Synthesis, characterization and antimicrobial activity of novel mannich bases of indol derivatives

J. Sreeramulu, P. Ashokgajapathiraju, K. Venugopal, L. K. Ravindranath and S. Muralikrishna

Department of Chemistry, S. K. University, Anantapuramu, A. P., India

ABSRTACT

New novel derivatives of 2-(5-chloro-3-(4-substituted phenylimino)methyl)-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N\textsubscript{1}-(2-oxo-1-(4-substituted piperidin-1-ylmethyl)indolin-3-ylidene)acetohydrazide 9(a-o) were prepared by the (mannich reaction) condensation of 2-(5-chloro-3-(4-substituted phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)N\textsubscript{1}-(2-oxoindolin-3-ylidene)acetohydrazide 8(a-e) with piper dine/morpholine/N-methyl piperizine and formaldehyde in DMF. The compounds 8(a-e) were obtained by the reaction of 2-(5-chloro-3-(4-substituted phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazaide 7(a-e) with isatin. Subsequently 7(a-e) were obtained by the reaction of ethyl-2-(5-chloro-3-(4-substituted phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate 6(a-e) with hydrazine hydrate. The synthesis 6(a-e) were obtained by the reaction of ethyl-2-(5-chloro-3-(4-formyl-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (5) with 4-substituted anilines. The compound (5) was obtained by the Vilsmeier-Haack reaction of 1-(5-chloro-1H-indol-3-yl)ethanone (1) with chloro ethyl acetate in K\textsubscript{2}CO\textsubscript{3} and DMF, the product obtained in the reaction is further treated with 4-hydrazino pyridine. Finally the title of the compounds 9(a-o) were subjected to antimicrobial studies and incorporated in the present studies. All the compounds were characterized by spectral studies (IR, \textsuperscript{1}H-NMR, \textsuperscript{13}C-NMR, Mass) and Elemental analysis.

Key words: Indol, piperidine, hydrazine hydrate, Isatin, anilines, antimicrobial activity.

INTRODUCTION

Heterocyclic compounds containing five and six-membered ring systems are successfully used as drugs. Synthesis of such compounds containing condensed rings or more than one heterocyclic nucleus is gaining more and more popularity due to their specific use in medicines. Indole is a fused aromatic heterocyclic ring, consisting of a six-membered benzene ring fused to a five membered pyrrole ring. Indole moiety is probably the most widely spread nitrogen heterocycle in nature. Indole and its derivatives found to possess interesting biological activities like antifungal[1-2], antimicrobial[3], anti-viral[4-7], antimalarial[8-9], anti-inflammatory[10-14], anticancer[15-17], antidepressant[18], anti-HIV[19-21], antibacterial[22-25], anticonvulsant[26-27], antitumor[28] antioxidant[29], anti-proliferative[30] and anti leukemic[31] agents. Large number of Indole derivatives are isolated from plant kingdom. It also acts as scavenger of free radicals. Indole nucleus is considered as “privileged scaffolds”[32] for the synthesis of number of novel alkaloids, agrochemicals, pharmaceuticals and perfumes[33]. Pyrazol derivatives are play an important role in biological activities like antimicrobial [34, 35], anti-inflammatory[36], anti-oxidant [37] agents. Therefore, in the present article a series of novel indole derivatives has been synthesised and evaluated for biological activity.
MATERIALS AND METHODS

All the chemicals used in the present investigation were purchased from Ark pharma, Inc. and Sigma-Aldrich Chemicals company, Inc. USA and used without further purification. TLC was performed on aluminium sheet of silica gel 60F254, E-Merk, Germany using iodine as visualizing agent. Melting points were determined in open capillary tubes on Mel-Temp apparatus and are uncorrected (in degree celsius). Column chromatography was performed on silica gel with different solvent systems as eluents to afford the pure compound. The Infra Red Spectra of the compounds were recorded in KBr pellets on FT-IR (perkin-Elmer 1000 units) instrument. All 1H and 13C-NMR spectra were recorded on a varian XL-300 Spectrometer operating at 400 MHz for 1H-NMR and 75 MHz for 13C-NMR. The 1H-NMR spectra were recorded using TMS as an internal standard (Chemical shifts in δ). The compounds were dissolved in DMSO-d6 and Chemical shifts were referenced to TMS (1H and 13C-NMR). Mass spectral data was recorded on FAB-MS instrument at 70ev with direct inlet system. Elemental analysis were recorded on a Carlo Erba 1108 elemental Analyser, Central Drug Research Institute, Lucknow, India.
Preparation of isatin
The required isatin was prepared by the procedure described by Marvel and Heirs[1]. In a one liter R.B. flask 22g of chloral hydrate and 300mL of water placed, to this 325g of crystallized sodium sulphate, 12g of aniline (in 75 mL of water), 12 mL of con. HCl and 27g of hydroxyl amine hydrochloride (in 25mL of water) were added. The flask was heated over a wire gauge for about 40-45 minutes. After one or two minutes of vigorous boiling, the reaction mixture was cooled and the separated solid was filtered and air-dried, yield 16g, m.p. 175°C. In a one liter R. B. flask, 8ml of con. H2SO4 was placed and warmed at 50°C, to this 18g of dry isonitroso acetanilide was added. The temperature was kept between 60°C-70°C using external cooling. After the addition of the isonitroso compound, the reaction mixture was heated to 80°C and kept at this temperature for about 10 minutes. Then the reaction mixture was cooled to room temperature and poured on to crushed ice. The precipitated Isatin was filtered and washed several times with cold water to remove the H2SO4 and then dried in the air, yield 12g. m.p.190°C.

