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SYNTHESIS AND EVALUATION OF ANTI-BACTERIAL POTENTIAL OF IMINE ANALOGS DERIVED FROM DIFFERENT SUBSTITUTED 3-ACETYL INDOLE AND AMINO PYRAZINE

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ABSTRACT

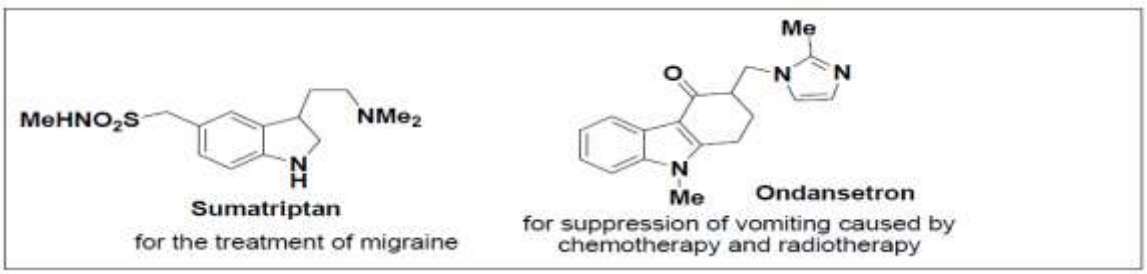
Despite the presence of large number of antibiotics, drug resistant species have become a major concern. The quest for better antimicrobial compounds against drug resistant species exhibiting minimum toxicity and higher therapeutic index is very relevant in present scenario. Indole and Pyrazine have been earlier explored for their biological activity in particular for their antimicrobial activity. In the present work, synthesis and characterization of a series of novel imine derivatives containing pyrazine and acetyl indole is reported along with results of *in vitro* studies for the determination of their activity against *Escherichia coli* and *Staphylococcus aureus*. Based on the data Compound 4 showed the most potent activity against the tested bacterial strains.

KEYWORDS: Indole, Pyrazine, Antibacterial, Imine derivatives.

INTRODUCTION

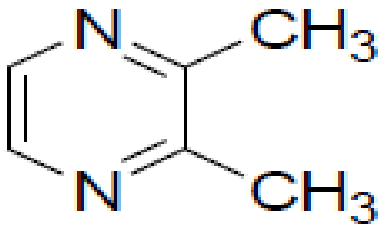
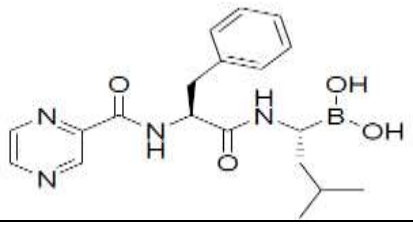
Earliest synthesis of indole has been reported by Baeyer and co-workers.[1] In the 1930s, indole gained immense popularity when substituted indole derivatives were found in many important alkaloids (e.g., tryptophan and auxins). Indole is widely distributed in the natural environment and can be produced by a variety of bacteria like *Bacillus alvei, Escherichia coli, Flavobacterium sp.* and *Haemophilus influenza*. It has been reported as an important signaling molecule in bacteria and it regulates various aspects of bacterial physiology, including resistance to drugs, spore formation, biofilm formation and plasmid stability.[2] The amino acid tryptophan is an indole derivative and the precursor of the neurotransmitter serotonin.[3] The indole-derived phytochemicals and bacterial metabolites are a result of biosynthesis via coupling of tryptophan with other amino acids. Chemically this heterocyclic

