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"Design, Synthesis, And Characterization Of Novel Benzimidazole Derivatives And Their Biological Evaluation"

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Article History

Abstract

Because of their various biological functions and possible therapeutic uses, benzimidazole derivatives have drawn a lot of interest in medicinal chemistry. Our goal was to create, manufacture, and assess a number of new benzimidazole derivatives with improved pharmacological properties in this work. In order to maximize target interactions with particular biological targets, the benzimidazole scaffold was logically modified during the design process. A multistep synthetic process was used to create the novel derivatives, allowing for the integration of various functional groups. The effective synthesis of the intended chemicals was confirmed by characterizing techniques such nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry, and infrared spectroscopy. The produced compounds were then evaluated using an in vitro techniques. Human cell lines were used in preliminary cytotoxicity tests to get knowledge about the derivatives' possible safety characteristics. This study's findings identified a number of benzimidazole compounds with significant biological properties and possible medicinal uses. In conclusion, a number of substances with remarkable therapeutic potential have been produced as a result of the design, synthesis, and assessment of novel benzimidazole derivatives.

Keywords: benzimidazole, mannich base, anticancer

1. Introduction:

A multi-step procedure which incorporates the Mannich reaction in combination with the benzimidazole architecture is required to create and synthesize novel Mannich bases that contain the benzimidazole ring (1,2). Typically, a primary or as secondary amine, a formaldehyde source, as well as a carbonyl molecule condense in the Mannich reaction. In this instance, the emphasis is on employing secondary amine to synthesize Mannich bases that contain the benzimidazole ring (3). Various biological effects can result from novel Mannich compounds of the benzimidazole ring, depending on the unique structural characteristics, substituents, and functional molecules that are present in the compounds.(4)
Modern techniques like the microwave-assisted synthesis for producing the benzimidazole ring have a number of benefits over older ones. The use of microwave radiation in this method speeds up chemical reactions,

increases yields, and frequently improves selectivity.(5,6) Due to its effectiveness and capacity to accelerate reaction rates by encouraging uniform heating and better regulated settings, microwave synthesis has grown in popularity. The identification of novel chemical pathways and transformations that could be challenging to accomplish using conventional techniques has been made possible by microwave-assisted synthesis. (7,8)
Because of their ability to interact with important proteins implicated in cancer processes, benzimidazole Mannich bases have drawn interest in anticancer research. The Mannich reaction, which involves amines, formaldehyde, and carbonyl compounds, produces molecules called mannich bases. On the other hand, benzimidazole derivatives have a variety of biological functions and are capable of being structurally altered to enhance their pharmacological qualities. (7,8)
Due to their various modes of action, benzimidazole Mannich bases show promise as potentially anticancer medications. It's crucial to stress that docking research investigations are computational hypotheses that require careful experimental validation in order to be confirmed. To convert these discoveries into efficient anticancer treatments, collaborations involving computational chemical researchers, medicinal chemists, researchers in biology, and physicians are necessary. (9,10)

2. Result and discussion :

2.1 Synthesis of compounds :

The compounds that were synthesized were scaled for yield, purified by recrystallization using an appropriate solvent system, reassigned for physical constant determination, and then subjected to spectrum analysis such as thin layer chromatography and IR spectroscopy, NMR spectroscopy and Mass spectroscopy. Utilizing diverse spectroscopic techniques, spectral analysis verifies the structure of produced substances. These methods shed light on the functional groups that are present, atom connectivity, and overall molecule structure.

The Scheme below shows the reaction paths for the produced chemicals. The synthesis of benzoimidazoles involved microwave-induced condensation of o-phenylenediamine with organic acid, followed by an acidic oxidation step in the presence of K2Cr2O7. When 2-acetyl benzimidazole is benzoylated with benzoyl chloride, a benzoyl group is introduced, which can give the molecule additional characteristics and reactivity. There are many uses for benzoylated benzimidazole derivatives, including the production of drugs. A main or secondary amines that varied a carbonyl molecule (such as formaldehyde or related derivatives), as well as either a basic or acidic catalyst condense in the Mannich reaction, an adaptable organic synthesis. Mannich bases, which are crucial intermediates in the chemical production of numerous chemicals, including medicines, are produced as a result of the process. In this instance, the formation of a Mannich base via the interaction of secondary amines and 2-acetyl benzimidazole is of interest. Mannich bases are flexible intermediaries that can be a.lactic acid, 20%MW, b. $K_2Cr_2O_7$, acetic acid , c. benzoyl chloride, $NaHCO_3$ sol, d. formaldehyde, dil. HCl.

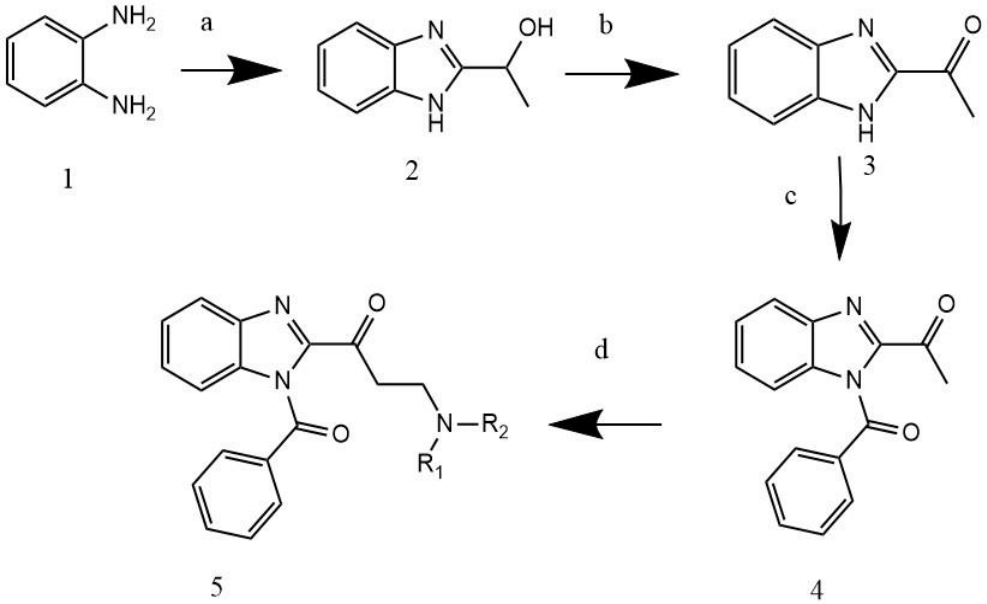


Fig. 2.1 Synthetic scheme 1

Compound	R_1	R_2
5a	$-Ch_3$	$-Ch_3$
5b	$-C_2h_5$	$-C2h_5$
5c	$-Ch_2OH$	$-CH_2OH$
5d	$-C_6H_5$	$-C_6H_5$
5e	$-CH_2C_6H_5$	$-CH_2C_6H_5$
5f	$-C_6H_5$	$-C_6H_5NH_2$

