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Original Article

SYNTHESIS, CHARACTERIZATION, AND BIOLOGICAL ACTIVITY OF NOVEL N-PHENYLPROPYL-3-SUBSTITUTED INDOLINE-2-ONE DERIVATIVES

PUSHPA[1,2], BASAVARAJ S. NARABOLI[1], J. S. BIRADAR[1*]

[1]Department of Studies and Research in Chemistry, Gulbarga University, Kalaburagi 585106, Karnataka, India, [2]Government First, Grade College, Raichur, Karnataka, India
Email: jsbiradar@rediffmail.com

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ABSTRACT

Objective: The objective of the present work deals with the synthesis, characterization and evaluation of antimicrobial and antioxidant activity of *N*-phenylpropyl-3-substituted indoline-2-one derivatives.

Methods: A series of new 3-hydroxy-3-(2-oxoethyl)-1-(3-phenylpropyl) indolin-2-one derivatives 3(a-l) and 3-(2-oxoethylidene)-1-(3-phenylpropyl) indolin-2-one derivatives 4(a-l) were synthesized by knoevenagel condensation of *N*-phenylpropyl–5-substituted indole-2,3-diones with various acetophenones analogues. The chemical structures of synthesized compounds were confirmed by IR, ^{1}HNMR and Mass spectroscopic and elemental data. These compounds were also screened for their *in vitro* antimicrobial and antioxidant activities.

Results: Novel compounds 3-hydroxy-3-(2-oxoethyl)-1-(3-phenylpropyl) indolin-2-one derivatives 3(a-l) and 3-(2-oxoethylidene)-1-(3-phenylpropyl) indolin-2-one derivatives 4(a-l) were synthesised and characterized using spectral and analytical data. The results of antibacterial and antifungal and antioxidant activities showed that some of the synthesized compounds exhibited promising results.

Conclusion: All the newly synthesized compounds were screened for antimicrobial activity by cup plate method and antioxidant activity by the DPPH method using Ciprofloxacin and Amphotericin B as standards against gram positive and gram negative bacteria and fungi respectively.

Keywords: Indole-2,3-diones, Pyridine, Thiophene, Antimicrobial, Antioxidant

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INTRODUCTION

Infectious diseases are the main cause of mortality in the world and rapid increase of antimicrobial resistance among pathogenic strains (bacterial and fungal) is becoming a serious public health problem because microbes replicate very rapidly and get mutated which help the microbes to survive in the presence of an antimicrobial drug, these will quickly become predominant throughout the microbial population.

Free radicals play important roles in many physiological and pathological conditions [1]. In, general, the generation and scavenging of oxygen free radicals is balanced and any imbalance or excessive amounts of active radicals may contribute to disease development. It has been found that free radical reactions can produce deleterious modifications in membranes, proteins, enzymes, and DNA [2], increasing the risk of diseases such as cancer [3], Alzheimer's [4], Parkinson's [5], angiocardiopathy [6], arthritis [7], asthma [8], diabetes [9] and degenerative eye disease [10].

Owing to increased microbial resistance, and various disorders caused by free radicals, and to develop more potent small molecules with enhanced antimicrobial and antioxidant properties, a series of *N*-phenylpropyl-3-substituted indoline-2-ones are synthesized by Knovenegal condensation.

Indole-1*H*-2, 3-dione or Indoline-2, 3-dione commonly known as Isatin is a well-known natural product found in plants of genus Isatis and in couropita guianancis aubl [11]. It has also been isolated as a metabolic derivative of adrenaline in humans [12]. The biological and pharmacological properties of isatin and its derivatives have led to extensive use of these compounds as key intermediate in organic synthesis [13]. It is a core constituent of many alkaloids [14], drugs [15], as well as dyes [16]. The literature survey reveals that various derivatives of isatin possess diverse activities such as antibacterial [17], antifungal [18], antiviral [19], HIV [20], antimycobacterial [21], anticancer [22], anti-inflammatory [23], anticonvulsant activities [24] and acts as a potent antagonist on atrial natriuretic peptide receptors *in vitro* [25].

It possesses an indole nucleus with two chemically distinct cyclic carbonyl groups keto and lactam. The structure of isatin has provoked tremendous interest in chemists to unfold the interesting aspects of organic reactions and mechanism. Isatins mainly react at three different sites, namely aromatic substitution at C-5, *N*-alkylation and carbonyl reaction at C-3. The most fascinating application of isatins in organic synthesis is undoubtedly due to the highly reactive C-3carbonyl group that is a prochiral center as well. At the C-3 carbonyl group of isatins, nucleophilic additions or spiro annulation takes place which transforms it into 2-oxoindole derivative. 2-oxoindoles especially those which are spiro fused to the other cyclic framework, have drawn tremendous interest of researchers in the area of synthetic organic chemistry and medicinal chemistry worldwide because they occur in many natural products such as spirotryprostatins, horsfiline, gelsemine, gelseverine, rhynchophylline and elacomine etc.