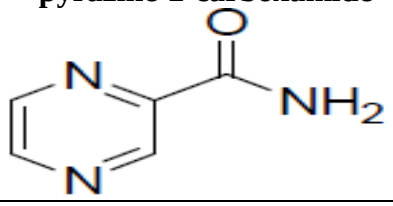
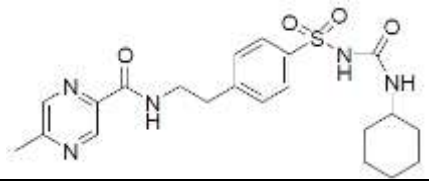
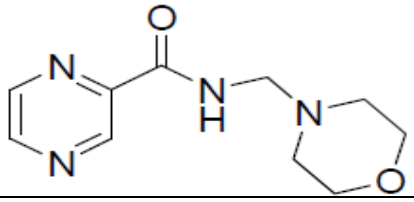
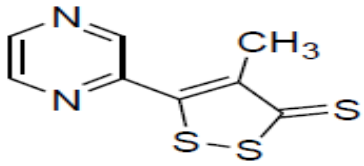
ring is a fusion of benzene and pyrrol ring system4. Indole is an important nitrogen heterocycle found in countless natural products, part of an essential amino acid (tryptophan), and a key structural component of many value added chemicals including pharmaceuticals. There are about 17 indole – containing drugs in all along with their disease indications. The indole core has seven positions that can be substituted. A survey of these 17 structural reveals that two (12%) are mono- substituted, ten (59%) are di-substituted and the remaining five (29%) are tri- substituted. A closer looks reveals there that are preferred substitution pattern with a vast majority of these drugs containing a substituent at C3 (88%) and /or C5 (71%). These strongly favored positions are followed by C2 (29%), N1 (18%), C4 and C7 (6%) respectively with no indole drug being substituted at C6. Three (frovatriptan, ondansetron and etodolaca) indole drug are decorated with a fused ring, which in all cases is a six – membered ring connected to the indole at C2 and C3. The blood pressure medicine pindolol is particularly interesting as it is one of the only two approved indole drugs that are mono-substituted, but more important, the only one that contains a substituent at C4, The largest drug class containing indoles in the form of a tryptamine core are analgesics (41%)5. Some naturally occurring indole alkaloids have gained FDA approval, including vincristine, vinblastine, vinorelbine and vindesine for anti-tumor activity, ajmaline for anti-arrhythmic activity and physostigmine for glaucoma and Alzheimer's disease. Taking inspiration from these natural compounds several synthetic drugs were synthesized that have reached the patient's bedside, such as indomethacin (NSAID), ondansetron (chemotherapy induced nausea and vomiting), fluvastatin (hypercholesterolemia), zafirlukast (leukotriene receptor antagonist). The success of the above mentioned compounds indicates the importance of the ring system in multi-disciplinary fields including pharmaceutical and agrochemical industry6. The chemistry and biological importance of this bicyclic arene is huge, accomplished and consolidated in literature that lead to synthesis of various indole derivatives through various electrophilic and nucleophilic reactions. This property of indole ring make better portfolio for synthetic manipulation.



In addition to this the other molecule pyrazine also exhibits good pharmacological activity. Pyrazine is a heterocyclic aromatic organic compound with the chemical formula C4H4N2.Pyrazine is a symmetrical molecule with point group D2h. Derivatives such as pyrazine, pyrimidines, phenazineare well known for their antitumor, antibiotic and diuretic activities. Pyrazine is less basic in nature than pyridine, pyridazine and pyrimidine. Tetramethyl pyrazine is reported to scavenge superoxide anion and decrease nitric oxide production in human poly morpho nuclear leukocytes, and is a component of some herbs in traditional Chinese medicine. Some of the pyrazine derivative contains various pharmacological effects. The pyrazine moiety has shown a wide spectrum of biological activities. The various substituted pyrazine are having significant Antianginals, Antidepressant, Antipsychotic, Ant diabetic, Antihistamines, hypolipidemic agent, Flavouring agent etc.[7] Some of the important marketed pyrazine nucleus containing drug having different biological or pharmacological activity were discussed in table below. The pyrazine nucleus based pharmaceutical are rapidly becoming very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in the very near future. The pyrazine based pharmaceuticals will be produced on a large scale by Modern Drug Discovery Company by different research development processes and will become available commercially for therapeutic use soon.[8] With the key benefits including favorable time to market and high rate of success in clinical trial compared with traditional pharmaceuticals due to diverse biological action with less toxicity, heterocyclic compounds will play a vital role in the treatment of different diseases. Table 1 gives representative examples containing pyrazine moiety and pharmaceutical activity.

