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SYNTHESIS AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME NOVEL INDOLES

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ABSTRACT

This research paper explores the synthesis and evaluation of biological activity associated with novel indole derivatives. Indoles are a class of organic compounds known for their diverse pharmacological properties, and this study focuses on the development of innovative indole- based compounds. The synthesis of these novel indoles involves efficient chemical transformations, and their biological activities are assessed through a comprehensive series of in vitro and in vivo experiments. The results reveal promising prospects for the development of new therapeutic agents, highlighting the potential applications of these novel indoles in the fields of medicinal chemistry and drug discovery.

KEYWORDS: Indoles, Novel compounds, Synthesis, Biological activity, Medicinal chemistry, Drug discovery, Pharmacological properties.

INTRODUCTION

The field of pharmaceutical chemistry encompasses a vast spectrum of research, ranging from the design and synthesis of biologically active molecules to the comprehensive study of drugs and their effects on living organisms. This multifaceted discipline plays a pivotal role in various related sciences such as drug technology, toxicological chemistry, pharmacognosy, and pharmacy organization.

Pharmaceutical chemistry primarily concerns itself with ensuring the quality of medicinal products by thorough analysis and evaluation according to stringent quality control standards. Its origins trace back to the 16th century, eventually giving rise to the emergence of

medicinal chemistry in the latter half of the 17th century. Pharmacists have been instrumental in nurturing and shaping the development of pharmaceutical chemistry. Medicinal chemistry, in the words of Berger, continually strives to establish a foundation grounded in the hope that biochemical rationales for drug discovery may emerge.

The earliest instances of synthetic molecules impacting biological processes can be traced to the introduction of chloroform and ether for anesthesia in the early 19th century. Phenacetin is regarded as one of the first drugs to be designed based on a comprehensive understanding of biochemical transformations.

Chemotherapeutic agents, when considered, are compounds that engage in ordinary chemical reactions with receptor sites on cells, albeit often involving a variety of bond formations. Paul Ehrlich concluded that drug resistance emerged when drugs failed to interact effectively with these cellular targets.

The field of pharmaceutical chemistry is constantly evolving, with innovative ideas, concepts, and trends driving the development of competent chemists and analysts. It emphasizes elements of pharmacology and bio-medicinal analysis, forging connections with disciplines like physical chemistry, pharmacology, biochemistry, and pharmacokinetics. The advancement in physiochemical properties analysis, including UV, IR, NMR, and X- ray analysis, has led to breakthroughs in drug design and synthesis.

In the realm of biochemical research, progress in enzymology and receptor interactions has brought about significant advancements. Isatins, a group of versatile substances, are extensively used as raw materials for the synthesis of various drugs. They are often extracted from mammalian tissues and have been shown to modulate numerous biochemical processes, thus finding utility in novel drug synthesis. Isatin's discovery dates back to 1841 when it was first identified by Erdman and Laurent, and it can be produced through the oxidation of indigo by nitric and chromic acids.

The synthetic versatility of isatins has made them a cornerstone of organic synthesis, offering opportunities for the creation of diverse heterocyclic compounds. Isatins have been found in nature in various plants, as well as in the secretions of Bufo frogs and as a metabolic derivative of adrenaline in humans. They are also found in coal tar.

Several methods exist for the synthesis of isatins. The Sandmeyer methodology, based on the

reaction of aniline with chloral hydrate and hydroxylamine hydrochloride, is one of the most widely used, resulting in isatins with high overall yields. The Martinet synthesis involves the reaction of an amino aromatic compound and an oxomalonate ester, while the Gassman procedure relies on the formation and oxidation of a 3-methylthio-2- oxindole intermediate. Metalation of anilide derivatives and metal-halogen exchange methods have also been developed for isatin synthesis.

These various methods have enabled the synthesis of a broad range of isatin derivatives, underpinning their significance in pharmaceutical and organic chemistry. The diverse applications of isatins continue to propel pharmaceutical chemistry into new frontiers of drug discovery and development.

METHODOLOGY AND MATERIALS

Starting Chemicals:

1. Isatin (98% purity)
2. 5-Chloroisatin (97% purity)
3. 2-Bromoacetophenone (phenacyl bromide) (98% purity)
4. p-Chlorophenacyl bromide (98% purity)
5. p-Bromophenacyl bromide (98% purity)

- All starting chemicals were obtained from Sigma Aldrich Chemical and used without further purification.