Preparation of Intermediates
2-(5-chloro-3-(4-substitutedphenylimino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)-N(2-oxoindolin-3-ylidene) acetohydrazide: (8a-e)
Equimolar quantities (0.01 mol) of Isatin and the corresponding 2-(5-chloro-3-(4-((phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide (7a) was dissolved in warm ethanol (40 mL) containing DMF (0.5 mL). The reaction mixture was refluxed for 1-4 hours and then kept at room temperature overnight. The progress of the reaction was monitored by TLC with Acetone: Ethylacetate (7:3) as mobile phase. The resulting solid was filtered and washed with ethanol, dried and recrystallized from ethanol to afford compound 2-(5-chloro-3-(4((phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N-(2-oxoindolin-3-yl idene) acetohydrazide(8a). The yield of 8a was 70% with m.p: 168-9°C. The similar procedure was adopted to synthesise 8(b-e) from 7(b-e) and Isatin. The structure of newly synthesized compounds 7(a-e) was established by IR, 1H-NMR and Elemental analysis.

RESULTS AND DISCUSSION
The target compounds were synthesised via the route as shown in Scheme above. The synthon required for the synthesis of the target molecules was prepared by a reported method, filtered and recrystallized from ethanol. For all the synthesized compounds, the progress of the reaction was monitored by TLC with cyclohexane, ethylacetate (7:3) as mobile phase. All the synthesized structures showed satisfactory result. The chemical shift values of the synthesized compounds were full agreement with the number of protons present in it.

Procedure for the synthesis of Ethyl-2-(3-acetyl-5-chloro-1H-indol-1-yl)acetate (2)
A mixture of 1-(5-chloro-1H-indol-3-yl)ethene(1) (0.02M) and anhydrous K2CO3 (0.03M), Chloro ethyl acetate (0.02M) and DMF(dimethyl formamide) was added and the mixture is stirred at room temperature for 8 hours. The progress of the reaction was monitored by TLC with cyclohexane: ethyl acetate (7:3) as mobile phase. The reaction mixture was diluted with ice-cold water. The separated solid was identified as ethyl-2-(3-acetyl-5-chloro-1H-indol-1-yl)acetate (2). This was collected by filtration and recrystallized from ethanol. The yield of (2) was 65% , M.P : 147°C.

The structure of the compound Ethyl-2-(3-acetyl-5-chloro-1H-indol-1yl) acetate (2) was established by IR and 1H NMR spectra.

Procedure for the synthesis of 2-(5-chloro-3-(2-pyridin-4-yl)hydrazono ethyl)-1H-indol-1-yl)acetate (4)
Equimolar quantities of 2-(5-chloro-3-(2-pyridin-4-yl)hydrazonoethyl)-1H-indol-1-yl)acetate(2) and 4-hydrazinopyridine (3) were dissolved in absolute alcohol, to this one drop of acetic acid is added then heated on a steam bath for 5-6h at 100°C. After standing for 24h at room temperature, the product was dried and recrystallised from warm absolute alcohol. The separated solid was identified as 2-(5-chloro-3-(2-pyridin-4-yl)hydrazonoethyl)-1H-indol-1-yl)acetate (4). This was collected by filtration and recrystallised from absolute ethanol. The yield of (4) was 70%, M.P: 172-174°C. The structure of 2-(5-chloro-3-(2-pyridin-4-yl)hydrazonoethyl)-1H-indol-1-yl)acetate (4) was established by IR, 1H-NMR and Elemental analysis.

