma and Osteosarcoma and Malignant Fibrous Histiocytoma)
Brain Tumors
Breast Cancer
Bronchial Tumors (Lung Cancer)
Burkitt Lymphoma - see Non-Hodgkin Lymphoma
C
Carcinoid Tumor (Gastrointestinal)
Carcinoma of Unknown Primary
Cardiac (Heart) Tumors, Childhood
Central Nervous System
Atypical Teratoid/Rhabdoid Tumor, Childhood (Brain Cancer)
Medulloblastoma and Other CNS Embryonal Tumors, Childhood (Brain Cancer)
Germ Cell Tumor, Childhood (Brain Cancer)
Primary CNS Lymphoma
Cervical Cancer
Childhood Cancers
Cancers of Childhood, Unusual
Cholangiocarcinoma - see Bile Duct Cancer
Chordoma, Childhood (Bone Cancer)
Chronic Lymphocytic Leukemia (CLL)
Chronic Myelogenous Leukemia (CML)
Chronic Myeloproliferative Neoplasms
Colorectal Cancer
Craniopharyngioma, Childhood (Brain Cancer)
Cutaneous T-Cell Lymphoma - see Lymphoma (Mycosis Fungoides and Sézary Syndrome)
D
Ductal Carcinoma In Situ (DCIS) - see Breast Cancer
E
Embryonal Tumors, Medulloblastoma and Other Central Nervous System, Childhood (Brain Cancer)
Endometrial Cancer (Uterine Cancer)

M
Male Breast Cancer
Malignant Fibrous Histiocytoma of Bone and Osteosarcoma
Melanoma
Melanoma, Intraocular (Eye)
Merkel Cell Carcinoma (Skin Cancer)
Mesothelioma, Malignant
Metastatic Cancer
Metastatic Squamous Neck Cancer with Occult Primary (Head and Neck Cancer)
Midline Tract Carcinoma With NUT Gene Changes
Mouth Cancer (Head and Neck Cancer)
Multiple Endocrine Neoplasia Syndromes
Multiple Myeloma/Plasma Cell Neoplasms
Mycosis Fungoides (Lymphoma)
Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms
Myelogenous Leukemia, Chronic (CML)
Myeloid Leukemia, Acute (AML)
Myeloproliferative Neoplasms, Chronic
N
Nasal Cavity and Paranasal Sinus Cancer (Head and Neck Cancer)
Nasopharyngeal Cancer (Head and Neck Cancer)
Neuroblastoma
Non-Hodgkin Lymphoma
Non-Small Cell Lung Cancer
O
Oral Cancer, Lip and Oral Cavity Cancer and Oropharyngeal Cancer (Head and Neck Cancer)
Osteosarcoma and Malignant Fibrous Histiocytoma of Bone
Ovarian Cancer
P
Pancreatic Cancer
Pancreatic Neuroendocrine Tumors (Islet Cell Tumors)
Papillomatosis (Childhood Laryngeal)
Paraganglioma
Paranasal Sinus and Nasal Cavity Cancer (Head and Neck Cancer)
Parathyroid Cancer
Penile Cancer
Pharyngeal Cancer (Head and Neck Cancer)
Pheochromocytoma
Pituitary Tumor
Plasma Cell Neoplasm/Multiple Myeloma
Pleuropulmonary Blastoma (Lung Cancer)
Pregnancy and Breast Cancer
Primary Central Nervous System (CNS) Lymphoma
Primary Peritoneal Cancer
Prostate Cancer
R
Rectal Cancer

