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Synthesis, Characterization And Biological Evalution Of 2-Aminoacetophenone Derived Benzothiazole Conjugated Chalcones Aspotential Antitubercular And Cytotoxic Agents

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Abstract

Because of its diverse spectrum of activities, research on molecules linked to benzothiazoles has significantly grown in recent years. Chalcones are 1,3-diphenylprop-2-en-1-one's structural derivatives. They have enormous compass of antimicrobial, anti-instigative, anti-neoplastic, antiviral, anti-diabetic, anti-malarial, and antioxidant properties, among many more derivatives that are being screened for. The compounds that are produced when these benzothiazoles are joined with chalcones have enhanced activity. A number of benzothiazole-linked chalcones have been created, as discussed here. These novel hybrid pharmacophore moieties contain anti-tubercular and anti-cancer compounds, according to well-known literature. In order to find the "in silico hit" against verified anticancer and antitubercular drug targets for additional exploitation utilizing molecular docking simulations, we conducted synthesis, spectrum analysis, and biological evaluation. FT-IR, 13C NMR, 1H NMR, and deconvoluted M+1 spectra were used to characterize the structures of the produced compounds. Using methotrexate and pyrazinamide as reference medications, the newly synthesized compounds were examined for cytotoxic and anti-tubercular properties in the second section of the study. The next part is the study regarding preliminary investigation of the possible molecular target or targets for the benzothiazole-linked chalcones (CH-1-CH-14) using the ligand-protein inverse docking[11] (LPID) simulation technique.The in vitro results and the laboratory results found from the docking simulation studies related to anticancer and antitubercular activities are less consistent. The poor correlation between in vitro and laboratory studies may be caused by the influence offuture implications of this research work would include the necessity to assess the hits utilizing ligand binding assay tests, followed by animal studies, employing biopharmaceutical descriptors like as logP, logD, and logS.

Key words: pharmacophore, chalcones, benzothiazoles, and docking simulation.

Introduction

Compounds connected to benzothiazoles[1] have a wide range of activities. Chalcone is a special kind of template with a wide range of biological characteristics, and its derivatives are widely used in the synthesis of different heterocyclic compounds.

Chalcones[2] are simple chemical compounds that have two aryl rings and an unsaturated carbonyl group (α, β) between

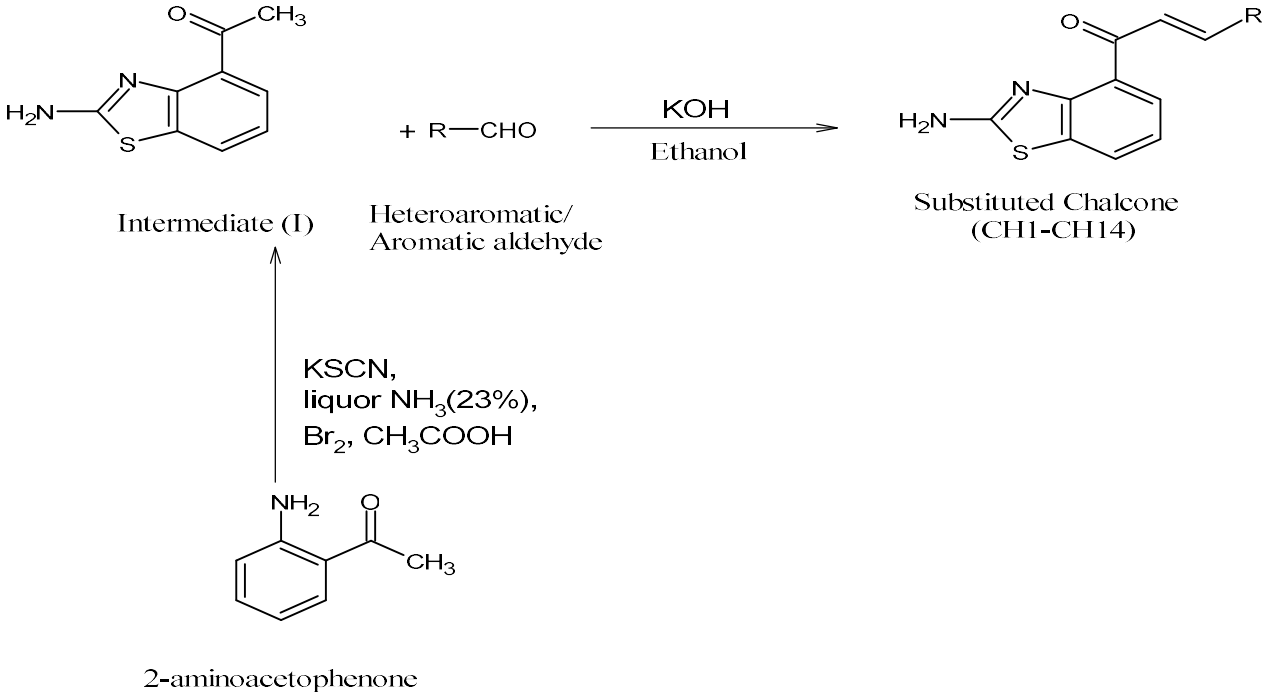
them.Both cis and trans forms are possible for these, with the trans form having greater thermodynamic stability.
Chalcone is a general word for substances that belong to the flavonoid family and have the 1,3-diphenyl-2-propen-1-one structure.They are open-chain flavonoids chemically, with a three-carbon, unsaturated carbonyl system linking the two aromatic rings.
Compounds with chalcone as their backbone component have been shown to exhibit a wide range of biological and ph armacological activities, including antimicrobial, anti-
inflammatory, analgesic, cytotoxic, antitumor, antimalarial14, antitubercular, antiviral, anti-
HIV, antiulcerative, antileishmanial, antioxidant, antiprotozoal, antihistaminic, antifedent, immunomodulatory, anticon vulsant, anti-
hyperglycemic, antihyperlipidemic15, and antiplatelet activities. Chalcones are therefore still of great scientific interest because of their association with numerous biological processes.