Procedure for the synthesis of Ethyl-2-(5-chloro-3-(4-formyl-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl) acetate (5)
2-(5-chloro-3-(1-(2-pyridin-4-yl)hydrazono) ethyl)-1H-indol-1-yl)acetate(4) on treatment with POCl3-DMF (dimethyl formamide)/SiO2 at 0-5 °C under microwave irradiation furnished the respective ethyl-2-(5-chloro-3 -(4-formyl-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate(5). In a typical procedure, to the Vilsmeier-Haack reagent, prepared from DMF and POCl3 at 0-5 °C, 2-(5-chloro-3-(1-(2-pyridin-4-yl)hydrazono)ethyl)-1H-indol-1-yl)acetate (4) and silicagel was added and is exposed to MicroWaves at 400W intermittently at 30sec intervals for 30min and extracted with dichloromethane followed by distillation of solvent under reduced pressure with

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rotaevaporator. The residue was recrystallised from absolute ethanol. The yield of ethyl-2-(5-cloro-3-(4-formyl-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (5) was 65% yield, with M.P: 152-154 °C.

The structure of Ethyl-2-(5-cloro-3-(4-formyl-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (5) was established by IR, ¹H-NMR and Elemental analysis.

Procedures for synthesis of Ethyl-2-(5-cloro-3-(4-substituted phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (6a-e)

Equimolar quantities of Ethyl-2-(5-cloro-3-(4-formyl-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (5) and 4-substituted aniline were dissolved in absolute alcohol and heated at 100 °C for 5 hours. The reaction mixture was kept overnight and evaporation of the solvent under reduced pressure with rotaevaporator. The residue was recrystallised from absolute ethanol. The yield of ethyl-2-(5-chloro-3-(4-substituted phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (6a) was 65% yield, M.P: 176-178 °C. The similar procedure was repeated with (5) and toulidine, anisidine, 4-nitroaniline, 4-tri fluoro methyl aniline. The structure of (6a-e) was established by IR, ¹H-NMR and Elemental analysis.

Procedures for the synthesis of 2-(5-cloro-3-(4-substituted phenylimino)methyl-1-pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide (7a-e)

A mixture of ethyl-2-(5-cloro-3-(4-(phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetate (6a) (0.01 M) and hydrazine hydrate (0.15 M) in ethanol 20ml was refluxed for 5 hours, C-NMR, Mass and Elemental analysis.

Physical, Analytical and Spectral data for the compounds:

Characterization of Ethyl-2-(3-acetyl-5-chloro-1H-indol-1-yl)acetate (2)

Yield 70%, M.P: 172-173 °C, IR(KBR): δ 3200 cm⁻¹ (stretching vibration of (-NH) sec. amine), 1690 cm⁻¹ (carbonyl group of ester), 1618 cm⁻¹ (stretching vibration of C≡N nucleus), 1450-1520 cm⁻¹ (characteristic of Indol nucleus), 1300 cm⁻¹ (C-O-C of ester group) and 677 cm⁻¹ (characteristic of C-Cl nucleus). ¹H-NMR(400MHz, DMSO-d₆) δ: 1.29(t, 3H, -CH₃ of ethyl group JAX=8.6 Hz), 2.50(s, 3H, CH₃ of ketone), 4.13(q, 2H, -CH₂ of ethyl group JAX=8.6 Hz), 4.74(s, 2H, N-CH₂ attached to indol nucleus), 7.20-7.75 (m, 4H of indol ring). Mass(m/z): 279.07, Anal. Calcd. For C₁₅H₁₃CINO₂: C 60.11%, H 5.04%, N 5.01%. Found: C 60.02%, H 4.95%, N 4.92%.

Characterization of 2-(5-chloro-3-(4-substituted phenylimino)methyl-1-pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide (9a-o)

A mixture of 2-(5-chloro-3-(4-(phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide (9a) (0.01 M), piperidine (0.15 M) and water was refluxed for 5 hours. The reaction mixture was cooled and poured onto ice cold water with stirring. The separated solid was filtered, washed with water and recrystallised from ethanol to afford 2-(5-chloro-3-(4-(phenylimino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)acetohydrazide (9a) with a yield of 70%, M.P: 144-145 °C. The similar procedure was adopted to synthesise (9b-o) from (8b-e) and hydrazine hydrate. The structure of newly synthesized compounds (9a-o) was established by IR, ¹H-NMR and Elemental analysis.
Characterization of Ethyl-2-(5-chloro-3-(4-(phenylimino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)acetate (6a)
yield 70%, M.P: 176-177°C. IR (KBr): δ 1690 cm⁻¹ (carbonyl group of ester), 1618 cm⁻¹ (stretching vibration of C=O group), 1450-1520 cm⁻¹ (stretching vibration of Indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1325 cm⁻¹ (O-C of ester group) and 677 cm⁻¹ (characteristic of C-Cl nucleus). ¹H-NMR (400MHz, DMSO-d₆) δ ppm: 1.29(t, 3H, CH₃ attached to phenyl ring), 4.13(q, 2H, -CH₂ of ethyl group, Jₓᵧ=8.6Hz), 4.74(s, 2H, N-CH₂ attached to indol nucleus), 6.95-7.25(m, 5H, of -C₆H₅ nucleus), 7.30-7.75(m, 4H, Indol nucleus), 7.80-8.07(m, 4H, of -C₆H₅N), 8.10(s, 1H, Pyrazole), 8.35(s, 1H, CH=N aldimeine). Mass(m/z): 483.15, Anal. Calcd. For C₂₉H₂₅ClN₂O₂: C 67.53%, H 4.86%, N 14.36% . Found: C 67.32%, H 4.58%, N 13.57%.