So as a part of our research in the area of heterocyclic compounds containing indole moiety, the main focus was on *N*-alkylation and nucleophilic addition at C-3 of isatin with various aromatic and heterocyclic acetophenone analogues. Herein we report the synthesis of some new *N*-phenylpropyl-3-substituted indoline-2-one derivatives, their characterization and antimicrobial and antioxidant activities.

The reaction of 5-substituted isatin (1) with 3-chloro propyl benzene in the presence of K2CO3 and *N,N*-dimethyl formamide gave 1-(3-phenylpropyl) indoline-2,3-dione (2a) and 5-flouro-1-(3-phenylpropyl) indoline-2,3-dione (2b). It was found that the K2CO3-DMF system is an effective promotion for this reaction [26]. Use of K2CO3 as a catalyst has inherent advantages including operational simplicity, low cost and suitability in industrial application. Reaction of 2(a-b) with acetophenone derivatives *viz*; acetyl naphthalene 2-acetyl thiophene, 3-acetylpyridine, 4-flouro acetophenone, 4-methoxy acetophenone, 4-benzonitrile gave 3-hydroxy-3-(2-oxoethyl)-1-(3-phenyl propyl) indoline-2-one derivatives (3a-l). The tertiary alcohol can easily be dehydrated under acidic conditions to yield 3-(2-oxoethylidene)-1-(3-phenylpropyl) indolin-2-one derivatives 4(a-l).

MATERIALS AND METHODS

Materials

All the chemicals and solvents were of laboratory reagent grade and used as received from Sigma Aldrich and SD fine. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC using silica gel-G coated aluminium plates (Merck) and spots were visualized by exposing the dry plates to iodine vapors. The IR (KBr) spectra were recorded on a Perkin-Elmer spectrometer on FT-IR spectrometer. The ^{1}H NMR (DMSO-*d6*) spectra recorded on a Bruker (400 MHz) and the chemical shifts were expressed in ppm (δ scale) downfield from TMS. Mass spectral data were recorded by electron impact method on JEOL GCMATE II GC-MS mass spectrometer. Elemental analysis was carried out using Flash EA 1112 series elemental analyzer. All the compounds gave C, H and N analysis within±0.5% of the theoretical values.

General procedure for the synthesis of 5-substituted-1-(3-phenylpropyl)indoline-2,3-dione (2a-b)

To a stirred solution of indoline-2,3-dione/5-flouro indoline-2,3-dione (33.9 mmol/9 mmol) in *N,N*-dimethylformamide (40 ml) were added K_2CO_3 (50 mmol), 3-chloropropyl benzene (13.6 mmol) and the reaction mixture was stirred at 80 °C for 16-18 h. Reaction mixture was poured into ice-cold water, precipitated solid was filtered, washed with water, and dried to obtain the desired product as a colorless solid.

1-(3-phenylpropyl)indoline-2,3-dione (2a)

IR (KBr) (λ_{max} in cm-1): 1604 (NHCO), 1702 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 2.93 (m, 2H, CH_2), 3.97 (t, 2H, N-CH_2), 7.29-7.9 (m, 9H,Ar-H). LCMS: m/z = 265 $[M]^+$.

5-fluoro-1-(3-phenylpropyl)indoline-2,3-dione (2b).

IR (KBr) (λ_{max} in cm-1): 1630 (NHCO), 1710 (C=O). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 2.93 (m, 2H, CH_2), 3.97 (t, 2H, N-CH_2), 7.29-7.9 (m, 8H, Ar-H). LCMS: m/z = 283 $[M]^+$.

General procedure for the Synthesis of 3-hydroxy-3-(2-oxoethyl)-1-(3-phenylpropyl)indolin-2-one derivatives (3a-l)

To a stirred solution of substituted indole-2,3-dione 1-(3-phenylpropyl)-indoline-2,3-dione (3.75 mmol) in ethanol (20 ml) were added piperidine (11.25 mmol), and various acetophenone derivatives like 1-(naphthalen-2-yl)ethan-1-one, 1-(thiophen-2-yl)ethan-1-one, 1-(pyridin-3-yl)ethan-1-one, 1-(4-fluorophenyl) ethan-1-one, 1-(4-methoxyphenyl)ethan-1-one(4 mmol), and 4-acetylbenzonitrile, the reaction mixture was stirred at room temperature for 6h. Reaction mixture was filtered, the solid was washed with ethanol, and dried to obtain the product.