Objective

Cancer is a serious disease that has long threatened human health and continues to pose a threat to the field of global public health. Cancer treatment still has numerous restrictions and difficulties, even with all of the modern advancements. Because of its efficacy, chemotherapy is frequently used to treat cancer. Aside from its efficacy, chemotherapy is frequently used to treat cancer. The primary elements influencing medication therapy are effects. As a result, the search for novel medications to treat cancer has accelerated recently, with a particular emphasis on current research.
Mycobacterium tuberculosis is the causative agent of tuberculosis, an infectious disease. Among infectious diseases, tuberculosis remains the most common cause of mortality, even after decades of progress in drug discovery. The diverse biological and pharmacological importance of compounds with unique or combined chalcone and benzothiazole pharmacophores[18] in their basic structure as possible cytotoxic and anti-tubercular medicines has been documented in a number of literature reviews. The crucial intermediate 1-(2-aminobenzo[d]thiazol-4-yl)ethanone(I) was synthesized and describe a series of benzothiazole conjugated chalcones, which were then tested for their potential as cytotoxic[17] and anti-tubercular drugs due to their therapeutic potential. Accordingly, the comprehensive process for identifying inhibitory data against certain mycobacterial and cancer cell lines.
Mycobacterium TB H37Rv strain was used to test the in vitro antitubercular activity[16] of the synthesized benzothiazole-linked chalcones (CH1-CH14) and assess their potential for in vitro cytotoxicity utilizing colon and breast cancer cell lines.
The database of compounds generated in this work is subjected to the Ligand–protein inverse docking approach in order to identify the "best fit" (hit identification) against specific established protein therapeutic targets that are anti-tubercular and anti-cancer. For additional research, the chemical with the lowest binding energy against each distinct target protein is taken into consideration. Understanding the chemicals' interactions with the target protein is feasible through these methods. The knowledge gained from the in silico established and validated protein drug can be applied to the findings of these research to find novel active ligands.

Method

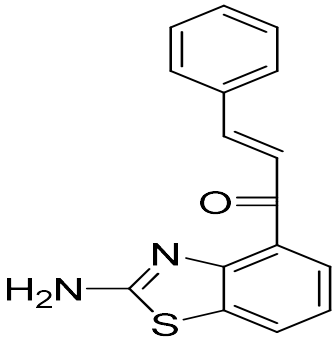
For the synthesis of benzothazole conjugated chalcones (CH1-CH14), is shown in the scheme



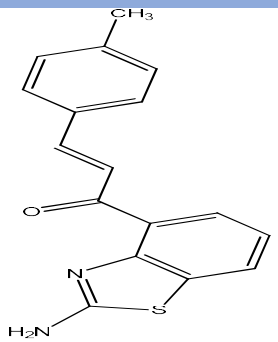
The primary intermediate in this investigation was 1-(2-aminobenzo[d]thiazol-4-yl)ethanone (I) 3, the molecule synthesized by dissolving 2-aminoacetophenone (2.0 g, 14.8 mmol) and KSCN (potassium thiocyanate) (4.0 g, 52.0 mmol) in CH_3COOH (glacial acetic acid) at 27.5 ± 2.5 °C. Subsequently, liquid bromine in glacial acetic acid (2.6 g, 16.3 mmol) was added dropwise, at maintaining the temperature below 8° for 160–180 minutes. After completion of addition, the reaction mass is monitored by checking TLC at timely manner. The starting material diappearence is the indication for the completion of the TLC. The solid product was isolated by filtration through Buchner funnel followed by washing with acetic acid and then the material is washed with chilled water[19]. The filtrate added with 300 ml of hot water (40-45°C), and the pH was adjusted to a range of 6.5 to 7.5 using ammonia, as measured with a pH meter. The reaction mass was kept at refrigeration temperature overnight and the product is precipitated appropriately. The resulting product was slowly added to crushed ice and finally washed with chilled water and applied vacuum until all the ML's is drained out before final drying. The dried residue underwent purification with activated carbon and was recrystallized from ethanol.

The following Claisen-Schmidt[5] condensation involves the intermediate (I) reacting with suitable aromatic or heteroaromatic aldehydes in a basic medium[20], specifically potassium hydroxide, within an ethanol solvent, resulting in the formation of the corresponding benzothiazole-linked chalcones (CH-1 to CH-14) with a favorable yield.