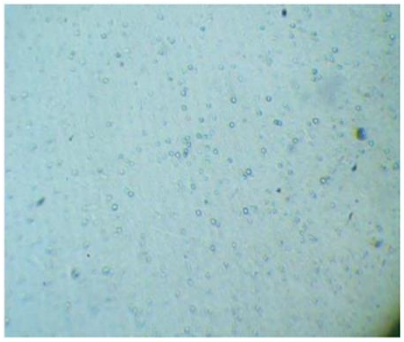
Where R_1 and R_2 are further altered to provide numerous functional categories and structures. The thereby produced Mannich base can be used in a variety of fields, such as medicine, agrochemicals, and materials research, depending on the type of secondary amine along with the presence of formaldehyde.

2.1 Biological activity :

2.1.1 Anticancer activity : [22-28]

The cell line that represent the most prevalent kind of human cancer, the human breast adenocarcinoma cell line (MCF7), were used to test the cytotoxicity of several substances. In order to compare, the potential cytotoxicity of 5Flourouracil, a common anticancer medication, was assessed under the identical circumstances. Tables display the IC50 and (dosage of the drug that resulted in a 50% reduction in survival values). According to the results of the MTT test, the compound's generated IC50 for 5-Flurouracil ought to equal 39.22 g/ml for MCF-7. By comparing the compound's IC50 value against the value of the standard compound, the drug shows a % (42.78 g/ml) inhibition, although the standard compound shows a % (39.22g/ml) inhibition, showing a considerable anti-cancer action.

Among the synthesized derivatives the 5d compound showed the more affection towords the receptor binding site as compared to others. The derivatives 5a, 5b, 5c, 5e and 5f showed comparatively less anticancer activity against the receptor (PDB code : 5NQR). Regarding all of the examined tumor cell lines, it was discovered that all of the substances investigated drugs had potential anticancer properties. In general, MCF7 activity was higher for all the investigated substances. The MCF7 cell line demonstrated the greatest resistance to compounds 5a and 5h. While substances 5b, 5c, 5e, and 5h have a lower level of activity against MCF7. And were only somewhat effective against MCF7. Nonetheless, are more potent than the typical medication used for testing.



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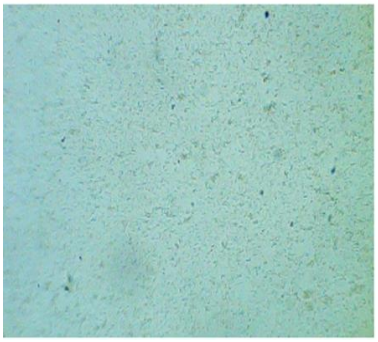
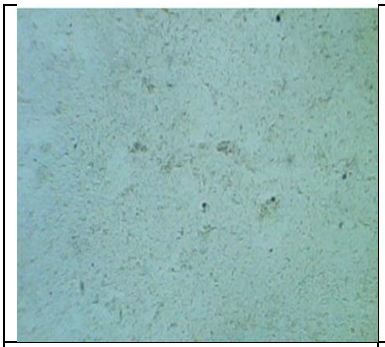
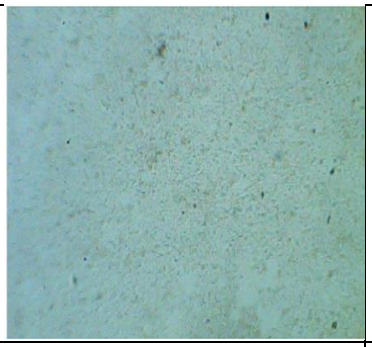


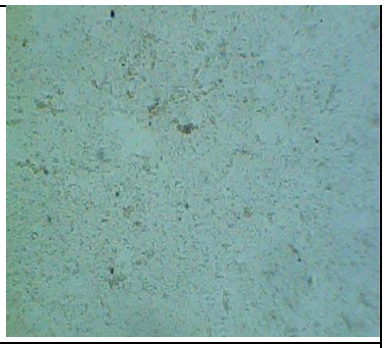
Standard



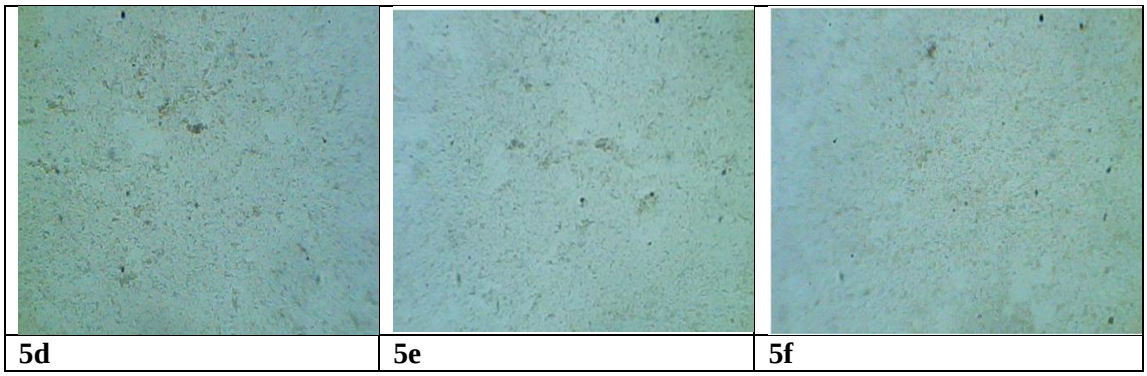
5a



5b



5c



5d	5e	5f

Fig. 2.2 Observations of anticancer activity on MCF7 cells

Sample	Concentration (µg/ml)	Absorbance Mean	Percentage inhibition (%)	IC50 (µg/ml)
Control	-	1.282	-	-
Standard 5FU	10	0.429	66.53	39.22
	40	0.341	73.40	
	100	0.263	79.48	
9a	10	0.651	49.21	42.39
	40	0.582	54.60	
	100	0.485	62.16	
9b	10	0.819	36.11	41.94
	40	0.736	42.58	
	100	0.629	50.93	
9c	10	0.542	40.12	40.53
	40	0.508	46.08	
	100	0.572	52.41	
9d	10	0.727	43.29	42.78
	40	0.637	50.31	
	100	0.501	60.92	
9e	10	0.612	37.16	39.48
	40	0.546	45.51	
	100	0.608	49.85	
9f	10	0.589	41.73	39.81
	40	0.541	48.46	
	100	0.574	52.90	