Synthesis of 1-(Morpholin-4-ylmethyl)- 1H-indole-2,3-dione (1c):

1. In a clean, dry round-bottom flask, dissolve isatin (0.4 mol) in 5 ml of ethanol.
2. Add a mixture of morpholine (0.13 mol) and formaldehyde 37% (0.5 ml) dissolved in 10 ml of ethanol to the flask.
3. Stir the reaction mixture for 3 hours at room temperature, and then let it crystallize overnight in the refrigerator.
4. Collect the separated crystalline product by filtration and dry it.
5. Recrystallize the obtained product from ethanol.

Synthesis of 3-Hydrazinylidene-1- (Substituted/Unsubstituted)-1,3-Dihydro- 2H-indol-2-one (3a-c):

1. In a clean, dry round-bottom flask, combine 1H-indole-2,3-dione (1a) (0.01 mol) and hydrazine hydrate (0.015 mol) in 20 ml of ethanol.

2. Reflux the contents on a water bath for 10-15 minutes and then allow it to cool at room temperature for 15 minutes.
3. Filter the yellow solid that separates, wash it with cold alcohol, and recrystallize it from ethanol.

Synthesis of 2-Chloro-N'-[1- (Substituted/Unsubstituted)-2-Oxo-1,2- Dihydro-3H-indol-3-ylidene]acetohydrazide (4 a-c):

1. In a clean, dry round-bottom flask, combine 3-hydrazonoindolin-2-one (3a) (0.01 mol), chloroacetyl chloride (0.01 mol), and dry benzene (20 ml).
2. Reflux the mixture for 30 minutes under anhydrous conditions using a calcium guard tube.
3. Evaporate the solution under reduced pressure, resulting in reddish-yellow solid.
4. Filter the solid, wash it with 10% sodium bicarbonate solution, water, and dry it at room temperature.
5. Recrystallize the obtained compound from chloroform.

Synthesis of (Benzoylamino) [1- (Substituted/Unsubstituted)-2-Oxo-1,2- Dihydro-3H-indol-3-ylidene]ethanoic Acid (2 a-c):

1. In a round-bottom flask, dissolve a mixture of 1H-indole-2,3-dione (1a) (0.03 mol), benzoyl glycine (0.03 mol), and anhydrous sodium acetate (0.03 mol) in 20 ml of acetic anhydride.
2. Stir the contents for 1 hour and then reflux on a water bath for 2 hours.
3. Leave the contents overnight, allowing the compound to separate as a yellow solid.
4. Filter and dry the obtained product.
5. Recrystallize the product from alcohol.

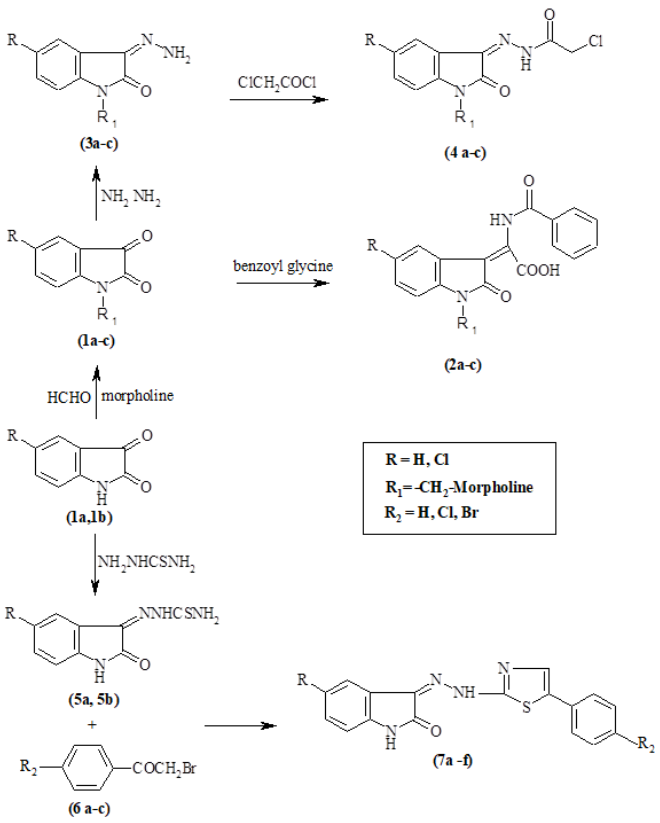
Synthesis of 2-(2-Oxo-1,2-Dihydro-3H- indol-3-ylidene)hydrazine Carbothioamide (5a, 5b):