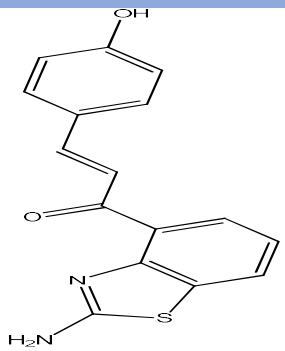
List of benzothiazole-linked chalcones[6]**CH1-CH14** produced via Scheme



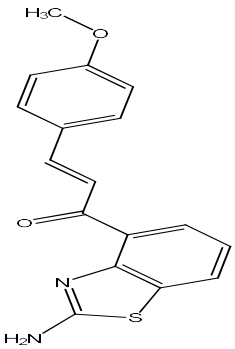
CH1
Yield:60%,
M.P.:264-266°C
M.F: $C_{16}H_{12}N_2OS$



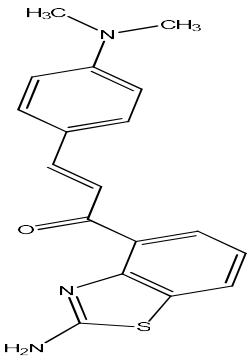
CH2
Yield: 72%,
M.P.:269-272°C
M.F: $C_{17}H_{14}N_2OS$



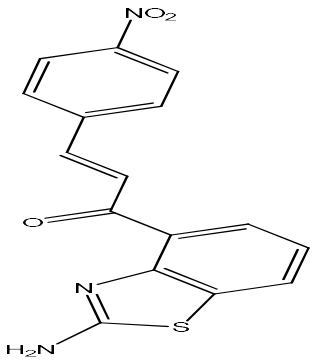
CH3
Yield: 63%,
M.P.: 248-249°C
M.F: $C_{16}H_{12}N_2O_2S$



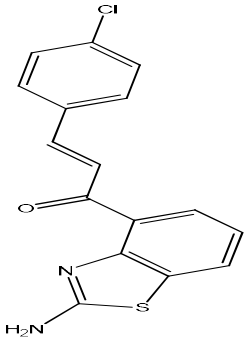
CH4
Yield: 45%,
M.P.:262-264°C
M.F: $C_{17}H_{14}N_2O_2S$



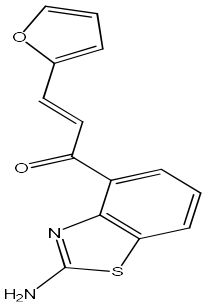
CH5
Yield: 52%,
M.P.:244-246°C
M.F: $C_{18}H_{17}N_3OS$



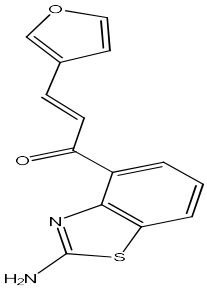
CH6
Yield: 61%
M.P.:244-246°C
M.F: $C_{16}H_{11}N_3O_3S$



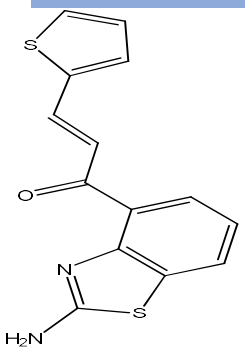
CH7
Yield: 58%,
M.P.: 276-278°C
M.F: $C_{16}H_{11}ClN_2OS$



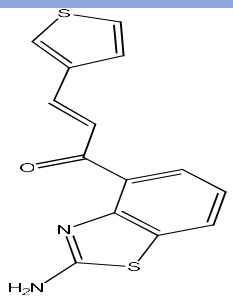
CH8
Yield: 78%,
M.P.: 284-287°C
M.F: $C_{14}H_{10}N_2O_2S$



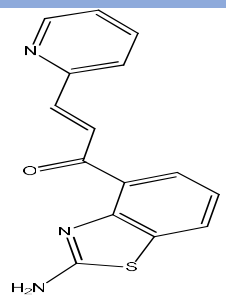
CH9
Yield: 62%,
M.P.:275-278°C
M.F: $C_{14}H_{10}N_2O_2S$



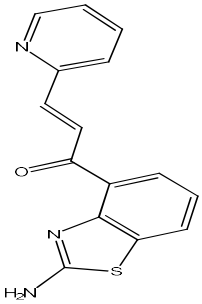
CH10
Yield:60%,
M.P.: 268-269ºC
M.F: $C_{14}H_{10}N_2OS_2$



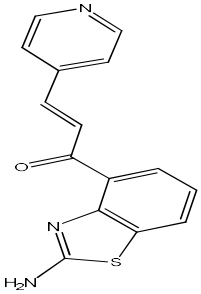
CH11
Yield:48%,
M.P.:260-265ºC
M.F: $C_{14}H_{10}N_2OS_2$



CH12
Yield:48%,
M.P.:286-288ºC
M.F: $C_{15}H_{11}N_3OS$



CH13
Yield: 65%,
M.P.: 293-295ºC
M.F: $C_{15}H_{11}N_3OS$



CH14
Yield:66%,
M.P.: 298-300ºC
M.F: $C_{15}H_{11}N_3OS$

Biological methods

The process of discovering natural or synthetic pharmaceuticals is primarily directed by bioassay methodologies. Bioassay is integral to each phase of the drug discovery process, serving to identify biological or pharmacological activities and assisting in the selection of lead compounds for subsequent investigation. In the context of natural products, bioassay is crucial during the isolation stages, as it helps determine which fractions of a crude sample are likely to yield pure isolated compounds. To be effective, bioassays must be rapid, straightforward, dependable, reproducible, and, most critically, predictive.