3-hydroxy-3-(2-(naphthalen-2-yl)-2-oxoethyl)-1-(3-phenylpropyl)indolin-2-one (3a)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (NHCO), 1706 (C=O), 3420 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 2 (m, 2H, CH_2), 3.5 (t, 2HN-CH_2,), 6.9-8.4 (m, 16H,Ar-H), 3.58 (s, 2H, CH_2), 4.02 (s, 1H, OH). LCMS: m/z = 435 $[M]^+$. Analysis: Calcd for $C_{29}H_{25}NO_3$ (435): C, 79.98; H, 5.79; N, 3.22. Found: C, 7 9.95; H, 5.75; N, 3.20.

3-hydroxy-3-(2-oxo-2-(thiophen-2-yl)ethyl)-1-(3-phenylpropyl) indolin-2-one (3b)

IR (KBr) (λ_{max} in cm^{-1}): 1630 (NHCO), 1710 (C=O), 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH2), 1.9 (m, 2H, CH2), 3.5 (t, 2H, N-CH_2), 6.9-7.9 (m, 12H, Ar-H), 4.20 (s, 1H, OH), 3.7 (s, 2H, CH_2). LCMS: m/z = 391 $[M]^+$. Analysis: Calcd for $C_{23}H_{21}NO_3S$ (391): C, 70.56; H, 5.4; N, 3.58. Found: C, 70.60; H, 5.5; N,3.6.

3-hydroxy-3-(2-oxo-2-(pyridin-3-yl)ethyl)-1-(3-phenylpropyl) indolin-2-one (3c)

IR (KBr) (λ_{max} in cm^{-1}): 1670 (NHCO), 1740 (C=O), 3523(OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH2), 1.9 (m, 2H, CH2), 3.8 (t, 2H, N-CH_2) 6.9-8.4 (m, 13H Ar-H), 3.3 (s, 2H, CH_2), 4.08 (s, 1H, OH). LCMS: m/z = 386 $[M]^+$. Analysis: Calcd for $C_{24}H_{22}N_2O_3$ (386): C, 74.59; H, 5.74; N, 7.25. Found: C, 74.60; H, 5.72; N, 7.20.

3-(2-(4-fluorophenyl)-2-oxoethyl)-3-hydroxy-1-(3-phenylpropyl)indolin-2-one (3d)

IR (KBr) (λ_{max} in cm^{-1}): 1640 (NHCO), 1720 (C=O), 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH2), 1.9 (m, 2H, CH2), 3.8 (t, 2H, N-CH_2), 6.91-8.15 (m, 13H, Ar-H), 3.50 (s, 2H, CH_2), 4.18 (s, 1H, OH). LCMS: m/z = 403 $[M]^+$. Analysis: Calcd for $C_{25}H_{22}FNO_3$ (403): C, 74.43; H, 5.50; N, 3.47. Found: C, 74.5; H, 5.5, N, 3.45

3-hydroxy-3-(2-(4-methoxyphenyl)-2-oxoethyl)-1-(3-phenylpropyl)indolin-2-one (3e)

IR (KBr) (λ_{max} in cm^{-1}): 1630 (NHCO), 1710 (C=O), 3500 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH_2), 3.8 (t, 2H, N-CH_2,) 6.9-7.8 (m, 13H, Ar-H), 3.3 (s, 2H, CH_2), 4.24 (s, 1H, OH), 3.83 (s, 3H, OCH_3). LCMS: m/z = 415 $[M]^+$. Analysis: Calcd for $C_{26}H_{25}NO_4$ (415): C, 75.16; H, 6.06; N, 3.37. Found: C, 75.16; H, 6.05, N, 3.4.

4-(2-(3-hydroxy-2-oxo-1-(3-phenylpropyl)indolin-3-yl)acetyl) benzonitrile (3f)

IR (KBr) (λ_{max} in cm^{-1}): 1645 (NHCO), 1720 (C=O), 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH2), 3.8 (t, 2H, N-CH_2,) 6.51-8.11 (m, 13H, Ar-H), 3.78 (s, 2H, CH_2), 4.20 (s, 1H, OH). LCMS: m/z = 411 $[M]^+$. Analysis: Calcd for $C_{26}H_{22}N_2O_3$ (411): C, 76.08; H, 5.40; N, 6.82; O, 11.69. Found: C, 76.07; H, 5.32; N, 6.84; O, 11.67.