Table. 2.1 Observation table for anticancer screening

The IC50 values determined at different concentrations of 10 g/ml, 40 g/ml and 100 g/ml. in this comparative evaluation of the synthesized derivatives derivative 5d found out to be having most potent at the each concentration of compound. The comparative study of synthesized compounds among themselves the derivative 5a and 5d also are found to be having the potent activity and the remaining compounds were found to be having moderate potent anticancer activity. The comparison of IC50 values of synthesized derivatives is shown in following table with the standard drug taken into consideration for examination.

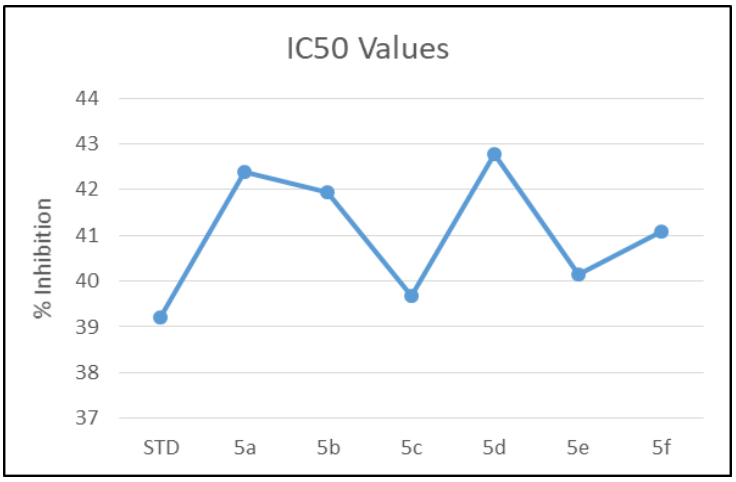


Fig. 2.3 Comparison of IC50 values

2.2.2. Anthelmintic activity: (29-32)

The bulk of recently synthesized chemicals were only mildly to moderately effective as anthelmintics against different types of earthworms. When the results of the activity data were compared, it was discovered that compound 9a possessed the highest activity at an average concentration regarding 20 mg/ml, exhibiting paralysis at 6.430.217 min and passing on at 29.531.80 min, when compared to the common drug albendazole, which exhibits paralysis at 0.410.298 min as well as death at 1.460.241min. Novel synthetic compounds 9b as well as 9g had only weak anthelmintic action against various earthworm specie.

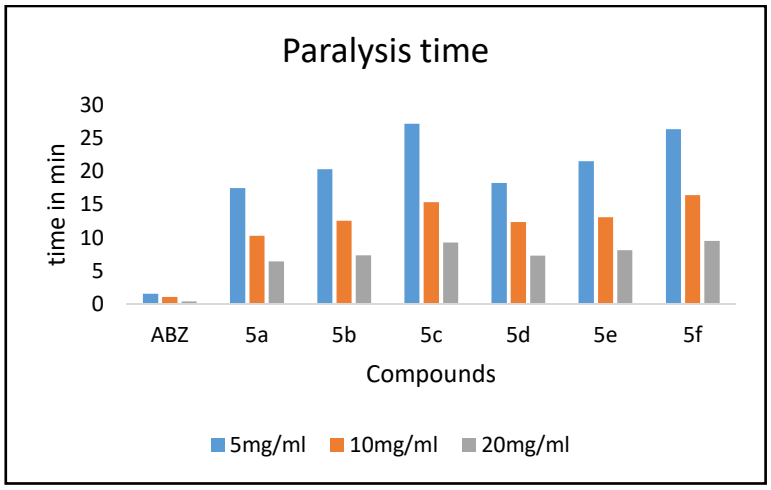


Fig. 2.4 paralysis time for anthelmintic activity

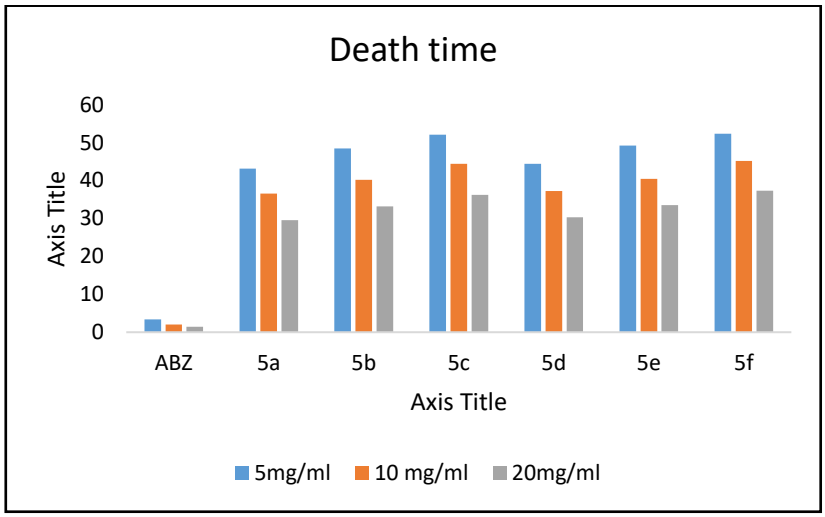


Fig. 2.5 death time for anthelmintic activity

When examined for through in vitro anthelmintic activity, newly synthesized compounds showed improved therapeutic efficacy compared to parent medication. Several of the synthesized compounds demonstrated significant or equal anthelmintic efficacy against the designated earthworm species. A few analogs have also shown promise in action.

2.3 Docking study:

The user might choose from a variety of ligands to dock in the chosen protein binding sites. The molecules may dock rigidly (in which case neither a molecule of protein nor the ligand itself has torsional adaptability) or flexible (in which case a ligand shares a stiff protein with a torsional flexible ligand).