The cytotoxic effects of the benzothiazole-linked chalcones (CH1-CH14) were assessed in vitro using the MTT assay. This colorimetric method quantifies the decrease in the yellowcolor of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by the mitochondrial succinate dehydrogenase[21]. Upon enter into the cells, MTT enters into the mitochondria, where MTT is converted into insoluble, dark purple product which is “formazan”. Following this, the cells are treated with DMSO to solubilize the formazan, which is then quantified spectrophotometrically at a wavelength of 570 nm. The reduction of MTT is indicative of metabolic activity, thus serving as a measure of cell viability. By comparing the quantity of dark purple formazan generated by cells exposed to the test agent with that produced by untreated control cells, one can infer the agent's efficacy in inducing cell death, as illustrated by the resulting dose-response curve[22].

REDUCTION OF MTT

Materials:

The National Centre for Cell Science (NCCS) in Pune, India, provided the HT-29 (colon cancer) and MCF-7 (breast

cancer) cell lines. DMEM (Dulbecco's Modified Eagle's Medium) and MEM (Minimum Essential Medium Eagle) were among the culture mediums utilized. Sigma Chemicals, located in St. Louis, MO, provided the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, and EDTA. Arrow Labs provided the fetal bovine serum (FBS), and Tarson provided the 96-well flat-bottom tissue culture plates.DMEM (Dulbecco's Modified Eagle's Medium) and MEM (Minimum Essential Medium Eagle) were among the culture mediums utilized. Sigma Chemicals, located in St. Louis, MO, provided the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], Trypsin, and EDTA. Arrow Labs provided the fetal bovine serum (FBS), and Tarson provided the 96-well flat-bottom tissue culture plates.

Method:

1. **Maintenance of cell lines:**

MCF-7 cells were kept in MEM media supplemented with 10% fetal bovine serum, whereas HT-29 cell lines were grown as adherent cells in DMEM media23. The cultures were maintained with 5% CO2 in a humidified atmosphere.

2. **Preparation of samples for cytotoxicity:**

The test chemicals were prepared as stock solutions at a concentration of 10 mg/mL in DMSO. The final drug concentrations were then 10, 50, 100, and 200 mg/mL after a series of dilutions were carried out using sterile water.

3. **Cytotoxicity evaluation:**

In the Trypan blue exclusion assay, cells were seeded into 96-well plates at a density of 1x10^4 cells per well. To support recovery, the cells were placed in an incubator for 24 hours. The medium was substituted with fresh media containing various dilutions of the test substances following this incubation phase. Subsequently, the plates were incubated for an additional 48 hours at 37°C in DMEM/MEM supplemented with 10% FBS. After the incubation, 90 µl of fresh DMEM without FBS24 was introduced, and the previous medium was discarded. Every well was subsequently filled with 10 µl of MTT reagent (5 mg/mL stock solution in DMEM lacking FBS), and the wells were incubated at 37°C for three to four hours[25]. Following the incubation, the medium was replaced by adding 200 µl of DMSO to each well to dissolve the blue formazan crystals, and then a 10-minute incubation at 37°C was carried out. Subsequently, a spectrophotometer was utilized to measure the absorbance at 570 nm. The drug used as a reference in the comparison was methotrexate. The assay was performed in triplicate for three different determinations. IC50 (µg/mL), representing the concentration of the compound that reduces the proliferation rate of tumor cells by 50% relative to the control group of untreated cells, was employed to assess cytotoxicity.The graph that charted the percentage of inhibition versus concentration was utilized to determine the IC50 values[8].

$$\% \text{ inhibition at the given concentration} = 1 - \frac{\text{(Absorbance average)}}{\text{(Control absorbance average)}} \times 100$$

IC_{50}=Inv.log (50-c)/m; c and m derived from y=mx+c of plot of % inhibition Vs log C.

***MYCOBACTERIUM TUBERCULOSIS* H37Rv (*Mtb* H37Rv) INHIBITORY ACTIVITY(*IN VITRO*ANTITUBERCULAR ACTIVITY)**

The microplate Alamar Blue assay (MABA) was utilized to evaluate the inhibitory effects of chalcones (benzothiazole-linked) (CH1-CH14) on Mycobacterium tuberculosis H37Rv (Mtb H37Rv) [27]. This method is recognized for its strong correlation with both proportional and BACTEC radiometric methods, its non-toxic characteristics, and its utilization of a thermally stable reagent. In summary, the outer perimeter wells of a sterile 96-well plate were filled with 200 µL of sterile deionized water to reduce medium evaporation during incubation. Once 100 µL of Middlebrook 7H9 broth was added to each well of the 96-well plate, the compounds underwent serial dilution on the plate. The medications assessed exhibited final concentrations between 100 and 0.2 µg/mL. Once sealed and covered with parafilm, the plates underwent incubation for five days at 37 °C. Following this incubation period, each well was treated with 25 µL of a freshly prepared

1:1 mixture of Alamar Blue reagent and 10% Tween 80, which was incubated for an additional 24 hours. A blue hue in the wells indicated no bacterial growth, while a pink tint suggested growth had occurred. The concentration that prevented the colour from altering from blue to pink was utilized to determine the IC50 value.