Characterization of Ethyl-2-(5-chloro-3-(1-(pyridin-4-yl)-4-((4-(trifluoromethyl)phenyl)imino)methyl)-1H-indol-1-yl)acetate (6b)
yield 70%, M.P: 192-3°C. IR (KBr): δ 1683 cm⁻¹ (carbonyl group of ester), 1620 cm⁻¹ (stretching vibration of C=O group), 1450-1520 cm⁻¹ (characteristic of Indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1325 cm⁻¹ (O-C of ester group) and 679 cm⁻¹ (characteristic of C-Cl nucleus). ¹H-NMR (400MHz, DMSO-d₆) δ ppm: 1.29(t, 3H, CH₃ of ethyl group, Jₓᵧ=8.6Hz), 2.34(s, 3H, -CH₃ attached to phenyl ring), 4.13(q, 2H, -CH₂ of ethyl group, Jₓᵧ=8.6Hz), 4.74(s, 2H, N-CH₂ attached to indol nucleus), 6.98-7.25(m, 4H, of -C₆H₅ nucleus), 7.30-7.75(m, 4H, Indol nucleus), 7.80-8.05(m, 4H, of -C₆H₅N), 8.10(s, 1H, Pyrazole), 8.35(s, 1H, CH=N aldimeine). Mass(m/z): 513.16, Anal. Calcd. For C₂₉H₂₅ClN₂O₂: C 65.43%, H 4.71%, N 13.63% . Found: C 65.32%, H 4.58%, N 13.51%.

Characterization of Ethyl-2-(5-chloro-3-(4-(4-methoxyphenylimino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)acetate (6c)
yield 60%, M.P: 138-9°C. IR (KBr): δ 1680 cm⁻¹ (carbonyl group of ester), 1615 cm⁻¹ (stretching vibration of C=O group), 1450-1520 cm⁻¹ (characteristic of Indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1325 cm⁻¹ (O-C of ester group) and 675 cm⁻¹ (characteristic of C-Cl nucleus). ¹H-NMR (400MHz, DMSO-d₆) δ ppm: 1.29(t, 3H, CH₃ of ethyl group, Jₓᵧ=8.6Hz), 3.83(s, 3H, -OCH₃ attached to phenyl ring), 4.13(q, 2H, -CH₂ of ethyl group, Jₓᵧ=8.6Hz), 4.74(s, 2H, N-CH₂ attached to indol nucleus), 7.0-7.25(m, 4H, of -C₆H₅ nucleus), 7.30-7.75(m, 4H, Indol nucleus), 7.80-8.05(m, 4H, of -C₆H₅N), 8.10(s, 1H, Pyrazole), 8.35(s, 1H, CH=N aldimeine). Mass(m/z): 528.13, Anal. Calcd. For C₂₉H₂₅N₂O₂: C 67.01%, H 4.58%, N 14.47% . Found: C 66.89%, H 4.47%, N 14.38%.