1. In a round-bottom flask, introduce isatin (1a) (0.01 mol), thiosemicarbazide (0.01 mol) dissolved in ethanol (25 ml), and add a catalytic amount of piperidine (0.5 ml).
2. Reflux the mixture for 10-15 minutes.
3. After the reaction is complete, cool the contents, and filter the precipitated yellow-colored compound.
4. Wash with chilled ethanol and recrystallize from ethanol.

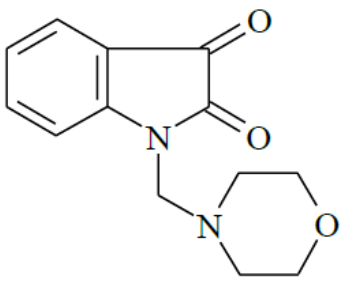
Synthesis of 3-[2-(5-Phenyl-1,3-Thiazol-2- yl)hydrazinylidene]-1,3-Dihydro-2H- indol-2-one (7 a-f):

1. In a round-bottom flask, dissolve an equimolar amount of 2-(2-oxo- 1,2-dihydro-3H-indol-3- ylidene)hydrazine carbothioamide (5a) (0.01 mol) and phenacyl bromide (6a) (0.01 mol) in ethanol (25 ml).
2. Reflux the contents for 6 hours.
3. As the reaction progresses, solid separates as orange-colored crystals.
4. Filter the product, wash it with a small quantity of ethanol.
5. Recrystallize 3-[2-(5-phenyl-1,3- thiazol-2-yl)hydrazinylidene]-1,3- dihydro-2H-indol-2-one (7a) from a mixture of DMSO and DMF.

SCHEME-1

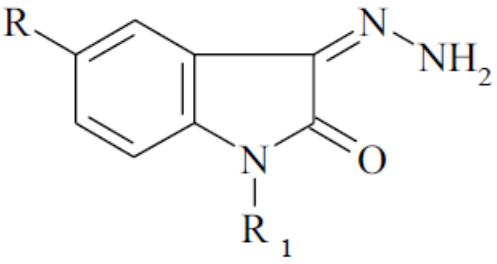


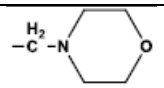
Compound 1c



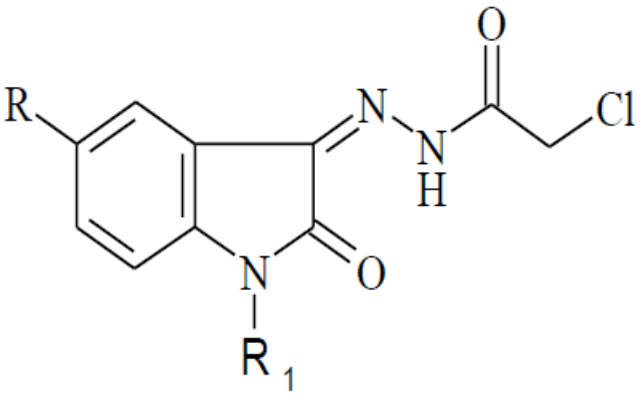
S.No	Compound Code	Molecular Formula	Molecular Weight	Melting Point (^{o}C)
1	**1c**	$C_{13}H_{14}N_2O_3$	246	196-98

: Physical characterization data of synthesized compounds (3a-c).



