RESEARCH PAPER

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL SCHIFF BASES OF ISATIN-PYRROLE CONJUGATES

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ABSTRACT:
A series novel Schiff's bases of Isatin-Pyrazole conjugates V(a-o) was synthesized by conventional method, and they were evaluated as a potential antioxidant and antimicrobial activity. The compounds were confirmed by TLC, melting point and spectral studies such as FT-IR, MS and ^{1}H NMR.The preliminary studies on antimicrobial activity of the new Isatin-Pyrazole conjugates have generated some interesting data. All the synthesized compounds were evaluated for antibacterial activity by using standard Moxifloxacin and antioxidant activity by using ascorbic acid. Newly synthesized compounds showed promising antimicrobial activity against Gram positive and Gram-negative bacteria. It is clearly concluded that the synthesized compounds with substitution at 5, 6th position with two halogens (5F,6Cl) on Isatin moiety showed potent antibacterial activity against Gram positive and Gram-negative bacteria. Newly synthesized compounds showed promising antioxidant activity.Compound Vo showed better antioxidant and antibacterial activity compared to all the synthesized compounds.
Keywords: Schiff's base, Isatin, Pyrrole, antibacterial activity, antioxidant activity, DPPH method.
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1. INTRODUCTION

Heterocyclic compounds comprise a substantial section of organic molecules with remarkable medicinal and pharmacological effects. Researchers are constantly exploring these heterocyclic moieties to unlock their potential as novel drug compounds due to their vast expanse of biological activity. Belonging to the class of such versatile moieties, Isatin (1H-indole-2,3-dione), was isolated in 1840 by Linné Erdman[1]. The nature of Isatin permits for creation and synthesis of libraries of therapeutically relevant compounds around the basic chemical scaffold. Schiff bases are an attempt to address medicinal gaps and enhance efficiency of the parent drugs. Infections and diseases caused by different types of organisms like bacteria, fungi and virus etc., in humans and animals[2]. The drug used to prevent the pathogenicity of microorganism is called an antimicrobial agent. Isatin (1H-indole-2,3-dione) was first isolated by Erdmann and Laurent as an oxidation product of indigo using nitric and chromic acids. It has a nitrogen atom at position 1 and two carbonyl groups at positions 2 and 3. Isatin is a biologically active heterocyclic moiety comprises two cyclic rings, one of the ring (A) is six-membered and the other ring (B) is five-membered. Both of the rings are planar. The six-membered ring has an aromatic character, whereas the five-membered ring possesses an anti-aromatic character.Isatin shows modifications at its C3 carbonyl functionalities, which lead to the formation of a number of biologically important Isatin-based Schiff bases derivatives.Isatin derivatives especially Schiff bases exhibit a wide array of biological activities, including antimicrobial, antiviral, anti-inflammatory, and anticancer properties. Moreover, Isatin's structure allows it to act as a versatile scaffold for designing new compounds with specific pharmacological

properties[3-5]. Consequently, it has garnered significant attention in medicinal chemistry and drug discovery research.Schiff bases of Isatin are a class of compounds formed by the condensation reaction between Isatin and a primary amine. The resulting Schiff bases exhibit diverse chemical and biological properties, making them valuable intermediates in the preparation of pharmaceuticals, dyes, and otherorganic compounds[6-9].Schiff base ligands and their respective compounds are pharmacologically active and are the most preferred compounds in several fields, including the environment, industrial, biological, material sciences and medicinal fields.

The current study focuses on the synthesis and evaluation of new Schiff bases of Pyrrole fused Isatin conjugates. These novel compounds where synthesized, characterized using their appearance, yield, melting point, thin layer chromatography (TLC), Infrared Spectroscopy (IR), 1HNMR, Mass and screened for their anti-bacterial, antioxidant activity through agar well diffusion and DPPH methods[10].

2. MATERIALS AND METHODS

All the materials and reagents were procured from commercial suppliers like Sigma Aldrich and S.D. Fine Chem Ltd and used without purification. The solvents were purified as per the standard protocol. Precoated TLC silica gel plates bought from S.D. Fine were used to validate the quality of the substances and progress of the reaction. To purify the reaction mixtures, silica gel 60 F254 was used. Capillary tube open type has been utilized to estimate melting points of all compounds with the help of the EZ Melt automated melting point device. All the synthesized compounds were characterized by spectral analysis like FTIR(Shimadzu), ^{1}HNMR (300 MHz, DMSO-d6), and Mass spectrometry (Shimadzu).