Characterization of Ethyl-2-(5-chloro-3-(4-((phenylimino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)acetate (6d)
yield 60%, M.P: 156-7°C. IR (KBr): δ 1685 cm⁻¹ (carbonyl group of ester), 1617 cm⁻¹ (stretching vibration of C=O group), 1450-1520 cm⁻¹ (characteristic of Indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1325 cm⁻¹ (O-C of ester group) and 677 cm⁻¹ (characteristic of C-Cl nucleus). ¹H-NMR (400MHz, DMSO-d₆) δ ppm: 1.29(t, 3H, CH₃ of ethyl group, Jₓᵧ=8.6Hz), 2.34(s, 3H, -CH₃ attached to phenyl ring), 4.13(q, 2H, -CH₂ of ethyl group, Jₓᵧ=8.6Hz), 4.74(s, 2H, N-CH₂ attached to indol nucleus), 6.98-7.25(m, 4H, of -C₆H₅ nucleus), 7.30-7.75(m, 4H, Indol nucleus), 7.80-8.05(m, 4H, of -C₆H₅N), 8.10(s, 1H, Pyrazole), 8.35(s, 1H, CH=N aldimeine). Mass(m/z): 586.15, Anal. Calcd. For C₂₉H₂₅N₂O₂: C 61.69%, H 4.19%, N 13.70% . Found: C 61.54%, H 4.04%, N 13.57%.
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C-Cl nucleus). δ H-NMR (400 MHz, DMSO-d6) δ ppm: 2.10 (s, 2H, amine), 4.74 (s, 2H, N-CH2), 6.98-7.25 (m, 5H, of -C6H5 nucleus), 7.30-7.75 (m, 4H, of indol), 7.80-8.05 (2d, 4H, of -CH2N), 8.10 (s, 1H, pyrazole), 8.35 (s, 1H, N=CH), 9.50 (s, 1H, -CONH). Mass (m/z): 469.14 Anal. Calcd. For C23H23ClN2O: C 63.90%, H 4.29%, N 20.86%. Found: C 63.67%, H 4.07%, N 20.62%.

2-(5-chloro-3-(pyridin-4-yl)-4-((p-tolylimino)methyl)-1H- pyrazol-3-yl)-1H-indol-1-yl)-N'-(2-oxo-1-(piperidin-1-yl) methyl) indolin-3-ylidine)acetohydrazide(9b)

Yield 60%, M.P: 164-5°C. IR (KBr): δ 3206 cm⁻¹ (NH stretching), 1680 cm⁻¹ (C=O of exo cyclic amide), 1651 cm⁻¹ (characteristic of C=N), 1450-1520 cm⁻¹ (characteristic of indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 767 cm⁻¹ (characteristic of C=Cl nucleus) respectively. δ H-NMR (400 MHz, DMSO-d6) δ ppm: 1.50-2.45 (m, 10H, piperidine), 2.83 (s, 3H, -CH3), 4.10 (s, 2H, N-CH2 attached to indolone nucleus), 4.74 (s, 2H, N-CH2CO attached to indol nucleus), 6.90-7.10 (m, 5H, of CH2N in C2H5N3O, 7.30-7.75 (m, 8H, 4H of indol nucleus). 7.80-8.07 (m, 4H, of -CH2N), 8.10 (s, 1H, pyrazole), 8.35 (s, 1H, aldimine), 9.70 (s, 1H, -CONH group). δ C-NMR spectra (75MHz, DMSO-d6): δ: 128, 112, 122, 126, 113, 131, 126.5, 137, 112.5, 157, 144, 124, 132, 147, 115, 147, 151, 145, 173, 132, 167, 148, 118, 129, 125, 131, 116, 73, 52, 25, 24 Corresponding to C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14 & C15, C16, C17, C18, C19, C20, C21 & C22, C23, C24, C25, C26, C27, C28, C29, C30, C31, C32, C33, C34, C35 & C36, C37 & C38, C37, C38, Mass (m/z): 709.27 (M+), 711.27 (M+2), Anal. Calcd. For C30H29ClN3O2: C 67.64%, H 5.11%, N 17.75%. Found: C 67.47%, H 4.91%, N 17.54%.

2-(2H-chloro-3-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(2-oxo-1-(piperidin-1-yl) methyl) indolin-3-ylidine)acetohydrazide(9d)

Yield 60%, M.P: 176-7°C. IR (KBr): δ 3206 cm⁻¹ (NH stretching), 1682 cm⁻¹ (C=O of exo cyclic amide), 1650 cm⁻¹ (characteristic of C=N), 1450-1520 cm⁻¹ (characteristic of indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 765 cm⁻¹ (characteristic of C-Cl nucleus) respectively. δ H-NMR (400 MHz, DMSO-d6) δ ppm: 1.50-2.45 (m, 10H, piperidine), 3.21 (s, 3H, -CH3), 4.10 (s, 2H, N-CH2 attached to indolone nucleus), 4.74 (s, 2H, N-CH2CO attached to indol nucleus), 6.85-7.05 (m, 5H, of -CH2N), 7.30-7.75 (m, 8H, 4H of indol nucleus), 7.80-8.07 (m, 4H, of -CH2N), 8.10 (s, 1H, pyrazole), 8.35 (s, 1H, aldimine), 9.70 (s, 1H, -CONH group). δ C-NMR spectra (75MHz, DMSO-d6): δ: 128, 112, 122, 126, 113, 131, 131, 112.5, 157, 144, 124, 132, 147, 115, 151, 145, 173, 132, 167, 148, 118, 129, 125, 131, 116, 75, 54, 25, 24 Corresponding to C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14 & C15, C16, C17, C18, C19, C20, C21 & C22, C23, C24 & C25, C26, C27, C28, C29, C30, C31, C32, C33, C34, C35 & C36, C37 & C38, C37, C38, Mass (m/z): 725.25, Anal. Calcd. For C28H27ClN3O2: C 66.15%, H 5.00%, N 17.56%. Found: C 65.92%, H 4.74%, N 17.14%.

