A New Approach for the Synthesis of Bis (Indolyl) Derivatives and the Evaluation of Antioxidant and Antiulcer Activity

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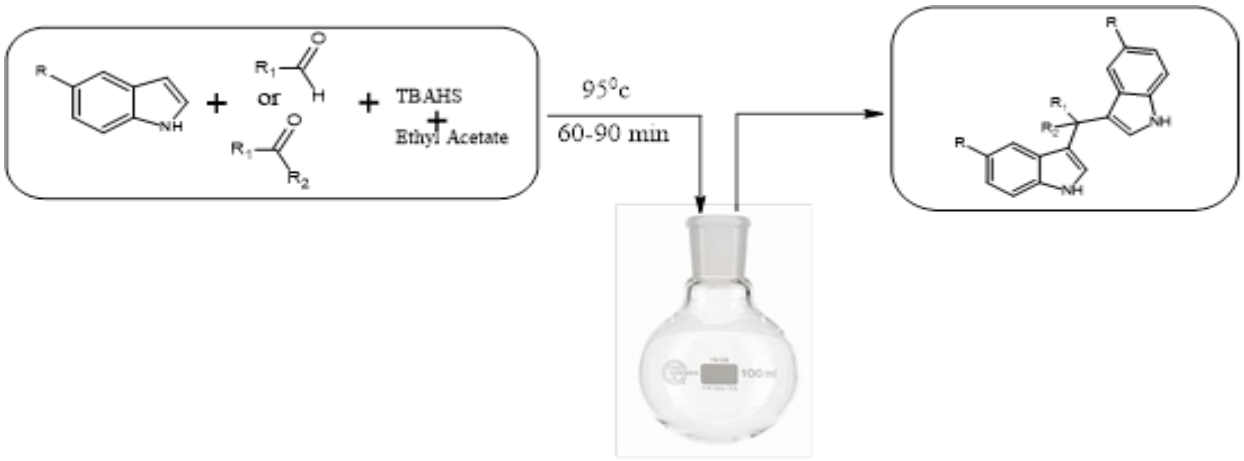


Abstract- This study introduces an efficient synthesis method for bis(indolyl) derivatives, highlighting their potential as anti-ulcer and antioxidant agents. The novel approach involves reacting indoles with aldehydes/ ketones in the presence of TBAHS as a catalyst and Ethyl Acetate as a solvent at 95°C for 60-90 minutes. This method offers advantages such as cost efficiency, minimal reagent consumption, one-pot synthesis, and high yield. The synthesized derivatives were characterized using IR to confirm their structures and purity. Subsequent in silico and in vitro experiments assessed their biological activities. The antioxidant activity, evaluated via phosphomolybdate assay, revealed that compounds 1, 2, and 5 exhibit strong antioxidant properties, which may contribute to their anti-ulcer effects by mitigating oxidative stress. The anti-ulcer activity was assessed using an acid neutralizing capacity assay, where compounds 1 and 2 at 50 mg, 1, 2, 3, and 5 at 100 mg, and 2 and 4 at 150 mg demonstrated the highest ANC. These findings suggest that several synthesized compounds could reduce ulcer formation and promote healing, indicating their potential as therapeutic agents.

Keywords: BisIndole, One pot, Anti-ulcer activity, Anti-oxidant activity

INTRODUCTION

Indole and its derivatives are part of a significant class of nitrogen heterocyclic compounds, known for their widespread presence in biologically active pharmaceuticals and natural products [(1)]. They serve as essential structural components in various pharmacological and agrochemical compounds, offering diverse biological properties [(2)]. The indole nucleus has demonstrated significant therapeutic potential in medicine, showcasing antimicrobial [(3-4)], anti-HIV [(5)], antioxidant [(6-8)], antimicrobial [(9)], antibiotic [(10)], cytotoxic [(11-12)], antitumor [(13)], anticancer [(14-17)], antiviral [(13)], and anti-inflammatory [(18-19)] activities. In recent years, there has been significant interest in the exploration of specific bis(indole) secondary metabolites due to their unique structural characteristics and diverse range of biological activities [(20)]. Bis-indole compounds have emerged as focal points in medicinal chemistry, serving as key structural scaffolds in both natural products and synthetic drugs [(21)]. These compounds, whether obtained synthetically or from natural sources, exhibit

broad pharmacological activities. The synthesis of bis(indolyl) methanes and their derivatives has become a prominent trend, garnering attention for their synthetic and biological applications [22]. Bis(indolyl)methanes (BIMs), characterized by the presence of two indole or substituted indole units within a single molecule, are prevalent in bioactive metabolites originating from terrestrial and marine environments [20, 23-24].

There are various synthetic procedures for synthesis of bis(indolyl) derivatives. Some of the most successful and widely used procedures are:-

(a) Various bis-indoles were successfully synthesized by reacting indole-carboxaldehydes with bifunctional amines (p-phenylenediamine or 4,4′-ethylenedianiline) under microwave irradiation, producing excellent outcomes. Glacial acetic acid was selected as the preferred catalytic agent for the synthesis. The optimal reaction conditions, identified as 30 minutes of reaction time at 65°C, led to a 98% yield of bis-indole [25].

(b) The study investigated optimal conditions for synthesizing Bis indolyl methanes (BIM) through the reaction of aldehydes with 1H-indole. Several catalytic systems, such as sulfuric acid, methanesulfonic acid, p-toluenesulfonic acid, and amberlyst-15, were evaluated. Sulfuric acid demonstrated poor performance in the opposite direction. Methanesulfonic acid, p-toluenesulfonic acid, and amberlyst-15 displayed effective catalytic activity. Among them, amberlyst-15 was preferred due to its easy removal as a heterogeneous catalyst [26].

(c) A new technique for producing bis-indoletriazoles was developed using a sequential process comprising four steps in a single pot. The process involved Friedel-Crafts reactions of indole with aldehyde, catalyzed by I_2 and H_2SO_4-SiO_2, followed by N-alkylation with propargyl bromide, azidation, and finally, copper(I)-catalyzed azide alkyne cycloaddition (CuAAC). These reactions efficiently took place at room temperature and were completed within a short period, resulting in a variety of bis-indoletriazoles in yields ranging from good to excellent, showcasing the broad applicability of this single-pot method [27].

