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Synthesis of some novel benzimidazole derivatives act as antibreast cancer activity

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Abstract

Benzimidazole, the benzo derivative of imidazole, is a class of bicyclic aromatic organic compound consisting of six-membered benzene ring fused to five membered imidazole at 4- and 5-positions of the imidazole ring. It is a vital pharmacophore of many biologically active heterocyclic compounds with a variety of pharmacological activities. Over the time, benzimidazole and its derivatives have evolved as vibrant heterocyclic systems due to their potency in a wide range of bioactive compounds like analgesics, antifungals, anti-inflammatory, antihypertensives, proton pump inhibitors, anti-HIV, antiviral, and so on. Multi-drug resistance in cancer that led to the failure of many chemotherapeutic drugs is a major concern. Various approaches are being developed to overcome this problem. One of them is target based drug discovery, which is an effective method to develop a novel anticancer drug. To develop newer drugs, previously reported work needs to be studied. Keeping this in mind, last 5 years literature on benzimidazole used as anticancer agent has been reviewed and summarized in the paper this research article along with the target of cancer also deal with structure activity relationship of various compounds having benzimidazole.

Keywords: Anticancer, benzimidazole, death, killing

Introduction

Cancer is a class of diseases in which group of cells display uncontrolled growth, invasion that intrudes upon and destroys adjacent tissues and sometimes metastasis, or spreading to other locations in the body via lymph or blood. Cancer is a major human health problem worldwide and is the second leading cause of death in the United State.

Breast cancer: Breast cancer is one of the most prevalent cancers and the leading cause of cancer death among women worldwide [1]. Apoptosis is an important biological process of cell death that is critical for elimination of unwanted, damaged or infected cells and is associated with diverse biological events including cell development, differentiation and proliferation [2].

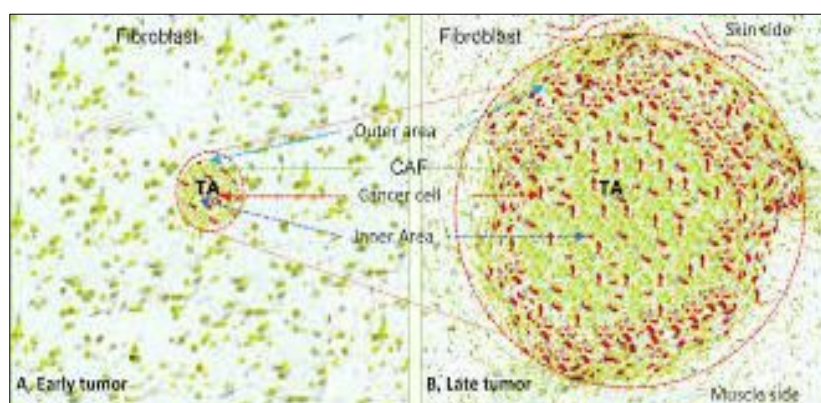


Fig 1: Formation of tumor

The epidermal growth factor receptor (EGFR) is one of the first identified important target of these novel antitumor agents. Approximately half of cases of Triple-Negative Breast Cancer (TNBC) and Inflammatory Breast Cancer (IBC) overexpress EGFR.

Cancer continues to pose a serious threat to human life, ranking as the second most common cause of death globally in 2022. According to estimates, 13.1 million individuals will pass away from cancer by 2030 Breast cancer is the most common kind among women in both developed and developing nations, accounting for one in ten of all new cancer diagnoses worldwide each year [3]. The regulation of tumor cell proliferation, differentiation, angiogenesis, and death is significantly influenced by EGFR. The majority of malignant epithelial cells in breast cancer overexpress EGFR and its receptors, EGFR indicating that plays a critical role in the angiogenic progression and metastasis of breast cancer. Reviews of the literature revealed that numerous heterocyclic scaffolds have been studied as potential treatments for breast cancer [4]. While several novel strategies, including as targeted medication therapy (e.g., atezolizumab, erlotinib, and Second, it's getting harder and harder to discover novel anti-cancer medication According to a recent study, the average cost of research and development for a new drug is \$985.3 million, with anti-cancer drugs costing even more [5].

Benzimidazole based marketed anticancer drugs

Binimetinib: The USFDA has approved binimetinib a chemical 5-((4-bromo-2-fluorophenyl) amino)-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-benzimidazole-6-carboxamide, in 2018 [6]. It is a strong, oral inhibitor of mitogen-activated protein kinase (MEK 1/2) that is selective. Array Biopharma is the developer of binimetinib, which is sold commercially under the brand Mektovi [7]. It is recommended as combination therapy with BRAF inhibitors Encorafenib for patients with metastatic melanoma with a BRAF mutation [8]. Currently, binimetinib is being developed clinically in different stages as an

immunotherapy or combination treatment for diseases such as non-small cell lung cancer with a mutant KRAS mutation [9].

Bendamustine

Bendamustine (2) is an alkylating agent that is widely recognized for its effectiveness and tolerance in treating a variety of hematologic malignancies. Its chemical formula is 4-(5-(bis (2-chloroethyl)amino)-1-methyl-1H-benzimidazol-2-yl) butanoic acid [10]. Bendamustine is recommended for the treatment of non-Hodgkin lymphoma and chronic lymphocytic leukemia For the treatment of patients with mantle cell lymphoma who are older than 60, bendamustine is now being investigated as a combination therapy in addition to Venetoclax, a Bcl-2 inhibitor, and Rituximab (NCT03834688) [11].

Selumetinib

Recently, on April 10, 2020, the US FDA approved selumetinib (3), which is chemically 5-((4-bromo-2-chlorophenyl) amino)-4-fluoro-N-(2-hydroxyethoxy)-1-methyl-1H-benzimidazole-6-carboxamide, for the treatment of plexiform neurofibromas and neurofibromatosis in pediatric patients AstraZeneca developed selumetinib, an oral MEK 1/2 kinase inhibitor that is marketed under the Koselugo brand [12].

Veliparib

Chemically speaking, veliparib is an oral PARP inhibitor, namely (R)-2-(2-methylpyrrolidin-2-yl)-1H-benzimidazole-4-carboxamide. In preclinical and clinical trials, the experimental medication veliparib demonstrated encouraging outcomes when used to treat ovarian cancer, including ovarian cancer with a mutant form that is BRCA-mutated [13].