2-(2H-chloro-3-(4-((4-methoxyphenyl)iminomethyl)-1H- pyrazol-3-yl)-1H-indol-1-yl)-N'-(2-oxo-1-(piperidin-1-yl)methyl) indolin-3-ylidine)acetohydrazide(9g)

Yield 65%, M.P: 203.4°C. IR (KBr): δ 3201 cm⁻¹ (NH stretching), 1690 cm⁻¹ (C=O of exo cyclic amide), 1657 cm⁻¹ (characteristic of C=N), 1450-1520 cm⁻¹ (characteristic of indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 679 cm⁻¹ (characteristic of C-Cl nucleus) respectively. δ H-NMR (400 MHz, DMSO-d6) δ ppm: 50-2.45 (m, 10H, piperidine), 4.10 (s, 2H, N-CH2 attached to indolone nucleus), 4.74 (s, 2H, N-CH2CO attached to indol nucleus), 7.1-7.28 (m, 5H, of -CH2N), 7.30-7.75 (m, 8H, 4H of indol nucleus), 7.80-8.07 (m, 4H, of -CH2N), 8.10 (s, 1H, pyrazole), 8.35 (s, 1H, aldimine), 9.70 (s, 1H, -CONH group). δ C-NMR spectra (75MHz, DMSO-d6): δ: 128, 112, 122, 126, 123, 113, 135, 131, 126.5, 137, 112.5, 157, 142, 123, 117, 154, 57, 147, 115, 151, 45, 173, 131.5, 167, 148, 118, 129, 125, 131.2, 116, 75, 54, 25, 24 Corresponding to C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14 & C15, C16, C17, C18, C19, C20, C21 & C22, C23, C24 & C25, C26, C27, C28, C29, C30, C31, C32, C33, C34, C35 & C36, C37 & C38, C37, C38, Mass (m/z): 740.24 Anal. Calcd. For C29H29ClN4O2: C 67.46%, H 4.35%, N 16.50%. Found: C 67.42%, H 4.16%, N 16.37%.
1H-NMR:

- 2-(5-chloro-3-(4-(((4-methoxyphenyl)imino)methyl)-1H-indol-1-yl)-N'-((1-(morpholino methyl)-2-oxoindolin-3-ylidene)acetohydrazide(9i)

Yield 70%, M.P: 178-9°C. IR(KBR): δ 3205 cm⁻¹(NH stretching), 1685 cm⁻¹(C=O of exo cyclic amide), 1652 cm⁻¹(cyclic >C=O), 1615 cm⁻¹(characteristic of C=N) 1450-1520 cm⁻¹, (characteristic of indol nucleus) 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1015°C-C of cyclic ether), 677 cm⁻¹ (characteristic of C=Cl nucleus)

- 2-(5-chloro-3-(4-((phenylimino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)-N'-(1-(morpholino methyl)-2-oxoindolin-3-ylidene)acetohydrazide(9i)

Yield 70%, M.P: 178-9°C. IR(KBR): δ 3205 cm⁻¹(NH stretching), 1685 cm⁻¹(C=O of exo cyclic amide), 1652 cm⁻¹(cyclic >C=O), 1615 cm⁻¹(characteristic of C=N) 1450-1520 cm⁻¹, (characteristic of indol nucleus) 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1015°C-C of cyclic ether), 677 cm⁻¹ (characteristic of C=Cl nucleus)

- 2-(5-chloro-3-(4-((p-tolylimino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)-N'-(1-(morpholino methyl)-2-oxoindolin-3-ylidene)acetohydrazide(9f)

Yield 65%, M.P: 163-4°C. IR (KBR): δ 3206 cm⁻¹(NH stretching), 1682 cm⁻¹(C=O of exo cyclic amide), 1652 cm⁻¹(cyclic >C=O), 1615 cm⁻¹(characteristic of C=N) 1450-1520 cm⁻¹, (characteristic of indol nucleus) 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1015°C-C of cyclic ether), 676 cm⁻¹(characteristic of C=Cl nucleus)