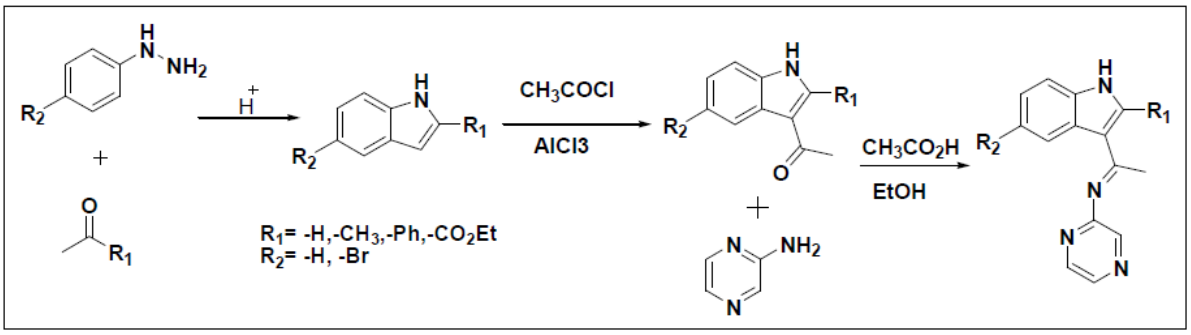
Table. 1: Molecules containing pyrazine moiety and their biological activity.

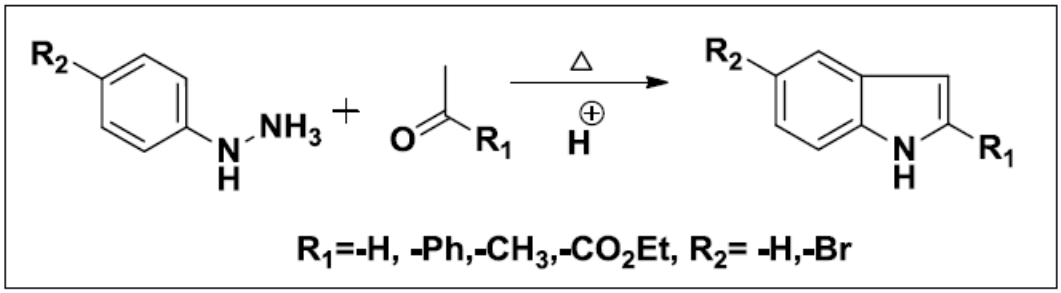
Molecule	Activity
	Contribute to the taste and aroma of various foods.[9]
Bortezomib 	Treating relapsed multiple myeloma.[10]

pyrazine-2-carboxamide 	Used in treatment of T.B.[11]
Glipizide 	Anti-diabetic drug.[12]
Morinamide 	Used in the treatment of tuberculosis.[13]
Oltipraz 	It is also used in tumor prevention.[14]

Over recent years there has been an increasing interest in the chemistry of pyrazine derivatives because of their Analgesic, Anti-allergic, Antibacterial, Anti- inflammatory, Antiviral, Diuretic, Anticancer, Anti-HIV, Anti-hypertensive, Cardiovascular, Antioxidant, Anti-mycobacterial activity.[15-25] Most of the bacterial disease constitutes potential threat to human health and these bacterial diseases not have been overcome.[26] More than one third of population is infected by bacteria and nearly two million people die from bacterial infection.[27] To overcome the emerging drug resistance, the discovery of new scaffolds is crucial against bacterial infections.