(d) The synthesis entails reacting indole with substituted aldehydes while employing an aluminium triflate catalyst in acetonitrile solvent. This process demonstrates efficient progress under mild conditions at room temperature, rendering it a practical and convenient approach for synthesizing the desired compounds [28].

The aim of our present investigation is to design a new way of approach for synthesis of bis(indolyl) derivatives based on one-pot synthesis principle and determining antioxidant and antiulcer activity (via invitro evaluation studies). In accordance with the synthesis principle compounds (1-8) were obtained

MATERIALS REQUIRED

The materials required for synthesis can be divided into 2 sections:

(i) APPARATUS

Round Bottom Flask, Beaker, Conical Flask, Separating Funnel, Tripod stand, Water Bath, Glass rod, Spatula, Butter Paper

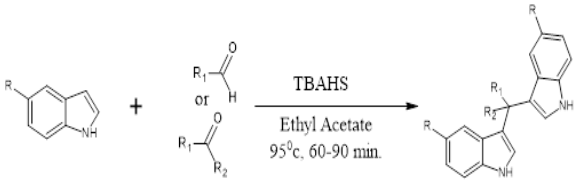
(ii) CHEMICALS

Indole, 5-methoxy Indole, Acetaldehyde, Benzaldehyde, 4-(dimethylamino) Benzaldehyde, Tert-butyl-ammonium-hydrogen-sulfate (TBAHS), Ethyl Acetate

EXPERIMENTAL PROCEDURE

(i) SYNTHETIC PROCEDURE

The reaction belongs to the class of one-pot synthesis methodology. In this methodology, the RBF is taken and all reactant [i.e Indole (and its derivatives), Aldehydes and Ketones (and its derivatives), catalyst (TBAHS) & solvent (Ethyl Acetate)] are placed inside. The top of RBF is sealed with cotton and is heated on a water bath for about 60-90 minutes. After the reaction is completed the RBF is allowed to cool at room temperature. All of these reactions were performed at about 95C°. After the optimum conditions obtained, different bisindoles were synthesized such as 3,3'-(Phenylmethylene)bis(1H-indole) as shown in scheme 1.



Scheme 1: Synthesis route of main reaction

(ii) IN-VITRO EVALUATION TEST

(b) ANTI-OXIDANT ACTIVITY

❖ Phosphomolybdate Assay

The total antioxidant activity of synthesized compounds was determined by phosphomolybdate assay using ascorbic acid as a standard drug. 0.1gm of the compound was combined with 1.0 ml of the reagent (0.6M sulphuric acid, 28mM sodium phosphate & 4mM ammonium Molybdate). The tubes were capped and incubated in a boiling water bath at 95^0c for 90 minutes. After the sample has cooled to room temperature, the absorbance was measured at 695nm against blank using an UV Spectrophotometer. The blank contained 1.0 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same condition as the rest of the sample. The total antioxidant activity is expressed as µg and the formula to calculate antioxidant activity is:

$$\text{TAC} \quad (\%) = \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}}\right] \times 100$$

(b) ANTI-ULCER ACTIVITY

❖ Acid Neutralizing Capacity

The total antiulcer activity of the synthesized compounds was assessed by evaluating their acid neutralizing capacity, using aluminum hydroxide ($Al(OH)_3$) as the standard drug. 8 compounds were tested at three different concentrations (50 mg, 100 mg, and 150 mg). Each compound was mixed with 30 mL of 1N hydrochloric acid (HCl). The mixture was thoroughly shaken, and 2-3 drops of phenolphthalein indicator were then added. This reaction mixture was titrated with 0.5N sodium hydroxide (NaOH) until a persistent pink color appeared, indicating the endpoint of the titration.

ANC = (vol. of HCl × Normality of HCl) – (vol. Of NaOH × Normality of NaOH) Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized divided by Grams of Antacid/Extract.

RESULT & DISCUSSION

(i) SYNTHESIS & OPTIMIZATION

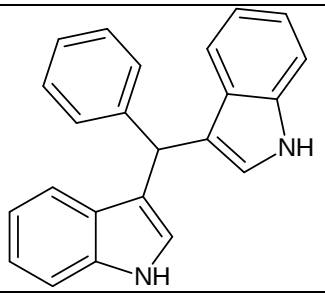
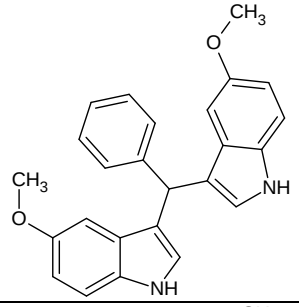
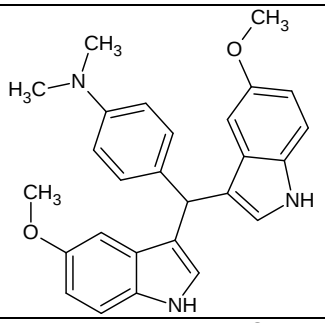
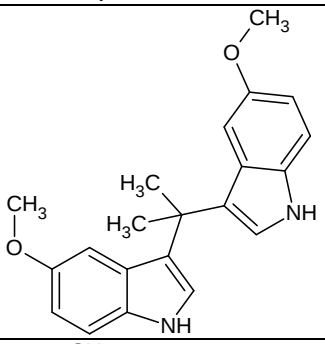
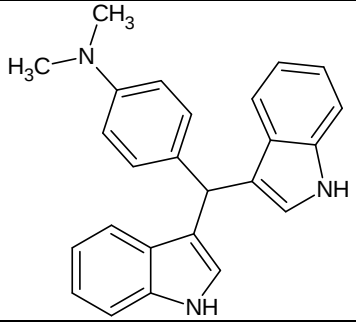
The optimal conditions for preparations of bis(indolyl) derivatives (BID) is by reacting indoles with aldehydes/ ketones to produce Bis-indoles. The catalytic effects of several catalytic systems were inspected in the reaction of aldehydes/ ketones and indoles, these catalytic systems included H_2o_2, chromic acid, ceric ammonium sulfate, potassium tert butoxide, PEG & TBAHS as shown in Table(1). Hydrogen peroxide did a remarkably bad job on this reaction , while TBAHS (at 0.5% equivalence) & cerric ammonium sulfate, TBAHS (at 1% equivalence) had good catalytic activities. However, TBAHS (at 1% equivalence) produced highest percentage of yield as compare to the rest. Therefore, TBAHS (at 1% equivalence) was determined to be optimal catalyst for the reaction of the indoles with aldehydes/ ketones. The solvent used for reaction will be ethyl acetate and the temperature used for synthesis will be between 60-90 min.