Dovitinib

This powerful oral pan tyrosine kinase inhibitor, dovitinib (6), is chemically 4-amino-5-fluoro-3-(5-(4-methylpiperazin-1-yl)-1H-benzimidazol-2-yl) quinolin-2(1H)-one It targets VEGFR, FGFR, and other tyrosine kinases [14].

Experiment work

Scheme of work

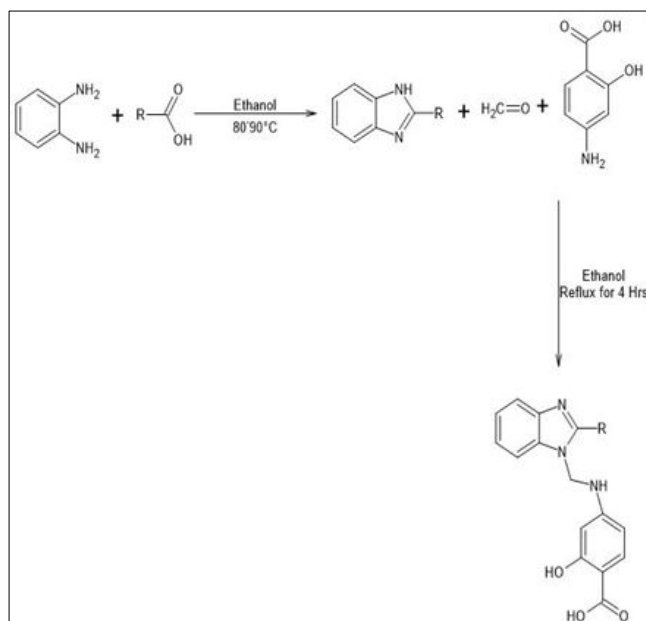
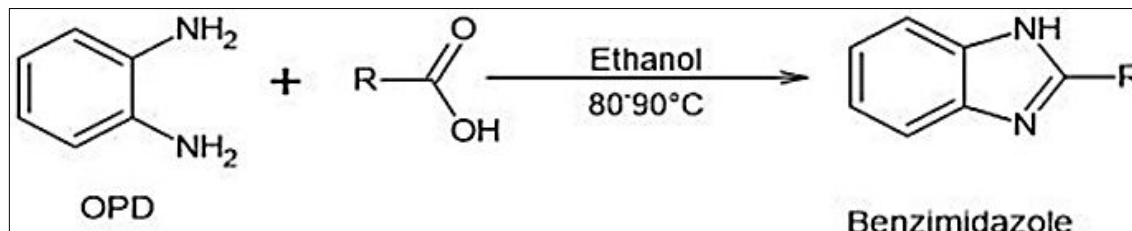


Table 1: Name of derivative

Name of derivative	-R
I	-H
II	-CH ₃
III	-CH ₃ CH ₂

General procedure**1. Synthesis of ben [1H] imidazole 1311**

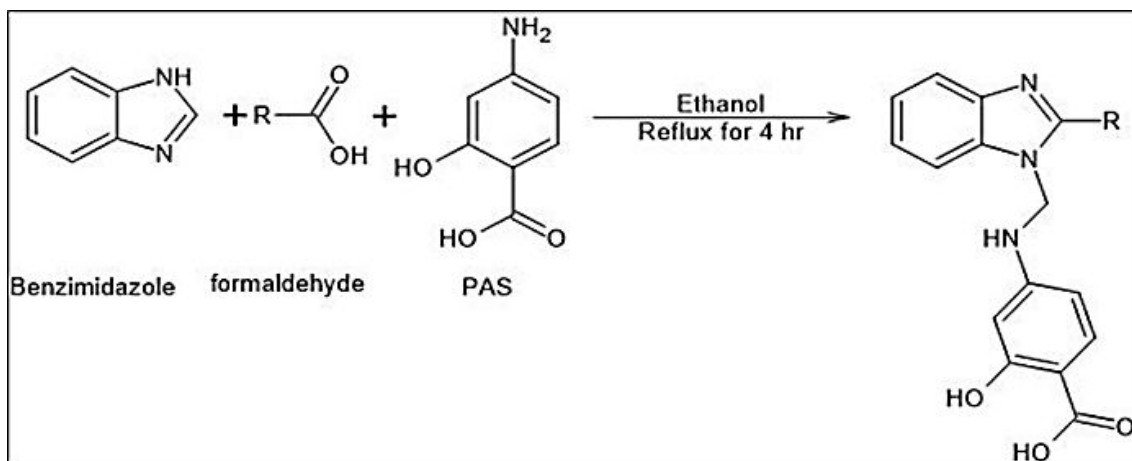
- 2.7 gm o-phenylenediamine was transfer to RBF into this 1.7gm (1.6ml), 0.034 mol of 90% Formic acid was added. The mixture was heated for 2hr at 100 °C.

**2. General procedure for synthesis of final compound**

A mixture of Benzimidazole and its derivatives (0.02mole), Formaldehyde (0.02) and Para-amino salicylic acid (0.02 mole) in ethanol (15 ml) was heated under reflux for 4hrs.

- The flask was removed and cooled, to this solution 10% NaOH is added until the mixture is just alkaline to litmus paper.
- Filter the product and wash it with cold water. The product can be purified by boiling it with 40 ml water and 0.2gm of charcoal for 15 minute.
- It was filtered in hot condition and filtrate was cooled to 10 °C, crystal will start to appear. Melting Point: 170-172 °C, Mobile Phase- Chloroform: Methanol = 3:2.