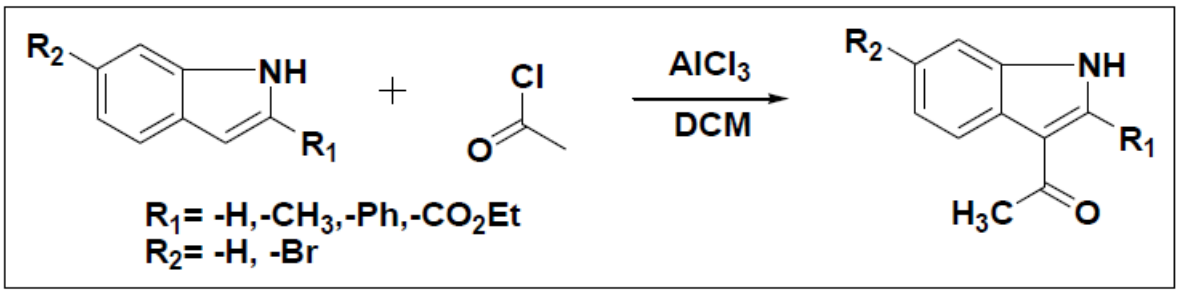
MATERIAL AND METHODS





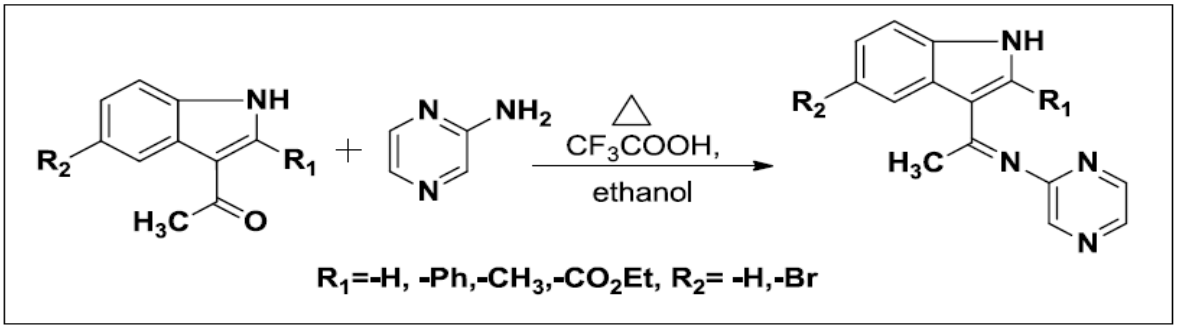
Step. I: Synthesis of 2- Substituted Indole.

In a solution of phenylhydrazine (1.25 mmole) dissolved in glacial acetic acid (15 mL), 1mM quantity of acetaldehyde, acetone, acetophenone and ethyl 2-oxopropanoate was added separately to carry out four separate reactions. The reaction mixtures were cooled to sub-zero temperature and solid was separated by filtration in each reaction. The products were recrystallized from methanol and water.



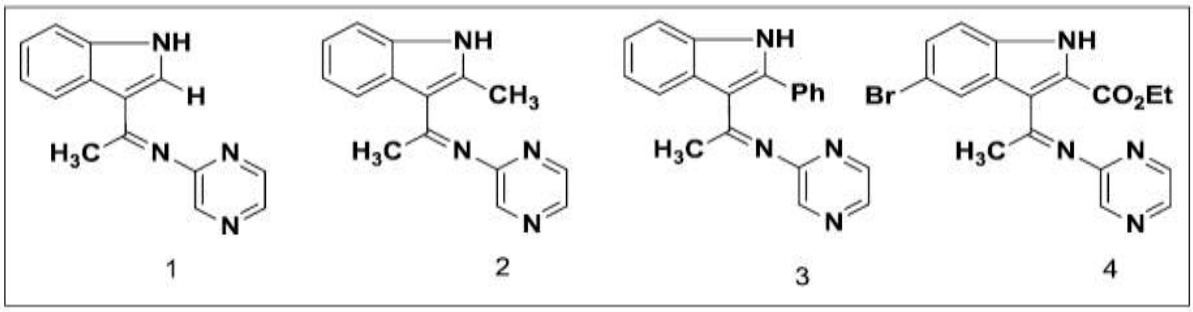
Step. II: Synthesis of 3-Acetyl-2-Substituted Indole.

In a small clean R.B. flask, mixture of substituted Indole (4.2 mmol) and AlCl3 (21 mmol) in 50 ml DCM was stirred for about 4 hours at 0°C. The reaction was monitored by TLC, all the reaction mass poured into a beaker containing ice and the product was filtered and recrystallized with aqueous ethanol.