S.no	Catalyst	Solvent	Equivalent	Temperature	Yield
1.	Hydrogen Peroxide	Ethyl Acetate	1	Room temp.	40%
2.	Chromic Acid	Ethyl Acetate	1	Room temp.	50%
3.	Cerric Ammonium Sulfate	Ethyl Acetate	1	Room temp.	60%
4.	Potassium Tert Butoxide	Ethyl Acetate	1	Room temp.	20%
5.	PEG	Ethyl Acetate	1	Room temp.	30%
6	TBAHS	Ethyl Acetate	1	Room temp.	80%
7.	TBAHS	Ethyl Acetate	0.5	Room temp.	60%

Table 1: Optimization table of different catalyst used for synthesis of different BID

Finally, when applying the previous conditions to prepare BID on the reaction of preparing some products, we had synthesized successfully 8 analogues as shown in in Table (**2**)

S.no	Product	Quantity of catalyst used	Quantity of Indoles used	Temperature	Reaction time

1.		0.159gm	0.5gm	95^0c	90 min.
2.		0.287gm	0.3gm	95^0c	90 min.
3.		0.203gm	0.3gm	95^0c	90 min.
4.		0.172gm	1.52gm	95^0c	90 min.
5.		0.067gm	0.47gm	95^0c	60 min.

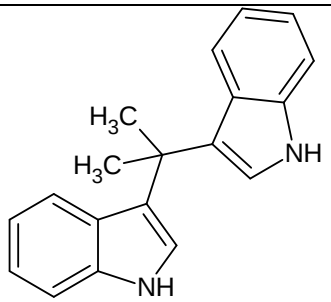
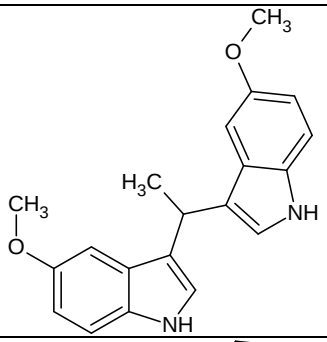
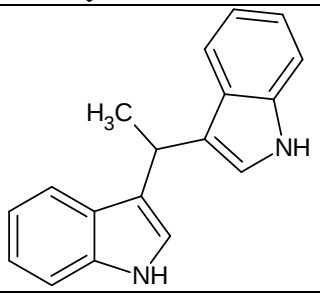
6.		0.172gm	0.2gm	95^0c	60 min.
7.		0.074gm	0.6gm	95^0c	60 min.
8.		0.159gm	1.06gm	95^0c	60 min.

Table 2: Different BID synthesis along with quantity and reaction conditions as well as time taken

(ii) CHARACTERIZATION

(a) IR ANALYSIS

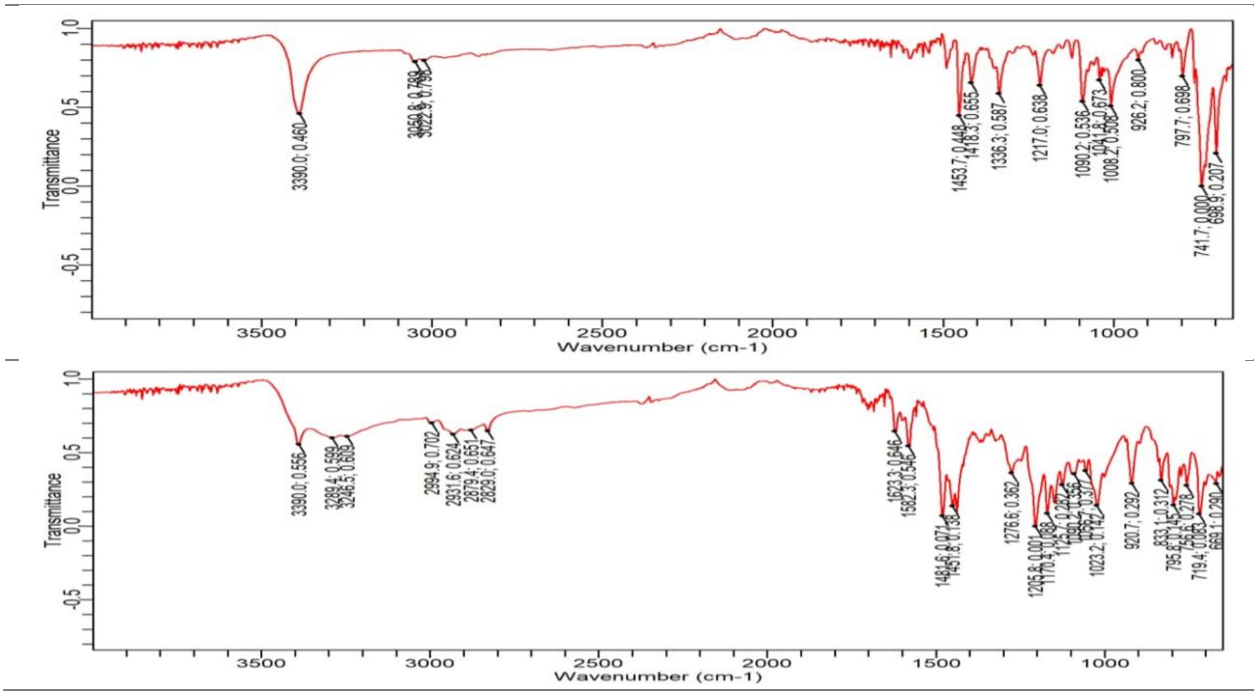
In order to elucidate the functional groups present in the synthesized compound, IR spectroscopy was employed. This technique provides insights into the vibrational modes of the molecular bonds, helping to confirm the chemical structure. The detailed IR bands absorption is given in Table 3.