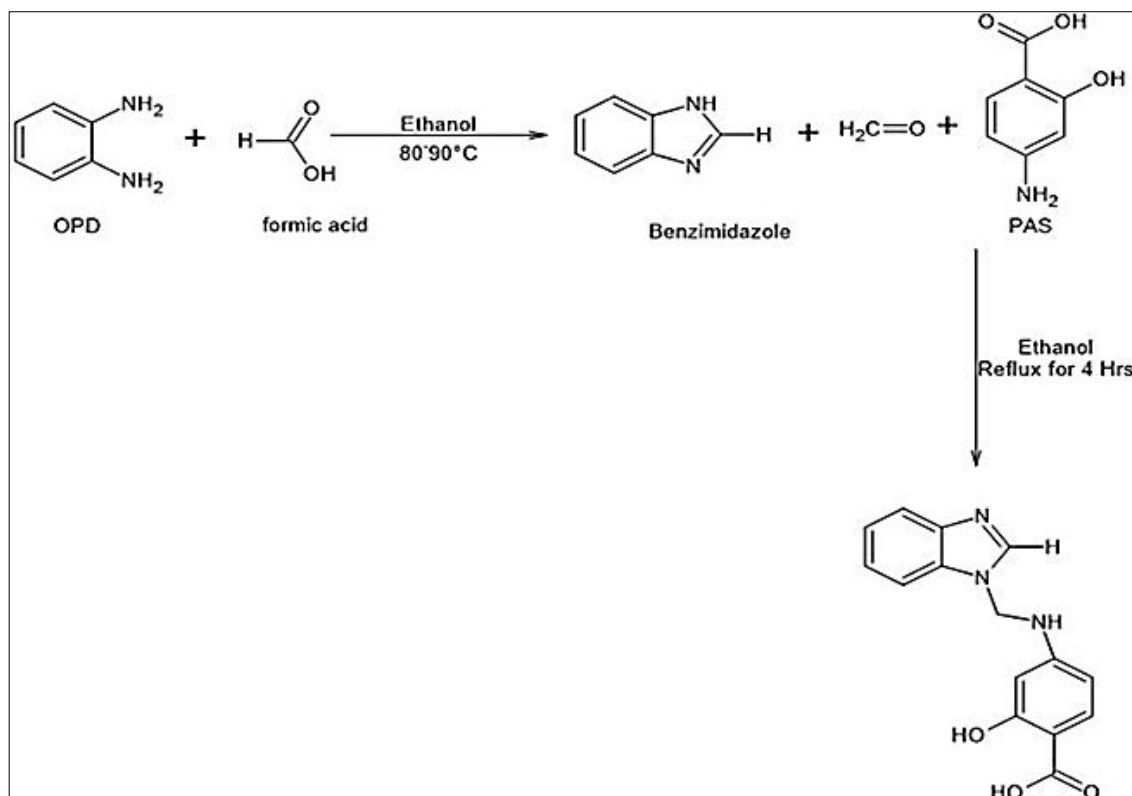
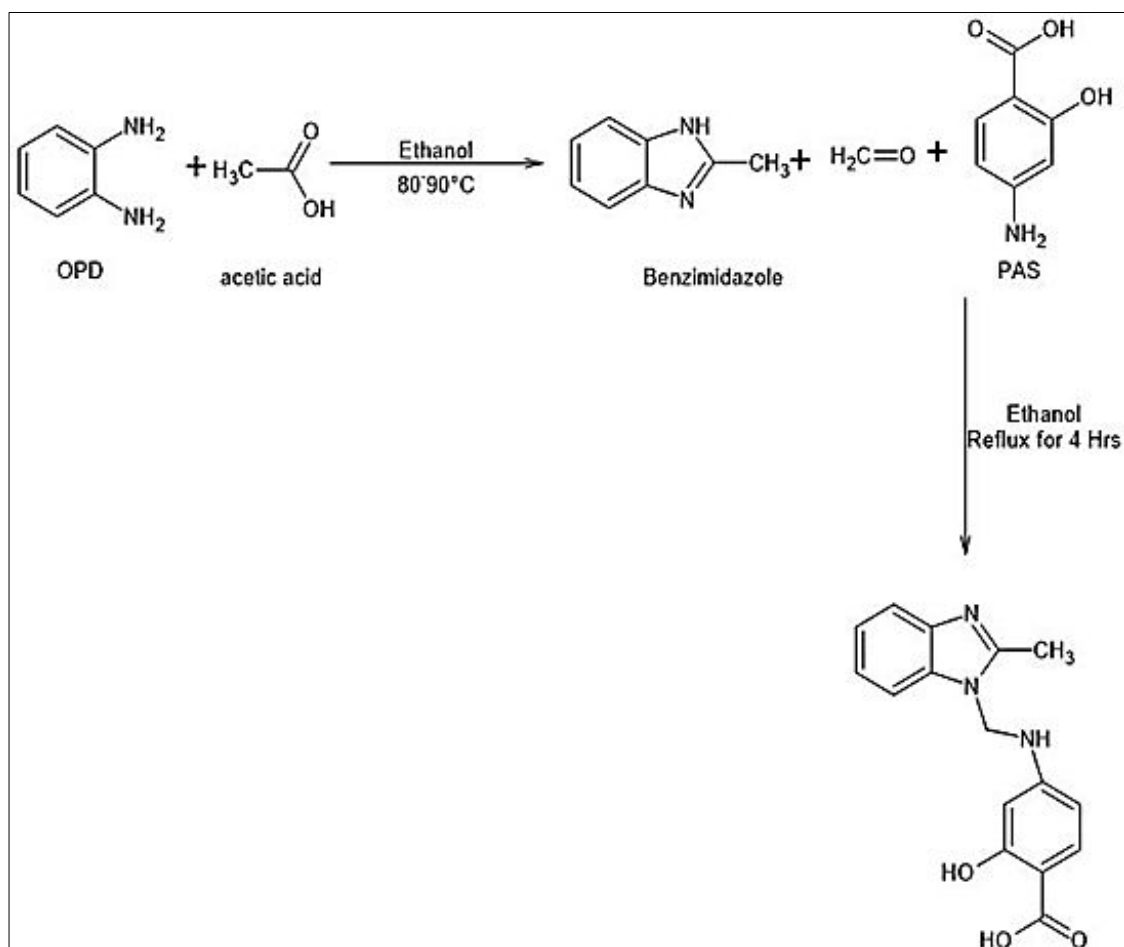
Subsequently ethanol was distilled off and the lump mass obtained. It was triturated with petroleum ether (40-60 °C). The solid was isolated and dried in air.