A	M
Ependymoma, Childhood (Brain Cancer)	Recurrent Cancer
Esophageal Cancer	Renal Cell (Kidney) Cancer
Esthesioneuroblastoma (Head and Neck Cancer)	Retinoblastoma
Ewing Sarcoma (Bone Cancer)	Rhabdomyosarcoma, Childhood (Soft Tissue Sarcoma)
Extracranial Germ Cell Tumor, Childhood	**S**
Extragonadal Germ Cell Tumor	Salivary Gland Cancer (Head and Neck Cancer)
Eye Cancer	Sarcoma
Intraocular Melanoma	Childhood Rhabdomyosarcoma (Soft Tissue Sarcoma)
Retinoblastoma	Childhood Vascular Tumors (Soft Tissue Sarcoma)
F	Ewing Sarcoma (Bone Cancer)
Fallopian Tube Cancer	Kaposi Sarcoma (Soft Tissue Sarcoma)
Fibrous Histiocytoma of Bone, Malignant, and Osteosarcoma	Osteosarcoma (Bone Cancer)
G	Soft Tissue Sarcoma
Gallbladder Cancer	Uterine Sarcoma
Gastric (Stomach) Cancer	Sézary Syndrome (Lymphoma)
Gastrointestinal Carcinoid Tumor	Skin Cancer
Gastrointestinal Stromal Tumors (GIST) (Soft Tissue Sarcoma)	Small Cell Lung Cancer
Germ Cell Tumors	Small Intestine Cancer
Childhood Central Nervous System Germ Cell Tumors (Brain Cancer)	Soft Tissue Sarcoma
Childhood Extracranial Germ Cell Tumors	Squamous Cell Carcinoma of the Skin - see Skin Cancer
Extragonadal Germ Cell Tumors	Squamous Neck Cancer with Occult Primary, Metastatic (Head and Neck Cancer)
Ovarian Germ Cell Tumors	Stomach (Gastric) Cancer
Testicular Cancer	**T**
Gestational Trophoblastic Disease	T-Cell Lymphoma, Cutaneous - see Lymphoma (Mycosis Fungoides and Sèzary Syndrome)
H	Testicular Cancer
Hairy Cell Leukemia	Throat Cancer (Head and Neck Cancer)
Head and Neck Cancer	Nasopharyngeal Cancer
Heart Tumors, Childhood	Oropharyngeal Cancer
Hepatocellular (Liver) Cancer	Hypopharyngeal Cancer
Histiocytosis, Langerhans Cell	Thymoma and Thymic Carcinoma
Hodgkin Lymphoma	Thyroid Cancer
Hypopharyngeal Cancer (Head and Neck Cancer)	Tracheobronchial Tumors (Lung Cancer)
I	Transitional Cell Cancer of the Renal Pelvis and Ureter (Kidney (Renal Cell) Cancer)
Intraocular Melanoma	**U**
Islet Cell Tumors, Pancreatic Neuroendocrine Tumors	Unknown Primary, Carcinoma of
K	Unusual Cancers of Childhood
Kaposi Sarcoma (Soft Tissue Sarcoma)	Ureter and Renal Pelvis, Transitional Cell Cancer (Kidney (Renal Cell) Cancer
Kidney (Renal Cell) Cancer	Urethral Cancer
L	Uterine Cancer, Endometrial
Langerhans Cell Histiocytosis	Uterine Sarcoma
Laryngeal Cancer (Head and Neck Cancer)	**V**
Leukemia	Vaginal Cancer
Lip and Oral Cavity Cancer (Head and Neck Cancer)	Vascular Tumors (Soft Tissue Sarcoma)
Liver Cancer	Vulvar Cancer
Lung Cancer (Non-Small Cell, Small Cell, Pleuropulmonary Blastoma, and Tracheobronchial Tumor)	**W**
	Wilms Tumor and Other Childhood Kidney Tumors
Lymphoma	**Y**
	Young Adults Cancer

Breast cancer

A cancer that forms in the cells of the breasts is known as Breast Cancer. Breast Cancer can occur in women and rarely in men. Symptoms of breast cancer include a lump in the breast, bloody

discharge from the nipple and changes in the shape or texture of the nipple or breast.

Breast cancers can start from different parts of the breast.

- Most breast cancers begin in the ducts that carry milk to the nipple (ductal cancers).
- Some start in the glands that make breast milk (lobular cancers).
- There are also other types of breast cancer that are less common like phyllodes tumor and angiosarcoma.
- A small number of cancers start in other tissues in the breast. These cancers are called sarcomas and lymphomas and are not really thought of as breast cancers.

Although many types of breast cancer can cause a lump in the breast, not all do. Many breast cancers are also found on screening mammograms, which can detect cancers at an earlier stage, often before they can be felt, and before symptoms develop.

Breast cancer can spread when the cancer cells get into the blood or lymph system and are carried to other parts of the body. The lymph system is a network of lymph (or lymphatic) vessels found throughout the body that connects lymph nodes (small bean-shaped collections of immune system cells). The clear fluid inside the lymph vessels, called lymph, contains tissue by-products and waste material, as well as immune system cells. The lymph vessels carry lymph fluid away from the breast. In the case of breast cancer, cancer cells can enter those lymph vessels and start to grow in lymph nodes **(Figure 4).**

Most of the lymph vessels of the breast drain into:

1. Lymph nodes under the arm (axillary nodes).
2. Lymph nodes around the collar bone (supraclavicular [above the collar bone] and infraclavicular [below the collar bone] lymph nodes).
3. Lymph nodes inside the chest near the breast bone (internal mammary lymph nodes).

If cancer cells have spread to your lymph nodes, there is a higher chance that the cells could have traveled through the lymph system and spread (metastasized) to other parts of your body. The more lymph nodes with breast cancer cells, the more likely it is that the cancer may be found in other organs. Because of this, finding cancer in one or more lymph nodes often affects your treatment plan. Usually, you will need surgery to remove one or more lymph nodes to know whether the cancer has spread [2].

The two main categories of breast cancer are invasive and noninvasive. Non-invasive breast cancers stay within the milk ducts and lobules of the breast. Invasive cancers spread beyond these areas and invade normal tissue. The main noninvasive type is ductal carcinoma *in situ* (DCIS), in which cancer develops in the ducts that carry milk to the nipple but does not spread beyond those ducts. A similar condition, lobular carcinoma *in situ,* begins in the lobules, the milk-producing glands, and does not grow further. Unlike DCIS, LCIS is not considered a cancer, but it does mean that a woman has a higher risk of developing breast cancer [3].

Stages of breast cancer

The stage of breast cancer depends upon pathology report, a report that details the size, shape and look of the cancer cells under a microscope. Most cancers, including invasive breast cancer, have four stages [4].

Stage 0 is abnormal cells that have not spread beyond the ducts or lobules of the breast, such as DCIS or LCIS, respectively.

Stage I cancer is invasive and spreading beyond where it started.