5-fluoro-3-hydroxy-3-(2-(naphthalen-2-yl)-2-oxoethyl)-1-(3-phenylpropyl)indolin-2-one (3g)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (NHCO), 1706(C=O), 3500 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 2 (m, 2H, CH_2), 3.5 (t, 2H, N-CH_2), 6.9-8.4 (m, 15H, Ar-H), 3.25 (s, 2H, CH_2), 4.24 (s, 1H, OH). LCMS: m/z = 453 $[M]^+$. Analysis: Calcd for $C_{29}H_{24}FNO_3$ (453): C, 76.80; H, 5.33; N, 3.09. Found: C, 76.81; H, 5.34; N, 3.08.

5-fluoro-3-hydroxy-3-(2-oxo-2-(thiophen-2-yl)ethyl)-1-(3-phenylpropyl)indolin-2-one (3h)

IR (KBr) (λ_{max} in cm^{-1}): 1630 (NHCO), 1710 (C=O), 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH_2), 3.5 (t, 2H, N-CH_2), 6.9-7.9 (m, 11H, Ar-H), 3.75 (s, 2H, CH_2), 4.20 (s, 1H, OH). LCMS: m/z = 409 $[M]^+$. Analysis: Calcd for $C_{23}H_{20}$ FNO_3S (409): C, 67.46; H, 4.92; N, 3.42. Found: C, 67.43; H, 4.95; N, 3.44.

5-flouro-3-hydroxy-3-(2-oxo-2-(pyridin-3-yl)ethyl)-1-(3-phenylpropyl)indolin-2-one (3i)

IR (KBr) (λ_{max} in cm^{-1}): 1670 (NHCO), 1740 (C=O), 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH_2), 3.8 (t, 2H, N-CH_2), 6.9-8.4 (m, 12H, Ar-H), 3.5 (s,2H, CH_2), 4.15 (s, 1H, OH). LCMS: m/z = 404 $[M]^+$. Analysis: Calcd for $C_{24}H_{21}FN_2O_3$ (404): C, 71.27; H, 5.23; N, 6.93. Found: C, 71.29; H, 5.24; N, 6.95.

5-flouro-3-(2-(4-fluorophenyl)-2-oxoethyl)-3-hydroxy-1-(3-phenylpropyl)indolin-2-one (3j)

IR (KBr) (λ_{max} in cm^{-1}): 1640 (NHCO), 1685 (C=O), 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH_2), 3.8 (t, 2H, N-CH_2), 6.91-8.15 (m, 12H, Ar-H), 3.78 (s, 2H, CH_2), 4.20 (s, 1H, OH). LCMS: m/z = 421 $[M]^+$. Analysis: Calcd for $C_{25}H_{21}F_2NO_3$ (421): C, 71.25; H, 5.02; N, 3.3. Found: C, 71.27; H, 5.04; N,3.35.

5-flouro-3-hydroxy-3-(2-(4-methoxyphenyl)-2-oxoethyl)-1-(3-phenylpropyl)indolin-2-one (3k)

IR (KBr) (λ_{max} in cm^{-1}): 1630 (NHCO), 1710 (C=O), 3500 (OH). ^{1}H NMR (400 MHz, $CDCl_3$) $^\delta$(ppm): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH_2), 3.8 (t, 2H, N-CH_2), 6.9-7.8 (m, 12H, Ar-H), 3.03 (s, 2H, CH_2), 4.65 (s, 1H, OH), 3.83 (s, 3H, OCH_3). LCMS: m/z = 435 $[M]^+$. Analysis: Calcd for $C_{26}H_{24}FNO_4$ (435): C, 72.04; H, 5.58; N, 3.23. Found: C, 72.06; H, 5.

5-flouro-4-(2-(3-hydroxy-2-oxo-1-(3-phenylpropyl)indolin-3-yl)acetyl)benzonitrile (3l)

IR (KBr) (λ_{max} in cm^{-1}): 1670 (NHCO); 1740 (C=O); 3523 (OH). ^{1}H NMR (400 MHz, $CDCl_3$): 2.62 (t, 2H, CH_2), 1.9 (m, 2H, CH_2), 3.8 (t, 2H, N-CH_2), 6.9-8.4 (m, 12H, Ar-H), 3.5 (s, 2H, CH_2), 4.15 (s, 1H, OH). LCMS: m/z = 428 $[M]^+$. Analysis: Calcd for $C_{26}H_{21}$ FN_2O_3 (428): C, 72.88; H, 4.94; N,6.54. Found: C, 71.89; H, 4.96; N, 6.55.