Step. III: Synthesis of Different Imine Derivatives.

3-acetylindole and 2-amino pyrazine (1:1mmol) in 25 ml ethanol with catalytic amount of trifluoro acetic acid was refluxed for 4 Hrs. The reaction was monitored by TLC. After completion, the reaction was quenched by addition of ice and washed with water. The solid was separated out by filtration and purified by column chromatography. Four compounds IP, MP, PP and EP were obtained and appropriately characterized.



Compound	R1	R2	Yield	M.P.
1	H	H	64%	1880C
2	CH3	H	42 %	2300C
3	Ph	H	60%	1600C
4	COOEt	Br	46 %	1380C

Componud 1; N-(1-(1H-indol-3-yl)ethylidene)pyrazin-2-amine: IR= 3742,1640,1520cm-1 1H NMR(DMSO-d6):2.45 δ (s,3H);7.20-7.3 δ 5(m,4H);7.05 δ (s,1H);8.30- 8.38 δ (m,3H);11.93 δ (bs,1H).

Componud 2; N-(1-(2-methyl-1H-indol-3- yl)ethylidene)pyrazin-2-amine
IR= 3623,1623,1513,3149cm-1; 1H NMR (DMSO-d6): 1.81 δ (s,3H);2.47 δ (s,3H);7.35-7.8 δ (m,4H);8.62- 8.69 δ (m,3H);12.2 δ (bs,1H).

Componud 3; N-(1-(2-phenyl-1H-indol-3-yl)ethylidene)pyrazin-2-amine
IR-3750, 1600,1518 cm-1; 1H NMR (DMSO-d6): 2.47 δ (s,3H);.96-7.41 δ (m,5H); 7.91-7.8 δ 3 (m,4H);8.82-8.88 δ (m,3H);11.54 δ (bs,1H).

Componud 4; ethyl 5-bromo-3-(1-(pyrazin-2-ylimino)ethyl)-1H-indole-2-carboxylate
IR-3742,3166,1721,1625,1512,570 cm-1;LCMS (M+H=387); 1H NMR (DMSO-d6): 1.25 δ (t,3H);1.4 δ (s,3H);4.40 δ (q,2H);7.4-7.6 δ (m,3H);8.2-8.3 δ (s,3H); 12.4 δ (bs,1H).

Determination of Antimicrobial Activity of Synthesized Compounds

Preparation of Bacterial Culture: Using aseptic techniques a single colony was transferred into a 100 mL of broth, capped and placed in incubator overnight at 35 °C. After 12–18 hours of incubation, using aseptic preparation and the aid of a centrifuge, a clean sample of bacteria was prepared. The broth was spun down using a centrifuge set at 4000 rpm for 5 min with appropriate aseptic precautions. The supernatant was discarded into an appropriately labeled contaminated waste beaker. The pellet was re suspended using 20 mL of sterile normal saline and centrifuged again at 4000 rpm for 5 min. This step was repeated until the supernatant was clear. The pellet was then suspended in 20 mL of sterile normal saline, and was labeled as Bs. The optical density of the Bs was recorded at 500 nm, and serial dilutions were carried out with appropriate aseptic techniques until the optical density was in the range of 0.5-1.0. The actual number of colony-forming units was calculated from the viability graph. The dilution factor needed was calculated and the dilution was carried out to obtain a concentration of 5×106 CFU/mL.

Preparation of Solution: 4 mM solution of different compounds was prepared in DMSO and then diluted to get 2 mM respectively.

Preparation of the Plates: Plates were prepared under aseptic condition. A sterile 96 well plate was labeled (Fig. 1). A volume of 100ml of test material in 10 % (v/v) DMSO or sterile water (usually a stock concentration of 1mg/ml for purified compound) was pippeted into the first row of the plate. To all broth 50 ml of nutrient saline was added. Serial dilutions were performed using a multi-channel pipette. Tips were discarded after use such that each well had 50 ml of the test material in serially descending concentration. To each well 10ml of solution were added. Using a pipette 30ml of 3.3x Strength broth were added to each well to ensure that the final volume was single strength of nutrient broth. Finally, 10ml of Bacterial suspension (5x106 CFU/ ml) was added to each well to achieve a concentration of (5x 105CFU/ ml). The plates were prepared in triplicate and place in an incubator set at 37oC for 18-24 hr. The color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive (Fig.1). The Lowest concentration at which color change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial Strain.