General procedures:

Step:1. Synthesis of methyl 2-(2,5-dimethyl-1H-pyrrol-1-yl)benzoate: Methyl anthranilate, Hexane 2,5 di one was taken in equimolar ratio, to that few drops of glacial acetic acid was added and the reaction mixture was refluxed for 24hrs.TLC was performed to the compound[11]. The mixture was then evaporated and then washed with Petroleum ether. The compound was then recrystallized and collected.

Step.2: Synthesis of 2—(2,5-dimethyl-1H-pyrrol-1-yl)benzamide

To the compound formed in step1 hydrazine hydrate was added in equi molar ratio, methanol was added as a solvent and then the reaction mixture was kept reflux for 48hrs and TLC was performed. Pet. Ether was added to the compound to remove the gummy matter in the compound and then evaporated. The compound was collected after recrystallizing it with methanol[12].

Step.3: Synthesis of 2--(2,5-dimethyl-1H-pyrrol-1-yl)-N-(6- methyl-2oxo-2,3 dihyd-1H-indole-3 ylidene)benzohyrazide

To that compound simple Isatin and its derivatives were added in equi molar ratio and kept reflux for 24hrs by using methanol as solvent. Then TLC was performed for the reaction mixture and then crushed ice was added to it and kept aside for 3-4 hrs for the precipitate formation. Filtered it and compound was collected after drying. The dried compound was then recrystallized by using methanol to get the pure compound [13].

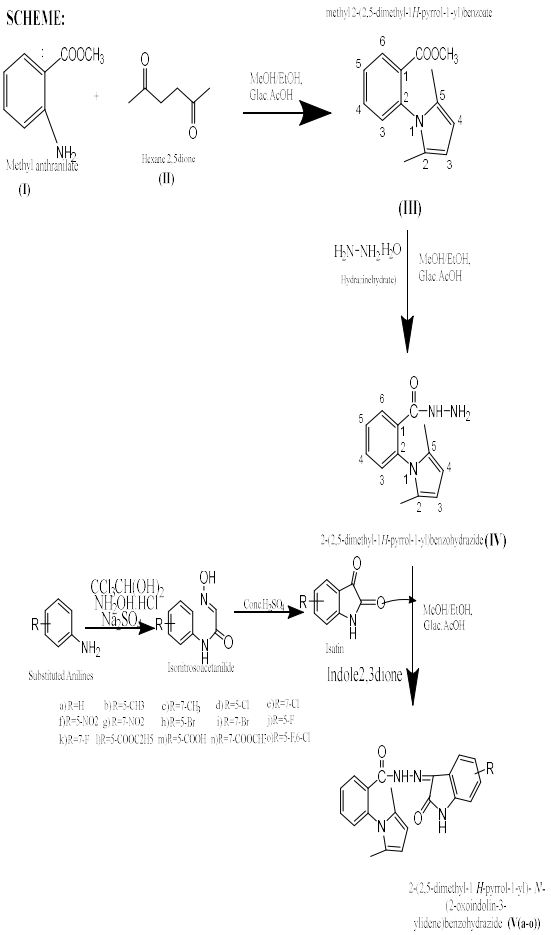

Figure 1: Schematic representation of Novel Schiff's bases of Pyrrole fused Isatin

conjugates-VI(a-o)