MOLECULAR DOCKING STUDIES

One useful tool in the field of drug design is the ligand–protein inverse docking (LPID) methodology. The goal of this strategy is to find possible ligands by docking one or more small molecules in different conformations to a receptor site[26]. Multi-conformer shape matching, genetic algorithms, evolutionary programming, simulated annealing, fragment-based docking, and other novel techniques are among the many adaptable docking algorithms that have been created. It has been shown empirically that these algorithms can recognize ligands[12] and bind conformations at receptor sites that closely mimic structures shown by experiments.The inverse-docking procedure, which looks for several possible protein targets for tiny compounds that may bind or show weak binding, is expected to benefit equally from these algorithms' ability to find candidate ligands and binding conformations. This strategy might make it easier to find unidentified and secondary therapeutic targets for medications, drug leads, natural products, and other ligands.

In this investigation, a graphical user interface (GUI)13 and computational drug discovery tools were used. ArgusLab version 4.0 was used for energy minimization, Accelrys Draw for molecular modeling, and iGemdock version 2.1 for molecular docking simulation protocols.

The series of CH1-CH-14 was evaluated using the molecular docking technique against known anticancer and anti-tubercular drug targets using iGEMDOCK software. Finding the synthesised ligands' binding energies, interactions, and modes within the active binding sites of possible therapeutic drug targets was the goal of this method. iGEMDOCK requires that the ligand and receptor coordinates be in either PDB or Mol-2 format. Partial charges of non-polar hydrogen atoms were transferred to the appropriate carbon atoms and removed from the receptor file. The crystallographic ligands from X-ray structures indicated the binding site, and the molecular docking was carried out using the ligand-protein inverse induced fit docking methodology. By using default settings for all calculations, the docking run took place out appropriately.

Anticancer activity

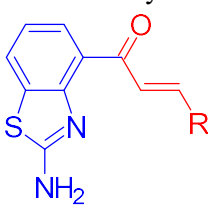
The benzothiazole-linked chalcones (CH-1-CH-14) synthesized in this study were designed to target cyclin-dependent kinase (CDK2) and histone deacetylase (HDAC). The involvement of CDK and HDAC as cancer targets has been clearly demonstrated in the development of colon and breast cancers.

Anti-tubercular activity

Possible targets for isoniazid (INH) are the enzymes InhA and 8an5. Consistent with the finding that INH disrupts the synthesis of mycolic acids, the very long-chain fatty acids forming the mycobacterial cell wall, two enzymes are crucial components of Mycobacterium TB's type II dissociated fatty acid biosynthesis pathway (FASII). Jacobs and team discovered InhA as a target enoylreductase that facilitates the long-chain breakdown of trans-2-enoyl-acyl carrier proteins (ACPs) in a manner dependent on NADH. It is widely recognized that INH hinders InhA. Additionally, Barry and his team have suggested that INH acts on 8an5, one of the three ketoacyl synthases involved in the FASII pathway, in vivo[28].

RESULTS OF CYTOTOXICITY STUDIES

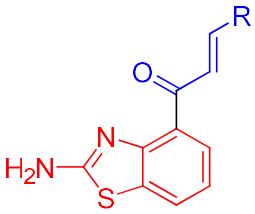
Table 1.1. Cytotoxicity data of benzothiazole-linked chalconesCH1-CH-14 against HT-29 colon cancer cell line.



Compound	R	IC_{50} (µg/mL)

Code		(Mean ± SEM)
CH1	C_6H_5	65.11 ± 0.5
CH2	4-MeC_6H_4	15.01 ± 0.2
CH3	4-OHC_6H_4	9.10 ± 0.9
CH4	4-$OMeC_6H_4$	28.41 ± 0.1
CH5	4-$NMe_2C_6H_4$	15.25 ± 0.1
CH6	4-$NO_2C_6H_4$	36.44 ± 0.9
CH7	4-ClC_6H_4	40.52 ± 0.1
CH8	Furan-2-yl	50.84 ± 0.1
CH9	Furan-3-yl	55.12 ± 0.3
CH10	Thiophen-2-yl	45.03 ± 0.3
CH11	Thiophen-3-yl	46.11 ± 0.5
CH12	Pyridin-2-yl	43.11 ± 0.5
CH13	Pyridin-3-yl	43.38 ± 0.1
CH 14	Pyridin-4-yl	44.37 ± 0.1
Methotrexate	-	0.8 ± 0.5

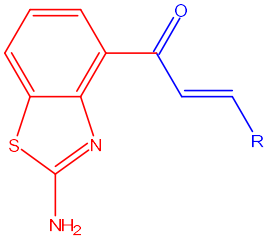
Table 1.2. Cytotoxicity data of benzothiazole-linked chalcones CH1-CH-14 against breast cancer cell line (MCF-7)**.**



Compound Code	R	IC_{50} (µg/mL) (Mean ± SEM)
CH1	C_6H_5	70.01 ± 0.7
CH2	4-MeC_6H_4	74.01 ± 0.5
CH3	4-OHC_6H_4	11.12 ± 0.2
CH4	4-$OMeC_6H_4$	30.14 ± 0.1
CH5	4-$NMe_2C_6H_4$	17.07 ± 0.9
CH6	4-$NO_2C_6H_4$	76.31 ± 0.1
CH7	4-ClC_6H_4	35.18 ± 0.2
CH8	Furan-2-yl	35.11 ± 0.9
CH9	Furan-3-yl	36.11 ± 0.9
CH10	Thiophen-2-yl	8.8 ± 0.1
CH11	Thiophen-3-yl	**NS**
CH12	Pyridin-2-yl	54.04 ± 0.3
CH13	Pyridin-3-yl	**NS**
CH 14	Pyridin-4-yl	44.01 ± 0.1
Methotrexate	-	2.8 ± 0.3

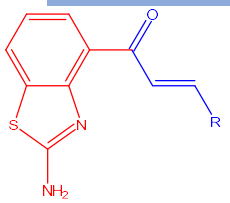
NS: Non-Sensitive

Table1.3 Benzothiazole-linked chalcones**CH-1-CH-14**docked along withi-GEMDOCK scores against anticancer protein drug targets.



