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Ligand based design, synthesis and SAR analysis of some novel 2-substituted-1H-Benzo[d]imidazoles as antineoplastic agents.

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Abstract: *Benzimidazole is a heterocyclic aromatic organic compound and well known as an important pharmacophore and a privileged structure in medicinal chemistry. Several newer benzimidazoles have been reported to possess cytotoxic activity against various cancer cell lines. In the present study, some novel 2-substituted 1H-Benz[d]imidazoles were synthesized and the structures were confirmed by spectral analysis like FTIR, ^{1}H-NMR and MS analysis. The compounds were screened for in silico cytotoxicity activity against HER2. The docking study showed that the compound U-1 and U-3, having 4-cholro-3-nitrophenyl and dimethoxy phenyl substitution at 2nd position in the benzimidazole ring system showed very good biding efficiency. Further, the compounds were tested for their antimicrobial activity against E.Coli at 50, 100, and 200 µg/ml concentrations. Similar results were obtained here also. Compound having the 4-chloro-3-nitro and dimethoxy phenyl substitution was found to be showing good zone of inhibition than the other compounds. The results revealed that the methoxy, chloro and nitro substitutions imparted crucial role in deciding the activity of the benzimidazole scaffold.*

Keywords: *^{1}H-Benzo[d]imidazoles, in silico cytotoxicity activity, HER2, E.Coli.*

1. INTRODUCTION :

Benzimidazole is an aromatic heterocyclic fused rings system containing of benzene and imidazole. It is an important pharmacophore and also a most privileged structure in medicinal chemistry. In recent days, it is a moiety of choice which possesses spectrum of pharmacological properties. The most prominent compound found in nature is N-ribosyl-dimethyl benzimidazole, which is benzimidazole ring system. It serves as an axial ligand for cobalt in Vitamin B12. Presence of benzimidazole nucleus in numerous categories of therapeutic agents were reported for antimicrobials (1), antivirals (2), antifungal (3, 4) anti-parasites (4), anticancer (5), anti-inflammatory (6), antioxidants(7), proton pump inhibitors (8), antihypertensive (9), anticoagulants (10), immunomodulators (11), hormone modulators (12), CNS stimulants, CNS depressants (13), lipid level modulators (14), antidiabetics (15), Anthelmintics (16), HIV inhibitors (17), antimalarial (18) etc. has made it an indispensable anchor for development of new therapeutic agents. Nowadays, it's becoming a challenging task in our field to come up with a newer drug like molecules and with more enzyme specificity of a particular cancer cell line (19-21), Recent developments in drug design and discovery majorly help us to understand the underlying mechanism of the cancer cell progression.

In most of the research studies it was reported that the heterocyclic possess broad range of therapeutic values, one such important heterocycle is benzimidazole. Its Derivatives are widely listed in united states Food and Drug administration. They are the nitrogen containing heterocyclic compounds and widely known for various biological and pharmacological activities like angiotensin II receptor blockers (azilsartan), anthelmintic agents (albendazole, ciclobenazole), antihistamines (astemizole, bilastine), fungicides(benomyl, carbendazim), opioids (bezitramide, brophine), Proton-Pump Inhibitors (dexlansoprazole, esomeprazole), antipsychotics (benperidol, clopimozide) etc., Further, the associations of computer in the drug design and discovery is found to be more essential in the drug research, The docking

study done previously revealed that the benzimidazole scaffold forms good interactions with the amino acid residues of the target proteins or receptors through hydrogen bond or pi-pi conjugation and hydrophobic interactions.
Number of growth factors and their receptors are involved in the development and metastasis of cancer, Tyrosine Kinase is one such enzyme receptor which plays a central role in cancer development, They are cell surface receptors which react by initiating appropriate signalling pathways which plays a key role in the regulation of cancer sternness, It makes them suitable in the breast cancer therapy, However, a lot of challenges are arising because of structural mutations, gene amplification and alternate pathway activation etc., In the light of the above facts, we thought it's worthwhile to propose this research work which includes the synthesis of 2-substituted-^{1}H-benzimidazoles and study of their SAR and antineoplastic activity with the help of computer applications.

2. MATERIALS AND METHODS :

Chemistry: General procedure for Synthesis of 2-substituted benzimidazole

Equimolar mixture of o-phenylenediamine (0.01M) and carboxylic acids (0.01M) in the presence of ammonium chloride and ethanol was refluxed in a reflux condenser for 2 hours at 80^{0}C. The products were then cooled and made alkaline using 10% NaOH until the red litmus turned blue. Then, Thin Layer Chromatography was performed to check out the purity of the products. Then the products were recrystallized and the melting points were recorded [22].