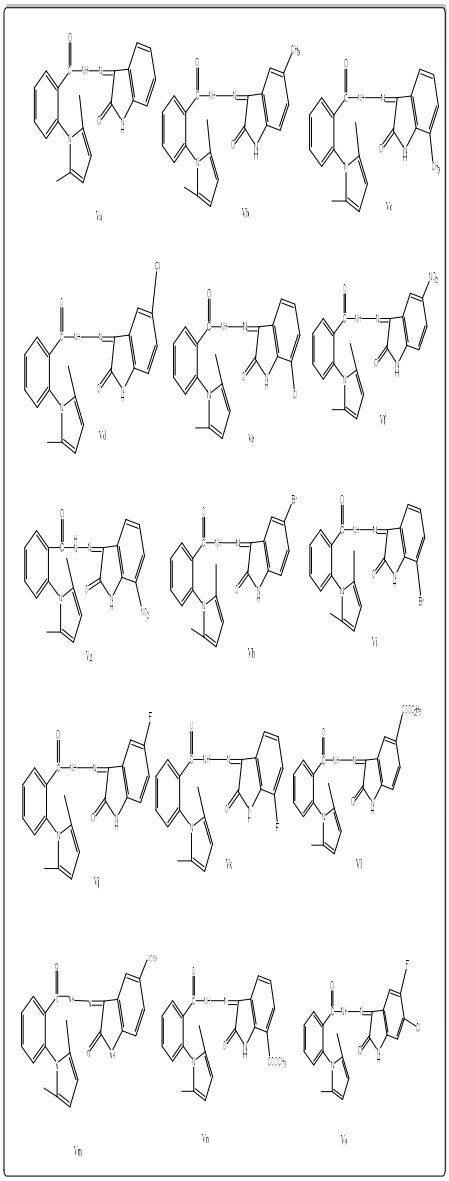

Figure 2: All synthetic compound structures-Va-Vo

Spectral Characterization

Compound. Va: 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-(2-oxoindolin-3-ylidene) benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58-(C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 359.40. (Table 1a and b)

Table 1a: Physical Data of synthetic compounds-Scheme-Va-Vo

S. No	Compound	-R	Molecular formula	Molecular weight
1	V-a	-H	C21H18O2N4	358.40
2	V-b	5-CH3	C22H20O2N4	372.43
3	V-c	7-CH3	C22H20O2N4	372.43
4	V-d	5-Cl	C21H17O2ClN4	392.84
5	V-e	7-Cl	C21H17O2ClN4	392.84
6	V-f	5-NO2	C21H17O4N5	403.13
7	V-g	7-NO2	C21H17O4N5	403.13
8	V-h	5-Br	C21H17O2BrN4	437.30
9	V-i	7-Br	C21H17O2BrN4	437.30
10	V-j	5-F	C21H17O2FN4	376.39
11	V-k	7-F	C21H17O2FN4	376.39
12	V-l	5-COOC2H5	C24H22O4N4	430.46
13	V-m	5-COOH	C22H18O4N4	402.41
14	V-n	7-COOCH3	C23H20O4N4	416.44
15	V-o	5-F,6-Cl	C21H16O2ClFN4	410.83

Table 1b: Physical Data of synthetic compounds-Scheme-Va-Vo

S.No	Compound	Melting Point (ºC)	Rf value (C:EA)	% Yield
1	V-a	193-195	0.8	67.56
2	V-b	188-190	0.57	76.31
3	V-c	200-202	0.61	65.78
4	V-d	149-151	0.87	57.5
5	V-e	199-201	0.73	95.23
6	V-f	160-162	0.85	61.9
7	V-g	89-91	0.66	69.24
8	V-h	148-150	0.82	52.42
9	V-i	180-182	0.68	82.55
10	V-j	232-234	0.58	76.9
11	V-k	70-72	0.66	91.33
12	V-l	120-122	0.72	68.18
13	V-m	228-230	0.55	65.11
14	V-n	221-223	0.49	90.69
15	V-o	158-160	0.68	86.23

Compound. Vb: 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-(5-methyl-2-oxoindolin-3 ylidene) benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 373.34.

Compound. Vc: 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-(7-methyl-2-oxoindolin-3-ylidene)benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 373.52.

Compound. Vd: N'-(5-chloro-2-oxoindolin-3-ylidene)-2-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum (EI-MS): M+1 Peak observed at 393.65.

Compound. Ve: N'-(7-chloro-2-oxoindolin-3-ylidene)-2-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 394.43.

Compound. Vf: N'-(5-nitro-2-oxoindolin-3-ylidene)-2-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide:IR (KBr, cm^{-1}): 3340.99-

(N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 404.34.

Compound. Vg: 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-(7-nitro-2-oxoindolin-3-ylidene)benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 404.35.

Compound. Vh: N'-(5-bromo-2 oxoindolin3-ylidene)-2-(2,5-dimethyl 1H-pyrrol-1-yl)benzohydrazide:IR (KBr, cm^{-1}): 3340.99-(N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum (EI-MS): M+1 Peak observed at 478.34.

Compound. Vi: N'-(7-bromo-2-oxoindolin-3-ylidene)-2-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide:IR (KBr, cm^{-1}): 3340.99-(N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum (EI-MS): M+1 Peak observed at 478.34.

