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SYNTHESIS, CHARACTERIZATION AND BIOASSAY OF (E)-N-((1H-INDOL-3-YL) METHYLENE)-BENZAMINE DERIVATIVES BY USING METHANE SULFONIC ACID

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ABSTRACT

We aimed to develop the yield and biological properties series of (E)-N-((1H-indol-3-yl) methylene)-benzamine derivatives were synthesized by assisted by Bronsted acid taking polar protic solvent. All the newly synthesized compounds were analyzed by spectra data viz; IR, 1H NMR and LCMS. All newly compounds were tested against three gram positive and two gram-negative bacterial strains and one in fungal strain. All newly compounds showed good activity against gram positive strains than against gram negative strains. The newly compounds were found more active against *S.aureus* and *B.subtilis*

KEYWORDS

Indole-3-aldehyde, Substituted aromatic primary amine, Methane sulfonic acid, (E)-N-((1H-indol-3-yl) methylene)-benzamine derivatives and Microbial activity.

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INTRODUCTION

A azomethine is a functional group that possesses a carbon-nitrogen double bond with the nitrogen atom connected to an aryl group but not hydrogen is called Schiff bases[1]. Schiff bases are usually synthesized from the condensation of active carbonyl group and primary amines[2]. Schiff bases have been reported to contain antimicrobial properties[3-9]. Schiff bases are characterized by the −N=CH− (imines) group which is most important for elucidating the mechanism of transamination

and racemisation reactions in biological systems and are also known to have biological activities such as antimicrobial[10] antifungal[11], antitumor[12] andherbicidal[13] activity. The derivatives of Indole found to possess antibacterial[14], anticonvulsant[15] and antihypertensive activity[16]. These observations led to the conception that Schiff bases ofindole-3-carbaldehyde would possess high potential antimicrobial properties. Schiff bases are crystalline that are immiscible water and miscible in organic solvents. They are weak bases, forming salts with acids in an anhydrous medium, in aqueous acid solutions, they undergo hydrolysis to yield an amine and carbonyl compounds. The majority of Schiff bases are stable in alkaline solutions. Schiff bases are valuable intermediate products of organic synthesis, for example, in the preparation of secondary amines and various heterocyclic compounds. The Schiff bases known as azomethine dyes are used for dyeing acetate and synthetic fibers; they are also used in color photography to reduce the photosensitivity of photographic emulsions[17]. In the present study a series of (E)-N-((1H-indol-3-yl) methylene)-benzamine derivatives Indole-3-aldehyde were synthesized by use of Methane sulfonic acid and characterized by IR, 1H NMR and mass spectroscopy. The compounds were screened for antibacterial and antifungal activities. The minimum inhibitory concentrations of the newly synthesized compounds were also determined by serial dilution method.Indole-3carbaldehyde on condensation with various substituted aromatic primary amines in presence of brownsted acid catalyst and ethanol as solvent yields of derivatives of (E)-N-((1H-indol-3-yl) methylene)-benzamine (Scheme No.1).

Experimental

A mixture of 2mmole of indole-3-aldehyde and 2mmole of different aryl substituted amines was taken in 100ml RB flask. Then the mixture was dissolved in ethanol as solvent. The catalyst amount of methane sulfonic acid added to the mixture. Then the mixture was stirred under reflux condition. After completion of the reaction was monitored by TLC. Then crude was dissolved in the ethyl acetate and washed with base. The solvent was evaporated, dried and recrystalized with ethanol. Structures, time in methane sulfonic acid assisted synthesis and % yield are given below.

Physicochemical and spectroscopic determination of synthesized compounds

The melting points of newly synthesized compound were checked in open capillary tube and were uncorrected. The reactions were monitored by thin layer chromatography using silica gel-GF254 as adsorbent on glass plate. The spots of the reaction mixture were applied on silica gel plate and the plate was run in (ethyl acetate: hexane (4:6)) in a closed chamber. UV cabinet is used for the identification of the spots reaction mixture on the silica gel coated plates. IR spectra were recorded on FTIR-8400F model in KBr. NMR spectra were recorded on Broker AVANCE 400 instrument using TMS as internal reference and chemical shift value are expressed in delta units. Molecular weights of compounds were identified by mass spectrometer. It showed fragmentation pattern as *m/z* values. All physical and spectral data are given in below.