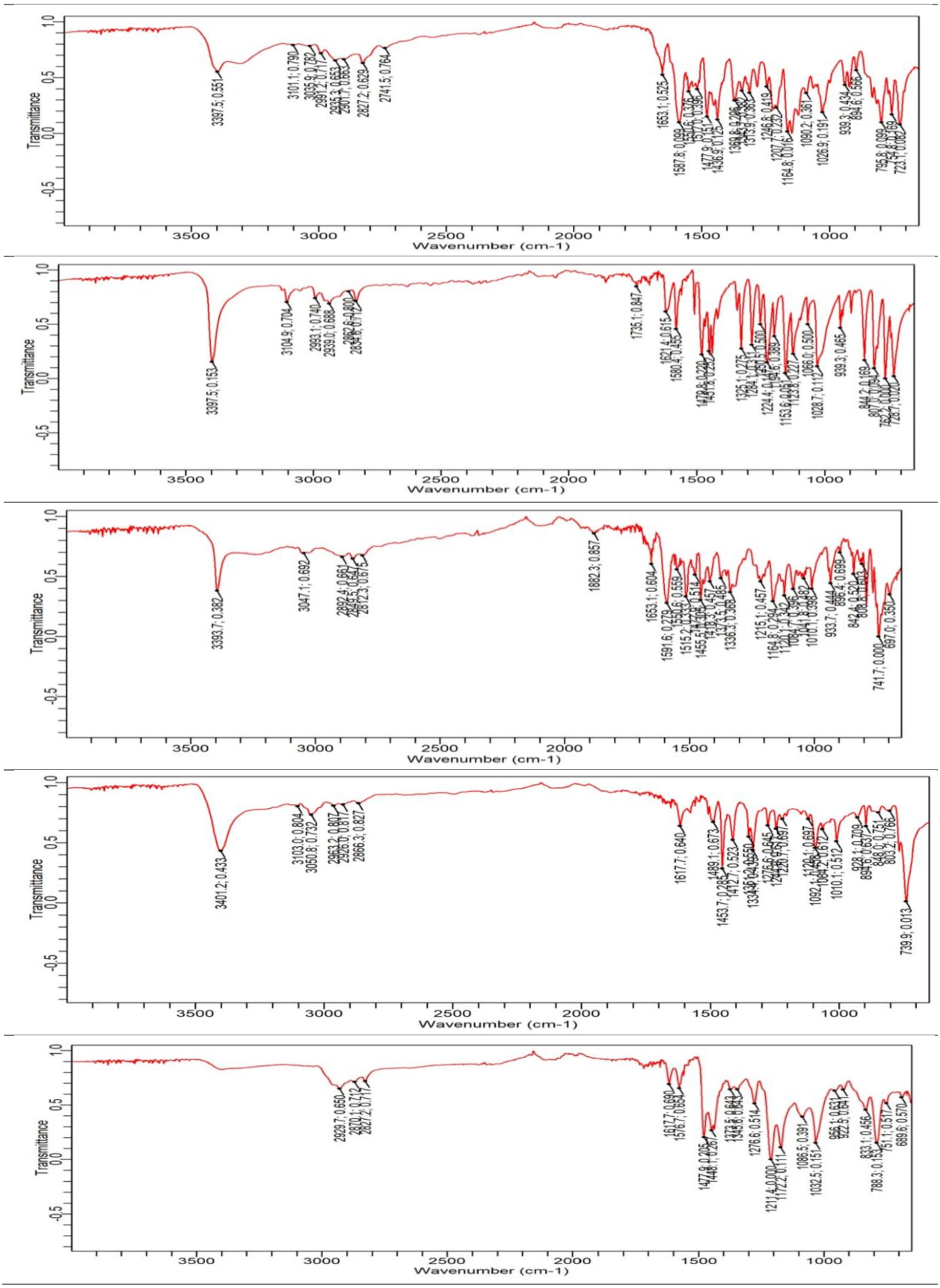
S.No	Name Of The Compound	Infra-Red Data			
		WaveNumber (cm^{-1})	Intensity	Functional Group	Description
1.	3,3'-(Phenylmethylene)bis(1H-indole) [1]	3390.01	Medium	N-H stretch	Indole N-H group
		3050.82	Weak	C-H stretch	Aromatic C-H stretch
		1453.66	Medium	C=C stretch	Aromatic ring
		1418.25	Weak	C=C stretch	Indole ring
		1216.97	Weak	C-N stretch	Indole ring
		741.74	Strong	C-H bend	Monosubstitued benzene
2.	3,3'-(Phenylmethylene)bis(5-methoxy-1H-indole) [2]	3090.01	Medium	Aromatic C-H stretch	Aromatic hydrogen atoms in indole and phenyl
		2931.55	Medium	Aliphatic C-H Stretch	Methylene groups
		1582.25	Strong	Aromatic C=C Stretch	Carbon-carbon bonds in aromatic rings
		1205.79	Medium	C-O Stretch	Methoxy groups

		1276.61	Medium	C-N Stretch	Carbon-nitrogen bonds in indole rings
		833.06	Medium	Aromatic C-H Bending	Aromatic hydrogen bending vibrations
3.	4-[Bis(5-methoxy-1H-indol-3-yl)methyl]-N,N-dimethylaniline [3]	3035.91	Medium	Aromatic C-H stretch	Aromatic hydrogen atoms in indole and phenyl
		2991.18	Strong	Aliphatic C-H Stretch	Methylene groups
		1587.84	Strong	Aromatic C=C Stretch	Carbon-carbon bonds in aromatic rings
		1343.70	Medium	C-N stretch	Aromatic Amines
		1246.79	Medium	C-O Stretch	Methoxy groups
		894.56	Medium	Aromatic C-H Bending	Aromatic hydrogen bending vibrations
4.	3,3'-(2,2-Propanediyl)bis(5-methoxy-1H-indole) [4]	3397.46	Medium	N-H Stretch	Indole N-H stretch
		2939.00	Medium	C-H Stretch (Alkane)	Propane bridge C-H stretch
		2993.05	Medium	C-H Stretch (Alkyl)	Methoxy C-H stretch
		1580.39	Medium	C=C Stretch (Aromatic)	Indole C=C stretch
		1580.39	Medium	N-H Bending (Secondary Amine)	Indole N-H bending
		1325.06	Medium	C-N Stretch	Indole C-N stretch
		1153.61	Strong	C-O Stretch	Methoxy C-O stretch
		1191.61	Medium	C-C Stretch (Aliphatic)	Propane bridge stretch
5.	4-(Di-1H-indol-3-ylmethyl)-N,N-dimethylaniline [5]	3393.74	Medium	N-H Stretch (Indole)	Stretching vibrations of indole N-H groups
		3047.09	Medium	C-H Stretch (Aromatic)	Aromatic C-H stretching vibrations
		2892.41	Medium	C-H Stretch (Alkyl)	Alkyl C-H stretching vibrations
		1591.57	Medium	C=C Stretch (Aromatic)	Aromatic ring C=C stretching vibrations
		1477.88	Medium	C=C Stretch (Aromatic)	Additional aromatic C=C stretching vibrations
		1336.25	Medium	C-N Stretch (Amine)	Stretching vibrations of the C-N bond in amines
		1215.11	Medium	C-N Stretch (Amine)	Another mode of C-N stretching vibrations
		741.74	Medium	C-H Bending (Aromatic)	Aromatic C-H out-of-plane bending vibrations
6.	3,3'-propane-2,2-diylbis(1H-indole) [6]	3401.19	Medium	N-H Stretch	Broad absorption due to N-H bonds
		3050.82	Medium	C-H Stretch (Alkanes)	Absorption bands due to C-H bonds in CH3 and CH2 groups
		1489.07	Medium	C=C Stretch (Aromatic)	Multiple bands from aromatic ring stretches
		1351.15	Medium	C-N Stretch (Aromatic)	Bands from C-N stretching in the indole rings