Compound	R	CDK2	HDAC
		GEMDOCK Score	GEMDOCK Score
		kcal/mol	kcal/mol
CH-1	C_6H_5	-121.260	-150.281
CH-2	4-MeC_6H_4	-131.663	-161.591
CH-3	4-OHC_6H_4	-118.345	-157.117
CH-4	4-$OMeC_6H_4$	-127.621	-158.695
CH-5	4-$NMe_2C_6H_4$	-126.405	-162.492
CH-6	4-$NO_2C_6H_4$	-147.153	**-206.714**
CH-7	4-ClC_6H_4	-128.107	-160.053
CH-8	Furan-2-yl	-120.370	-158.149
CH-9	Furan-3-yl	-116.935	-153.711
CH-10	Thiophen-2-yl	-133.377	-174.143
CH-11	Thiophen-3-yl	**-159.625**	-189.191
CH-12	Pyridin-2-yl	-117.013	-131.580
CH-13	Pyridin-3-yl	-103.460	-130.922
CH-14	Pyridin-4-yl	-107.241	-134.857

Table 1.4. Benzothiazole-linked chalcones CH-1-CH-14 with their iGEMDOCK scores against validated anti-tubercular protein drug target.



Compound	R	8an5
		iGEMDOCK Score
		kcal/mol
CH-1	C_6H_5	-14.525
CH-2	4-MeC_6H_4	-28 .155
CH-3	4-OHC_6H_4	-25.322
CH-4	4-$OMeC_6H_4$	-35.222
CH-5	4-$NMe_2C_6H_4$	-21.058
CH-6	4-$NO_2C_6H_4$	-25.212
CH-7	4-ClC_6H_4	-24.565
CH-8	Furan-2-yl	-50.121
CH-9	Furan-3-yl	-49.223
CH-10	Thiophen-2-yl	**-98.522**
CH-11	Thiophen-3-yl	**-98.245**
CH-12	Pyridin-2-yl	-32.252
CH-13	Pyridin-3-yl	-38.657
CH-14	Pyridin-4-yl	-42.121

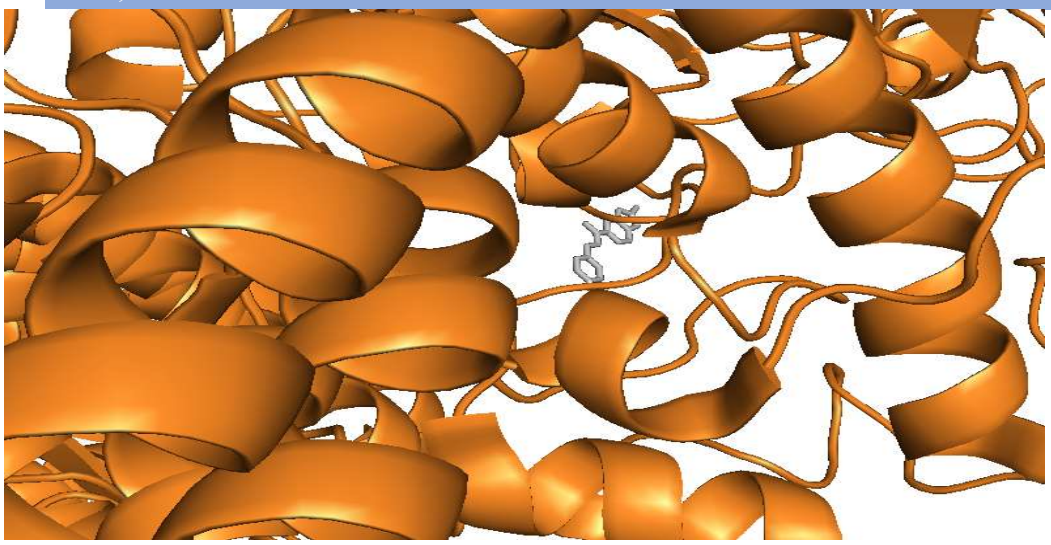
DISCUSSION ON THE RESULTS

The investigation of the inhibitory activity screening data on the HT-29 colon cancer cell line in vitro showed that the molecules CH-3, CH-2, and CH-5 established the most potent inhibitory action, with IC50 values of 9.10 ± 0.9, 15.01 ± 0.2, and 15.25 ± 0.1µg/mL. The compound CH4 also exhibited appreciable inhibitory action with IC-50 value of 28.41 ± 0.1µg/mL. On the other hand, compounds such as CH-6, CH-7, and CH-12 showed a moderate level of activity at concentrations (IC50) ranging from 36.44 ± 0.9 to 43.11 ± 0.5µg/mL. Comparatively, the compounds CH13, CH14, CH10, CH11, CH8, CH9, and CH1 displayed less activity with IC50 values ranging from 43.38 ± 0.1 to 65.11 ± 0.5