Reaction**Reaction Involve in the Synthesis****Mechanism Reaction**

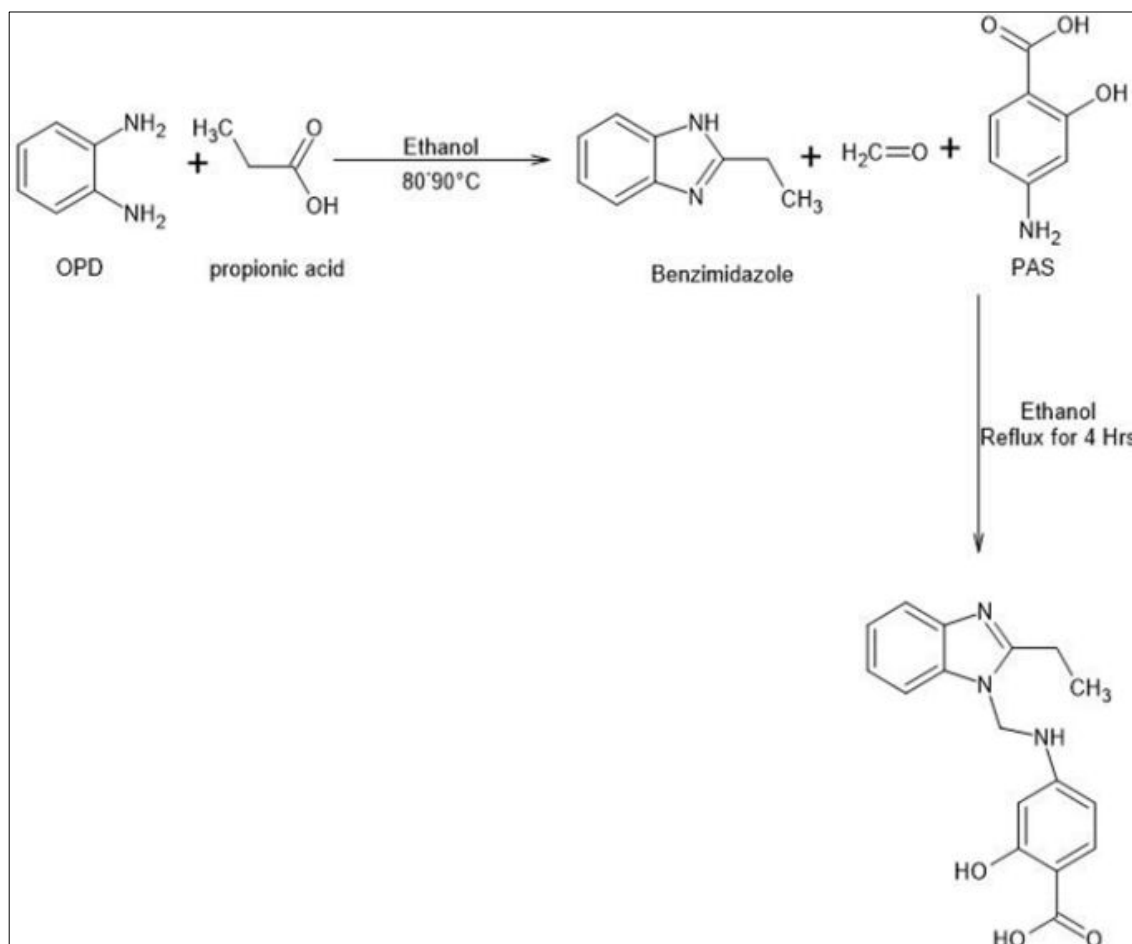
- The Mannich reaction is an organic reaction which consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group by Formaldehyde and a Primary or secondary amine/ammonia
- The final product is a beta-amino-carbonyl compound also known as "Mannich base".
- The reaction is named after "Chemist Carl Mannich".

- The Mannich reaction is an example of nucleophilic addition of an amine to a carbonyl group followed by dehydration to the Schiff base.
- The Schiff base is an electrophile which reacts in the second step in an electrophilic addition with a compound containing an acidic proton.

The Mannich reaction is also considered a Condensation reaction

Synthesis of derivatives**1. Synthesis of 4-(((1H-benzo [dimidazole-1-yl) methyl) amino)-2-hydroxybenzoic acid****2. Synthesis of 2-hydroxy-4-(((2-methyl-1H-benzo[d]imidazole-1-yl) methyl) amino) benzoic acid**

3. Synthesis of 4-(2-ethyl-1H-benzo[d]imidazol-1-yl)methyl)amino)-2-hydroxybenzoic acid



General procedure

1. Synthesis of Ben [1H] imidazole 1311

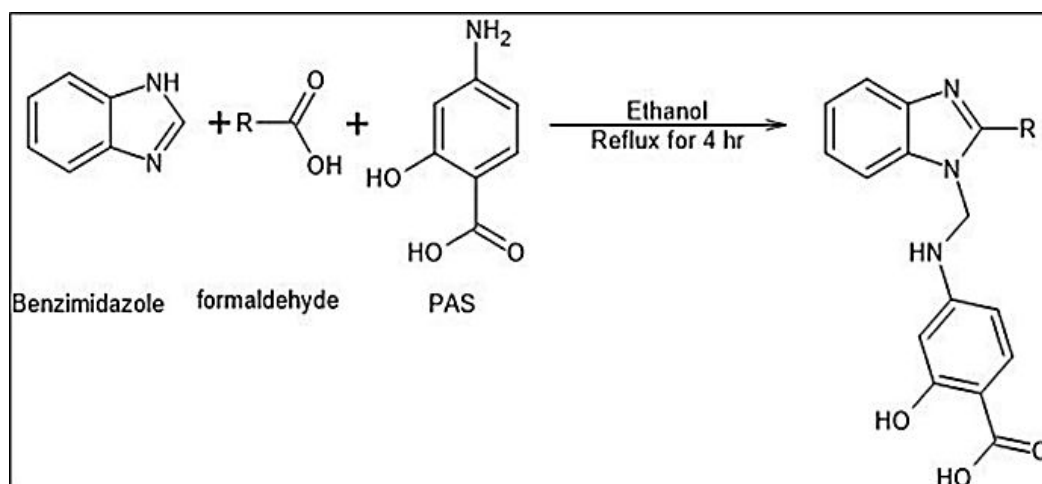
- 2.7 gm o-phenylenediamine was transfer to RBF into this 1.7 gm (1.6 ml), 0.034 mol of 90% Formic acid was added. The mixture was heated for 2hr at 100 °C.
- The flask was removed and cooled, to this solution 10% NaOH is added until the mixture is just alkaline to litmus paper.
- Filter the product and wash it with cold water. The product can be purified by boiling it with 40 ml water and 0.2 gm of charcoal for 15 minute.