Compound. Vj: 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-(5-fluoro-2-oxoindolin-3-ylidene)benzohydrazide:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 378.34.

Compound. Vk: 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N'-(7-fluoro-2-oxoindolin-3-ylidene)benzohydrazide: IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 378.34.

Compound. Vl: ethyl 3-(2-(2-(2,5-dimethyl-1H-pyrrol-1-yl)benzoyl)hydrazineylidene)-2-oxoindoline-5-carboxylate:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 431.46

Compound. Vm: 3-(2-(2-(2,5-dimethyl-1H-pyrrol-1-)benzoyl)hydrazineylidene)-2-oxoindoline-5-carboxylic acid:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum(EI-MS): M+1 Peak observed at 403.26.

Compound. Vn: methyl 3-(2-(2-(2,5-dimethyl-1H-pyrrol-1-yl)benzoyl)hydrazineylidene)-2-oxoindoline-7-carboxylate:IR (KBr, cm^{-1}): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58-

(C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 7.40-7.43(t, 1H, Ar-H),7.25-7.29 (m, 1H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3). Mass Spectrum (EI-MS): M+1 Peak observed at 417.43.

Compound. Vo: N'-(6-chloro-5-fluoro-2-oxoindolin-3-ylidene)-2s-(2,5-dimethyl-1H-pyrrol-1-yl)benzohydrazide: IR (KBr, cm^{-1}):): 3340.99- (N-H, str.), 3058.56- (C-H Aromatic, str.), 2976.58- (C-H Aliphatic, str.), 1742.84-(C=O, str.),1660.28-(C=N, str.), 1591.59-(C=C, str.), 1398.20- (S-CH, str.),813.12(C-F),744.90(C-Cl). ^{1}H NMR (CDCl3) 400 MHz, δ ppm): 8.31 (s, 1H, -NH-indole), 8.30 (s, 1H,-NH amide), 7.91-7.93(d, 2H, Ar-H, *J=4HZ*), 7.50-7.62 (m, 4H, Ar-H), 5.59 (s, 2H, CH_2), 2.27 (s, 6H, CH_3).Mass Spectrum (EI-MS): M+4 Peak observed at 411.83.

Pharmacological activity

Antioxidant activity-DPPH method[14-15]: An antioxidant is the substrate that prevents the oxidation of molecules inside a cell. It is a well-known chemical process that allows the removal of electrons or hydrogen from a substance. Free radicals are produced during the biological oxidation reaction. Because the radicals are reactive, they start the chain reaction simultaneously. This can lead to the damage or even the death of a cell. Antioxidant agents are capable of terminating a chain reaction by eliminating free radical intermediates. Hence, they are also called as free radical scavengers. They perform the antioxidant behavior by being oxidized, hence antioxidants can be considered reducing agents. Some examples are ascorbic acid, thiols or polyphenols. Antioxidants are commonly used as supplements in food and have been examined for inhibition of diseases such as heart disease and cancer. Antioxidants are free radical scavengers which neutralize reactive oxygen species (ROS) produced during aerobic cellular metabolism: superoxide (O_2^-), hydrogen peroxide (H_2O_2) and peroxynitrite (OONO−). Also, antioxidants exert protective effects on cells against the deleterious effects of ROS on cell membranes, mitochondria, DNA, lipids or proteins.

Natural antioxidants are important elements in health maintenance and prevention of atherosclerosis, aging, neurodegenerative diseases, carcinogenesis, hematological disorders or chronic inflammation. Endogenous antioxidants (catalase, superoxide dismutase, peroxidase and glutathione) exert their activity by scavenging oxygen free radicals and thereby are important in preventing oxidative stress. Exogenous antioxidants (vitamin C, vitamin E, flavonoids, carotenoids and -plant-derived products) however, are useful to counterbalance a deficient total antioxidant capacity. Fruits, vegetables and grains contain numerous free radicals scavenging molecules including polyphenols, flavonoids, vitamins, that have a significant antioxidant activity.