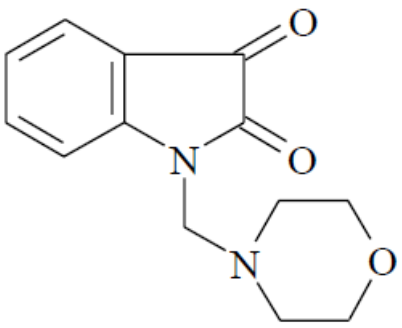
S.No	Compound Code	R	R_1	Molecular Formula	Molecular Weight	Melting Point (oC)
1	3a	H	H	$C_8H_7N_3O$	161	216-18
2	3b	Cl	H	$C_8H_6N_3OCl$	195	220-22
3	3c	H		$C_{13}H_{16}N_4O_2$	260	227-30

Physical characterization data of synthesized compounds (4a-c).



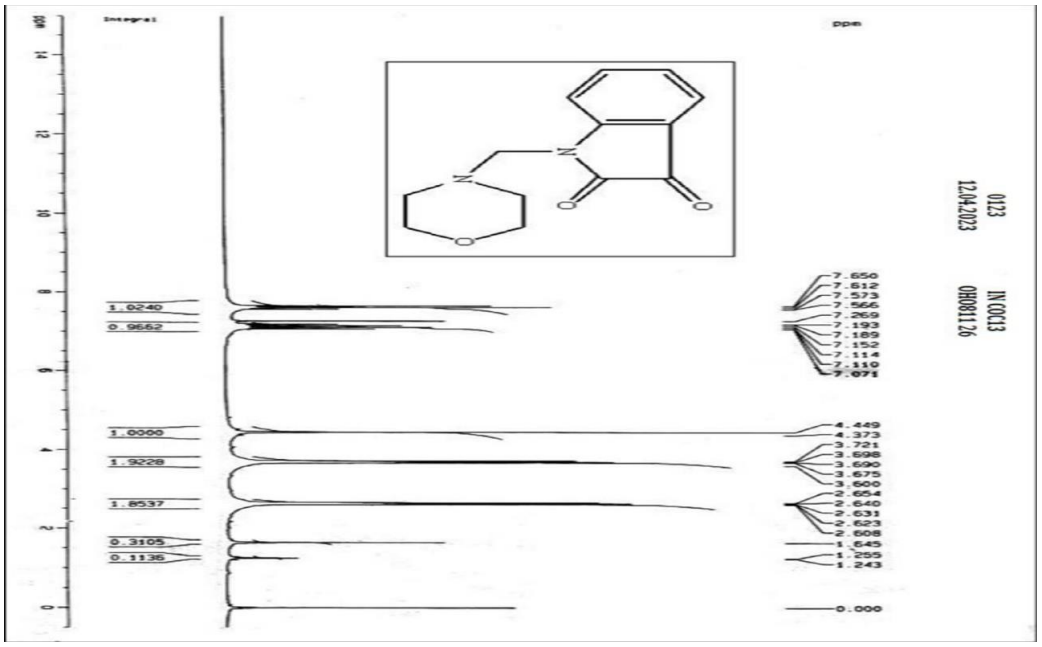
SPECTRAL DATA

Compound 1c

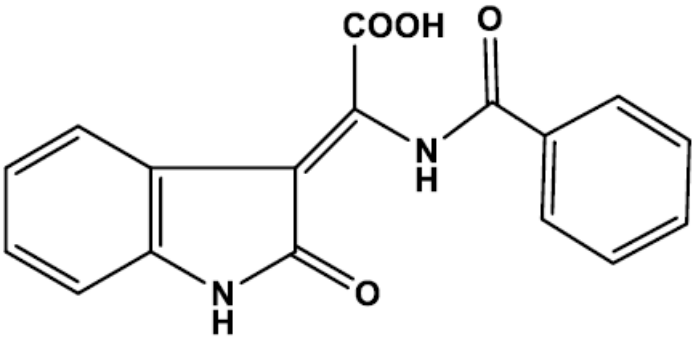


^{1}H NMR spectrum (CDCl3, δ ppm)

Value in δ ppm	Nature of segments	No of protons	Type of proton
7.0-7.6	multiplet	4H	4H of Ar-H
4.3-4.4	singlet	2H	2H of bridge –N-CH2-N-
3.6-3.7	doublet of doublet	4H	4H of (2xO-CH2) of morpholine
2.6-2.7	doublet of doublet	4H	4H of (2xN-CH2) of morpholine