General procedure for the synthesis of 3-(2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one derivatives 4(a-l)

To a stirred solution of 3(a-l) (0.91 mmol) in ethanol (15 ml) was added concentrated HCl (5 ml) and the reaction mixture was refluxed for 6h. The progress of the reaction was monitored on TLC using several solvent systems of different polarity. Reaction mixture was filtered, dried and purified by recrystallization from ethanol to obtain the desired product as bright red needles.

3-(2-(naphthalen-2-yl)-2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one (4a)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (NHC=O), 1706 (C=O), 3059 (Ar C-C stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.07-2.79 (m, 2H, CH_2), 2.78 (t, 2H, CH_2), 3.85 (t, 2H, N-CH_2), 6.74 (s, 1H, CH), 7.04-8.6 (m, 16H, Ar-H). LCMS: m/z = 417 $[M]^+$. Analysis: Calcd for $C_{29}H_{23}NO_2$ (417): C, 83.43; H, 5.55; N, 3.35. Found: C, 83.93; H, 5.58; N, 3.39.

3-(2-oxo-2-(thiophen-2-yl)ethylidene)-1-(3-phenylpropyl) indolin-2-one (4b)

IR (KBr)(λ_{max} in cm^{-1}): 1649 (NHC=O), 1711 (C=O), 3080 (Ar C-H stretch). 650 (C-S). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.06 (m, 2H, CH_2), 2.75 (t, 2H, CH_2), 3.82 (t, 2H, N-CH_2), 6.71 (s, 1H. CH), 7.07-8.5 (m, 12H, Ar-H). LCMS: m/z = 373 $[M]^+$. Analysis: Calcd for $C_{29}H_{19}NO_2S$ (373): C, 73.97; H, 5.13; N, 3.75. Found: C, 73.97; H, 5.14, N, 3.79.

3-(2-oxo-2-(pyridin-3-yl)ethylidene)-1-(3-phenylpropyl) indolin-2-one (4c)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (R-C=O), 1706 (C=O), 3018 (Ar C-H stretch), 1608 (C=N).

^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.05 (m, 2H, CH_2), 2.75 (t, 2H, CH_2), 3.81 (t, 2H, N-CH_2), 6.72 (s, 1H, CH_2) 7.03-8.85 (m, 13H, Ar-H). LCMS: m/z = 368 $[M]^+$. Calcd for $C_{24}H_{20}N_2O_2$ (368): C, 78.24; H, 5.47; N, 6.70. Found: C, 78.28; H, 5.57; N, 6.72.

3-(2-(4-fluorophenyl)-2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one (4d)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (NHC=O), 1706 (C=O), 3054 (Ar C-H stretch), 844 (C-F stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.08 (m, 2H, CH_2), 2.75 (t, 2H, CH_2), 3.82 (t, 2H, N-CH_2), 7.1 (s, 1H, CH), 7.3-7.6 (m, 13H, Ar-H). LCMS: m/z = 385 $[M]^+$. Calcd for $C_{25}H_{20}FNO_2$ (385): C, 77.90; H, 5.23; N, 3.63. Found: C, 77.92; H, 5.25; N, 3.69.

3-(2-(4-methoxyphenyl)-2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one (4e)

IR (KBr) (λ_{max} in cm^{-1}): 1655 (NHC=O), 1706 (C=O), 3059(Ar C-H stretch), 1598 (C=C stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.09 (m, 2H, CH_2), 2.75 (t, 2H, CH_2), 3.82 (t, 2H, N-CH_2), 3.92 (s, 3H. OCH_3), 6.72 (s, 1H, CH), 7.27-8.1 (m, 13H, Ar-H). LCMS: m/z = 397 $[M]^+$. Analysis: Calcd for $C_{26}H_{23}NO_3$ (397): C, 78.57; H, 5.83; N,3.52. Found: C, 78.59; H, 5.85, N, 3.54.

4-(2-(2-oxo-1-(3-phenylpropyl)indolin-3-ylidene)acetyl) benzonitrile (4f)

IR (KBr) (λ_{max} in cm^{-1}): 1650 (NHC=O), 1710 (C=O), 3090 (Ar C-H stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.06 (m, 2H, CH_2), 2.74 (t, 2H, CH_2), 3.81 (t, 2H, N-CH_2), 7.75 (s,1H, CH), 6.9-8.1 (m, 13H, Ar-H). LCMS: m/z = 392 $[M]^+$. Analysis: Calcd for $C_{26}H_{20}N_2O_2$ (392): C, 79.57; H, 5.14; N, 7.14. Found: C, 79.59; H, 5.15; N, 7.16.