- 2-(5-chloro-3-(1-(pyridin-4-yl)-4-((p-tolylimino)methyl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1-(morpholino methyl)-2-oxoindolin-3-ylidene)acetohydrazide(9g)

Yield 65%, M.P: 163-4°C. IR (KBR): δ 3206 cm⁻¹(NH stretching), 1682 cm⁻¹(C=O of exo cyclic amide), 1652 cm⁻¹(cyclic >C=O), 1615 cm⁻¹(characteristic of C=N) 1450-1520 cm⁻¹, (characteristic of indol nucleus) 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1015°C-C of cyclic ether), 676 cm⁻¹(characteristic of C=Cl nucleus)

C-NMR SPECTRA (75MHz, DMSO-d₆):

- δ 7.1-7.6 (m, 5H, of -C=O of exo cyclic amide), 7.3-7.35 (m, 8H, of indol nucleus, 4H of indolone nucleus), 7.8-8.07 (m, 4H, of -C=O of exo cyclic amide)

- δ 4.15 (s, 2H, N-CH₂ attached to morpholin ring), 4.74 (s, 2H, N-CH₂ attached to indol nucleus), 6.7-7.0 (m, 5H, of -C=O of exo cyclic amide)

- δ 2.3-3.7 (m, 8H, morpholin ring), 3.2 (s, 3H, -OCH₃)

2-(5-chloro-3-(1-pyrindin-4-yl)-4-(((4-trifluoromethyl)phenylimino)methyl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N'-(1-(morpholinomethyl)-2-oxoindolin-3-ylidene)acetohydrazide (9j)

Yield 65%, M.P: 185-8°C. IR (KBr): δ 3206cm⁻¹(NH stretching), 1685cm⁻¹(C=O of exo cyclic amide), 1650cm⁻¹ (cyclic >C=O), 1615cm⁻¹(characteristic of C=N) 1450-1520 cm⁻¹ (characteristic of indol nucleus) 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 1015(C=O of cyclic ether), 680cm⁻¹ (characteristic of C-Cl nucleus) respectively. **¹H-NMR (400MHz, DMSO-d₆)** δ ppm: 2.37(s, 3H, N-CH₃ of piperazine ring), 2.46(s, 8H, -CH₂ of piperazine ring ), 4.15(s, 2H, N-CH₂ attached to morpholin ring), 4.74(s, 2H, N-CH₂CO attached to indol nucleus), 7.60-7.75(m, 8H, 4H of indolone nucleus), 7.80-8.07(m, 4H, of -C₆H₄ nucleus), 7.80-7.95(m, 4H, of indole nucleus), 8.10(s, 1H, pyrazole), 8.35(s, 1H, aldimine), 9.70(s, 1H, -CONH group). **¹³C-NMR spectra (75 MHz, DMSO-d₆)** δ: 128, 112, 122, 126, 123, 135, 121, 126, 137, 112.5, 157, 149, 123, 120, 124, 145, 114, 149, 45, 173, 131.5, 167, 147, 118, 129, 124, 131, 116, 75, 53, 66 corresponding to C₁₂, C₂, C₄, C₅, C₆, C₇, C₈, C₉, C₁₀, C₁₁, C₁₂, C₁₃, C₁₄ & C₁₅. δ(C₆H₅ nucleus), 7.30-7.75 (m, 8H, 4H of indole nucleus), 7.80-8.07(m, 4H, of -C₆H₄N), 8.10 (s, 1H, pyrazole), 8.35 (s, 1H, aldimine), 9.70(s, 1H, -CONH group). **¹³C-NMR spectra (75 MHz, DMSO-d₆)** δ: 128, 112, 122, 126, 123, 113, 135, 121, 126, 137, 112.5, 157, 149, 123, 120, 124, 145, 114, 149, 45, 173, 131.5, 167, 147, 118, 129, 124, 131, 116, 53, 58, 44 Corresponding to C₁₂, C₁₃, C₁₄, C₁₅, C₁₆, C₁₉, C₂₀, C₂₁, & C₂₂, C₂₃, C₂₄, C₂₅, C₂₆, C₂₇, C₂₈, C₃₀, C₃₁, C₃₂, C₃₃, C₃₄, C₃₅ & C₃₇ & C₃₈. Mass(m/z): 710.26, Anal. calcd. For C₆₃H₄₁ClF₃N₃O₂: C 61.14%, H 4.08%, N 16.45%. Found: C 60.85%, H 3.84 %, N 16.20%.


