In-Silico Analysis of 2-(Substituted-2h-Chromen-3-Yl)-5-Aryl-1H-imidazole Derivatives as an Anti-Angiogenesis Agents in Breast Cancer

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ABSTRACT

Based on earlier proven pharmacophore analogues of cancer a novel 2 (substituted-2H-chromen-3-yl)-5-aryl-1H-imidazoles (13–16) were rationally designed. Compounds 13–16 were screened in silico for the inhibition of KRAS/Wnt and their anti-angiogenesis properties. Compound 16f has been identified as a potent anti-angiogenesis molecule, which can be considered as a new lead structure. The molecular docking analysis displayed the higher binding affinity of 16f with KRAS, Wnt and VEGF.

Keywords: Chromene derivatives, angiogenesis, Breast cancer, VEGF, KRAS, Wnt

INTRODUCTION

Breast cancer is cancer that develops from breast tissue. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin. Risk factors for developing breast cancer include: female sex, obesity, lack of
physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, having children late or not at all, older age, and family history. About 5–10% of cases are due to genes inherited from a person's parents, including BRCA1 and BRCA2 among others. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ductal carcinomas, while those developing from lobules are known as lobular carcinomas. In addition, there are more than 18 other sub-types of breast cancer. Some cancers develop from pre-invasive lesions such as ductal carcinoma in situ. The diagnosis of breast cancer is confirmed by taking a biopsy of the concerning lump. Once the diagnosis is made, further tests are done to determine if the cancer has spread beyond the breast and which treatments it may respond to. The balance of benefits versus harms of breast cancer screening is controversial. A 2013 Cochrane review stated that it is unclear if mammographic screening does more good or harm. The medication tamoxifen or raloxifene may be used in an effort to prevent breast cancer in those who are at high risk of developing it. Surgical removal of both breasts is another preventative measure in some high risk women. In those who have been diagnosed with cancer, a number of treatments may be used, including surgery, radiation therapy, chemotherapy, hormonal therapy and targeted therapy. Types of surgery vary from breast-conserving surgery to mastectomy. Breast reconstruction may take place at the time of surgery or at a later date. In those in whom the cancer has spread to other parts of the body, treatments are mostly aimed at improving quality of life and comfort. Outcomes for breast cancer vary depending on the cancer type, extent of disease, and
person's age. Survival rates in the developed world are high, with between 80% and 90% of those in England and the United States alive for at least 5 years.

In developing countries survival rates are poorer. Worldwide, breast cancer is the leading type of cancer in women, accounting for 25% of all cases. In 2012 it resulted in 1.68 million cases and 522,000 deaths. It is more common in developed countries and is more than 100 times more common in women than in men.

**Genetics:** Some genetic susceptibility may play a minor role in most cases. Overall, however, genetics is believed to be the primary cause of 5–10% of all cases. Women whose mother was diagnosed before 50 have an increased risk of 1.7 and those whose mother was diagnosed at age 50 or after has an increased risk of 1.4. In those with zero, one or two affected relatives, the risk of breast cancer before the age of 80 is 7.8%, 13.3%, and 21.1% with a subsequent mortality from the disease of 2.3%, 4.2%, and 7.6% respectively. In those with a first degree relative with the disease the risk of breast cancer between the age of 40 and 50 is double that of the general population. In less than 5% of cases, genetics plays a more significant role by causing a hereditary breast–ovarian cancer syndrome. This includes those who carry the **BRCA1** and **BRCA2** gene mutation. These mutations account for up to 90% of the total genetic influence with a risk of breast cancer of 60–80% in those affected. Other significant mutations include: **p53** (Li–Fraumeni syndrome), **PTEN** (Cowden syndrome), and **STK11** (Peutz–Jeghers syndrome), **CHEK2**, **ATM**, **BRIP1**, and **PALB2**. In 2012, researchers said that there are four genetically distinct types of the breast cancer and that in each type, hallmark genetic changes lead to many cancers.

**Medical conditions:** Breast changes like atypical ductal hyperplasia and lobular
carcinoma *in situ*, found in benign breast conditions such as fibrocystic breast changes, are correlated with an increased breast cancer risk. Diabetes mellitus might also increase the risk of breast cancer.

**Pathophysiology:** Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time. Normal cells will commit cell suicide (apoptosis) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT pathway; another is the RAS/MEK/ERK pathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed. This is one of the steps that causes cancer in combination with other mutations. Normally, the PTEN protein turns off the PI3K/AKT pathway when the cell is ready for cell suicide. In some breast cancers, the gene for the PTEN protein is mutated, so the PI3K/AKT pathway is stuck in the "on" position, and the cancer cell does not commit suicide.

Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure. Abnormal growth factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth. In breast adipose tissue, overexpression of leptin leads to increased cell proliferation and cancer. In the United States, 10 to 20 percent of people with breast cancer and people with ovarian cancer have a first- or second-degree relative with
one of these diseases. The familial tendency to develop these cancers is called hereditary breast–ovarian cancer syndrome. The best known of these, the BRCA mutations, confer a lifetime risk of breast cancer of between 60 and 85 percent and a lifetime risk of ovarian cancer of between 15 and 40 percent. Some mutations associated with cancer, such as p53, BRCA1 and BRCA2, occur in mechanisms to correct errors in DNA. These mutations are either inherited or acquired after birth. Presumably, they allow further mutations, which allow uncontrolled division, lack of attachment, and metastasis to distant organs. However, there is strong evidence of residual risk variation that goes well beyond hereditary BRCA gene mutations between carrier families. This is caused by unobserved risk factors. This implicates environmental and other causes as triggers for breast cancers. The inherited mutation in BRCA1 or BRCA2 genes can interfere with repair of DNA cross links and DNA double strand breaks (known functions of the encoded protein). These carcinogens cause DNA damage such as DNA cross links and double strand breaks that often require repairs by pathways containing BRCA1 and BRCA2. However, mutations in BRCA genes account for only 2 to 3 percent of all breast cancers. Levin et al. say that cancer may not be inevitable for all carriers of BRCA1 and BRCA2 mutations.
Figure 1: Overview of signal transduction pathways involved in apoptosis. Mutations leading to loss of apoptosis can lead to tumorigenesis.

**Diagnosis:** Most types of breast cancer are easy to diagnose by microscopic analysis of a sample—or biopsy—of the affected area of the breast. Also, there are types of breast cancer that require specialized lab exams. The two most commonly used screening methods, physical examination of the breasts by a healthcare provider and mammography, can offer an approximate likelihood that a lump is cancer, and may also detect some other lesions, such as a simple cyst. When these examinations are inconclusive, a healthcare provider can remove a sample of the fluid in the lump for microscopic analysis (a procedure known as fine needle aspiration, or fine needle aspiration and
cytology—FNAC) to help establish the diagnosis. The needle aspiration may be performed in a healthcare provider's office or clinic using local anaesthetic if required. A finding of clear fluid makes the lump highly unlikely to be cancerous, but bloody fluid may be sent off for inspection under a microscope for cancerous cells. Together, physical examination of the breasts, mammography, and FNAC can be used to diagnose breast cancer with a good degree of accuracy. Other options for biopsy include a core biopsy or vacuum assisted breast biopsy, which are procedures in which a section of the breast lump is removed; or an excisional biopsy, in which the entire lump is removed. Very often the results of physical examination by a healthcare provider, mammography, and additional tests that may be performed in special circumstances (such as imaging by ultra sound MRI) are sufficient to warrant excision biopsy as the definitive diagnostic and primary treatment method.

METHODOLOGY

Molecular docking studies

With a view to find a correlation between the anti-angiogenesis activity of the 5- (substituted aryl)-2-(5-methoxy-2H-chromen-3-yl)-1H-imidazoles (16a–f, 15h, 14f, h, j, 13f, g, i, j) and their binding with the target proteins viz.; KRAS, Wnt and VEGF, the docking studies were performed employing the AutoDock software. The receptor–ligand interactions and the corresponding interaction energies were identified after the docking studies. Since the Human KRAS protein is a target in anti-angiogenesis, we selected the crystal structure of the Human KRAS receptor protein with PDB ID: (4EPX.pdb) from the protein data bank. The docking studies were performed with fourteen molecules (16a–f, 15h, 14f, 14h, 14j, 13f, 13g, i, j) to investigate the receptor interactions. All of
the fourteen molecules were constructed using the tools of the SYBYL software and the structures were minimized using 500 steps of the steepest descent followed by 500 steps of the conjugate gradient methods and the structure was stabilized at 1100 steps.

Later the binding/catalytic sites of protein 4EPX.pdb were identified using the biopolymer module of the SYBYL-X (Tripos) software. Further, the molecules were loaded into the AutoDock software, and converted into pdb structures by applying the charge assigning method of Kolman and Gasteiger Huckle, and then saved into a pdbqt form. Later, polar atoms were added to the protein and the protein structure was maintained in the grid N-dimension (Grid sizes for receptor X = 11.393, Y = 1.498, Z = 9.595; ligand X = 40, Y = 36, Z = 38) for fourteen molecules. The virtual screening studies were performed using the AutoDock 4.0 software to predict the interactions based on the Genetic and Lamarckian algorithms.