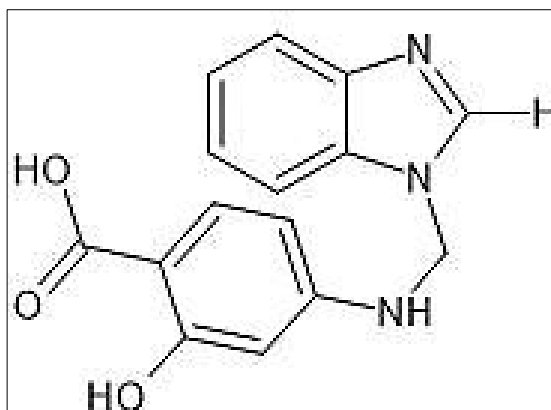
- It was filtered in hot condition and filtrate was cooled to 10 °C, crystal will start to appear. Melting Point: 170-172 °C, Mobile Phase- Chloroform: Methanol = 3:2

2. General procedure for synthesis of final Compound

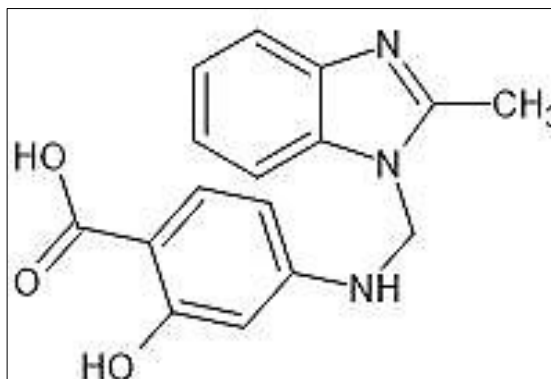
A mixture of Benzimidazole and its derivatives (0.02 mole), Formaldehyde (0.02) and Para-amino salicylic acid (0.02 mole) in ethanol (15 ml) was heated under reflux for 4 hrs. Subsequently ethanol was distilled off and the lump mass obtained. It was triturated with petroleum ether (40-60 °C). The solid was isolated and dried in air.

Reaction



Results**Physicochemical Characteristics****Table 2:** Physicochemical Characterization of 1st Derivative

Sr. No.	Content	Observation
1)	Color of Product	Brown
2)	Yield	0.25
3)	Melting Point	145-150 °C
4)	TLC	Ethyl Acetate: Pet. Ether = 4:1
	Mobile phase	
	RF value	
5)	Starting material	0.53
	Final product	0.61

**Table 3:** Physicochemical Characterization of 2nd Derivative

Sr. No.	Content	Observation
1)	Color of Product	Brown
2)	Yield	0.27
3)	Melting Point	120-125 °C
4)	TLC Mobile phase	Chloroform: Ethanol 3:2
	RF value	
5)	Starting material	0.43
	Final product	0.54