J. Sreeramulu

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2-(5-chloro-3-(4-(((4-nitrophenyl)imino)methyl)-1-(pyridin-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl)-N’-(1-((4-methylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)acetohydrazide (9n)

Yield 70%, M.P: 195-6°C. IR (KBr): δ 3210 cm⁻¹ (NH stretching), 1690 cm⁻¹ (C=O of exo cyclic amide), 1657 cm⁻¹ (cyclic >C=O), 1615 cm⁻¹ (characteristic of C=N), 1450-1520 cm⁻¹ (characteristic of indol nucleus), 1410-1460 cm⁻¹ (stretching vibration of pyridine ring), 679 cm⁻¹ (characteristic of C-CI nucleus) respectively. ¹H-NMR (400 MHz, DMSO-d₆) δ ppm: 2.2 (s, 3H, N-CH₃ of piperazine ring), 2.46(s, 8H, -CH₂ of piperazine ring), 4.15(s, 2H, N-CH₂ attached to morpholin ring), 4.74(s, 2H, NCH₂CO attached to indol nucleus), 7.10-7.28(m, 5H, of -C₆H₅ nucleus), 7.30-7.75 (m, 8H, 4H of indol nucleus, 4H of indolone nucleus), 7.80-7.95(m, 4H, of -C₆H₄Cl group). Mass(m/z): 755.25. Anal. Calcd. For C₃₉H₃₅ClN₅O₂: C 61.94%, H 4.53%, N 20.37%. Found: C 61.72%, H 4.35%, N 17.96%. Mass(m/z): 788.25. Anal. Calcd. For C₃₉H₃₅ClF₅N₅O₂: C 61.66%, H 4.40%, N 17.96%. Found: C 61.45%, H 4.18%, N 17.59%.

Biological Activity

The newly synthesized compounds 2-(5-chloro-3-(((4-((trifluoromethyl)phenyl)imino)methyl)imino)methyl)-1-(pyridin-4-yl)-1H-indol-1-yl)-N’-(1-((4-methylpiperazin-1-yl)methyl)-2-oxoindolin-3-ylidene)acetohydrazide (9a-o) were screened for their antimicrobial studies against antibacterial and anti fungal activity by Disc Diffusion method[38]. The synthesized compounds were used at the concentration of 250µg/ml DMF as a solvent [39].

<table>
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<th>Staphylococcus aureus NCCS 2079</th>
<th>Bacillus cereus NCCS 2106</th>
<th>Escherichia coli NCCS 2065</th>
<th>Pseudomonas aeruginosa NCCS 2200</th>
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Antibacterial activity

The antibacterial activity of 9(a-o) were screened against the Staphylococcus aureus (gram positive), Bacillus cereus, Escherichia Coli (gram negative) and Pseudomonas aeruginosa organisms. In a given series of piperazine/morpholine/N-methyl piperazine manich bases having nitro (9d,9i,9n) and trifluoromethyl (9e,9j,9o) exhibit high bacterial activity[40,41], when compared to other substituents. The structural activity relationship for different substituents is in the order i.e. -NO₂ > -CF₃ > -H > -CH₃ > -OCH₃. For a given substituents the order of...
antimicrobial activity in each series of mannich bases follows the order -morpholin mannich bases > -piperizine mannich bases > -N-methyl piperizine mannich bases. Here amoxicillin and cefaclor are tested as reference compounds to compare the activity. The antibacterial activity of 9(a-o) was shown in the above given table.

**Antifungal activity**

Antifungal activity of final compounds 2-(5-chloro-3-(4-((4-substituted phenylimino)methyl)-1H-pyrazol-3-yl)-1H-indol-1-yl) N1-(2-oxo-1-(4-substituted piperi din-1-yl methyl) indolin-3-ylidene) acetohydrazide (9a-o) were screened against *Aspergillus niger*, *Candida albicans*. In a given series of piperizine / morpholine / N-methyl piperizine mannich bases containing chloro and nitro groups in their structures has shown increased effect on their antifungal activity. Here ketoconazole is tested as reference compound to compare the antifungal activity. Antifungal activity of 2-(5-chloro-3-(4-((4-substituted phenylimino)methyl)-1-(pyridine-4-yl)-1H-pyrazol-3-yl)-1H-indol-1-yl) N1-(2-oxo-1-(4-substituted piperi din-1-yl methyl) indolin -3-ylidene) acetohydrazide (9a-o).

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**CONCLUSION**

Indol mannich bases bearing pyrazole ring, Isatin moiety besides azomethine group were prepared by adopting Vilsmemeire-Haack reaction and mannich reaction. These synthons were purified & characterized by chromatographic and spectral techniques. Indol mannich bases were subjected to antimicrobial evaluation and some of these compounds were found to possess good anti bacterial and anti microbial activity.

**Acknowledgements**

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**REFERENCES**