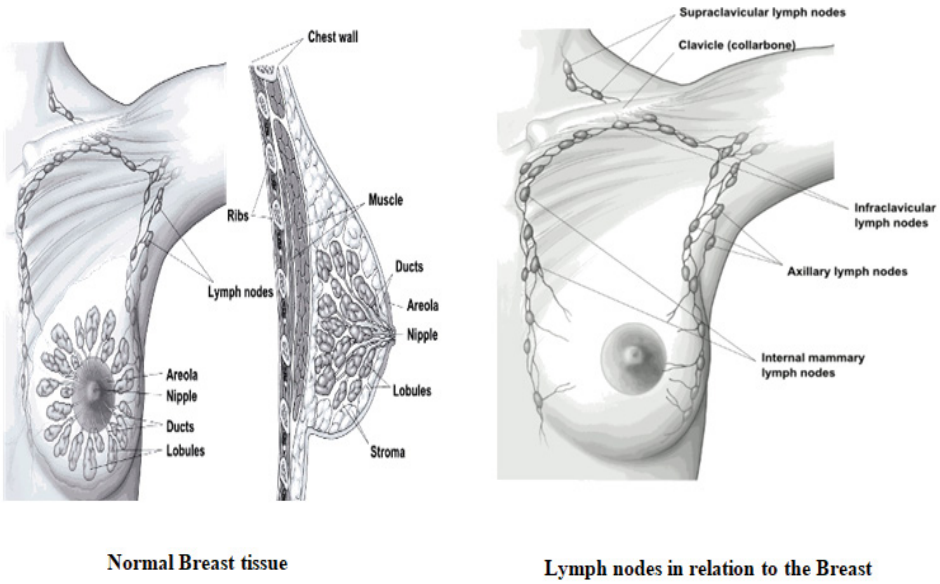


Figure 4 The normal Breast tissue and lymph nodes in relation to the breast (Credits: https://www.cancer.org/cancer/breast-cancer/about/what-is-breast-cancer.html).

In Stage IA, the cancer is 2 cm or smaller and has not spread into the lymph nodes or outside of the breast. In Stage IB, small clumps of cancer cells ranging from 0.2 to 2 mm exist in the lymph nodes. There may not be a tumor in the breast, but if there is, it measures no bigger than 2 cm.

Stage II cancer also has two subcategories. Stage IIA describes a cancer that has spread to 1 to 3 lymph nodes under your arms (axillary lymph nodes) with or without a tumor up to 2 cm large in the breast, or the breast tumor measures 2 to 5 cm without cancer cells in the axillary lymph nodes. Stage IIB refers to a tumor between 2 and 5 cm along with cancer in 1 to 3 axillary lymph nodes or lymph nodes near the breastbone, or the tumor is larger than 5 cm when no cancer cells exist in the axillary lymph nodes **(Figure 5)**.

Stage III breast cancer includes Stage IIIA, IIIB, and IIIC. In stage IIIA, the tumor may be any size or may not exist at all. In addition, the cancer has spread to 4 to 9 lymph nodes close to the breastbone or in the axilla. The tumor may also be larger than 5 cm with small clumps of breast cancer cells in the lymph nodes or the tumor is larger than 5 cm and has spread to 1 to 3 axillary lymph nodes or nodes near the breast bone. In Stage IIIB, the tumor (any size) has reached the skin of your breast and/or your chest wall and up to 9 lymph nodes under your arms or near your breastbone.

Inflammatory breast cancer is automatically **Stage IIIB** or a later stage.

Stage IIIC involves three behaviors of the cancer:

- The cancer may not be found in the breast at all, or may be any size and has spread to the chest and breast skin as in stage IIIB.
- 10 or more axillary lymph nodes.
- The lymph nodes above or below your collarbone contain the cancer.
- The axillary lymph nodes and those near your breastbone may contain the cancer.

Stage IV breast cancer has spread beyond the breast and lymph nodes to other areas in the body. Breast cancer is not curable at Stage IV, but therapies can help treat it so that a woman can live many years while managing breast cancer as a chronic condition.

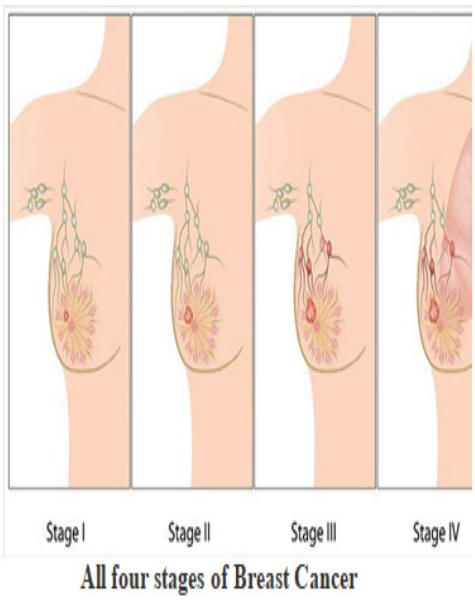
In fact, more than 154,000 women are estimated to be living with stage IV breast cancer right now. About one quarter of them were diagnosed with stage IV cancer, and the other three quarters were diagnosed with an earlier stage that advanced to stage IV [4]. Survival from or with breast cancer has continued to increase over the past decade as rates of death from the disease have fallen. Some experts estimate that the 5-year survival rate of women under age 50 who are diagnosed with stage IV breast cancer will double from 18% to 36%.

Michigan Cancer Foundation-7 (MCF-7)

MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman [5]. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert Soule and co-workers **(Figure 6)** [6].