Docking was performed and 140 results were generated on the basis of their energy values. The results were arranged in chronological order and analyzed. Similarly the above procedure was also applied to perform the docking studies for the Wnt receptor PDB ID: (3FOA.pdb) and PDB ID: Vegf.

RESULTS & DISCUSSION

Binding of 16f to Human KRAS receptor

The docking of compound 16f in the binding site of the Human KRAS receptor illustrated that the chromene and imidazole ring play a decisive role in inhibiting angiogenesis activity. The docking results showed hydrogen bonding interactions between the oxygen of the chromene ring and (i) the amine group of Asn 26 with a bond distance of 1.1 Å, (ii) ND2 of Asn 26, d = 2.5 Å and (iii) His 27, d = 3.2 Å. The methoxy oxygen
in the chromene ring also showed hydrogen bonding with His 27, d = 2.8 Å. Further hydrogen bonding interactions were also observed involving the two nitrogen atoms of the imidazole ring with the backbone carbonyl of Gln 25 (d = 2.6 Å and d = 2.3 Å) and Ile 24 (d = 3.4 Å). The chromene aromatic ring of 16f was surrounded by the Asn 26, His 27, Gln 25 and Ile 24 amino acid residues.

**Binding of 16f to Wnt receptor**

Docking of compound 16f in the active site of the Human Wnt receptor showed hydrogen bonding between the nitrogen atom of the imidazole ring with the backbone carbonyl of Gln 25 CB (d = 1.66 Å) and His 27 (d = 3.03 Å). The oxygen atom of the chromene ring showed interactions with the amine group of Tyr 100 (d = 1.905 Å).

**Binding of 16f to VEGF receptor**

16f in the active site of the VEGF receptor showed two hydrogen bonding interactions between the nitrogen atom of the imidazole ring with Arg 82 (d = 2.37 Å and d = 2.05 Å). Further hydrogen bonding interactions from the oxygen atom of the chromene ring and amine group of Arg 105 (d = 1.93 Å) was observed. For the other chromenyl imidazoles synthesized their docking studies did not show closer interactions with the KRAS, Wnt and VEGF receptors.
Figure 2: H-bonding interactions between amino acid residues at the active site of the Human KRAS target and compound 11f.

![Figure 2](image)

Figure 3: Surface representation of the KRAS receptor and ligand complex 11f.

![Figure 3](image)

Figure 4: H-bonding interactions between amino acid residues at active site of the Wnt target and compound 11f.

![Figure 4](image)
Figure 5: Surface representation of the Wnt receptor and ligand complex 11f.

Figure 6: H-bonding interactions between amino acid residues at the active site of the VEGF target and compound 11f.
Table 1: Binding of 16f to human KRAS receptor

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<th>Binding of 16f to human KRAS receptor: Chromene and imidazole ring interacts with the receptor by forming hydrogen bonds</th>
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Table 2: Binding of 16f to Wnt receptor

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**CONCLUSION**

Several new 2-(substituted-2H-chromen-3-yl)-5-aryl-1H-imidazoles (13–16) were rationally designed, synthesized and subjected to in vitro primary anticancer screening against several cancer cell lines (SW480, DLD-1, HCT116, GSK 3b in pretreated viability HCT116, HT-29, RKO, SW837, Colo320 and SNU-C1). Among them four compounds (13g, 16a, 16e and 16f) were subjected to the basal TCF/TK Luciferase DLD-1 assay. The results of the in vitro cancer assay indicated that all the compounds showed considerable cancer inhibition. However, the substitution of the methoxy group at the 5th position of the chromene and the 4th position of the benzyloxy group on the phenyl ring of the imidazole was found to be more favorable for cancer inhibition. Whereas, the compounds with no methoxy group at the 5th position on the chromene showed diminished cancer activity. Among all the screened compounds, 16f was found to be
the most potent inhibitor of cancer with the highest selectivity. Furthermore, in the anti-angiogenesis assay compound 16f showed potent activity in inhibiting the VGF_ADSC/CFC Angiotube area. The molecular docking analysis also exhibited the higher binding affinity of 16f with the KRAS, Wnt and VEGF ligands. Hence, 16f could be considered as a lead structure in the development of a new series of antiangiogenesis/anticancer agents.

**REFERENCES**