		1453.66	Strong	CH2 Bend	Absorption due to bending in methylene groups
		1351.15	Strong	CH3 Bend	Absorption due to bending in methyl groups
		894.56	Weak	Out-of-plane C-H Bend	Out-of-plane bending vibrations in the aromatic ring
7.	3,3'-(1,1-Ethanediyl)bis(5-methoxy-1H-indole) [7]	3397.46	Medium	NH Stretch (Indole)	Secondary amine in indole
		3090.01	Weak	Aromatic C-H Stretch	Aromatic ring in indole
		2929.68	Medium	Alkyl C-H Stretch	Ethylene bridge
		1576.66	Medium	Aromatic C=C Stretch	Aromatic ring in indole
		1276.61	Strong	Methoxy C-O Stretch	Methoxy groups
8.	3-[1-(1H-indol-3-yl)ethyl]-1H-indole [8]	3404.92	Medium	N-H Stretch	N-H stretching in the indole ring
		3050.82	Medium	Aromatic C-H Stretch	C-H stretching in aromatic rings
		2961.36	Medium	Aliphatic C-H Stretch	C-H stretching in the ethyl group
		1544.98	Strong	Aromatic C=C Stretch	C=C stretching in aromatic rings
		1338.11	Medium	C-N Stretch	C-N stretching vibrations
		846.10	Medium	Aromatic C-H Bending	Out-of-plane bending of aromatic C-H bonds

Table 3: IR band absorption of all compounds

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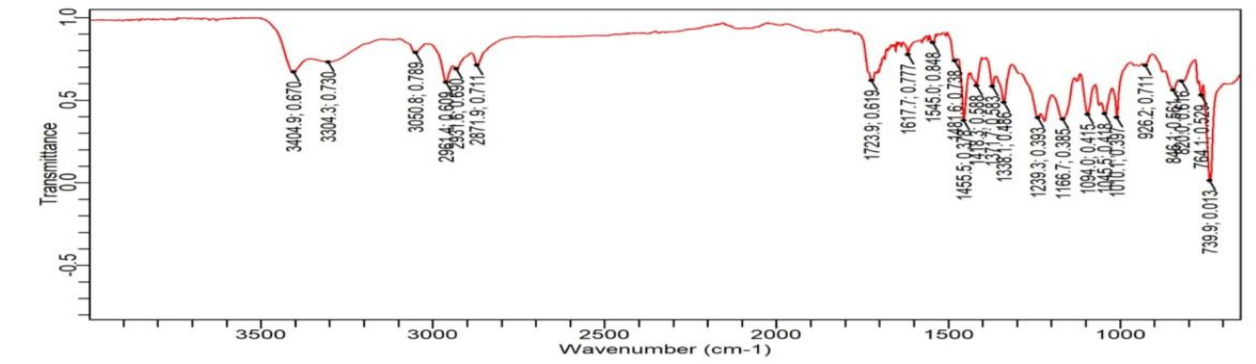


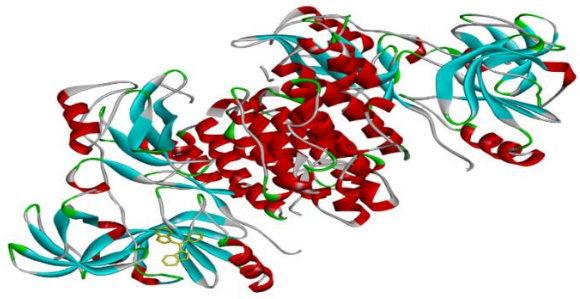
(b) DOCKING STUDIES

The molecular docking studies were conducted to evaluate the binding affinities and interaction profiles of the target protein with various ligands. 2hck was protein used for docking. The docking scores, which indicate the binding energies, were used to rank the ligands. The results of the docking simulations are summarized in Table 4.

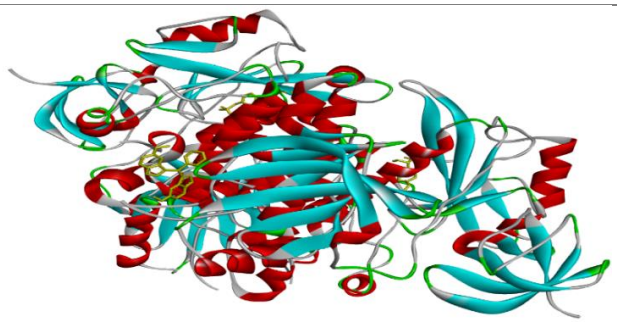
S.No	Sample Code	Binding Energy	Amino Acids	Interactions
1.	1	-7.91	GLU117, LYS324, LEU325, LYS315, TRP260, HIS326, TRP254	Conventional Hydrogen Bond Pi cation Pi anion Pi-Pi stacked Pi-Pi T shaped Pi-Alkyl
2.	2	-6.04	PHE278, ALA422, LYS423, PRO425, PHE424, LEU407, ARG409	Carbon-Hydrogen Bond Pi-Pi T shaped Alkyl Pi-Alkyl
3.	3	-5.72	VAL199, LEU163, PRO361, ASP197, TYR520	Conventional Hydrogen Bond Carbon-Hydrogen Bond Pi-Pi T shaped Alkyl
4.	4	-3.96	GLN195, ASP197, LEU163, THR198, GLU159, PRO361, TYR520	Conventional Hydrogen Bond Carbon-Hydrogen Bond Pi anion Pi sigma Pi-Pi T shaped Alkyl Pi-Alkyl
5.	5	-5.91	PHE212, LEU223, LEU143, SER142, ASP141	Conventional Hydrogen Bond Pi-Pi T shaped Amide-Pi Stacked Pi-Alkyl
6.	6	-4.91	LYS343, GLY344	Van der waals Amide-Pi Stacked Pi-Alkyl
7.	7	-5.01	LEU89, TYR90, LYS151, GLY152, SER247, SER248	Conventional Hydrogen Bond Carbon-Hydrogen Bond Alkyl Pi-Alkyl
8.	8	-4.37	VAL399, ALA164, PRO165, ARG160, LYS362, LEU163	Pi cation Pi sigma Alkyl Pi-Alkyl