Using a variety of scoring algorithms, including BioPredicta, which generates 85% of hinding models and the fewest incorrect postures coming from native co-crystallized structures, the development of this molecular docking tool is shooting to identify the ideal geometry of contact between the the complexes of ligand and receptor with low interaction energy. In the docking assays, potent NUDTS inhibitors (PDB Code-5NQR) that prevent hormone signaling in breast tissue have been utilized. A 2D illustration of the ligand preparation producing benzimidazole derivatives was produced using ChemDraw. Selecting the proper protein receptor as well as enzyme from the proteins data bank is part of the preparation process for receptors. Co-crystalline ligands which have been artificially separated by software are also added to the structure.

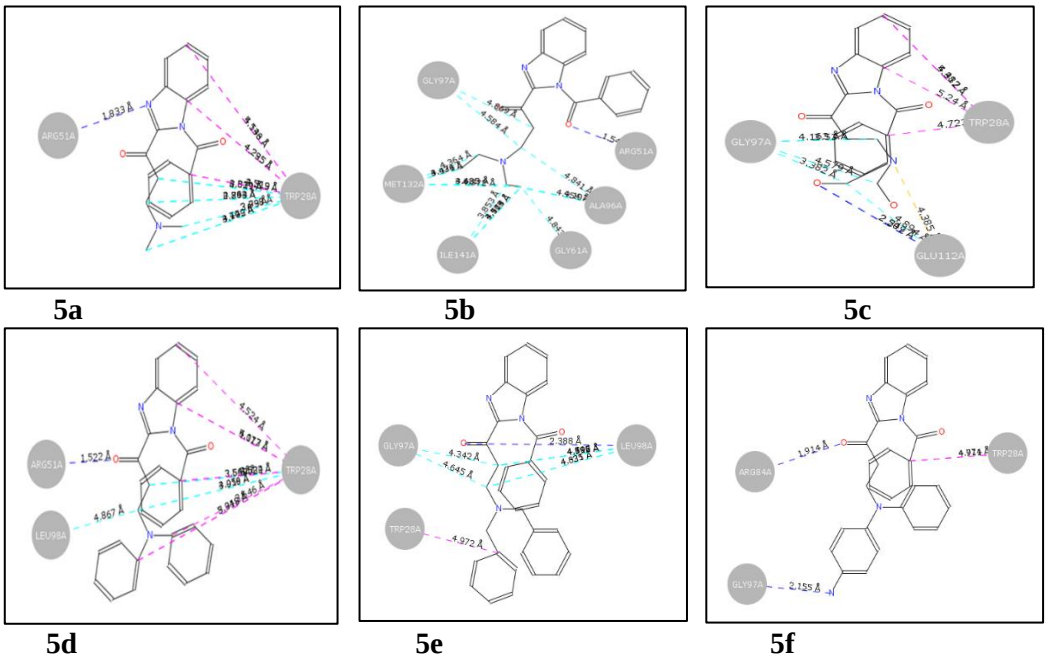
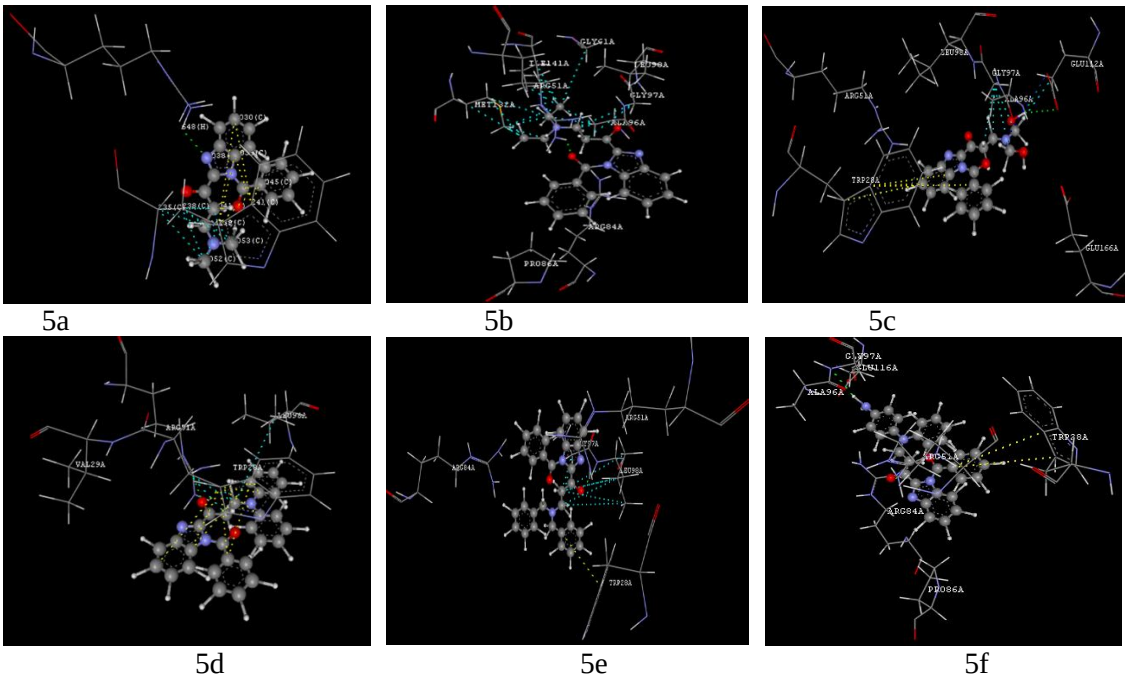


Fig. 2.6 2D Representation of derivatives (5a-5f)

The synthesized derivatives were evaluated for their anticancer activity. The dock score of compounds are shown in table and dock score is found to be -5.042 shown minimum dock score than other compounds.

As we compared result of compound 9g to the literature this docking score indicated that designed compounds have good binding affinity for binding to receptor (PDB Code-5NQR). The best pose obtained by docking results is reported, where main interaction between ligand and receptor can be observed.

All designed compound adopt a very similar conformation at binding pocket, showing Hydrogen bond interaction with amino acid of ARG51A with amino group containing benzimiazole moieties and a hydrophobic interaction with the amino acid of LEU98A, TRP28A containing alkyl molecule, as shown by a 2D representation diagram.