5-flouro-3-(2-(naphthalen-2-yl)-2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one (4g)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (NHC=O), 1711 (C=O), 3059 (Ar C-H stretch), 1678(C=C stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.09 (m, 2H, CH_2), 2.74 (t, 2H, CH_2), 3.8 (t, 2H, N-CH_2), 6.6 (s, 1H, CH), 7.27-8.5 (m, 15H, Ar-H), LCMS: m/z = 435 $[M]^+$. Analysis: Calcd for $C_{29}H_{22}FNO_2$ (435): C, 79.98; H, 5.09; N, 3.22. Found: C, 79.99; H, 5.10; N, 3.25.

5-fluoro-3-(2-oxo-2-(thiophen-2-yl)ethylidene)-1-(3-phenylpropyl)indolin-2-one (4h)

IR (KBr) (λ_{max} in cm^{-1}): 1649 (NHC=O), 1706 (C=O), 3090 (Ar C-H stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.06 (m, 2H, CH_2), 2.74 (t, 2H, CH_2), 3.81 (t, 2H, N-CH_2), 6.63 (s, 1H, CH), 7.07-8.4 (m, 11H, Ar-H). LCMS: m/z = 391 $[M]^+$. Analysis: Calcd for $C_{23}H_{18}FNO_2S$ (391): C, 70.58; H, 4.63; N, 3.58. Found: C, 70.59; H, 4.65; N, 3.60.

5-flouro-3-(2-oxo-2-(pyridin-3-yl)ethylidene)-1-(3-phenylpropyl) indolin-2-one (4i)

IR (KBr) (λ_{max} in cm^{-1}): 1660 (R-C=O), 1706 (C=O), 3018 (Ar C-H stretch), 1608 (C=N). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.02 (m, 2H, CH_2), 2.74 (t, 2H, CH_2), 3.79 (t, 2H, N-CH_2), 6.7 (s, 1H. CH), 7.03-8.85 (m, 12H, Ar-H). LCMS: m/z = 386 $[M]^+$. Analysis: Calcd for $C_{24}H_{19}FN_2O_2$ (386): C, 74.60; H, 4.96; N, 7.25. Found: C, 74.64; H, 4.99; N,7.28.

5-fluoro-3-(2-(4-fluorophenyl)-2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one (4j)

IR (KBr) (λ_{max} in cm^{-1}): 1665 (NHC=O), 1713 (C=O), 3070 (Ar C-H stretch). 699 (C-F). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.02 (m, 2H, CH_2), 2.6 (t, 2H, CH_2), 3.79 (t, 2H, N-CH_2), 7 (s, 1H, CH), 7.2-7.8 (m, 12H, Ar-H). LCMS: m/z = 403 $[M]^+$. Analysis: Calcd for $C_{25}H_{19}F_2NO_2$ (403): C, 74.34; H, 4.75; N, 3.47. Found: C, 74.38; H, 4.71; N, 3.48.

5-flouro-3-(2-(4-methoxyphenyl)-2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one (4k)

IR (KBr) (λ_{max} in cm^{-1}): 1655 (NHC=O), 1700 (C=O), 3020 (Ar C-H stretch), 690 (C-F). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.09 (m, 2H, CH_2), 2.75 (t, 2H, CH_2), 3.7 (t, 2H, N-CH_2), 6.7 (s, 1H, CH), 7.27-8.1(m, 12H, Ar-H), 3.83(s, 3H, OCH_3). LCMS: m/z = 415 $[M]^+$. Analysis: Calcd for $C_{26}H_{22}FNO_3$ (415): C, 75.17; H, 5.34; N, 3.37. Found: C, 75.17; H, 5.34; N, 3.37.

5-flouro-4-(2-(2-oxo-1-(3-phenylpropyl)indolin-3-ylidene)acetyl)benzonitrile (4l)

IR (KBr) (λ_{max} in cm^{-1}): 1650 (NHC=O), 1710 (C=O), 3090 (Ar C-H stretch). ^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 2.06 (m, 2H, CH_2), 2.74 (t, 2H, CH_2), 3.81 (t, 2H, N-CH_2), 6.72 (s, 1H, CH), 6.9-8.1 (m, 12H, Ar-H). LCMS: m/z = 410 $[M]^+$. Analysis: Calcd for $C_{26}H_{19}FN_2O_2$ (410): C, 76.08; H, 4.67; N, 6.83. Found: C, 76.08; H, 4.67; N, 6.83.