From the results obtained, the highest docking score was observed in sample 1 & 2 with the binding energy of -7.91 & -6.04, the average docking scores were observed in samples 3, 5 & 7 with the binding energy of -5.72, -5.91 & -5.01 while the lowest docking scores was observed in samples 4, 6 & 8 with the docking scores of -3.96, -4.91 & -

4.37 : Hence proving sample 4 with lowest binding energy proving it having poorest binding affinity . In case sample 1, the compound formed Conventional-Hydrogen Bonds with TRP254, Pi-Pi stacked and Pi-Pi T shaped bonds with TRP260 as well as Pi-Alkyl Bonds with LYS324, LYS315 & LEU325. In case of sample 2, the compound formed Carbon-Hydrogen bonds with LYS423, Pi-Pi T shaped bonds with PHE424 & PHE278 as well as alkyl and Pi-Alkyl bonds with ALA422, ARG409, PRO425 & LEU407.



(1)



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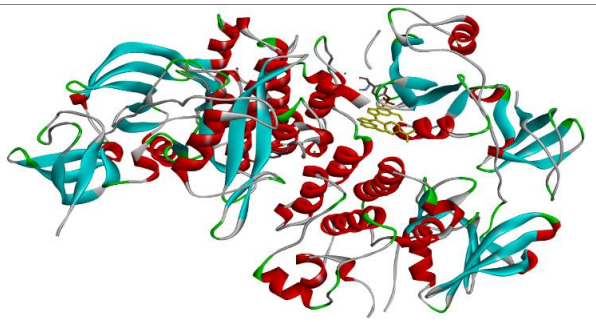

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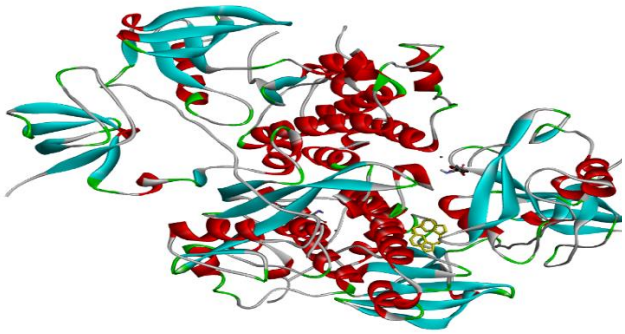

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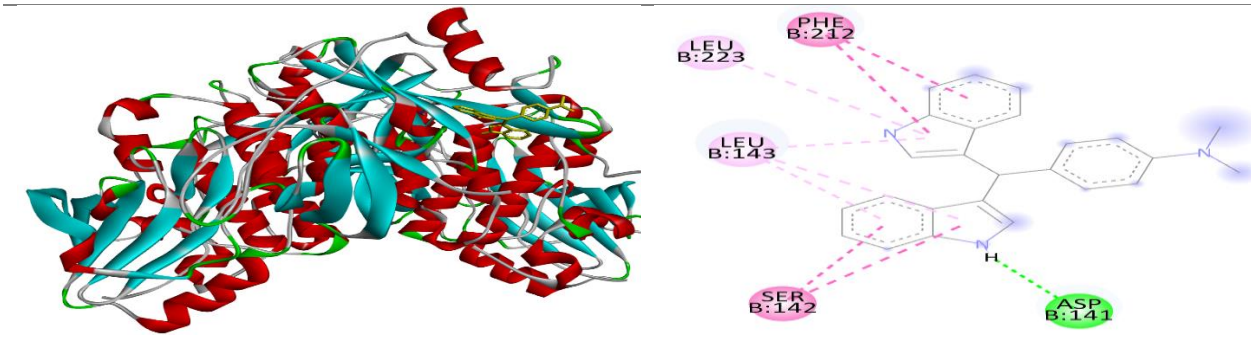

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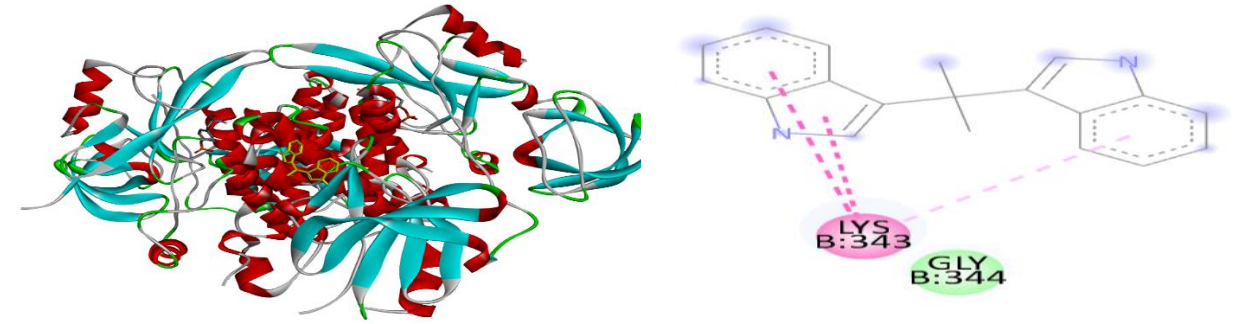