Coumarin (A natural product)

Coumarin, the name is coming from the word coumarou. It is the French word for the tonka beans. The word tonka for the tonka bean is taken from the Galibi (Carib) tongue spoken by natives of French Guiana (one source for the plant); it also appears in Old Tupi, another language of the same region, as the name of the tree. The old genus name, Coumarouna, was formed from another Tupi name for tree, kumarú **(Figure 7)** [7].



All four stages of Breast Cancer

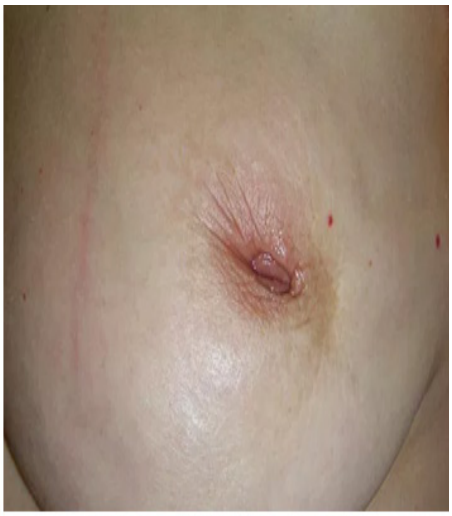

Real Picture of inverted nipple
(Creadits: Dermnet New Zealand)

Figure 5 Stages of breast cancer and real picture of inverted nipple.

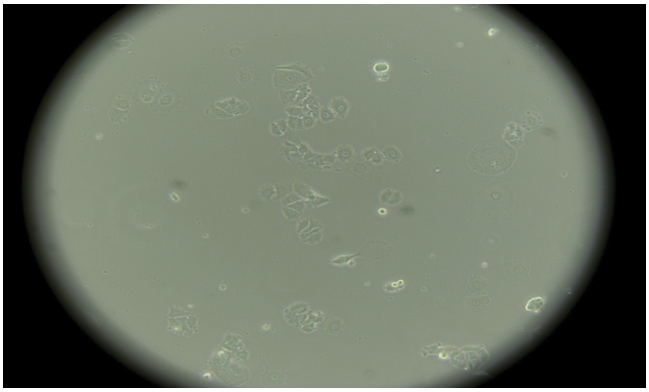


Figure 6 MCF-7 cell lines.

Figure 7 Tonka Bean and chemical structure of natural product coumarin.

Coumarins are the best known aromatic lactones. The isolation of coumarin was first reported by Vogel in Munich in 1820. He associated the pleasant odour of the tonka bean from Guiana with that of clover, *Melilotous officinalis,* which gives rise to the characteristic aroma of new-mown hay. Vogel then concluded that the long colorless crystals which he discovered on slicing open Tonka Beans and which crystallized as glistening needles from aqueous alcohol were identical with similar crystals he obtained, albeit in much lower yield, by extracting fresh clover blossoms.

A large number of valuable species used commonly as medicinal plants, aromatic plants, and edible plants for human and animal feeding belongs to coumarin-rich plant families. Among them are species with well-documented biological activity, in which coumarins are part of the active principles [8].

Novobiocin **(Figure 8)** is a natural product isolated from soil samples containing Streptomyces spheroids [9] and has clinical use for the treatment of bacterial infection [10,11] and more recently some forms of cancer [12]. Neckers and coworkers proposed that Novobiocin also possess anti-tumor activity [13].

Furo[3,2-c]coumarin

The chemical structure of furo[3,2-c]coumarin has been described in **Figure 9**. Among the heterocyclic fused coumarins, furocoumarins form a distinct class of coumarin derivatives. Numbers of furo-coumarins are naturally occurring and show a wide range of biological properties.

Yeh et al. [14] isolated Neo trans-chinlactone, a furano coumarin derivative from the CH_3CH_2 extract of *S. mycorhiza*. It was evaluated *in vitro* against several human cancer cell lines. It showed significant inhibition against two ER^+ human breast cancer cell lines. Also, a discovery of coumarin-monastrol hybrid as potential anti-breast tumor-specific agent has been found [15].

Many furocoumarins are reported to have phototoxic effects to insects, fungi, viruses and bacteria [16-19] and cytotoxic property [20]. Recently, synthesis, natural occurrence and biological activity of furocoumarins have been reviewed by Santana et al. [21]. Furocoumarins like psoralen, xanthotoxin and angelicin have interesting therapeutic properties [22-24]. If one restricts the fusion of furan ring with the lactone ring of coumarin, three structural isomers; furo[2,3-c]coumarin, furo[3,2-c]- coumarin and furo[3,4-c]coumarin are possible. Among these three structural isomers, furo[3,2-c]coumarins have been widely studied. Many furo[3,2-c]coumarins are naturally occurring and possess variety of physiological activities. Neotanshin lactone which is isolated from the rhizome of Salvia miltiorrhiza Bunge is an anti-breast cancer agent [25]. In continuation of such interest in synthesizing newer furo[3,2-c]coumarin derivatives, it was thought worthwhile to incorporate pyrazole moiety in furo[3,2-c]

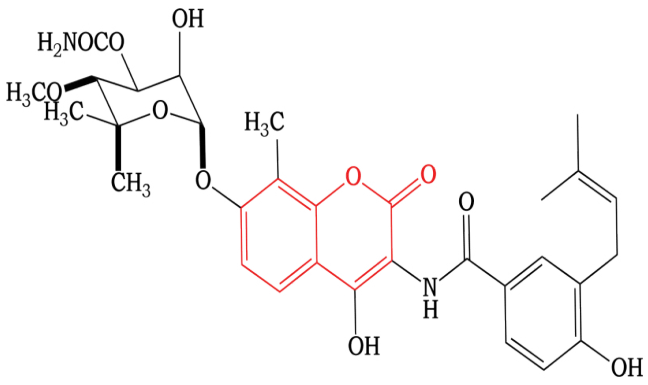


Figure 8 Natural product Novobiocin.