Compound 2a



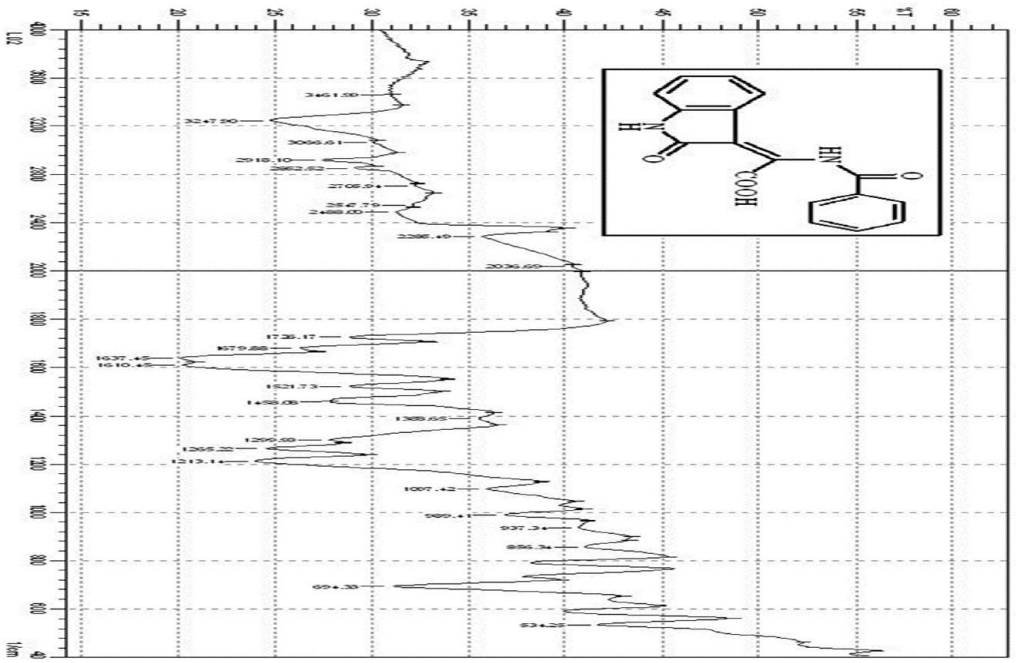
IR spectra (cm^{-1})

Types of Vibrations	Group frequency in Wave number (cm^{-1})
OH of COOH Stretching	3247
NH Stretching	3190
Ar-CH=CH Stretching	2918, 2852 1726

C=O of COOH Absorption band	1679
C=O Absorption band	

NMR spectrum (DMSO, δ ppm)

Value in δ ppm	Nature of segments	No of protons	Type of proton
14.4-14.5	broad singlet	1H	1H of OH of COOH
12.7-12.8	singlet	1H	1H of indole -NH
11.0-11.2	singlet	1H	1H of –NH of -CONH-
6.9-8.0	multiplet	9H	9H of Ar-H



BIOLOGICAL ACTIVITY ANTIBACTERIAL ACTIVITY

The compounds synthesized during the present investigation were screened for their antibacterial activity. The antibacterial tests were conducted on four common microorganisms such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Klebsiella pneumonia*, which are the representative types of gram positive and gram negative organisms respectively. The antibacterial activity of the compounds was assessed by agar-well diffusion method 68.

Procedure: The following protocol was used for the determination of antibacterial activity.

Media Used: Sabouraud agar medium.

Temperature: The Bring the agar plates to room temperature before use.

Inoculum preparation

- Using a loop or swab, transfered the colonies to the plates.
- Visually adjusted turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardized the suspension with a photometric device.

Inoculation of Agar plate

- Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, dipped a sterile cotton swab into the inoculum and rotated it against the wall of the tube above the liquid to remove excess inoculum.
- Swabbed entire surface of agar plate three times, rotating plates approximately 60º between streaking to ensure even distribution.
- Allowed inoculated plate to stand for at least 3 minutes before applying disks.

Addition of test compound into plate

- By using a sterile borer wells (5 mm in diameter) were cut from the agar by pressing. Likewise, made five well on each plate.
- Added 5 µl, 10 µl, 25 µl, 50 µl and 75 µl of compound into the respective wells on each plate.

Incubation

- Incubated plates within 15 min of compound application.
- Inverted the plates, and stacked them not more than five high.
- Incubated the plates for 18-24 h at 37 ºC in incubator.