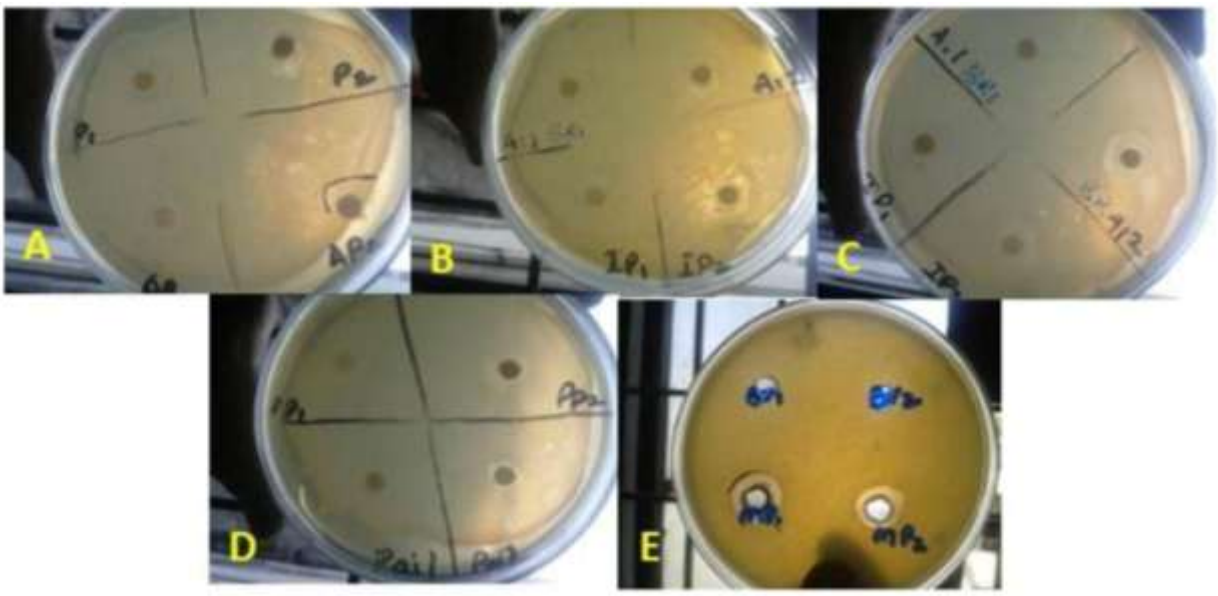


Figure. 1: Images (A - E) of Antimicrobial Activity.

Result of antimicrobial activity.

Compound	*Escherichia coli*		*Staphylococcus aureous*	
	Zone of Inhibition in cm and Concentration in mM			
	4 mM	2 mM	4 mM	2 mM
2-H indole pyrazone (1)	1.25	1.3	1.4	1.25
2-methyl indole pyrazone (2)	----	----	**1.65**	1.2
2-phenyl indole pyrazone (3)	0.95	1.1	1.0	0.8
2-ester indole pyrazone (4)	1.1	**1.9**	1.0	0.95

DISCUSSIONS AND CONCLUSION

The synthesized Schiff bases have shown potent antimicrobial activity against *Staphylococcus aureous* and *Escherichia coli.* The antimicrobial study showed that, compound 4 (Fig. 1C) (2mM) and compound 2 (Fig. 1E) (4mM) exhibited better inhibitory potential against both micro-organisms. The findings warrant more investigations for the development of successful candidates as antimicrobial agents.

Although the activity of compounds reported here are comparable, and these derivatives representing starting point for further investigations to optimize activity of these imine derivatives. The results obtained here indicate that it is possible to design different analogs that could be more effective on introducing structural modifications.

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