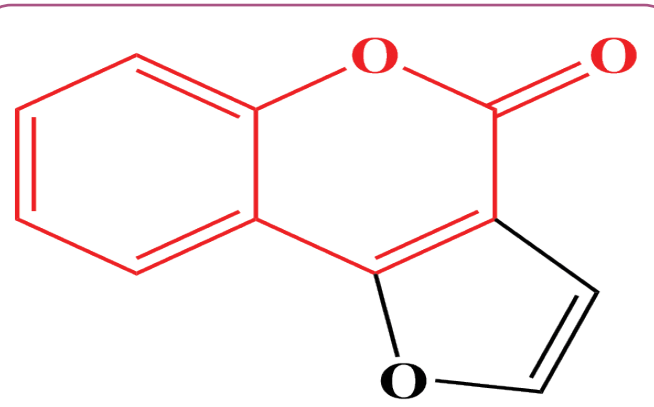


Figure 9 Furo[3,2-c]coumarin.

coumarins the synthesis, characterization and antimicrobial activities has been reported earlier [26,27]. Now, as an extension of previous work, the synthesized compounds were screened for MCF-7 MTT Assay. The proposed compounds have been described in **Figure 10**.

Materials and Methods

Synthesis and characterisation

All chemicals were purchased from Sigma-Aldrich, Germany. Melting points were determined by the open capillary method and were uncorrected. FTIR spectra of the synthesized compounds were recorded on a Shimadzu-8400S, using KBr pellets in 10-4 resolution and 30 scans. ^{1}H NMR spectra were recorded on a Varian spectrometer, USA at 400 MHz at room temperature. Samples were prepared in $CDCl_3$ containing TMS as an internal standard. Splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values were given in parts per million (ppm). ^{13}C NMR were recorded on a Varian 400 spectrometer, operating at 400 MHz. The Liquid Chromatography Mass Spectra (LC-MS) were recorded on a Varian Inc, USA, 410 Prostar Binary LC with 500 MS IT PDA detectors.

Synthesis of 4-hydroxy coumarin (1a), 6-methyl-4-hydroxy coumarin (1b) and 8-methyl-4-hydroxy coumarin (1c)

The following general procedure was used:

In a 500 mL round bottom flask attached with a reflux condenser and gas absorption trap, a mixture of an appropriate phenol (0.2 mole), malonic acid (0.1 mole), anhydrous zinc chloride (0.6 mole) and phosphorous oxychloride (0.4 mole) were heated with stirring at 60-65°C for 35 hours.

The yellow colored mixture was cooled and decomposed with water and left overnight. The resulting crude 4-hydroxy coumarin was filtered out, washed with water and dried. This crude product was purified by dissolving it in 10% sodium bicarbonate solution, filtering and reprecipitating by adding dilute HCl solution. The solid product was separated out which was filtered out, washed with water, dried and recrystallized from ethanol **(Figure 11)**.

4-Hydroxy coumarin (1a): R_1=R_2=H; Yield: 60%, m.p. 202-204°C (lit. [28] m.p. 206°C).

6-Methyl-4-hydroxy coumarin (1b): R_1=CH_3, R_2=H; Yield: 43%, m.p. 236-238°C (lit. [28] m.p. 240°C).

8-Methyl-4-hydroxy coumarin (1c): R_1=H, R_2=CH_3; Yield: 53%, m.p. 222°C(lit. [28] m.p. 223°C).

Preparation of 1-phenyl-3-aryl-1H-pyrazole-4-carbaldehydes (2a-b)

The following general procedure was used:

In a 100 mL round bottom flask, a mixture of an appropriate acetophenone (0.01 mol), phenyl hydrazine (0.01 mol) and ethanol (5 mL) containing 1-2 drops of glacial acetic acid was warmed on the steam cone for 15 minutes. The separated phenyl hydrazone was filtered off, washed with cold ethanol (5 mL) and dried. It was recrystallized from ethanol **(Figure 12)**.

The freshly prepared acetophenone phenyl hydrazone (0.06 mol) was taken in a 250 mL three necked round bottom flask fitted with addition funnel and guard tube. Then anhydrous dimethyl formamide (DMF) (0.6 mol) was added and the reaction mixture was cooled to 0°C with stirring. To this reaction mixture, phosphorous oxychloride ($POCl_3$) (0.18 mol) was added dropwise with stirring during one hour at 0°C. The reaction mixture was further stirred at 0°C for one hour and then heated at 65-70°C for two hours. It was then poured into crushed ice (200 g) and left overnight in refrigerator, during which a solid product was separated out which was filtered off, washed with sodium carbonate (5%, 3 × 30 mL) and water. It was then dried and recrystallized from ethanol.

1,3-Diphenyl-1H-pyrazole-4-carbaldehyde(2a): R_3=H; Yield: 97%, m.p. 139°C (lit. [29] m.p. 140°C).