Characterization of synthesized compounds

Yield: 82.3%; M.P:122-124°C; IR(KBrcm-1): 1572(C=C), 1685(C=N), 3044(C-H), 1245 (C-N)2850(N=CH); NMR:^{1}HNMR (400MHz, CDCl3) (δppm): 6.85-7.24(m, 10H, ArH), 8.53(s, 1H; N=CH), 7.64(s, 1H, CH), ^{13}CNMR (400MHz, CDCl3) (δppm): 159.27, 143.36, 135.75, 130.06, 129.83, 128.71, 125.44, 122.00, 121.45, 120.62, 118.91, 110.02, 106.78; Molecular formulae: $C_{15}H_{12}N_2$, LCMS: 220.43.

Yield:85.6%; M.P:127-129°C; IR(KBrcm-1):1574 (C=C), 1685 (C=N), 3044 (C-H), 1243 (C-N), 2855 (N=CH); NMR: 1HNMR (400MHz, $CDCl_3$) (δppm):6.85-7.24(m, 9, ArH), 8.48 (s, 1H;N=CH), 7.58(s, 1H, CH)^{13}CNMR (400MHz, CDCl3) (δppm): 161.98, 155.43, 138.66, 135.85, 127.68, 123.03, 122.47, 121.77, 120.36, 118.93, 116.75, 110.63, 104.85, 65.12, 13.36; Molecular formulae: $C_{17}H_{16}N_2O$, LCMS: 266.09 (m+2).

Yield:76.6, M.P:117-119°C; IR (KBr cm-1) 1628 (C=C), 1684 (C=N), 29443, 2904 (C-H), 1245 (C-N), 2855 (N=CH), 1071 (C-O); NMR: 1H NMR (400MHz, CDCl3) δppm: 6.82-7.31 (m, 9H, ArH), 8.55 (s, 1H; N=CH), 7.70 (s,1H,CH), 3.64 (s, 3H, CH3):^{13}CNMR (400MHz, $CDCl_3$) (δppm); 161.08, 157.92, 141.34, 135.66, 125.38, 122.91, 121.48,

120.88, 120.04, 116.42, 109.92, 101.59, 56.05. Molecular formulae: $C_{16}H_{14}N_2O$, LCMS: 250.53.

Yield: 79.6%; M.P:146-148°C; IR (KBr cm-1): 1564 (C=C), 1655 (C=N), 3103 (C-H), 1249 (C-N), 2850 (N=CH), 3541 (-OH), 745 (C-N); ^{1}HNMR (400MHz, $CDCl_3$) δppm: 8.94 (s, H,-OH), 6.37-7.35 (m, 7H, ArH), 9.15(s, 1HN=CH), 7.64(s, 1H, CH,)3.18 (s, 6H, 2-CH3):^{13}CNMR (400MHz, $CDCl_3$) δppm: 161.64, 150.07, 149.25, 138.68, 134.71, 127.35, 125.64, 124.36, 122.82, 121.8, 121.14, 119.72, 104.42, 102.58, 101.32, 41.45.

Molecular formulae: $C_{17}H_{17}N_3O$, LCMS: 279.45.

Yield: 74.5%; M.P:142-145°C; IR (KBr cm-1): 1569 (C=C), 1658 (C=N), 3113 (C-H), 1245 (C-N), 2856 (N=CH), 755 (C-Cl);^{1}HNMR (400MHz, CDCl3) δppm: 7.20-7.34 (m, 9H, ArH), 8.55(s, 1H;N=CH), 7.64 (s,1H, CH):^{13}CNMR (400MHz, $CDCl_3$) δppm; 161.36, 143.65, 135.78, 131.42, 130.82, 129.66, 127.18, 123.46, 122.08, 121.39, 120.05, 112.57, 103.64: Molecular formulae: $C_{15}H_{11}ClN_2$, LCMS: 256.31 (m+2).