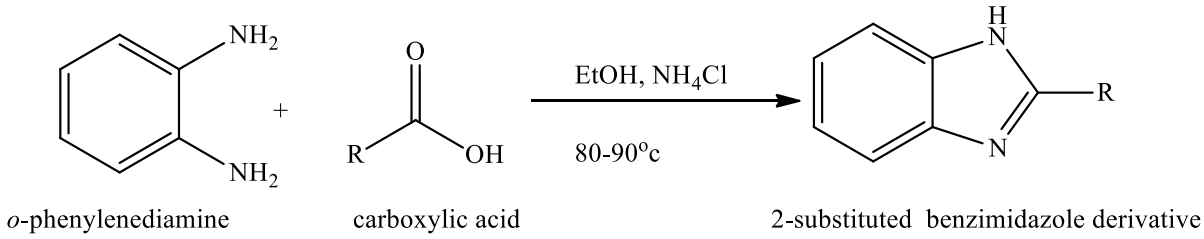

Figure 1. Synthesis of 2-substituted benzimidazole derivatives

All the chemicals used were of AR grade. The reaction was monitored using TLC method. The melting point of all synthesized compound were recorded and are uncorrected. IR Spectra were recorded on Perkin-Elmer-1800 FTIR Spectrophotometer. ^{1}H-NMR spectra ($CDC1_3$) were recorded on Bruker Advance 400 NMR spectrophotometer using TMS as internal standard. Mass spectra were recorded on LC-MS Q-T of Micro Mass analyser (shimadzu).

In Silico Screening of 2-substituted Benzimidazole Derivatives on HER2

Molecular docking is a tool of computational investigation of ligand binding to a receptor. This *in silico* approach reduces the laboratory works as well as justifies chemical/physical explanations. In addition, it helps to predict the biological activity of a given ligand and for lead optimization. The X-ray crystal structure of the protein, HER2 was downloaded from the RSCB Protein Data Bank (RCSB PDB) for the docking studies.

Steps Involved in Docking Studies

To get the docking score value, at first ligand structure were drawn on the chemdraw software in mdl (.sdf) file format. And next, PDB format of protein structure were downloaded from the RCSB website i.e., HER2. Then pre-processed protein in Swiss PDB Viewer software and make a new folder in the desktop with attaching both the ligand along with the pre-processed macromolecule in the same. Open PyRx software and click the following (Edit-preferences-workspace-browse-ok) and close the software. Again, open PyRx software and click the following (file-import-chemical table file-next) and then choose the ligand which will display on the screen. At the bottom row, the selected option will be seen just right click and choose to minimize all and again do right click and choose to convert all to auto dock option. Then choose vina wizard option and press start, add macromolecule. Afterwards, select the ligand and macromolecule shown onto the screen, choose forward option, the docking process will start and shows the highest score value at the first and can be saved in .csv format (23-25), *in silico* molecular design of heterocyclic benzimidazole scaffolds as prospective anticancer agents.

In Vitro antimicrobial activity on E.coli

In this study, all the synthesized compounds were screened for antimicrobial activity by the disk diffusion method. The antibacterial activity of the compounds was evaluated against Gram-negative bacteria: *Escherichia coli.* In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface.

The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured. Nutrient agar plates were prepared by mixing agar, peptone, NaCl and beef extract in a conical flask and gently heating the mixture. The mixture was then poured onto the petri dishes which harden on cooling. Then, the agar plates were inoculated with *Escherichia coli*. Antimicrobial activity was studied using ciprofloxacin as a standard. 6mm discs were prepared using Whatmann filter paper. The discs used for standard were loaded with 1% concentration of ciprofloxacin. The discs used for test were loaded with 200µg, 100µg and 50µg of the benzimidazole compounds. One disc was used as solvent control. The discs were then, gently placed on the bacteria containing agar plates. The agar plates were then placed in the incubator for 24 hours to measure the zone of inhibition (26).

3. RESULTS AND DISCUSSION :

Chemistry

In the present study, 2-substituted benzimidazole derivatives were synthesized from o-phenylenediamine with different carboxylic acids in the presence of ethanol and 10% sodium hydroxide under reflux condensation in a water bath at 100^0C for 2 hours. Then the crude products were cooled and sodium hydroxide solution was mixed slowly with continuous stirring to make the products alkaline until the red litmus was turned blue. Then the recrystallization of the crude products was carried out by dissolving the crude products in the boiling water. The filtrates were then cooled and the crystals of benzimidazole were separated, washed with cold water and dried. The dried crystals were used to determine the melting point.