µg/mL compared to the standard drug Methotrexate (IC50: 0.8 ± 0.5 µg/mL). The Structure-Activity Relationship (SAR) of these compounds clearly demonstrated that the benzothiazole and chalcone moieties, which make up the basic nucleus, are associated with the inhibitory activity on the HT-29 colon cancer cell line, as observed in the case of compounds CH1-CH-14. In some cases, the cytotoxicity potential was enhanced by certain substituents, while decreased by others. Specifically, the order of activity was CH3 (4-OHC6H4) > CH2 (4-MeC6H4) > CH5 (4-NMe2C6H4) > CH4 (4-OMeC6H4) > CH6 (4-NO2C6H4) > CH7 (4-ClC6H4) > CH12 (Pyridin-2-yl) > CH13 (Pyridin-3-yl) > CH14 (Pyridin-4-yl) > CH10 (Thiophen-2-yl) > CH11 (Thiophen-3-yl) > CH8 (Furan-2-yl) > CH9 (Furan-3-yl) > CH1 (C6H5). On the other hand, it was discovered that there are different aliphatic, aromatic, and heteroaromatic functional group substitutions that can occur at position C-3 of the α,β-unsaturated carbonyl system.

The analysis of the inhibitory activity screening data for the in vitro breast cancer cell line (MCF-7) as presented in Table 1.2, indicated that the compounds CH10 and CH3 exhibited most prominent inhibitory effects, with IC50 results of 8.8 ± 0.1 and 11.12 ± 0.2 µg/mL, respectively. Notably, the compound CH5 also demonstrated considerable inhibitory activity, with an IC50 value of 17.07 ± 0.9 µg/mL. In contrast, the other compounds, including CH4, CH7, CH8, CH9, and CH14, displayed moderate activity, with IC50 values ranging from 30.14 ± 0.1 to 44.01 ± 0.1 µg/mL. The compounds CH12, CH1, CH2, and CH6 showed lower activity, with respect to IC50 values between 54.04 ± 0.3 and 76.31 ± 0.1 µg/mL, particularly when compared to the standard drug Methotrexate, which has an IC50 of 2.8 ± 0.3 µg/ mL. The SAR (Structure-Activity Relationship) analysis of the related compounds highlighted the essential role of the benzothiazole and chalcone moieties in cell lines regarding breast cancer (MCF-7) inhibitory activity, as observed in compounds CH1 through CH14. Furthermore, the activity was influenced positively by certain substituents while being diminished by others, with the following order of activity: CH10 (Thiophen-2-yl) > CH3 (4-OHC6H4) > CH5 (4-NMe2C6H4) > CH4 (4-OMeC6H4) > CH7 (4-ClC6H4) > CH8 (Furan-2-yl) > CH9 (Furan-3-yl) > CH14 (Pyridin-4-yl) > CH12 (Pyridin-2-yl) > CH1 (C6H5) > CH2 (4-MeC6H4) > CH6 (4-NO2C6H4).

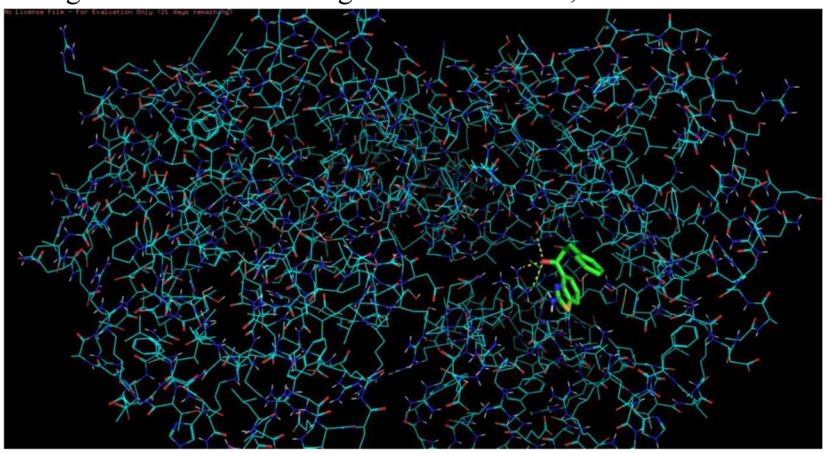
A series of **14 benzothiazole-linked chalcones, designated CH-1 to CH-14**, underwent ligand-protein inverse induced fit docking simulations utilizing the iGEMDOCK version 2.1 software. These compounds were evaluated against the validated drug targets CDK2 and HDAC. The findings, as presented in Table 1.3, may facilitate the preliminary identification of potential target-based hits (best fit) in relation to the observed cytotoxicity. The identification of the hit (best fit molecule) for each target was determined by analyzing its binding energy and interactions within the active binding sites. According to the results from the LPID studies on the benzothiazole-linked chalcones CH-1 to CH-14, compound CH-11 demonstrated a stable binding phenomenon having the lowest energy of binding of **-159.625 kilocal/mol** against **CDK2 proteindrug target.** Similarly, compound **CH-6** exhibited the lowest binding energy and **active binding site region of HDAC** having themost stable binding orientation within, recorded at **-206.714 kilo cal/ mol.**