Yield: 72.9%; M.P: 208-210°C; IR (KBrcm-1) 1570C=C), 1667(C=N), 3078 (C-H), 1329 (C-N), 2859 (N=CH), 3320 (-OH) Hbonded, 1498.71(RNO2); ^{1}HNMR (400MHz, $CDCl_3$) δppm: 7.72-8.15 (m, 9H, ArH), 8.45 (s, 1H; N=CH), 7.34 (s, 1H, CH), 6.26(d, 1H; ArOH).^{13}CNMR (400MHz, $CDCl_3$) δppm; 162.45, 153.62, 145.74, 141.38, 138.42, 130.14, 125.68, 124.15, 122.83, 121.42, 120.36, 118.42, 116.76, 103.42. Molecular formulae: $C_{15}H_{11}N_3O_3$, LCMS: 281.61.

Yield: 70.7%; M.P:155-157°C; IR (KBr cm-1): 1533 (C=C), 1660 (C=N), 3089 (C-H), 1015 (C-N), 2844 (N=CH); 1HNMR (400MHz, $CDCl_3$) δppm: 6.59-7.01 (m, 9H, ArH), 8.65 (s, 1H; N=CH), 7.16 (s, 1H, CH), 4.41(s, 1H, CH):^{13}CNMR (400MHz, $CDCl_3$) δppm; 159.72, 144.39, 138.43, 136.64, 129.88, 128.36, 127.42, 126.77, 124.54, 122.83, 121.73, 120.49, 118.76, 110.47, 101.89. Molecular formulae: $C_{15}H_{13}N_3$, LCMS: 235.52.

Yield: 63.3%; M.P: 200-203°C; IR (KBr cm-): 1555 (C=C), 1670 (C=N), 3072 (C-H), 1214 (C-N), 2850.(N=CH) 3329 (-OH) H bonded, 1476 (R-NO2); ^{1}H NMR (400MHz, $CDCl_3$) δppm: 7.78-8.13(m, 8H, ArH), 8.47(s, 1H; N=CH), 7.36 (s, 1H, CH), 6.71(d, 1H, ArOH): ^{13}CNMR (400MHz, $CDCl_3$) δppm; 162.08, 155.76, 142.15, 138.42, 136.72, 131.74, 125.86, 124.92, 122.77, 121.91, 121.69, 120.89, 119.84, 113.36, 104.45. Molecular formulae: $C_{15}H_{11}N_3O_3$, LCMS: 281.28.

Yield: 81.5%; M.P: 131-134°C; IR(KBr cm-1): 1533 (C=C), 1654 (C=N), 3089(C-H), 1206 (C-N), 2851(N=CH), 1556(R-NO_2); NMR:^{1}HNMR (400MHz, $CDCl_3$)) δppm: 7.78-8.3 (m, 9H, ArH), 8.50 (s, 1H; N=CH), 7.71 (s, 1H, CH); ^{13}CNMR (400MHz, $CDCl_3$) δppm, 161.29, 136.78, 131.16, 129.43, 127.71, 122.57, 121.40, 120.06, 118.91, 111.12, 103.78; molecular formulae: $C_{16}H_{12}N_2O_2$, LCMS: 264.12.

Yield: 74.4%; M.P: 211-214°C; IR(KBr cm-1): 1492 (C=C), 1676 (C=N), 3097 (C-H), 1220 (C-N), 2856 (N=CH), 1320 (C-F); 1HNMR (400MHz, $CDCl_3$)) δppm: 7.55-7.79 (m, 9H, ArH), 8.54 (s, 1H; N=CH), 7.25 (s, 1H, CH), 3.57 (s, 1H, ArNH); ^{13}CNMR (400MHz, $CDCl_3$) δ ppm: 161.87, 159.65, 145.23, 136.78, 134.28, 130.59, 127.48, 124.87, 1211.89, 121.08, 120.71, 1107.49, 105.18, 101.56. Molecular formulae: $C_{15}H_{12}FN_3$LCMS: 255.39 (m+2).