Reading plates

- Plates were read only if the lawn of growth is confluent or nearly confluent.
- Measured the diameter of inhibition zone to nearest whole millimeter by holding the measuring device.

The extent diameter of inhibition zone after 24 h was measured in millimeters and the results were shown in **Table No VI-IX**.

Table No VI: Antibacterial activity data of newly synthesized compounds (2a, 2c and 7a-f) against *Staphylococcus aureus*.

Compound / Conc	*Inhibition zone diameter in mm				
	75 µg	50 µg	25 µg	10 µg	5 µg
2a	12	R	R	R	R
2c	11	R	R	R	R
7a	11	10	R	R	R
7b	12	10	**8**	R	R
7c	8	R	R	R	R
7d	10	R	R	R	R
7e	12	10	R	R	R
7f	13	8	R	R	R
Ciprofloxacin (10 µg)	--	--	--	26	R

Table No VII: Antibacterial activity data of newly synthesized compounds (2a, 2c and 7a-f) against *Enterococcus faecalis.*

Compound / Conc	*Inhibition zone diameter in mm				
	75 µg	50 µg	25 µg	10 µg	5 µg
2a	R	R	R	R	R
2c	13	10	R	R	R
7a	12	10	**8**	R	R
7b	R	R	R	R	R
7c	9	8	R	R	R
7d	13	11	**10**	R	R
7e	15	9	R	R	R
Ciprofloxacin (10 µg)	--	--	--	27	R

Table No VIII: Antibacterial activity data of newly synthesized compounds (2a, 2c and 7a-f) against *Escherichia coli*.

Compound / Conc	*Inhibition zone diameter in mm				
	75 µg	50 µg	25 µg	10 µg	5 µg
2a	10	R	R	R	R
2c	10	R	R	R	R
7a	11	10	**8**	R	R
7b	10	R	R	R	R
7c	10	8	R	R	R
7d	12	11	**9**	8	R
7e	R	R	R	R	R
Ciprofloxacin (10 µg)	--	--	--	32	--

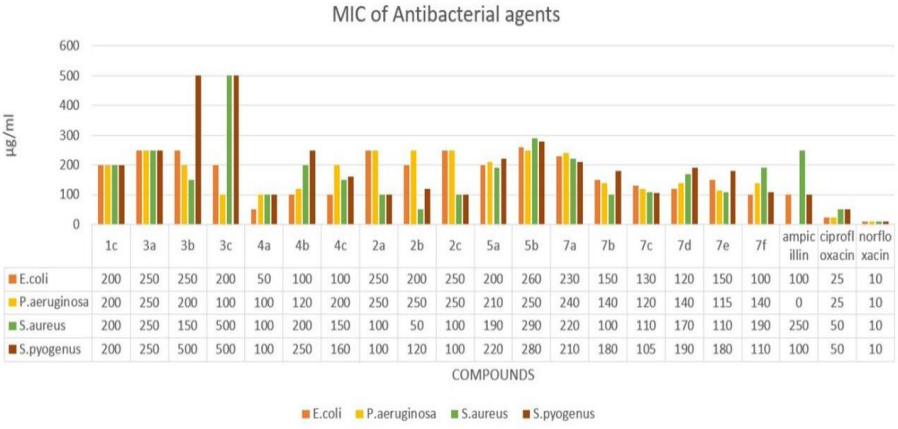
RESULTS AND DISCUSSION

Antibacterial Activity: All the synthesized compounds were subjected to antibacterial activity screening at various concentrations, and the results are presented in Tables VI, VII, and VIII. Ciprofloxacin was used as the standard reference for both Gram- positive and Gram-negative bacteria. Compound 7b exhibited moderate activity against Staphylococcus aureus at a concentration of 25 µg/ml (Table VI). Compounds 7a and 7d showed moderate activity against Enterococcus faecalis at 25 µg/ml (Table VII). Additionally, compounds 7a and 7d exhibited moderate activity against Escherichia coli at 25 µg/ml, while compound 7d also displayed moderate activity against Escherichia coli at 10 µg/ml (Table VIII).