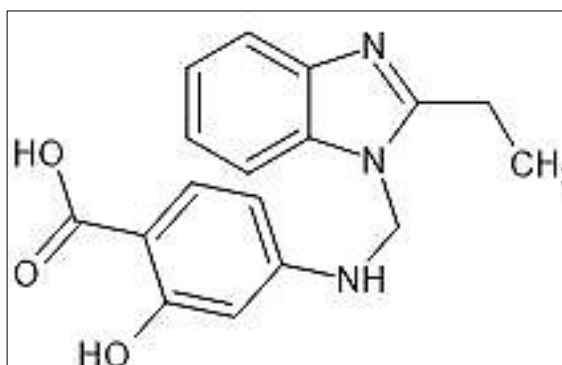
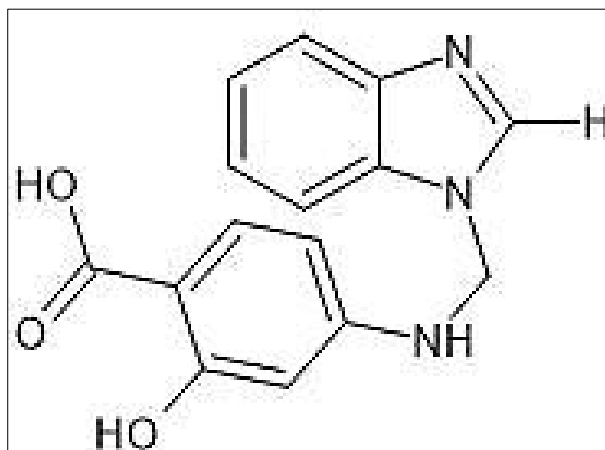
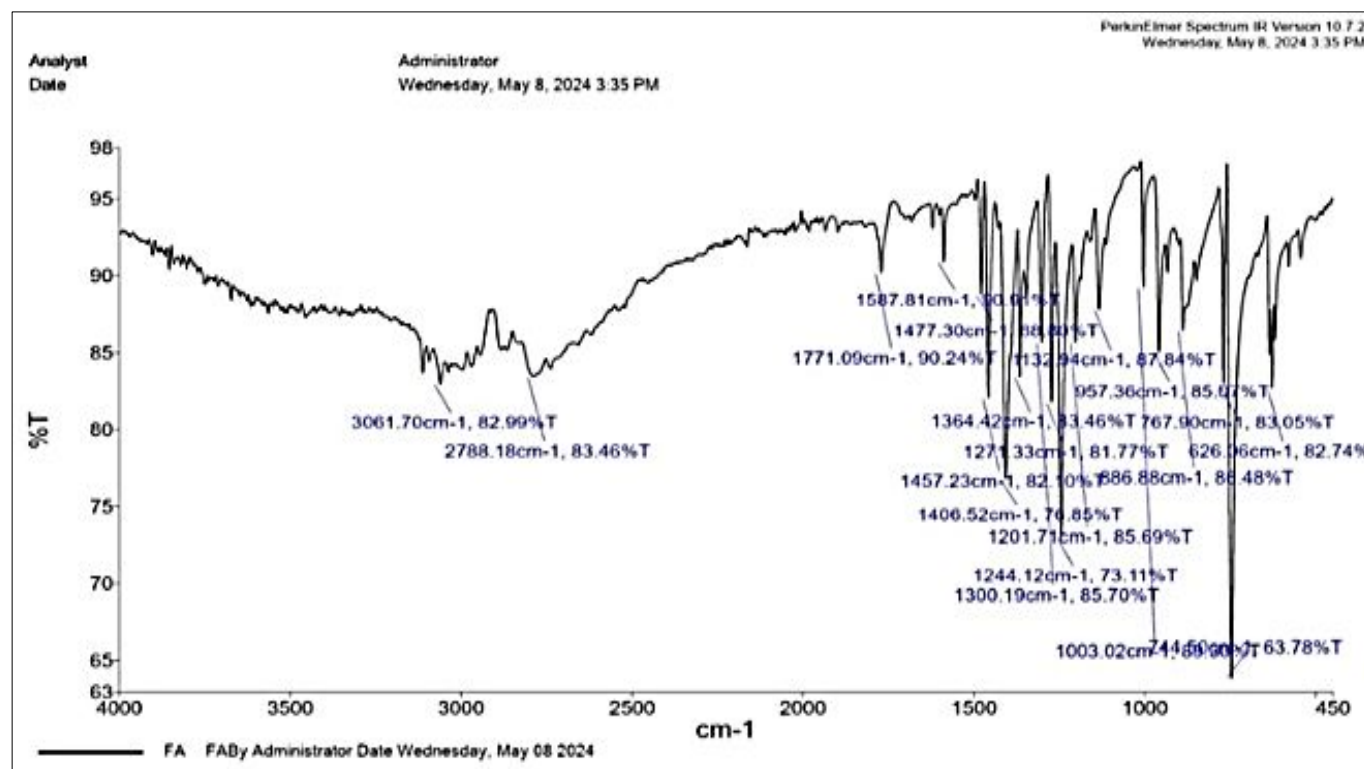
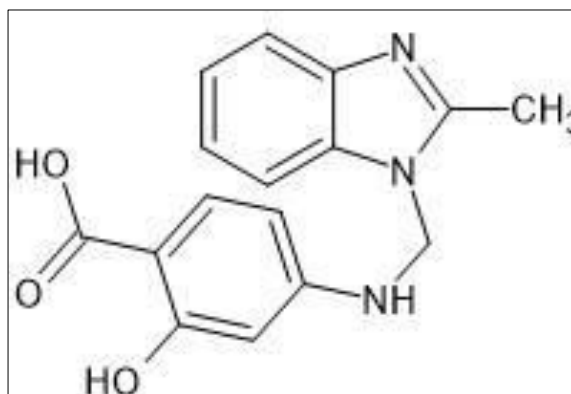
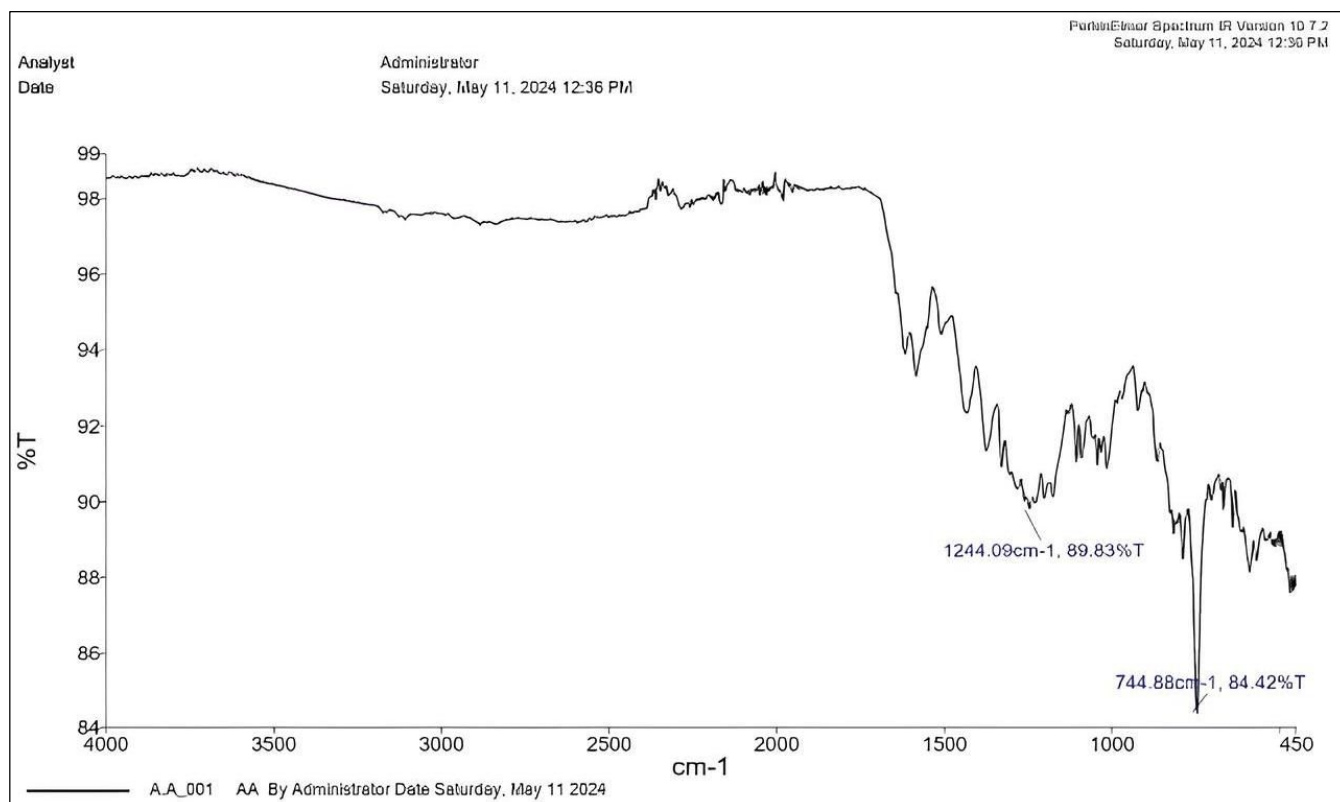