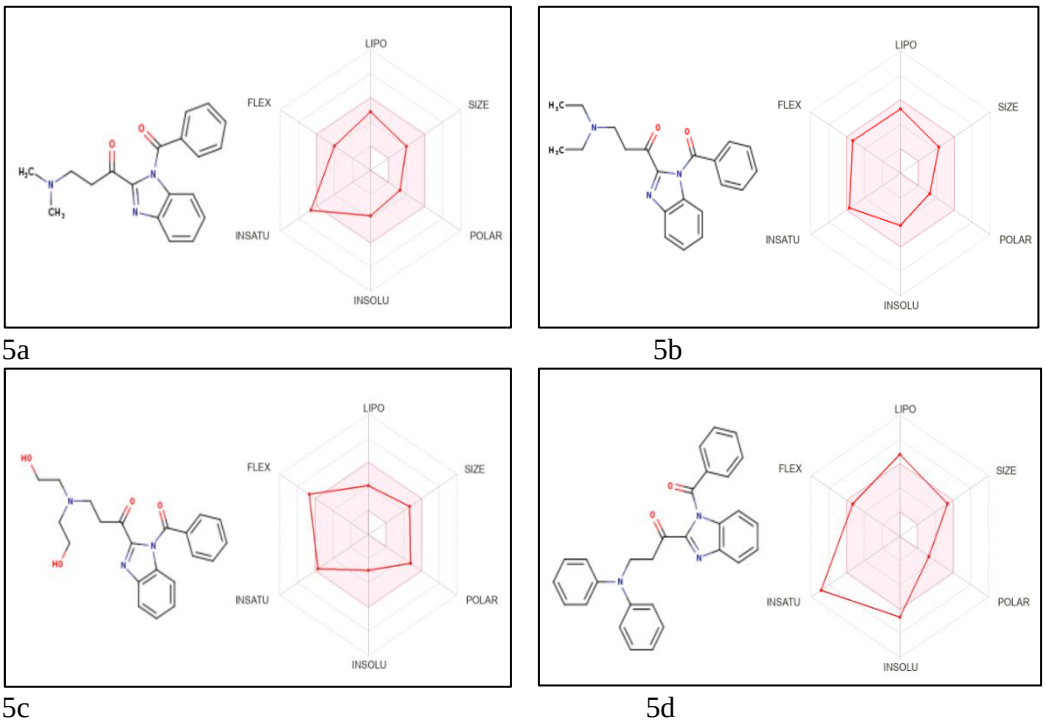
5a 5b 5c

5d 5e 5f

Fig. 2.7 3D representation of derivatives (5a-5f)

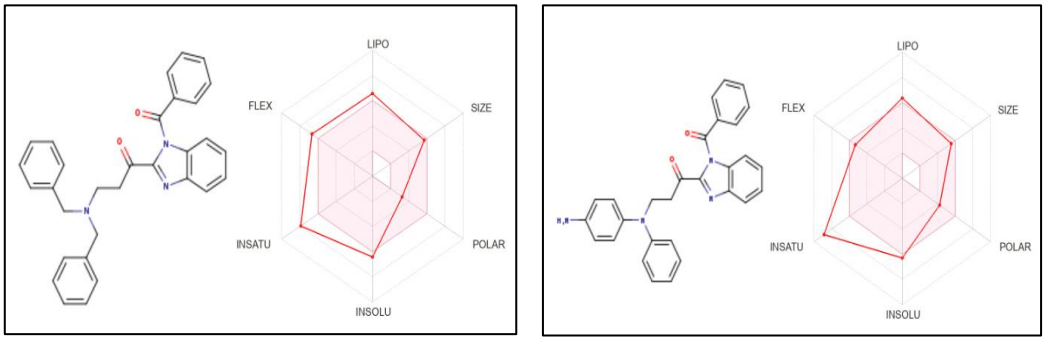
2.4 ADME STUDY:

These six derivatives underwent additional testing for in silico drug-likeness prediction and ADMET research. Only 15 phytochemicals out of the rest adhere to the ADMET restrictions and drug-like characteristics LogP readings. These substances met the requirements for polarity, hydrophobicity, and lipophilicity. Based on TPSA (topological polar surface area) and miLogP (molinpiration LogP) values, the drug-likeness features are screened. This study aids in the selection of the best phytochemicals that are drug-like and biologically permeable in terms of polarity. Tables describe the findings from the ADMET characteristics and BOILED-Egg models of the 15 phytochemicals, respectively.



5a 5b

5c 5d



5e 5f

Fig 2.8 ADME docking study of derivatives

The compounds synthesized from 5a to 5f were found to be having molecular weight between 320-460 gm/ml according to spectral data analysed. The rotatable bonds in 5c and 5e were found to be 10 whereas the remaining derivatives were found to be having 5a with 6 and 5b, 5d and 5f with 8 rotatable bonds. Meanwhile these derivatives where found to be having 3-6 H-bond acceptors whereas derivative 9c found with maximum 6 H-bond acceptor groups. The H-bond donor groups only found in derivative 5c and 5f with 2 and 1 H-bond acceptor groups respectively. The other data like MR, heavy atoms as well as aromatic heavy atoms as mentioned in table no.

Compound	Formula	MW	Rotatable bonds	H-bond acceptors	H-bond donors	MR	Heavy atoms	Aromatic heavy atoms
9a	$C_{19}H_{19}N_3O_2$	321.37	6	4	0	93.42	24	15
9b	$C_{21}H_{23}N_3O_2$	349.43	8	4	0	103.03	26	15
9c	$C_{21}H_{23}N_3O_4$	381.43	10	6	2	105.35	28	15
9d	$C_{29}H_{23}N_3O_2$	445.51	8	3	0	134.87	34	27
9e	$C_{31}H_{27}N_3O_2$	473.56	10	4	0	142.39	36	27
9f	$C_{29}H_{24}N_4O_2$	460.53	8	3	1	139.28	35	27

Table No. 2.2 ADME STUDY

Compound	iLOGP	XLOGP3	WLOGP	MLOGP	Silicos-IT Log P	Consensus Log P
9a	3	2.97	2.86	2.39	2.67	2.78
9b	3.64	3.7	3.64	2.84	3.46	3.46
9c	2.81	1.6	1.58	1.23	2.26	1.9
9d	3.78	6.31	6.14	4.35	4.81	5.08
9e	4.11	5.96	5.7	4.46	5.62	5.17
9f	3.26	5.63	5.73	3.8	4.09	4.5

Table no. 2.3 ADME STUDY

Compound	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
9a	High	✓	×	✓	✓	✓	✓	×
9b	High	✓	×	✓	✓	✓	✓	✓
9c	High	×	✓	×	×	×	✓	×
9d	High	×	×	×	✓	✓	×	✓
9e	High	×	✓	×	✓	✓	×	✓
9f	High	×	×	×	✓	✓	×	✓