Table 1. Physico-Chemical Properties of the Synthesized Compounds

Sl. No	IUPAC Name	Molecular Weight	Molecular Formula	Melting Point (º C)	Percentage Yield (%)
1.	2-(4-Chloro-3-Nitrophenyl)-14-Benzoimidazole	273	$C_{13}H_8CIN_30_2$	120º C	85
2.	2-(4-Chloro benzyl) Benzimidazole	242	$C_{14}H_{11}CIN_2$	140	67
3.	2-(14-Benzo(d)Imidazol-2-yl)-4,5-demethoxy aniline	269	$C_{15}H_{15}\ N_30_2$	128 º C	55

Characterization of the Compound by spectral analysis:

IR Spectroscopy

The major use of infrared spectroscopy is to determine the functional group of molecules, relevant to both organic and inorganic chemistry. The compound U-3 showed the presence of the following functional groups:

Aromatic -CH: 3010cm^{-1}
-NH: 3450cm^{-1}
Aromatic C=C: 1550cm^{-1}
Alkene C=C: 1640cm^{-1}

NMR Spectroscopy

NMR spectroscopy provides detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. The synthesized compound, U-3 was soluble in DMSO. The spectral results showed that,
Aromatic protons: 6.5 – 6.8, multiplet
CH=CH linkage: 6.46 – 6.75, doublet
Benimidazole NH: 7.67 – 7.8, doublet

Mass Spectroscopy

A mass spectrum is a type of plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical identity or structure of molecules and other chemical compounds. The mass spectroscopy of the compound U-3, showed the molecular ion peak at m/z = 269 and M+ = 270. The spectral results were satisfactorily proving the structure of the compound.

***In Silico* Screening of 2-substituted Benzimidazole Derivatives on 3ERT**

The docking studies was carried out by using PyRx software along with different other software were used for screening. The software includes BIOVIA discovery studio, Swiss PDB viewer, Chemdraw software.

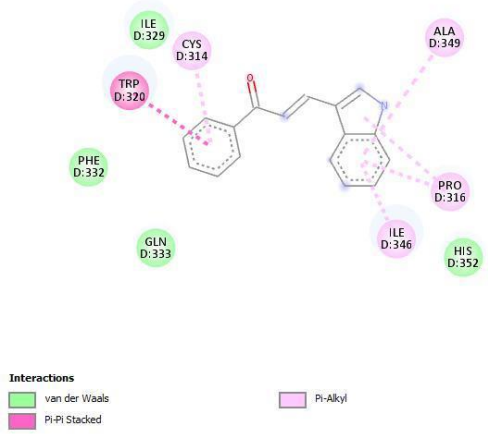

Figure 2. Binding interaction of compound U-3 with the protein HER2.

The synthesized compounds with significant potential α-amylase and α-glucosidase showed better interactions for the superimposed complex. Bonded functional groups at various positions on the aromatic ring provided analogs with a strong affinity. 4-Chloro-3-Nitrophenyl and 4,5-dimethoxy aniline group containing molecules showed stronger binding energy with the receptor. Tryptophan, Cysteine, Proline, Isoleucine and Alanine were involved in the hydrogen bond formation, Vander walls force of attraction with the molecule. The attached substituents have contributed to the good interactions with the receptor.

Table 2. Docking score value of the compounds tested

S. No.	Compound Code	Score Value
1	U-1	-6.6
2	U-2	-6.2
3	U-3	-6.8

In vitro* antibacterial activity of the synthesized compounds on *E.coli

The antimicrobial activity of the compounds U-1, U-2 and U-3 were carried out by disc diffusion method. In the disc diffusion method, the zones of inhibition of the compounds were studied. The zone of inhibition shows the resistance against the bacteria. The bacterium Escherichia coli was used for the antimicrobial study. Ciprofloxacin was used as standard. 1% concentration of ciprofloxacin was used on one disc. The concentrations of 200µg, 100µg and 50µg of each of the compounds U-1, U-2 and U-3 were used on separate discs. A control was placed. After incubation of about 24hours, all the five compounds showed maximum zone of inhibition for the concentration of 200µg and the least zone of inhibition for the concentration of 50µg. The disc for the standard showed zone of inhibition of 18mm. The control showed no zone of inhibition. The compounds U-2 and U-3 showed the good zone of inhibition of about 15mm for the concentration of 200µg.

Table 3. Zone of inhibition of the compounds tested on *E.Coli*

Sl.No.	Compound Code	Zone of Inhibition (mm) 200ug	100ug	50ug
1.	U-1	13	8	5
2.	U-2	15	8	6
3.	U-3	15	7	4

4. CONCLUSION :

An attempt was made to synthesize some novel 2-substitued benzimidazole derivatives through the condensation reaction of *o*-phenylene diamine and various carboxylic acids. The compounds were characterized by Thin Layer

Chromatography, Melting Point, IR, NMR and Mass Spectral Analysis. The spectral study reports were satisfactory, and they confirm the structure of the compounds. The docking study showed that the compound, having 4-Chloro benzyl and dimethoxy phenyl substitution at 2nd position in the benzimidazole ring system showed very good biding efficiency among the compounds tested. Further, the compounds were tested for their antimicrobial activity against *E.Coli* at 50 and 100 ug/ml concentrations. Compound having the 4-Chloro benzyl and dimethoxy substitutions were found to be showing good zone of inhibition than the other compounds. The results revealed that the compounds containing Chloro, Nitro and 4,5-dimethoxy aniline played a crucial role in determining the antibacterial activity of the benzimidazole scaffold.

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