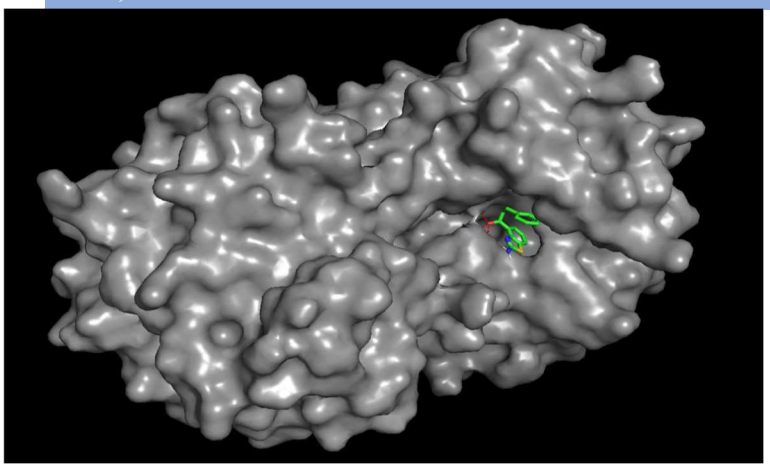
H-bond interactions along with active binding site, binding mode of CH-6 against protein HDAC.

ANTITUBERCULAR DRUG TARGET

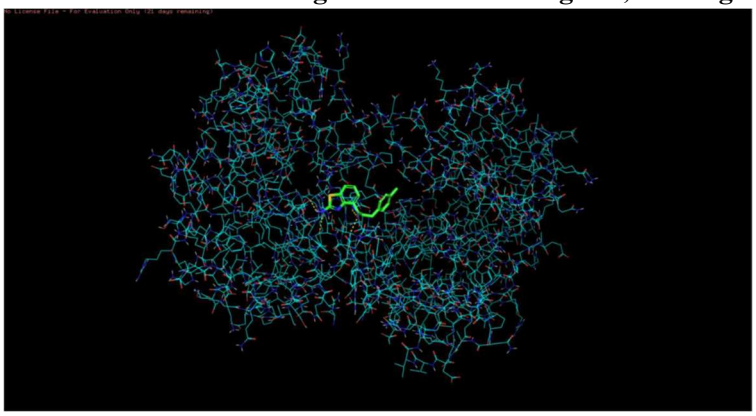
A series of 14 benzothiazole-linked chalcones, designated CH-1 to CH-14, underwent ligand-protein inverse induced fit docking simulations utilizing the iGEMDOCK version 2.1 software. These compounds were evaluated against the validated drug targets CDK2 and HDAC. The findings, as presented in **Table 1.3**, may facilitate the preliminary identification of potential target-based hits (best fit) in relation to the observed cytotoxicity. The identification of the hit (best fit molecule) for each target was determined by analyzing its binding energy and interactions within the active binding sites. According to the results from the LPID studies on the benzothiazole-linked chalcones**CH-1 to CH-14**, compound CH-11 demonstrated a stable binding phenomenon having the lowest energy of binding of **-159.625 kilo cal/ mol** against **CDK2 protein drug target**. Similarly, compound **CH-6** exhibited the lowest binding energy and **active binding site region of HDAC** having themost stable binding orientation within, recorded at **-206.714 kilo cal/ mol.**



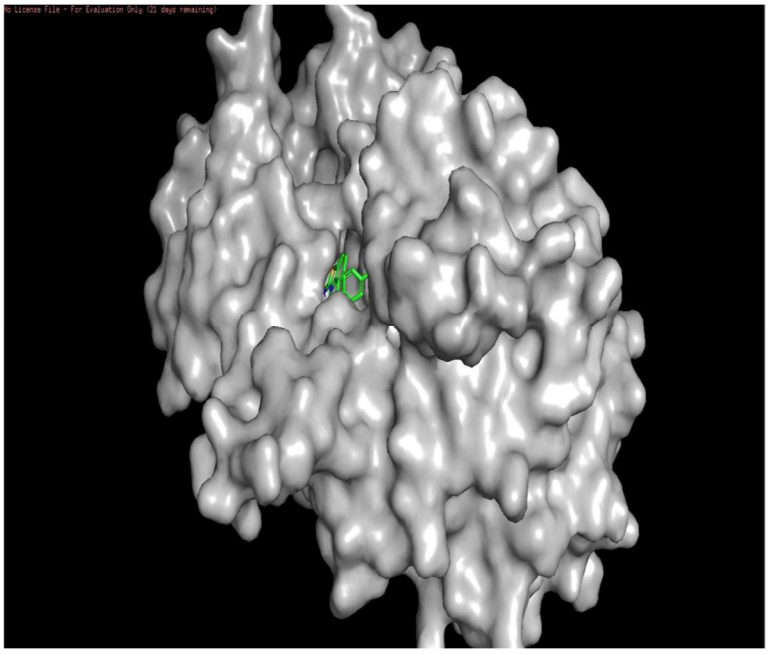
H-bond interactions along with active binding site, binding mode of CH-10 against protein 8an5 (sticks).



H-bond interactions along with active binding site, binding mode of CH-11 against protein 8an5 (sticks).



Active binding site, binding mode and H-bond interactions of CH-11 against protein 8an5 (sticks).



H-bond interactions along with active binding site, binding mode of CH-11 against protein 8an5 (surface-1).

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