Table No. 2.4 ADME STUDY

2.5 Experimental Procedure

A. Procedure for synthesis of 1-hydroxyethylbenzimidazole:

A mix of 10 gm of O-phenylenediamine and 9 ml of lactic acid refluxed in microwave for 7 min. then resulting compound is cooled down and added 10% NaOH sol until basicity to litmus. Then it filtered, dried and recrystalized in hot water. (12-14)

B. Procedure for synthesis of 2-acetylbenzimidazole:

Taken and combined are 10 mmol as part of 1-hydroxybenzimidazole over 10 ml as part of 5% acetic acid sol in addition 10 mmol of K2Cr2O7 in 10 ml of the 5% acetic acid sol. Using a magnetic stirrer, this liquid is swirled for 3 to 5 hours. The finished product is filtered, water washed, and dried. Recrystalized in alcohol. (12-14)

C. Synthesis of 1-(1-benzoyl-1H-benzo-[d]-imidazol-2-yl)ethan-1-one

0.5 gm of 2nd step product taken in stoppered test tube having 10 ml of 10% sol of NaHCO3 and 1 gm of benzoyl chloride. Mixture was shaken vigorously. Afrer the odour of benzoyl chloride vanished the mixture is acidified with dil. HCl to congored colour and filtered. Extracted with cold ether and recrystalized from ethanol. (15-17)

D. Procedure for synthesis of 5a-f :

0.01 mol 3rd step product, 1.5 ml of 40% and 10 ml of DMF is taken in conical flask and mixture is stirred for 20 min at rt. Then 20 amine is added in mixture and again stirred for 6 hours to obtain a mannich base. Filter out content, a cold water wash, completion of drying, and recrystalized with ethanol. (18-21)

2.4.1 compound 9a : 1-(1-benzoyl-1H-benzo-[d]-imidazol-2-yl)-3-(dimethylamino) propan-1-one : Yield : 63%, mp : 274°C, IR (cm^{-1}) : 1693(C=N), 1724,1730 (C=O resp.), 1634 (C=C), H1 NMR (DMSO) δ 2.28 (6H, s), 2.63-2.84 (4H, 2.69 (t, J = 7.0 Hz), 2.79 (t, J = 7.0 Hz)), 7.39-7.78 (7H, 7.46 (d, J = 8.5, 7.5, 1.4, 0.4 Hz), 7.54 (d, J = 8.1, 2.6, 0.5 Hz), 7.59 (d, J = 7.6, 7.5, 2.6 Hz), 7.70 (t, J = 7.5, 1.5 Hz), 7.72 (d, J = 8.5, 1.5, 1.4, 0.4 Hz)), 7.92 (1H, d, J = 8.1, 7.5, 1.8 Hz), 8.14 (1H, d, J = 7.6, 1.8, 0.5 Hz). , MS: m/z 321(M+); Calculated for $C_{19}H_{19}N_3O_2$.

2.4.2 compound 9b : 1-(1-benzoyl-1H-benzo-[d]-imidazol-2-yl)-3-(diethylamino) propan-1-one : Yield : 58%, mp : 292°C, IR (cm-1) : 1696(C=N), 1748,1737 (C=O resp.), 1634 (C=C), H1 NMR (DMSO) 1H NMR: δ 0.98 (6H, t, J = 7.2 Hz), 2.58-2.85 (8H, 2.64 (q, J = 7.2 Hz), 2.70 (t, J = 7.0 Hz), 2.79 (t, J = 7.0 Hz)), 7.39-7.78 (7H, 7.46 (d, J = 8.5, 7.5, 1.4, 0.4 Hz), 7.54 (d, J = 8.1, 2.6, 0.5 Hz), 7.59 (d, J = 7.6, 7.5, 2.6 Hz), 7.70 (t, J = 7.5, 1.5 Hz), 7.72 (d, J = 8.5, 1.5, 1.4, 0.4 Hz)), 7.92 (1H, d, J = 8.1, 7.5, 1.8 Hz), 8.14 (1H, d, J = 7.6, 1.8, 0.5 Hz). , MS: m/z 349 (M+); Calculated for $C_{21}H_{23}N_3O_2$.

2.4.3 compound 9c : 1-(1-benzoyl-1H-benzo-[d]-imidazol-2-yl)-3-(bis (hydroxyl ethyl)amino) propan-1-one : Yield : 54%, mp : 288°C, IR (cm-1) : 3424-3342 (C-OH), 1692(C=N), 1741,1732 (C=O resp.), 1634 (C=C), 1H NMR: δ 2.70 (2H, t, J = 7.0 Hz), 2.77-2.94 (6H, 2.83 (t, J = 7.0 Hz), 2.89 (t, J = 2.7 Hz)), 3.54 (4H, t, J = 2.7 Hz), 7.39-7.78 (7H, 7.46 (d, J = 8.5, 7.5, 1.4, 0.4 Hz), 7.54 (d, J = 8.1, 2.6, 0.5 Hz), 7.59 (d, J = 7.6, 7.5, 2.6 Hz), 7.70 (t, J = 7.5, 1.5 Hz), 7.72 (d, J = 8.5, 1.5, 1.4, 0.4 Hz)), 7.92 (1H, d, J = 8.1, 7.5, 1.8 Hz), 8.15 (1H, d, J = 7.6, 1.8, 0.5 Hz). , MS: m/z 381 (M+); Calculated for $C_{19}H_{19}N_3O_4$.

2.4.4 compound 9d : 1-(1-benzoyl-1H-benzo-[d]-imidazol-2-yl)-3-(diphenylamino) propan-1-one : Yield : 46%, mp : 312°C, IR (cm-1) : 1686 (C=N), 1736,1728 (C=O resp.), 1635 (C=C), 1H NMR: δ 2.81 (2H, t, J = 6.1 Hz), 3.59 (2H, t, J = 6.1 Hz), 6.52 (4H, d, J = 8.2, 1.2, 0.5 Hz), 6.92 (2H, t, J = 8.1, 1.2 Hz), 7.22 (4H, d, J = 8.2, 8.1, 1.3, 0.5 Hz), 7.39-7.78 (7H, 7.46 (d, J = 8.5, 7.5, 1.4, 0.4 Hz), 7.54 (d, J = 8.1, 2.6, 0.5 Hz), 7.59 (d, J = 7.6, 7.2, 2.6 Hz), 7.70 (tt, J = 7.5, 1.5 Hz), 7.72 (d, J = 8.5, 1.5, 1.4, 0.4 Hz)), 7.92 (1H, d, J = 8.1, 7.2, 1.8 Hz), 8.15 (1H, d, J = 7.6, 1.8, 0.5 Hz)., MS : m/z 451 (M+); Calculated for $C_{29}H_{23}N_3O_2$.