Antimicrobial activity

The antimicrobial activities of newly synthesized (E)-N-((1H-indol-3-yl)methylene)-benzamine derivatives were evaluated against six different strains of microorganism (three gram positive, two gram negative bacteria and one fungus) using nutrient agar medium (Hi-Media Laboratories, India) and sabouraud dextrose agar medium (Hi-Media Laboratories, India) respectively. Zone of inhibition of compounds were determined by Cup plate method and minimum inhibitory concentration of the test compounds were determined by two fold serial dilution technique. Dimethyl sulfoxide (DMSO) was used as solvent for both techniques. Paper disc diffusion method for zone of inhibition and minimum inhibitory concentrations of the synthesized compounds were determined by serial dilution method. Amoxicillin, Ciprofloxacin and fluconazole were used as reference standards for antibacterial and antifungal activity respectively. The lowest concentration of the screened compounds exhibited no visible microbial growth were considered as minimum inhibitory concentration. The observed zones of inhibition and MIC values for bacterial and fungal strains are

given in Table No.1, Table No.2 and Table No.3 respectively.

RESULTS AND DISCUSSION

We have newly synthesized a series (E)-N-((1H-indol-3-yl) methylene)-benzamine derivatives of indole-3-aldehyde by methane sulfonic acid as catalyst synthesis. This protocol presented several advantages, such as moderate to good yields, much low reaction time (3-15 min) and also simple purification procedure.

The bioassay results indicated that most of the synthesized compounds exhibited well. Methane sulfonic acid assisted organic synthesis accelerates the course of several organic reactions, this catalyst promoted the yield .producing high yields and lower quantities of side products and consequently, easier work-up and purification of the products[15]. (E)-2-((1H-indol-3-yl) methyleneamino)-5-(dimethylamino) phenol(E)-N1-((1H-indol-3-yl) methylene) bezene-1, 2-diamine showed highest antibacterial activity with MIC against gram positive, gram-negative and fungi.

Table No.1: *In vitro* activity-zone of inhibition in mm (MIC in µg/mL) for gram positive strains

S.No	Compound Code and standard Antibiotic	*S. aureus*	*B. subtilis*	*S. epidermidis*
1	3a)	15	20	18
2	3b)	19	21	17
3	3c)	22	24	18
4	3d)	12	22	16
5	3e)	12	18	14
6	3f)	20	20	17
7	3g)	15	16	12
8	3h)	21	18	15
9	3i)	17	14	16
10	Amoxicillin	30	35	25

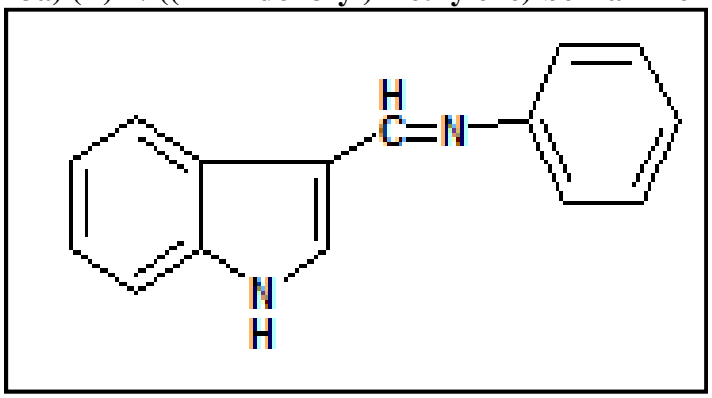
Table No.2: *In vitro* activity-zone of inhibition in mm (MIC in µg/mL) for gram negative strains

S.No	Compound Code and standard Antibiotic	*E. coli*	*K. pneumonae*
1	3a)	18	13
2	3b)	18	17
3	3c)	22	21
4	3d)	19	19
5	3e)	16	16
6	3f)	20	19
7	3g)	12	15
8	3h)	17	19
9	3i)	15	14
10	Ciprofloxacin	25	30