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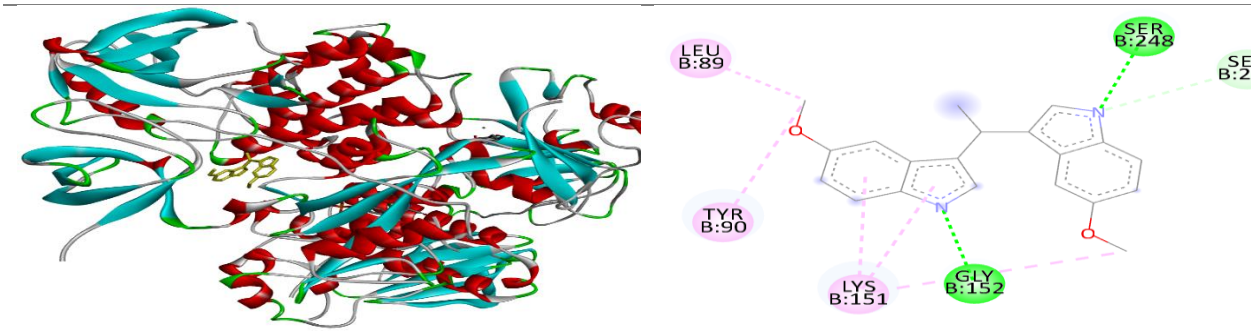

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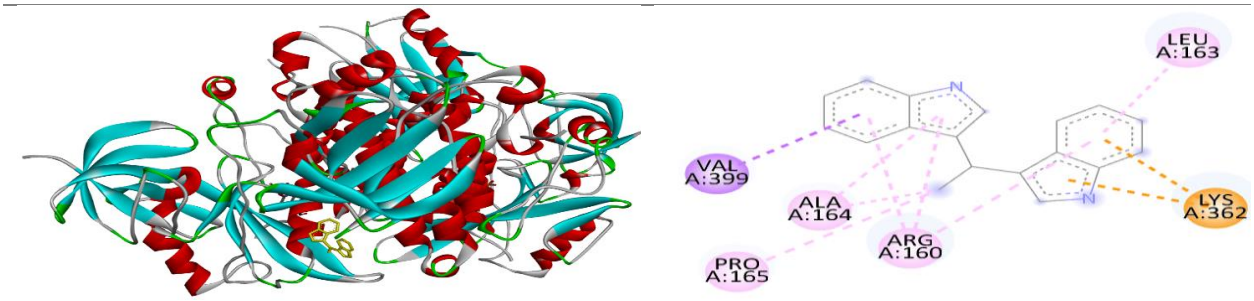

(8)

In conclusion, it can be said that the molecular docking results indicate that sample 1 has the highest binding affinity and forms stable interactions with the target protein, making it a promising candidate for further experimental validation. The consistent involvement of key residues across different ligands underscores their potential importance in designing new inhibitors. Overall, the docking analysis provides valuable

insights into the binding mechanisms and can guide the development of potent therapeutic agents.

(iii) IN-VITRO EVALUATION TEST

(a) ANTI-OXIDANT ACTIVITY

❖ Phosphomolybdate Assay

The antioxidant activities of various test samples were evaluated using the phosphomolybdate assay. In this assay, ascorbic acid was used as the standard reference compound, with a concentration of 1000 µg/mL. For consistency, the same concentration (1000 µg/mL) was also used for each of the eight test samples included in the analysis. The summarized results of these evaluations are presented in Table 5, and a graphical representation is provided in Figure 1.

S.no	Standard Absorbance (1000µg/ml)	Sample Code	Sample Absorbance (1000µg/ml)	Antioxidant Activity (%)	Standard Deviation
1.	0.707	1	0.021	97.02	0.4
2.	0.707	2	0.077	89.10	0.3
3.	0.707	3	0.224	68.31	0.8
4.	0.707	4	0.507	28.71	0.9
5.	0.707	5	0.129	81.75	1.1
6.	0.707	6	0.269	61.95	0.4
7.	0.707	7	0.226	68.03	0.7
8.	0.707	8	0.416	41.15	1.2

Table 5: Result data of phosphomlybdate assay

In our study evaluating antioxidant activity at a concentration of 1000 µg/mL, we observed distinct differences among the sample compounds. Sample codes 1, 2, and 5 stood out by exhibiting the highest levels of antioxidant activity, indicating their strong potential in scavenging free radicals or inhibiting oxidative processes. Conversely, sample codes 3, 6, and 7 displayed moderate antioxidant activity, positioning them as average performers in our assay. Meanwhile, sample codes 4 and 8 were at the lower end of the spectrum, demonstrating the least antioxidant activity among all the compounds tested. These results highlight the variability in antioxidant capacity among the different samples, which could be attributed to their distinct chemical compositions or structural properties.

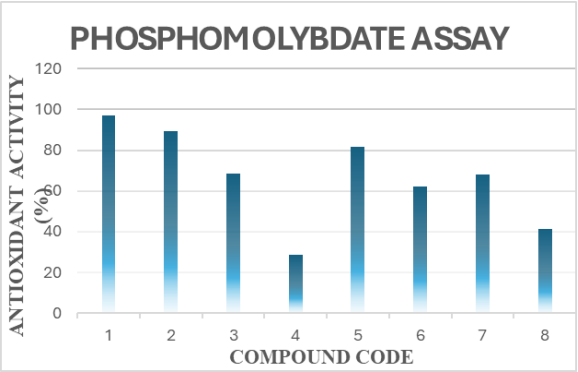

Fig 1: Graphical Representation of data of Phosphomolybdate assay

(i) Results at a concentration of 50mg

(b) ANTI-ULCER ACTIVITY

❖ Acid Neutralizing Capacity

In this investigation, we conducted an assessment of the antiulcer properties exhibited by eight distinct test compounds, denoted as Compound 1 through Compound 8. This evaluation was accomplished through the utilization of the Acid Neutralizing Capacity (ANC) assay. ANC values were ascertained for each compound and juxtaposed against those of a control substance, specifically a standard drug.

Within the confines of this assay, aluminium hydroxide (Al(OH)3) served as the benchmark reference compound. Three varying concentrations of Al(OH)3, specifically 50mg, 100mg, and 150mg, were employed. To maintain uniformity and ensure consistency across the experimental setup, identical concentrations of 50mg, 100mg, and 150mg were administered for each of the eight test samples scrutinized in this analysis.

The summative outcomes derived from these evaluations have been meticulously collated and are delineated in Tables 6-8. Moreover, for enhanced comprehension and visual clarity, a graphical illustration is provided in Figures 2-4.