Antioxidative activity All the synthesized compounds were screened for their antioxidative activity by the 2,2-diphenylpicrylhydrazyl (DPPH) assay. DPPH method. A methanolic DPPH solution (0.037 mg mL-1) was prepared and kept in the dark before analysis. Methanolic solutions of the compounds were prepared in various concentrations, depending on the examined compound. 200 µL of each sample was added to 2.8 mL of DPPH solution and reaction mixture was kept in the dark for 20 min. A blind test was performed by adding 200 µL of methanol in 2.8 mL of DPPH solution and the absorbance of both the blind check and the investigated samples was measured at 517 nm.

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of Blank - Absorbance of Test X 100}}{\text{Absorbance of Blank}}$$

The 50 % decline in absorbance of the DPPH solution was derived from the graph with the concentration (µM) plotted against the absorbance. These concentration values were used to determine the IC_{50} values in µM.

Antibacterial activity[16-18]:

The compounds were tested in vitro for their antibacterial activity against two gram positive bacteria viz ***Staphylococcus aureus, Bacillus subtilis***and two gram negative bacteria viz. ***Escherichia coli***and ***Klebsiellapneumoniae*** are pathogenic in human beings. Cup plate agar diffusion method using Muller Hinton agar. Nutrient agar was prepared by, 18-24h growth culture in nutrient agar, sterile Petri dishes, sterile micropipettes, and sterile cotton swabs sterile cork borer (8mm) and sterile test tubes. Preparation of nutrient agar was prepared by

taking Peptone0.6g, yeast extracts 0.15g, dipotassium dehydrogenate phosphate 0.13g was dissolved in 100ml distilled water and pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 p.s.i for 20 min. Preparation of subculture: One day prior to these testing's, inoculation of the above bacterial cultures was made in the nutrient agar and incubated at 370C for 18-24h.Preparation of medium (Muller-Hinton Agar): The 3.8g of agar was dissolved into 100ml of distilled water and the pH was adjusted to 7.4 ±0.2It was sterilized by autoclaving at 15 p.s.i for 20 minutes. Preparation of test solutions: Each test compound (5g) was dissolved in diethyl formamide (5ml) to give stock solution of concentration 1000µg/ml. Then 0.1ml of this solution was used for testing. Preparation of standard solution: Standard drug norfloxacin was used. The concentration was 100µg/ml. Method of testing Muller – Hinton agar plates were prepared by pouring 10-15ml of the medium into each sterilized Petridis and were allowed to set at room temperature. The cell suspension was standardized to the density of 530nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The eight cups were scooped in each plate using a sterile cork borer of 8mm diameter the solution of test compounds (0.10ml) were added in cups by using micropipettes and these were incubated at 37^0C for 48h. The zone of inhibition was measured in mm of each organism.

3. RESULTS AND DISCUSSION

Synthesis:

A series of some novel Pyrrole fused-Isatin conjugates (V(a-o)) were synthesized by a conventional method via scuff's base mechanism in four steps. In step one, Methyl anthranilate, Hexane 2,5 di one reacts to give methyl 2-(2,5-dimethyl-1H-pyrrol-1-yl) benzoate. It can be reacts with hydrazine hydrate to form 2--(2,5-dimethyl-1H-pyrrol-1-yl)benzamide in step-II. Finally, substituted Isatin derivatives were reacts with 2--(2,5-dimethyl-1H-pyrrol-1-yl)benzamide to form the title compounds 2--(2,5-dimethyl-1H-pyrrol-1-yl)-N-(6- methyl-2oxo-2,3 dihyd-1H-indole-3 ylidene)benzohyrazide-Va-Vo. All the synthesized compounds gave a good yield and structures were confirmed by FT-IR, ^{1}HNMR, and LC-MS spectral analysis.

Antioxidant activity:

The DPPH reducing capabilities of all synthetic compounds (V(a-o)) were evaluated by determining their IC_{50} values. The compound VIId, VIIh and VIIn are showing better antioxidant activity were compared with standard drug. IC_{50} values of all compounds obtained in the DPPH assays were presented in Table.4. The IC_{50} value corresponds to the concentration of a sample, which has ability to scavenge 50% of the free radicals present in the reaction mixture. Low IC_{50} values indicate a high antioxidant activity of the sample compound (table 2 and figure 3).