Biological activities

Antimicrobial activity

The antibacterial activities of compounds 4(a-l), were carried out using the Cup plate diffusion method [25]. This method depends on the diffusion of the antibiotic from a cavity through the solidified agar layer in a petri dish to an extent such that the growth of the added microorganism is prevented in a circular zone around the cavity containing a solution of the antibiotic. For antibacterial activity, antibacterial species used are two Gram negative species, *Escherichia coli* (ATCC 9637), *Salmonella typhi* (ATCC 6539) and two Gram-positive species, *Bacillus subtilis (*ATCC 6633), *Staphylococcus aureus* (ATCC 29737). Two fungal strains *Aspergillus niger* (ATCC 16509), *Aspergillus fumigates (*ATCC16406) were used for antifungal activity. Solution of each compound at a concentration of 1000µg/ml in DMSO was prepared and the inhibition zone diameter in millimeter was used as the criterion for measuring the microbial activity after 24h for bacteria and 72h for fungi. Ciprofloxacin is used as bacterial standards and Amphotericin B is used as fungal standards for references to evaluate the efficacy of the tested compounds under the same conditions. DMSO used as control and solvent to prepare compound solutions. Measurements of results are shown in table 2

Table 1: Physical Properties of 3-(2-oxoethylidene)-1-(3-phenylpropyl)indolin-2-one derivatives 4(a-l)

Comp. No	R	R_1	M. For.	M. Wt.	% Yield	M. P. °C
4a	H		$C_{29}H_{23}NO_2$	417	67%	149-51
4b	H		$C_{23}H_{19}NO_2S$	373	60%	172-73
4c	H		$C_{24}H_{20}N_2O_2$	368	63%	160-62
4d	H		$C_{25}H_{20}FNO_2$,	385	67%	173-75
4e	H		$C_{26}H_{23}NO_3$	397	67%	146-47
4f	H		$C_{26}H_{20}N_2O_2$	392	58%	187-89
4g	F		$C_{29}H_{22}FNO_2$	436	83%	204-05
4h	F		$C_{23}H_{18}FNO_2S$	391	62%	154-56
4i	F		$C_{24}H_{19}FN_2O_2$,	386	63%	210-12
4j	F		$C_{25}H_{19}F_2NO_2$	403	56%	194-95
4k	F		$C_{26}H_{22}FNO_3$	415	64%	140-42
4l	F		$C_{26}H_{19}FN_2O_2$	410	61%	197-99

Comp. No.–Compound number. M. for.-Molecular formula, M. wt.-Molecular weight, M. pt.-Melting point.

Antioxidant activity assay

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

The free radical scavenging activity (RSA) of all the compounds at concentrations of 25, 50, 75 and 100 µg/ml was carried out in the presence of a freshly prepared solution of stable free radical DPPH (0.04% w/v) following a Hatano's method [26,27]. Ascorbic acid (AA) is used as standards. All the test analyses were performed on three replicates and the results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence of test compounds and absorption of DPPH in the absence of test compounds at λ 517 nm on ELICO SL 171 Mini Spec, spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation:

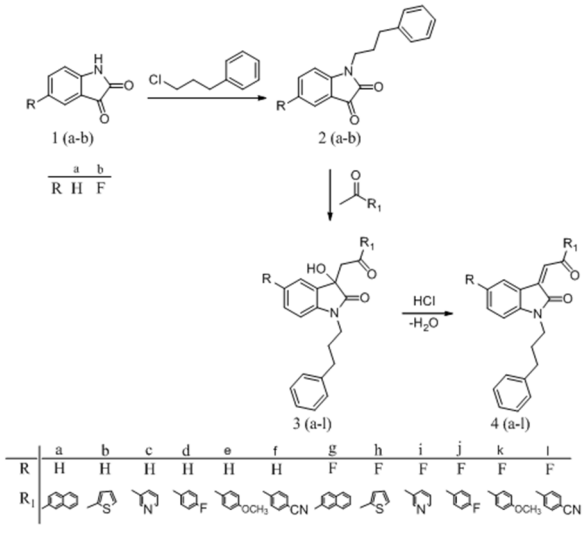
$$\% \text{ of DPPH RSA} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 10$$

RESULTS AND DISCUSSION

Chemistry

The Synthesis of title compounds was on account of the biological activity of indole and was carried out using a general, simple and straight forward pathway. 5-Substituted-1*H*-indole-2,3-dione was used as basic material for the synthesis of resultant derivatives. The treatment of 5-substituted 1*H*-indole-2,3-dione and (3-chloropropyl) benzene in *N*,*N*-Dimethyl formamide with K_2CO_3 yield substituted *N*-phenyl propyl isatins 2(a-b). These on condensation with acetphenone analogues resulted into compounds 3(a-l) which on dehydration yielded compounds 4(a-l) (Scheme 1).