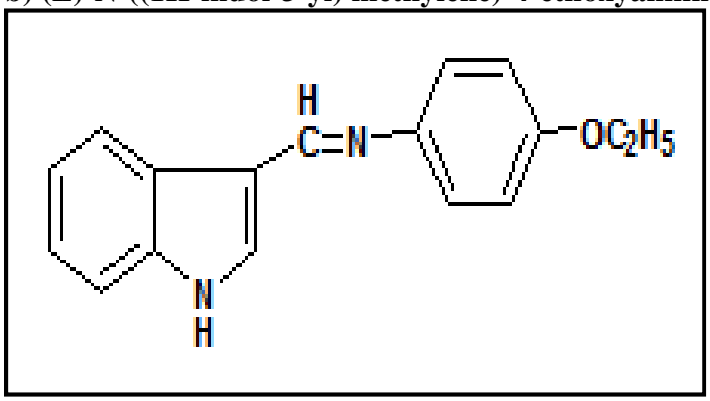
Table No.3: *In vitro* activity-zone of inhibition in mm (MIC in µg/mL) for *candida albicans*

S.No	Compound Code and standard Antifungal drug	*Candida albicans*
1	3a)	15
2	3b)	16
3	3c)	20
4	3d)	13
5	3e)	17
6	3f)	19
7	3g)	12
8	3h)	11
9	3i)	10
10	Fluconazole	25

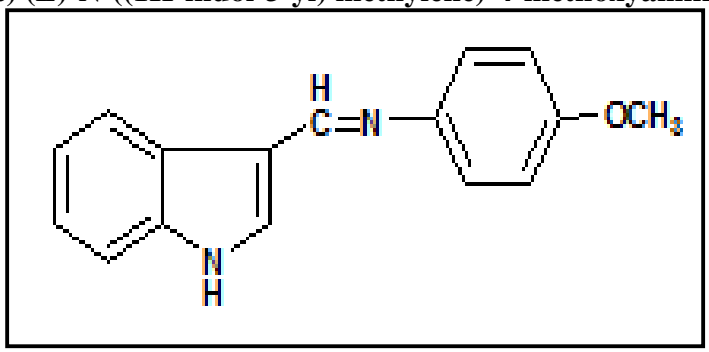
3a) (E)-N-((1H-indol-3-yl) methylene)-benzamine



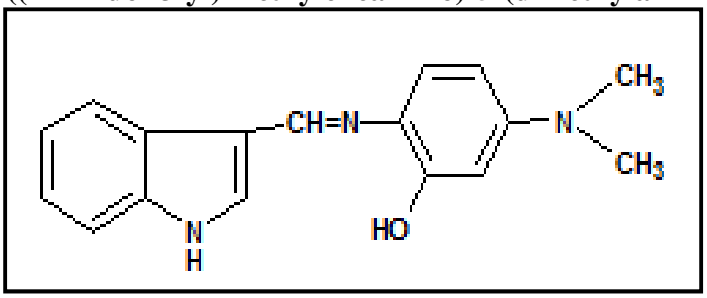
3b) (E)-N-((1H-indol-3-yl) methylene)-4-ethoxyaniline



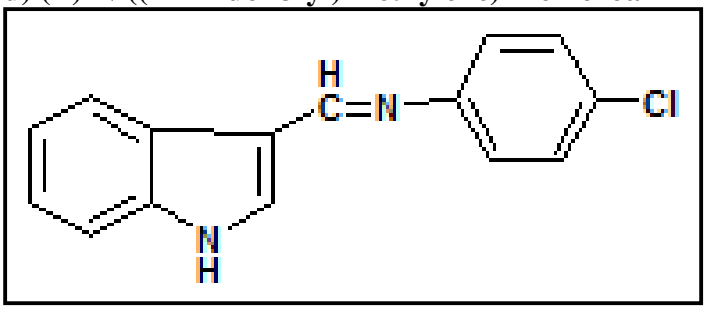
3c) (E)-N-((1H-indol-3-yl) methylene)-4-methoxyaniline



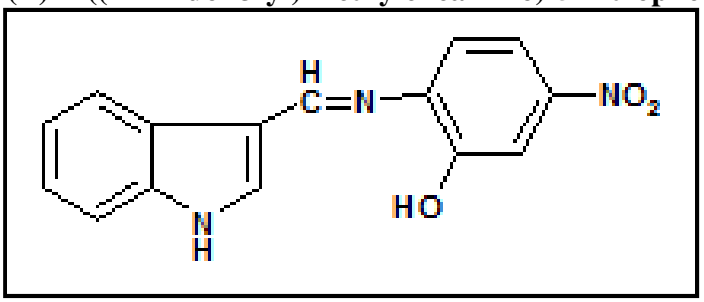
3d) (E)-2-((1H-indol-3-yl) methyleneamino)-5-(dimethylamino) phenol