Table 4: Physicochemical Characterization of 3rd Derivative

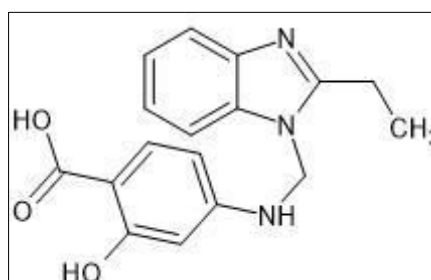
Sr. No.	Content	Observation
1)	Color of Product	Brown
2)	Yield	0.31
3)	Melting point	55-60 °C
4)	TLC Mobile Phase	Chloroform: Ethanol 3:2
5)	RF value	
	Starting Material	0.8
	Final Product	0.6

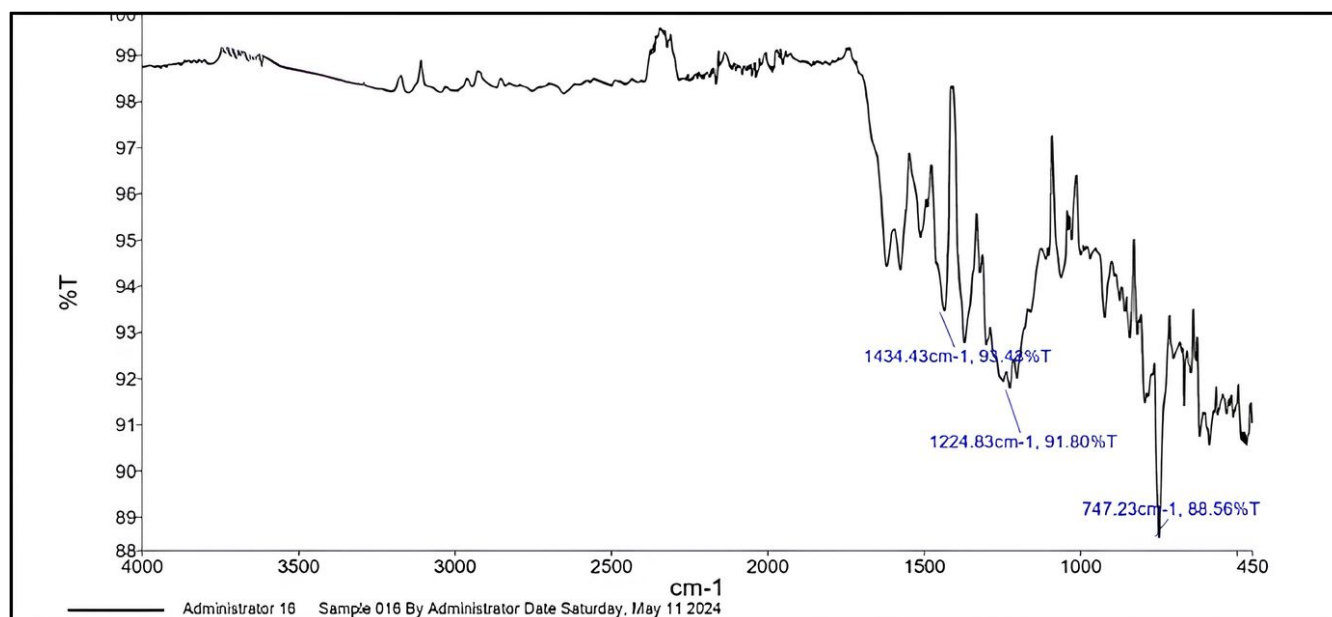
Fourier Transform Infrared Spectroscopy (FTIR) Spectra of 1st Derivative**Molecular Formula:** C₁₅N₃O₃H₁₃**IR Interpretation Spectra**

Sr. No.	Functional group	Wave number (cm ⁻¹)
1)	C-H (Aromatic)	3061.70
2)	C-N stretching of tertiary amine	1364.42
3)	R-COOH	2788.18

Fourier Transform Infrared Spectroscopy (FTIR) Spectra of 2nd Derivative**Molecular Formula: C₁₆N₃O₃H₁₅****IR Interpretation Spectra**

Sr. No.	Functional group	Wave number (cm ⁻¹)
1)	C-O	1244
2)	Cis & trans alkene group present	744

Fourier Transform Infrared Spectroscopy (FTIR) Spectra of 3rd Derivative

Molecular Formula: C₁₇N₃O₃H₁₇**IR Interpretation Spectra**

Sr. No.	Functional group	Wave number (cm ⁻¹)
1)	C-H (Alkyl group present)	1434
2)	C-O	1224
3)	Cis & trans alkene group present	747

Anti-breast cancer screening**Table 5:** Effects of compound against MCF-7 Cell line (Breast cancer cell line) by MTT assay

Sr. No	Sample	Concentration (µg/m)	Absorbance (nm)	% inhibition	IC50
1	Control		3.893		
2	Std. 5 FU	10	1.583	59.33	81.91
		30	1.383	64.47	
		100	1.145	70.58	
3	FA I	10	2.463	36.73	184.56
		30	2.638	32.23	
		100	2.215	43.10	
4	AA II	10	2.770	28.84	>100
		30	2.683	31.08	
		100	2.588	33.52	
5	PA III	10	2.158	44.56	>100
		30	2.169	44.28	
		100	2.109	45.82	

Discussion**Pharmacophore**

Benzimidazole pharmacophore has been used in many compounds which exhibit anti-breast cancer activity. Benzimidazole attached with various types of cap group and linker, shows good anti-breast cancer activity: Hence, Benzimidazole derivatives were prepared for anti- breast cancer activity.