Table 2: Antioxidant activity of 2-(2,5-dimethyl-1H-pyrrol-1-yl)-N-(6- methyl-2oxo-2,3 dihyd- 1H-indole-3 ylidene)benzohyrazide derivatives(Va-Vo)

S. No	Sample Code	IC_{50} µg/Ml
1	V-a	134±3.02
2	V-b	112±1.53
3	V-c	148±0.37
4	V-d	112±1.45
5	V-e	148±1.92
6	V-f	115±1.23
7	V-g	95±3.12
8	**V-h**	**80±0.01****
9	V-i	110±2.09
10	V-j	154±3.12
11	**V-k**	**84±0.02****
12	V-l	92±1.02
13	V-m	137±2.54
14	**V-n**	**74±0.01****
15	V-o	105±2.76
16	**Ascorbic acid**	**68.23±0.01****

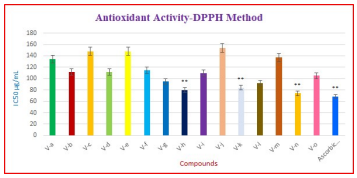

Figure 3: Antioxidant activity of some novel Schiff's Bases by DPPH radical Scavenging activity

Antimicrobial activity:
Among the synthesized compoundsVa-Vo, compound Vo were found to be more active, and the zone of inhibitions were also high compared with other derivatives. Compound Vd, compound Vj were found to be least biologically active amongst the synthesized title compounds. The results of the agar diffusion tests are tabulated in Table 3 and Figure 4.

Table 3a: Antibacterial activity against *Staphylococcus aureus* (zone of inhibition in mm)

		Zone of Inhibition in mm		
		Staphylococcus aureus		
	R	**50µg/ml**	**100µg/ml**	**300µg/ml**
V-a	H	09	17	20
V-b	5-CH_3	13	18	20
V-c	7-CH_3	10	15	21
V-d	**5-Cl**	**15**	**19**	**25**
V-e	**7-Cl**	**14**	**20**	**27**
V-f	5-NO_2	09	12	16
V-g	7-NO_2	11	12	17
V-h	5-Br	09	16	17
V-i	7-Br	09	13	17
V-j	5-F	11	13	17
V-k	7-F	09	15	20
V-l	5-$COOC_2H_5$	09	12	19
V-m	**7-COOH**	**14**	**20**	**27**
V-n	7-$COOCH_3$	12	18	21
V-o	6-Cl, 5-F	**15**	**21**	**26**
Standard	**Moxifloxacin**	**15**	**23**	**29**
control		00	00	00

Table 3b: Antibacterial activity against *Bacillus subtilis* (zone of inhibition in mm)

Compound	**R**	**Zone of Inhibition in mm**		
Compound	**R**	***Bacillus subtilis***		
		50µg/ml	**100µg/ml**	**300µg/ml**
V-a	H	09	15	18
V-b	5-CH_3	13	18	20
V-c	7-CH_3	**18**	**22**	**26**
V-d	**5-Cl**	09	13	17
V-e	**7-Cl**	**16**	**21**	**27**
V-f	5-NO_2	13	19	20
V-g	7-NO_2	11	13	18
V-h	5-Br	09	12	18
V-i	7-Br	11	15	18
V-j	5-F	14	18	20
V-k	7-F	09	12	19
V-l	5-$COOC_2H_5$	11	14	19
V-m	**7-COOH**	**18**	**21**	**27**
V-n	7-$COOCH_3$	11	16	20
V-o	6-Cl, 5-F	**10**	**18**	**23**

Standard	Moxifloxacin	17	21	34
control		00	00	00

4. CONCLUSION

Newly synthesized compounds (Va-Vo) were characterized by Melting point,TLC, IR, 1H NMR and Mass spectral analysis.The preliminary studies on antimicrobial activity of the new pyrrole and Isatin derivatives have generated some interesting data. An attempt has been made to infer the ultimate outcome of the present studies basing on this data.All the synthesized compounds were evaluated for antibacterial activity by using standard Moxiflaxacin and antioxidant activity by using ascorbic acid.Synthetic work of the studies could go positively as per the planning and as such in all the reactions carried out. The expected compounds alone could be obtained.

Newly synthesized compounds showed promising antimicrobial activity against Gram positive and Gram negative bacteria. It is clearly concluded that the synthesized compounds with substitution at 5, 6th position with two halogens(5F,6Cl) on Isatin moiety showed potent antibacterial activity against Gram positive and Gram negative bacteria.Newly synthesized compounds showed promising antioxidant activity.Compound **Vo** showed better antioxidant activity compared to all the synthesized compounds.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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