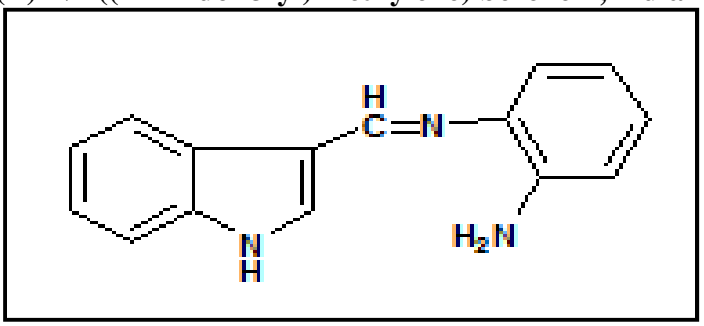
3d) (E)-N-((1H-indol-3-yl) methylene)-4-chloroaniline



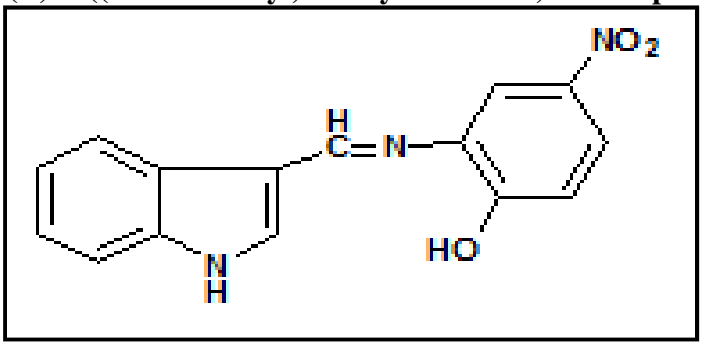
3e) (E)-2-((1H-indol-3-yl) methyleneamino)-5-nitrophenol



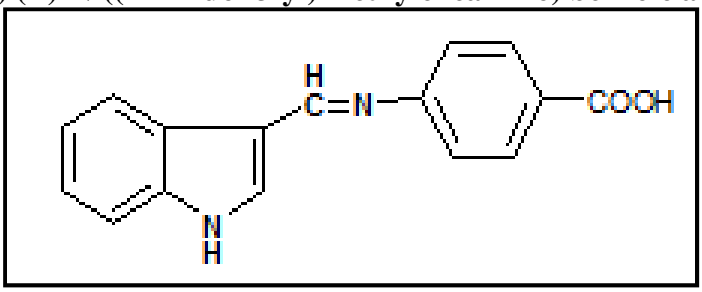
3f) (E)-N1-((1H-indol-3-yl) methylene) bezene-1, 2-diamine



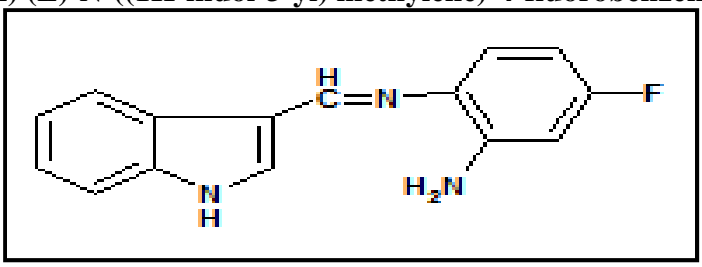
3g) (E)-2-((1H-indol-3-yl) methyleneamino)-4-nitrophenol