The results of the acid neutralizing capacity assay are segmented into three distinct categories based on the concentrations utilized:

S.No	Compound	Volume of NaOH consumed	mEq of Acid consumed	ANC per gram of antacid
1.	Standard	8.9	25.55	511
2.	Sample- 1	10.2	24.9	498
3.	Sample- 2	12.4	23.8	476
4.	Sample- 3	14.6	22.7	454
5.	Sample- 4	23.7	18.15	363
6.	Sample- 5	15.3	22.35	447
7.	Sample- 6	19.4	20.3	406
8.	Sample- 7	21.1	19.45	389
9.	Sample- 8	23.7	18.15	363

Table 6: Result data of ANC (at 50mg concentration)

In the discussion of results at a 50 mg concentration, samples 1 and 2 demonstrated the highest Acid Neutralizing Capacity (ANC) compared to all other samples. Notably, sample 1 exhibited ANC values that were closest to those of the standard when measured per gram of antacid. Conversely, samples 3, 5, and 6 displayed moderate ANC levels. In contrast, samples 4, 7, and 8 showed the lowest ANC values in comparison to the standard.

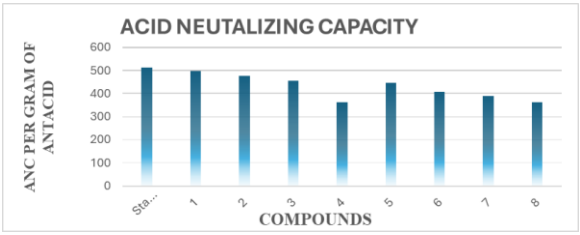

Fig 2: Graphical Representation of data of ANC (at 50mg concentration)

(ii) Results at a concentration of 100mg

S.No	Compound	Volume of NaOH consumed	mEq of Acid consumed	ANC per gram of antacid
1.	Standard	14.4	22.8	228
2.	Sample- 1	15.9	22.05	220.5
3.	Sample- 2	19.3	20.35	203.5
4.	Sample- 3	18.6	20.7	207
5.	Sample- 4	25.2	17.4	174
6.	Sample- 5	17.1	21.45	214.5
7.	Sample- 6	20.3	19.85	198.5
8.	Sample- 7	24.8	17.6	176
9.	Sample- 8	27.1	16.45	164.5

Table 7: Result data of ANC (at 100mg concentration)

In the discussion of results at a 100 mg concentration, samples 1, 2, 3 and 5 demonstrated the highest Acid Neutralizing Capacity (ANC) compared to all the other samples. Notably, sample 1 & 5 exhibited ANC values that were closest to that of the standard when measured ANC per gram of antacid where 1 showed better ANC results as compare to 5. Conversely, samples 4 and 7 displayed moderate ANC levels. In contrast, sample 8 showed the lowest ANC values in comparison to the standard.

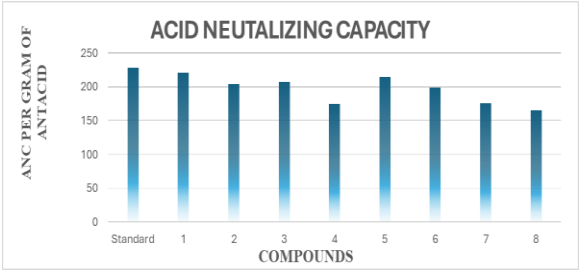

Fig 3: Graphical Representation of data of ANC (at 100mg concentration)

(iii) Results at a concentration of 150mg

S.No	Compound	Volume of NaOH consumed	mEq of Acid consumed	ANC per gram of antacid
1.	Standard	35.9	12.05	80.3
2.	Sample- 1	39.2	10.4	69.3
3.	Sample- 2	38.7	10.65	71

4.	Sample- 3	40.1	9.95	66.3
5.	Sample- 4	36.3	11.85	79
6.	Sample- 5	45	7.5	50
7.	Sample- 6	39.1	10.45	69.6
8.	Sample- 7	38.8	10.6	70.6
9.	Sample- 8	44	8	53.3

Table 8: Result data of ANC (at 150mg concentration)

In the discussion of results at a 150 mg concentration, samples 2, 4 and 7 demonstrated the highest Acid Neutralizing Capacity (ANC) compared to all the other samples. Conversely, samples 1, 3 and 6 displayed moderate ANC levels. In contrast, sample 5 & 8 showed the lowest ANC values in comparison to the standard.

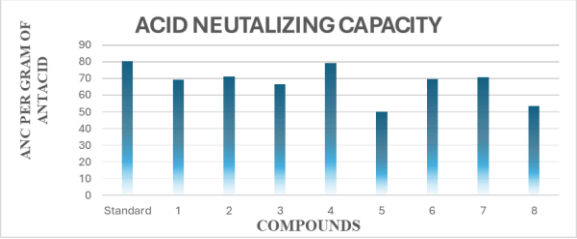

Fig 4: Graphical Representation of data of ANC (at 150mg concentration)

CONCLUSION

In conclusion, our study presents a novel and efficient approach for the synthesis of bis(indolyl) derivatives, demonstrating their potential as both anti-ulcer and antioxidant agents. The innovative synthesis approach involves the reaction of indoles and aldehydes/ketones in the presence of TBAHS as a catalyst and Ethyl Acetate as a solvent, conducted at 95°C for 60-90 minutes to yield BID, which offers advantages such as cost efficiency, less consumption, one-pot synthesis and high yield of product. The synthesized bis(indolyl) derivatives were characterized using IR and 1H_NMR confirming their structures and purity. Following synthesis, we assessed their biological activities through a series of in vitro experiments. We tested the antioxidant activity of the bis(indolyl) derivatives using phosphomolybdate assay. The results revealed that compounds 1, 2 & 5 possess strong antioxidant properties, which may contribute to their anti-ulcer effects by mitigating oxidative stress. Our findings indicate that several of the synthesized compounds exhibit significant anti-ulcer activity too, which was evaluated using acid neutralizing capacity assay. Many compounds like 1 & 2 (at 50mg concentration), 1, 2, 3 & 5 (at 100mg concentration) & 2, 4 (at 150mg concentration) showed highest ANC (based on different concentration used). These compounds were likely be able to reduce ulcer formation and promote healing, demonstrating their potential as therapeutic agents.

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