The antibacterial activity of the synthesized compounds was evaluated, and the results demonstrated varying degrees of activity against the tested bacteria. Compound 7b displayed moderate activity against Staphylococcus aureus, indicating its potential as an antibacterial agent. In the case of Enterococcus faecalis, both compounds 7a and 7d exhibited moderate antibacterial activity. Furthermore, these two compounds demonstrated moderate activity against Escherichia coli, with compound 7d displaying this effect even at a lower concentration of 10 µg/ml.

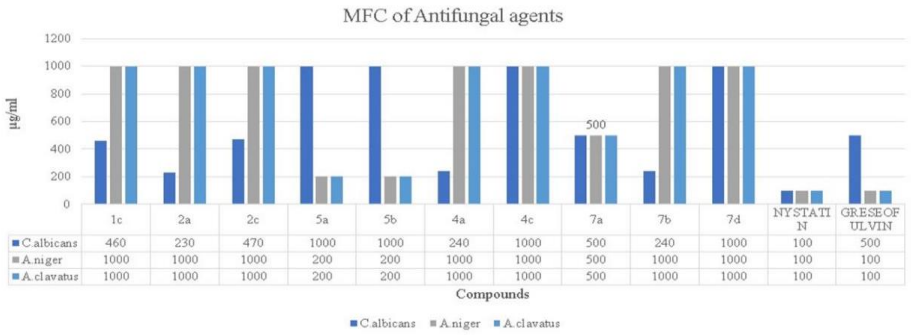
The structure-activity relationship suggests that the presence of the indole ring and specific aromatic substituents (R groups) is crucial for antibacterial activity. Compounds with electron-donating groups, such as OH, tended to exhibit higher activity compared to those with electron- withdrawing groups like Cl and F. Among the compounds, 1c, 2a, 2c, 4a, 7b, and 7d demonstrated the highest antifungal activity against Aspergillus niger and Aspergillus clavatus.

Graph No. 1:MIC of Antibacterial activity (MIC µg/ml) (1c-7f Derivatives)



	1c	3a	3b	3c	4a	4b	4c	2a	2b	2c	5a	5b	7a	7b	7c	7d	7e	7f	ampicillin	ciprofloxacin	norfloxacin
E.coli	200	250	250	200	50	100	100	250	200	250	200	260	230	150	130	120	150	100	100	25	10
P.aeruginosa	200	250	200	100	100	120	200	250	250	250	210	250	240	140	120	140	115	140	0	25	10
S.aureus	200	250	150	500	100	200	150	100	50	100	190	290	220	100	110	170	110	190	250	50	10
S.pyogenus	200	250	500	500	100	250	160	100	120	100	220	280	210	180	105	190	180	110	100	50	10

Graph No 2: MFC of Antifungal activity(MFC µg/ml) (1c,2a,2c,5a,5b,4a,4c,7a,7b,7d Derivatives)



SUMMARY

The research aimed to synthesize various indole derivatives and investigate their antibacterial activity. The results indicate that these compounds exhibit less strong to weak antibacterial activity against both Gram-positive and Gram-negative bacteria when the two moieties, indole and thiazole, are combined for antibacterial studies. The antibacterial activity of these compounds is influenced by the presence of thiazole, and when thiazole is linked with other moieties, it demonstrates a broad spectrum of antibacterial activity. However, when thiazole is linked with indole, it fails to exhibit significant activity. This suggests that indolyl thiazoles hold potential for further exploration, and combining or adding different moieties may enhance their potency.

CONCLUSION

In conclusion, the synthesized indole derivatives showed limited antibacterial activity, ranging from less strong to weak. It is important to note that antibacterial activity is influenced by the specific moieties present in the compounds. This research underscores the need for a more detailed study on the toxicity of these compounds. It is crucial to recognize that no drug is entirely free from potential side effects, and drugs are chemical substances that can alter physiological processes, both positively and negatively. To harness the full benefits of these compounds, it is essential to consider their potential harm. Some compounds exhibited antibacterial activity at low dosages, indicating their potential, and molecular modeling may offer valuable insights into the development of new drugs. While not all molecules synthesized for biological testing may lead to the discovery of new drugs, they serve as models for evaluation, prototypes, or the testing of hypotheses and theories. Further research in this area could contribute to the development of the next generation of antibiotics.

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