2.4.5 compound 9e : 1-(1-benzoyl-1H-benzo-[d]-imidazol-2-yl)-3-(dibenzylamino) propan-1-one : Yield : 64%, mp : 345°C, IR (cm-1) : 1678 (C=N), 1746,1738 (C=O resp.), 1642 (C=C), 1H NMR: δ 2.70 (2H, t, J = 7.1 Hz), 2.88 (2H, t, J = 7.1 Hz), 3.62 (4H, s), 7.18-7.78 (17H, 7.24 (d, J = 7.7, 1.4, 0.9, 0.5 Hz), 7.27 (t, J = 7.7, 1.4 Hz), 7.33 (d, J = 7.7, 1.8, 0.5 Hz), 7.46 (d, J = 8.5, 7.5, 1.4, 0.4 Hz), 7.54 (d, J = 8.1, 2.6, 0.5 Hz), 7.59 (d, J = 7.6, 7.5, 2.6 Hz), 7.70 (tt, J = 7.5, 1.5 Hz), 7.72 (d, J = 8.5, 1.5, 1.4, 0.4 Hz)), 7.92 (1H, d, J = 8.1, 7.5, 1.8 Hz), 8.15 (1H, d, J = 7.6, 1.8, 0.5 Hz)., MS : m/z 481 (M+); Calculated for $C_{21}H_{23}N_3O_2$.

2.4.6 compound 9f : 3-((4-aminophenyl)-(phenyl)amino)-1-(1-benzoyl-1H-benzo-[d]-imidazol -2-yl)propan-1-one : Yield : 56%, mp : 340°C, IR (cm-1) : 1663 (C=N), 1732,1742 (C=O resp.), 1646 (C=C), 1H NMR: δ 2.85 (2H, t, J = 6.1 Hz), 3.63 (2H, t, J = 6.1 Hz), 6.50 (2H, d, J = 8.2, 1.2, 0.5 Hz), 6.71 (2H, d, J = 8.5, 1.9, 0.5 Hz), 6.86-7.10 (3H, 6.92 (tt, J = 8.1, 1.2 Hz), 7.04 (d, J = 8.5, 1.7, 0.5 Hz)), 7.22 (2H, d, J = 8.2, 8.1, 1.3, 0.5 Hz), 7.39-7.78 (7H, 7.46 (d, J = 8.5, 7.5, 1.4, 0.4 Hz), 7.54 (d, J = 8.1, 2.6, 0.5 Hz), 7.59 (d, J = 7.6, 7.2, 2.6 Hz), 7.70 (t, J = 7.5, 1.5 Hz), 7.72 (d, J = 8.5, 1.5, 1.4, 0.4 Hz)), 7.92 (1H, d, J = 8.1, 7.2, 1.8 Hz), 8.15 (1H, d, J = 7.6, 1.8, 0.5 Hz)., MS : m/z 460 (M+); Calculated for $C_{29}H_{24}N_4O_2$.

The creation of mannich base derivatives products with benzimidazole moiety was the main objective of the research. Formation of 2-ethanolbenzimidazole is the first step. The second stage involves the oxidation of 2-acetylbenzimidazole with $K_2Cr_2O_7$.Benzoylation of 2-acetylbenzimidazole is done in the third stage. In the last step, secondary amino acids are used to create mannich bases while formaldehyde is present. These recently created compounds were produced at a respectable yield.
To track the progress of the reaction and the purity of intermediary substances and end products, TLC was carried out using several solvent systems. The physicochemical properties of the synthesized compounds show superior solubility and an acceptable range of melting points.
The IC50 for compound 5a (42.39), 5b (41.94), 5g (42.78), and 5-Flurouracil for MCF-7 should be 39.22g/ml, according to the MTT test. The most potent anti-cancer activity is shown by compound 5d, which has a 43.29% (10 g/ml) inhibition compared to the standard compound's 66.53% (10 g/ml) inhibition.

The majority of recently created derivatives have proven modestly to moderately effective against various kinds of earthworms as anthelmintics. Comparing the outcome of the activity data, it was found that compound 5a had the greatest degree of activity at a median concentration of 20 mg/ml, causing paralysis beginning at 6.430.217 min and death at 29.531.80 min, as opposed to the widely used drug albendazole, which causes paralysis that 0.410.298 min and causes death at 1.460.241 min. Novel synthesized compounds 5b and 5d only demonstrated somewhat effective anthelmintic effects on different earthworm species.

2.6 Summary and Conclusion:

The disclosed synthetic methods in this study design provide new examples of heterocyclic synthesis. The present study describes a novel, straight forward procedure for synthesizing mannich bases made of benzimidazole derivatives. O-phenylenediamine is combined with lactic acid to create mannich bases, which are subsequently produced with an average yield of 40% to 70% by oxidation, benzoylation, and mannich base synthesis. The structures of all the generated compounds were predicted by IR, H1NMR, as well as mass data. All synthesized compounds exhibited anthelmintic and anticancer properties.

The ability of the synthesized chemicals to fight cancer was assessed. The addition of the diphenylamine group to compound 5d, which possesses an IC50 value around 42.78 g/ml, significantly increased its activity against the cancer cell line MCF7. Surprisingly, the activity is markedly increased by substituting the secondary amine group. The diphenylamine group is necessary to deliver anticancer effect that is relevant. Compounds 5a and 5b, with an inhibitory concentration (IC50) of 42.78 g/ml, are less active than compound 5d.
When examined for through in vitro anthelmintic activity, newly synthesized compounds showed improved therapeutic efficacy compared to parent medication. Several of the synthesized compounds demonstrated stronger or equal antihelmintic efficacy against the designated earthworm species. Additionally, a chemical 5d has shown potential activity.
Conclusive research is required to comprehend the mechanisms and elements that underlie certain activities. Investigating fresh synthetic substances with proven activity has been our goal.