1-Phenyl-3-p-tolyl-1H-pyrazole-4-carbaldehyde(2b): R_3=CH_3; Yield: 93%, m.p. 145-147°C (lit. [29] m.p. 148°C).

Preparation of 2-(1-phenyl -3-aryl-1*H*-pyrazol-4-yl)-1-

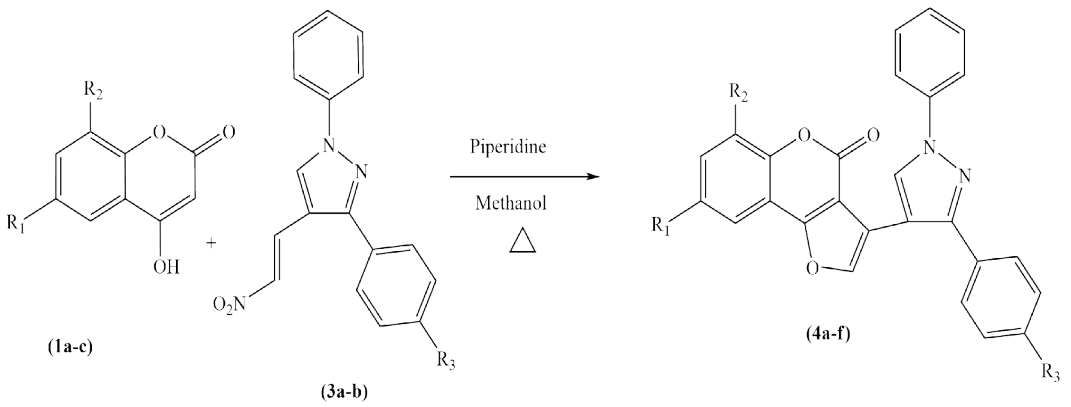


Figure 10 Synthesized Furo[3,2-c]coumarins.

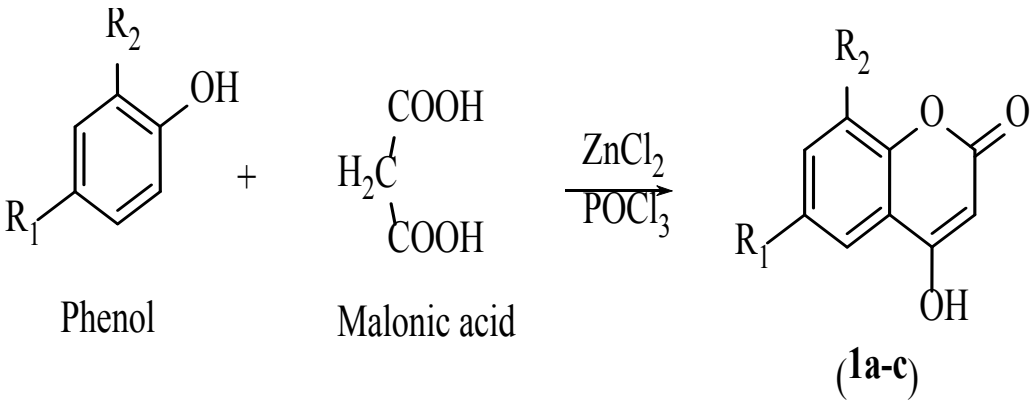


Figure 11 Synthesis of 4-hydroxy coumarin, 6-methyl-4-hydroxy coumarin, 8-methyl-4-hydroxy coumarin.

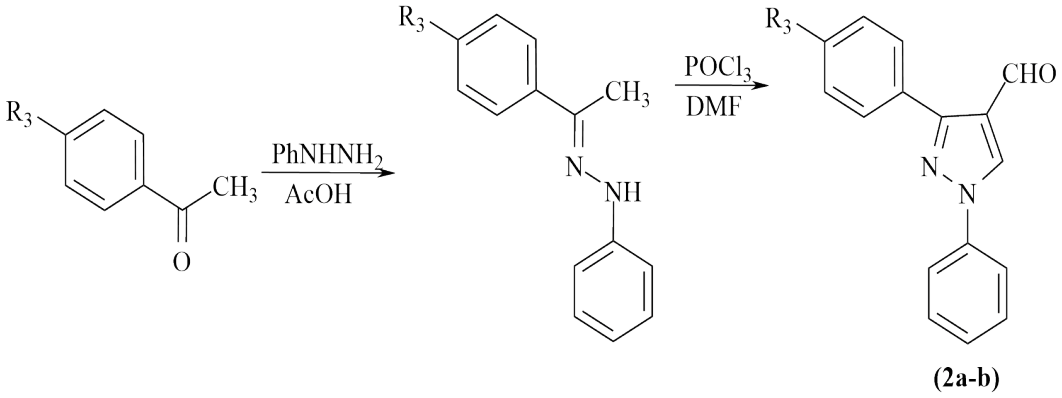


Figure 12 Preparation of 1-phenyl-3-aryl-1H-pyrazole-4-carbaldehydes.

nitroethenes (3a-b)

1-Phenyl-3-aryl-1H-pyrazole carbaldehyde (0.16 mol), nitro methane (0.1 mol), ammonium acetate (0.16 mol) and glacial acetic acid (100 mL) were mixed in a 250 mL round bottom flask fitted with a reflux condenser.