Chemical Studies**1. Synthesis**

- Synthesis of 4-(((1H-benzo [dimidazole-1-yl)methyl) amino)-2-hydroxybenzoic acid
- Synthesis of 2-hydroxy-4-(((2-methyl-1H-benzo [d] imidazole-1- yl) methyl) amino) benzoic acid
- Synthesis of 4-((2-ethyl-1H-benzo [d]imidazol-1-yl) methyl) amino)-2-hydroxybenzoic acid

Benzimidazole Derivatives were synthesized as per the established route of synthesis. Their melting points were taken in open capillaries on scientific melting point apparatus and were quoted as uncorrected values. Thin layer chromatography (Revalue) confirmed the purity of these compounds the synthesis data along with their melting points, % yield and R_f values is compiled.

2. Infrared spectroscopy study

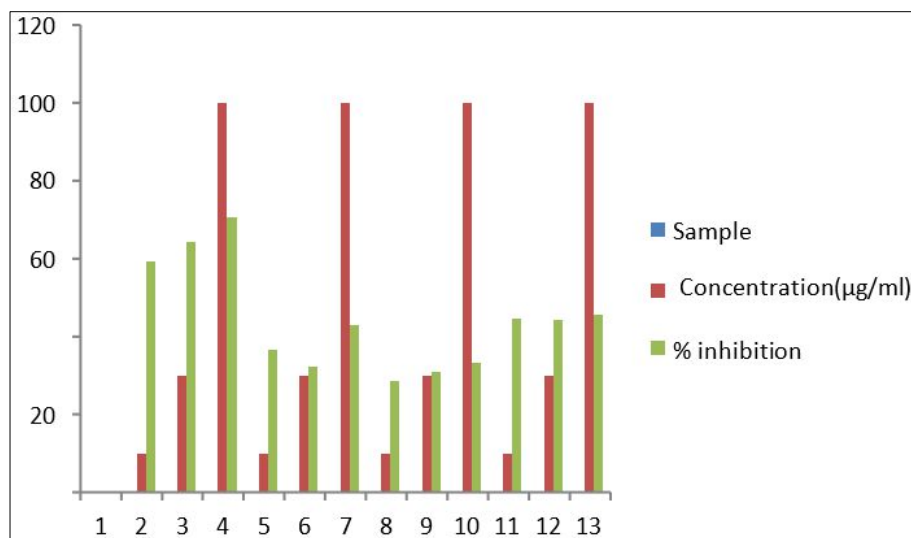
The infrared spectra of the first, second and the final representative compound FAI, AAI, PAII using JASCO FT IR Spectrometer 4000 1 was obtained in KBr and peaks are expressed in terms of wavenumber (cm) The FT IR spectra are presented in spectra 1,2,3. The FT-IR interpretation is satisfied and the entire compounds contain to their anticipated structure

3. Biological Studies

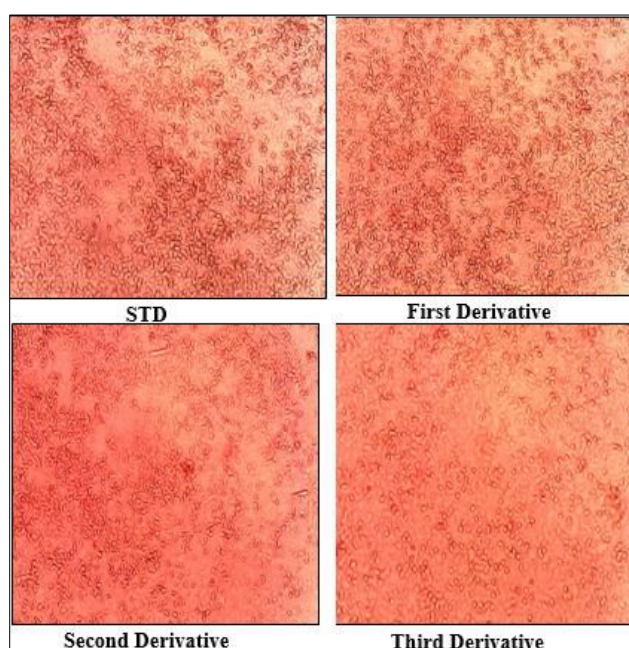
The biological screening was done by using MCF-7 cell line culture the synthesize series of compound has generated certain compounds that can inhibit % growth which were screen on MCF-7 cell line. The result suggested that synthesized compound FAI, AAI, PAII has given activity as compared to standard drug.

Conclusion

At the Concentration 100 µg/ml, HI and HAI samples showed good percent inhibition MCF-7 cell line as compared to standard drug.



Screening of anti-breast cancer activity



Conclusion

Although cancer has traditionally been viewed as a genetic disease the perspective of cancer as an epigenetic disease is rapidly emerging thus in addition to mutation in the DNA it code increasingly clear that cancer can result from aberration in chromatin and chromatin modifying proteins such as aEGFR result EGFR inhibitors represent one promising subgroup of compound for epigenetic therapy this all has been summarised in the section introduction.

The experimental method consists of synthesis of

1. Synthesis of 4(1H-Benzo [dimidazole-1-ymethylamino]-2-hydroxybenzoic acid
2. Synthesis of 2-hydroxy-4-[2 methyl-1H-benzo[d]imidazole-1-methyl amino]benzoic acid
3. Synthesis of 4-[2 ethyl-1-H-benzo[d]imidazole-1yl]amino]-2-hydroxybenzoic acid

The total 3 final compounds were synthesized using scheme presented in the section; Material an method

The melting point thin layer chromatography [RF value] FT-IR spectroscopy conform the structure and purity of

synthesized compound this has briefly summarized in section

Results: physical and spectral characterization

The synthesized series of compound has generated certain compound that can inhibit % growth which were screen on MCF-7 cell line the observations has been well tabulated in section result Anti-Breast cancer screening the synthesized compound FA-I, AA-II, PA-III has good activity as compare to standard drug.

From the research work undertaken and the result obtained in project initiated design and synthesis benzimidazole derivative antibreast cancer activity was confirmed in result.

Acknowledgement

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