3h) (E)-N-((1H-indol-3-yl) methyleneamino) benzoic acid



3i) (E)-N-((1H-indol-3-yl) methylene)-4-fluorobenzene



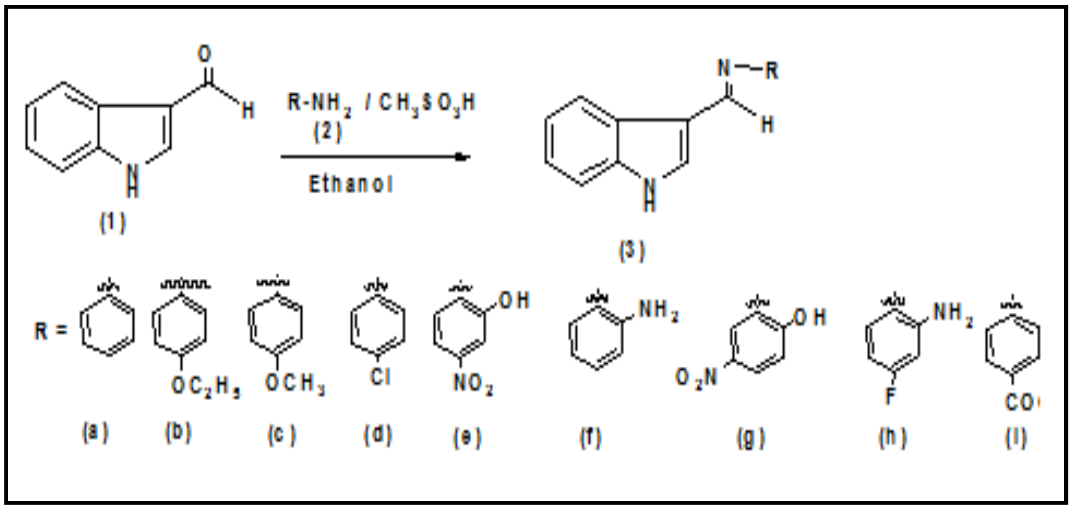


Figure No.1: Scheme for the synthesis of Schiff bases

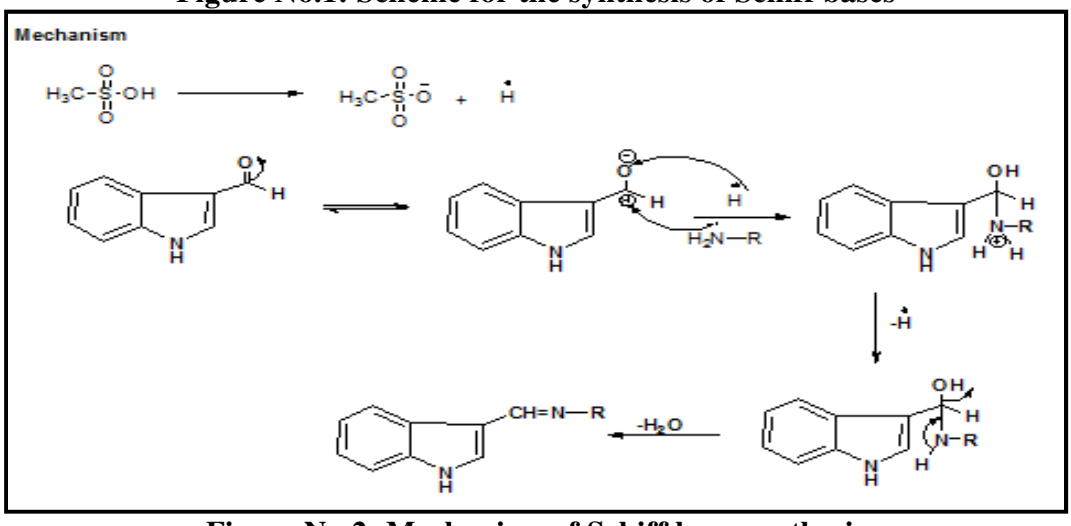


Figure No.2: Mechanism of Schiff base synthesis

CONCLUSION

The structures of newly synthesized a series (E)-N-((1H-indol-3-yl) methylene)-benzamine derivatives of indole-3-aldehydeand substituted (electron donating group and electron with drawing group) of primary amines in the presence of Bronsted acid and compounds were confirmed by IR, NMR and LCMS. All compounds exhibited significant antibacterial activity but they showed moderate antifungal activity. The titled compounds are underway further optimization, structure-activity relationship and bioassay.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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