The reaction mixture was refluxed for 4 to 5 hours in an oil bath. It was then poured into crushed ice. Fine yellow crystals irritating to the skin obtained were filtered out and were recrystallized from rectified spirit **(Figure 13)**.

Compound 3a: R_3 = H, Yield = 90%, m.p. 172-173˚C (lit. [27] m.p. 173°C)

Compound 3b: R_3= CH_3 , Yield = 92%, m.p. 185˚C (lit. [27] m.p. 185°C)

Synthesis of 3-(1'-phenyl-3'-aryl-1H-pyrazol-4'-yl)-furo[3,2-c] coumarins (4a-f)

The following general procedure was used:

To a mixture of an appropriate 4-hydroxy coumarin (1a-c) (0.002 mol) and 2-(1-phenyl-3-aryl-1H-pyrazol-4-yl)-1-nitroethene (3a-b) (0.002 mol) in methanol (15 mL) was added 1-2 drops of piperidine at room temperature. The reaction mixture was stirred for 10 minutes and then refluxed in an oil bath for four hours and cooled to room temperature. The reaction mixture was poured into ice cold water (75 mL) and the crude product obtained was extracted with chloroform (3 × 30 mL). The combined chloroform extract was washed with dil. HCl and then with water (3 × 20 mL).

It was dried over anhydrous sodium sulfate. The removal of chloroform under vacuum gave a solid product. This was purified by column chromatography using silica gel and pet-ether(60-80)-chloroform (3:7) as an eluent to afford the compounds (4a-f). The compounds thus obtained were recrystallized from chloroform-haxane **(Figure 14)**.

Compound 4a: R_1=R_2=R_3=H, Yield=69%, m.p. 214˚C (lit. [27] m.p. 173°C)

Compound 4b: R_1=CH_3, R_2=R_3=H, Yield=70%, m.p. 233-235˚C (lit. [27] m.p. 173°C)

Compound 4c: R_1=R_3=H, R_2=CH_3, Yield=74%, m.p. 218˚C (lit. [27] m.p. 173°C)

Compound 4d: R_1=R_2=H, R_3=CH_3, Yield=73%, m.p. 215-217˚C (lit. [27] m.p. 173°C)

Compound 4e: R_2=H, R_1=CH_3, R_3=CH_3, Yield=67%, m.p. 200-202˚C

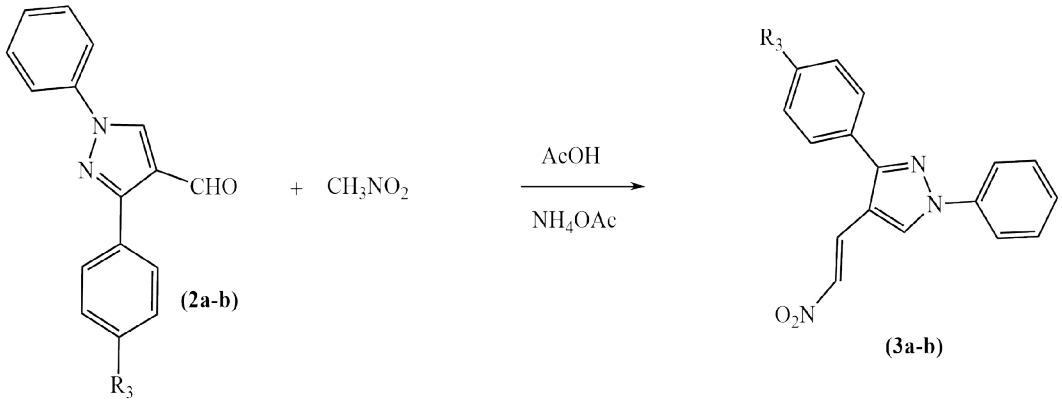


Figure 13 Preparation of 2-(1-phenyl -3-aryl-1H-pyrazol-4-yl)-1-nitroethenes.

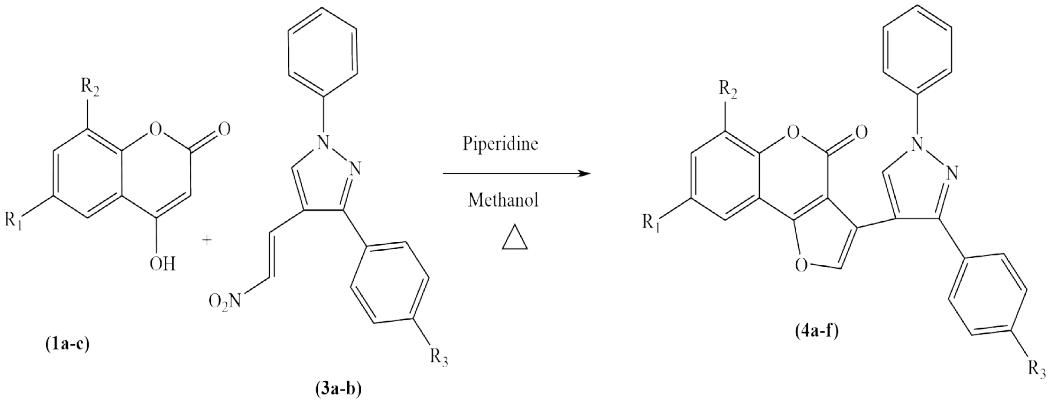


Figure 14 Synthesis of 3-(1'-phenyl-3'-aryl-1H-pyrazol-4'-yl)-furo[3,2-c] coumarins.