Reference:

1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6864827/#:~:text=The%20applicatioa%20of%20heterocycles%20provides,of%20drugs%20or%20drug%20candidates
2. Al-Mulla A. A review: biological importance of heterocyclic compounds. Der Pharma Chemica. 2017;9(13):141-7.
3. Roman G. Mannich bases in medicinal chemistry and drug design. European journal of medicinal chemistry. 2015 Jan 7;89:743-816.
4. Bala S, Sharma N, Kajal A, Kamboj S, Saini V. Mannich bases: an important pharmacophore in present scenario. International journal of medicinal chemistry. 2014;2014.
5. Keri RS, Hiremathad A, Budagumpi S, Nagaraja BM. Comprehensive review in current developments of benzimidazole-based medicinal chemistry. Chemical biology & drug design. 2015 Jul;86(1):19-65.
6. Bansal Y, Silakari O. The therapeutic journey of benzimidazoles: A review. Bioorganic & medicinal chemistry. 2012 Nov 1;20(21):6208-36.
7. Sharma S, Abuzar S. The benzimidazole anthelmintics—chemistry and biological activity. Progress in Drug Research/Fortschritte der Arzneimittelforschung/Progrèsdes recherches pharmaceutiques. 1983 Jan 1:85-161.
8. Yadav G, Ganguly S. Structure activity relationship (SAR) study of benzimidazole scaffold for different biological activities: A mini-review. European journal of medicinal chemistry. 2015 Jun 5;97:419-43.
9. https://encyclopedia.pub/entry/31419#:~:text=Among%20the%20BZs%2C%20benzimidazole%20(BI,because%20of%20the%20annular%20tautomerism.
10. Srestha N, Banerjee J, Srivastava S. A review on chemistry and biological significance of benzimidazole nucleus. IOSR J Pharm. 2014 Dec;4(12):28-41
11. Padhy GK, Panda J, Raul SK, Behera AK. 2-Acetylbenzimidazole: a Valuable Synthon for the Synthesis of Biologically Active Molecules.
12. Wu L, Yang Y, Wang Z, Wu X, Su F, Li M, Jing X, Han C. Design, Synthesis, and Biological Evaluation of Aromatic Amide-Substituted Benzimidazole-Derived Chalcones. The Effect of Upregulating TP53 Protein Expression. Molecules. 2020 Mar 5;25(5):1162.
13. Chawla A, Kaur G, Sharma AK. Green Chemistry as a Versatile Technique for the Synthesis of Benzimidazole Derivatives. Int. J. Pharm. Phytopharmacol. Res.. 2012;2:148-59.
14. Kalirajan R, Rathore L, Jubie S, Gowramma B, Gomathy S, Sankar S. Microwave assisted synthesis of some novel pyrazole substituted benzimidazoles and evaluation of their biological activities.
15. Eswayah A, Khaliel S, Saad S, Shebani N, Fhid O, Belaid A, Alsharif T, Elforjane H, Saadalla Y, Baga E. Synthesis and analgesic activity evaluation of some new benzimidazole derivatives. American Journal of Chemistry and Application. 2017;4(5):30-5.
16. Hashem HE, El Bakri Y. An overview on novel synthetic approaches and medicinal applications of benzimidazole compounds. Arabian Journal of Chemistry. 2021 Nov 1;14(11):103418.
17. Rathee PS, Dhankar R, Bhardwaj S, Gupta M, Kumar R. Synthesis and antimicrobial studies of novel benzimidazole derivatives. Journal of Applied pharmaceutical science. 2011 Jun 30(Issue):127-30.
18. Lai Z, Wu R, Li J, Chen X, Zeng L, Wang X, Guo J, Zhao Z, Sajiki H, Cui S. Multicomponent double Mannich alkylamination involving C (sp2)–H and benzylic C (sp3)–H bonds. Nature Communications. 2022 Jan 21;13(1):435.
19. Gul HI, Tugrak M, Gul M, Mazlumoglu S, Sakagami H, Gulcin I, Supuran CT. New phenolic Mannich bases with piperazines and their bioactivities. Bioorganic chemistry. 2019 Sep 1;90:103057.
20. Saranya S, Harry NA, Krishnan KK, Anilkumar G. Recent developments and perspectives in the asymmetric Mannich reaction. Asian Journal of Organic Chemistry. 2018 Apr;7(4):613-33.
21. Sujith KV, Rao JN, Shetty P, Kalluraya B. Regioselective reaction: synthesis and pharmacological study of Mannich bases containing ibuprofen moiety. European journal of medicinal chemistry. 2009 Sep 1;44(9):3697-702
22. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Antineoplastic Agents. [Updated 2023 Feb 13]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548022/
23. Strober W. Trypan Blue Exclusion Test of Cell Viability. Curr Protoc Immunol. 2015 Nov 2;111:A3.B.1-A3.B.3. doi: 10.1002/0471142735.ima03bs111. PMID: 26529666; PMCID: PMC6716531.
24. https://www.testing.com/tests/lactatedehydrogenaseldh/#:~:text=Lactate%20dehydrogenase%20(LDH)%20is%20a,evaluate%20certain%20types%20of%20cancer.

25. https://www.sigmaaldrich.com/IN/en/technical-documents/protocol/cell-culture-andcell-culture-analysis /cell-counting-and-health-analysis/cell-proliferation-kit-i-mtt
26. https://www.sigmaaldrich.com/IN/en/technical-documents/protocol/cell-culture-andcell-culture-analysis/ cell-counting-and-health-analysis/cell-proliferation-kit-xtt-assay
27. Orellana EA, Kasinski AL. Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Proliferation. Bio Protoc. 2016 Nov 5;6(21):e1984. doi: 10.21769/BioProtoc.1984. PMID: 28573164; PMCID: PMC5448418.
28. Sawant VJ, Bamane SR, Shejwal RV, Patil SB. Comparison of drug delivery potentials of surface functionalized cobalt and zinc ferrite nanohybrids for curcumin in to MCF7 breast cancer cells. Journal of Magnetism and Magnetic Materials. 2016 Nov 1;417:222-9.
29. https://www.yourgenome.org/facts/what-are-helminths/
30. Shahare MB, Kadam VJ, Jagdale DM, Gandhi PS, Gaikwad PL. Synthesis and evaluation of novel anthelmentic benzimidazole derivatives. Int. J. Res. Pharm. Chem. 2012;2(1):132-6.
31. Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of Ficus benghalensis. International Journal of Green Pharmacy (IJGP). 2008;2(3).
32. Islam, S., Fahad, F.I., Sultana, A., Sayem, S.A.J., Roy, S.B., Islam, M.N., Roy, A. and Sayeed, M.A., 2022. Evaluation of antioxidant, cytotoxic, anti-inflammatory, antiarthritic, thrombolytic, and anthelmintic activity of methanol extract of Lepidagathis hyalina Nees root. Evidence-Based Complementary and Alternative Medicine, 202