(lit. [27] m.p. 173°C)

Compound 4f: R_1=H, R_2=R_3=CH_3, Yield=71%, m.p. 222˚C (lit. [27] m.p. 173°C)

Cell lines

MCF-7 (breast cancer) cell were cultured in DMEM medium and supplemented with 10% of fetal bovine serum (FBS) then the culture flasks were incubated for 3-4 days at 37˚C in 5% CO_2 incubator.

Analysis of cell viability by MTT assay

Cell viability was measured quantitatively by using MTT, showed the activity of living cells. MCF-7 was seeded into 24 well plates and treated with 100 µl/ml, 150 µl/ml, 200 µl/ml, 250 µl/ml and 300 µl/ml of various furo[3,2-c]coumarins mixture dissolved in Ethanol. The treated mixture was then incubated at 37ºC with 5% CO_2 for 24 hours. After incubation, 2 µl/ml of the labeled reagent was added to each well followed by incubation for 3 hours at 37°C with 5% CO_2 and then the medium was discarded and the crystals were dissolved in 1.0 ml of 0.04 N HCl. The absorbance of cells was measured at 570 nm with an ELISA reader. MTT assay was performed in the Department of Microbiology, SSR College of Arts, Commerce and Science, Silvassa.

Statistical analysis

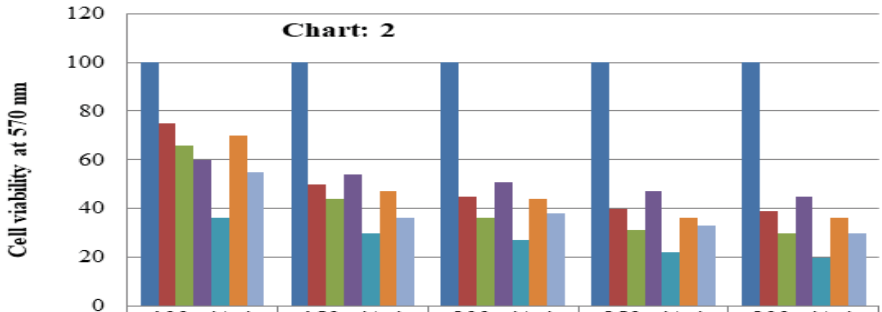
Each data point was obtained by making at least 3 independent measurements. All data are expressed as mean + S.D. Data were analyzed by an analysis of variance ($p<0.05$) and the means separated by one way ANOVA.

Results and Discussion

Various furo[3,2-c] coumarins (4a-c) and (5a-c)were screened at concentration of 100 µl/ml, 150 µl/ml, 200 µl/ml, 250 µl/ml and 300 µl/ml. The most of compounds significantly reduced the growth of MCF-7 cell line. The evaluation of reduction for MCF-7 cell line treated with furo[3,2-c]coumarins mixture at 570 nm using ELISA reader data is shown in **Figure 15**.

The following conclusions can be made on the basis of obtained viability results,

a) The compound 4d at 250 µl/ml and 300 µl/ml shows excellent cell viability 22 and 20 which are the highest among all the compounds as it has reduced MCF-7 cell line as much.



	100 µl/ml	150 µl/ml	200 µl/ml	250 µl/ml	300 µl/ml
Control	100	100	100	100	100
Cell viability (570 nm) 4a	75	50	45	40	39
Cell viability (570 nm) 4b	66	44	36	31	30
Cell viability (570 nm) 4c	60	54	51	47	45
Cell viability (570 nm) 4d	36	30	27	22	20
Cell viability (570 nm) 4e	70	47	44	36	36
Cell viability (570 nm) 4f	55	36	38	33	30

Figure 15 The evaluation of reduction for MCF-7 cell line treated with furo[3,2-c]coumarins.

b) The rest of compounds 4a, 4b, 4c, 4e and 4f show moderated viabilities from 75 to 30 at various concentrations.

c) The concentration 250 µl/ml and 300 µl/ml has almost similar viability among all concentrations. Thus, minimum concentration should be 250 µl/ml as even rise in concentrations has no further viabilities.

Conclusion

Thus, to gain a better understanding of the beneficial biological activities of furo[3,2-c] coumarins upon breast cancer prevention, a greater knowledge of the metabolism of furo[3,2-c]coumarins is needed. More research is clearly needed. If such studies succeed in identifying an active furo[3,2-c]coumarins derivative, a potent breast cancer agent can be introduced.

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Declarations

- The synthesis and characterizations has been carried out by author.
- The cancer cell viability study has been conducted in Department of Microbiology, SSR College of Arts. Commerce and Science, Pune, India.
- The syntheses and physical analysis were performed in Department of Chemistry, Sardar Patel University, Vidya Nagar, Anand, Gujarat, India. The spectroscopic characterizations were performed in Centre of Excellence, Vapi, Gujarat, India.

Funding

- The chemicals and apparatus were provided by of Department of Chemistry, Sardar Patel University, Vidya Nagar, Anand, Gujarat, India.
- The expanses of spectroscopic analysis were paid by author only.
- The cell were collected and analyzed by Department of Microbiology, SSR College of Arts, Commerce and Science with funding of SSR Memorial Trust.

Conflicts of Interest

No conflicts of interest. Academic progress only.

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