The structure elucidation of the final products was carried out by IR, ^{1}H-NMR and Mass spectral data. IR peaks of the compound were recognized from 1700-1720 cm^{-1} for C=O stretching, 1640-1660 $cm^{-1}$for NHCO stretching, 3075-2850 cm^{-1} for C-H aliphatic and aromatic correspondently, some stretching bands were also found for C=C at1575-1490 cm^{-1}. In ^{1}H-NMR spectra typical proton signals for C-H aliphatic and aromatic were observed between δ 2.36-3.68, and δ 8.06-6.30 respectively.



Scheme 1: Synthesis of compounds 3 (a-l) and 4 (a-l)

Table 2: Antibacterial activity, size of inhibition zone (mm) formed at concentration 1000 µg/ml of synthesized compounds 4(a-l)

Zone of inhibition in mm						
Compound	**Antibacterial activity**				**Antifungal activity**	
	Gram positive		**Gram negative**			
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Salmonella typhi***	***Aspergillus fumigates***	***Aspergillus niger***
4a	32±.47	31.5±.23	31.8±.13	34.5±.23	30.8±.38	25.6±.24
4b	31.6±.27	32.96±.12	30.66±.27	33±.27	31±.471	22.2±.34
4c	22±.47	28.6±.27	----------	28.77±.32	32.53±.24	30.76±.32
4d	20.67±.54	----------	22.83±.13	20.33±.272	33.4±.169	29.33±.27
4e	34.6±.27	32.56±.24	33.66±.272	33.5±.23	34.9±.42	27.66±.45
4f	32±.47	28.2±.16	25.26±.15	25.73±.30	32.33±.27	28.66±.27
4g	24.6±.27	22.8±.13	20.66±.27	----------	----------	21.83±.36
4h	21.33±.27	21.7±.32	21.33±.27	----------	22.5±.235	19.6±.25
4i	19±.47	21.33±.27	----------	----------	29.5±.235	22.33±.27
4j	18.33±.27	19.5±.23	----------	----------	33.86±.38	21.56±.24
4k	19.34±.47	21.6±.25	15.5±.23	----------	31.63±.25	27±.47
4l	17.66±.28	24.5±.24	19.8±.36	17±.09	26.76±.32	24.76±.32
Ciproflaxcin	40	34	37	39		
AmphotericinB					40	38

No activity =----------, Note: Values are expressed in mean±SD (n=3)

Antimicrobial activity

The results of antimicrobial activities revealed that the synthesized compounds having H in the 5th position of isatin ring and compounds with aromatic substitution at the R_1 have shown good activity when compared with the synthesized compounds having F at the 5th position. Moreover, when the aromatic ring has electronegative atom either in the ring or as a substituent, such compounds were found to be less active. Highest activity is shown by a aromatic ring with methoxy as a substituent.

All the final synthesized derivatives were taken for preliminary screening to evaluate antibacterial activity by cup plate method, in the nutrient agar medium against two gram-positive and two gram-negative bacterial strains at concentration of 1000 µg/ml. The zone of inhibition (mm) of each derivative was ascertained and compared with Ciprofloxacin taken as standard drug for antibacterial activity. DMSO was used to prepare stock solutions of test compounds. The findings of antibacterial evaluation revealed that most of the compounds have variable activity against bacterial strains. Compounds 4a, 4b, 4e and 4f were the active compounds which exhibited excellent activity against the bacteria in comparison to standard drug Ciprofloxacin. 4e was found to exhibit excellent activity against bacterial strains. All the final compounds were examined for antifungal activity using cup plate method, in the agar medium against two pathogenic fungal strains. The area of inhibition (mm) of each derivative was ascertained and compared with Amphotericin B standard drug. The compounds 4c, 4d, 4e, 4f and 4j were found to be active against the fungal strains used. All the synthesized products were found to be active against *A. fumigatus* than *A. Niger*. However none of the compounds exhibited zone of inhibition more than that of standard.

Antioxidant activity

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity (RSA)

In vitro method of scavenging of the stable DPPH radical is extensively used to evaluate the antioxidant activity in less time than other methods. DPPH is a stable free radical that can accept hydrogen radical or an electron and must thus be converted to a stable diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons or hydrogen atoms taken up. The DPPH antioxidant assay measures the hydrogen donating capacity of the molecules under study. When the free-radical DPPH is reduced by the sample, its colour changes from violet to yellow. Based on the structure activity relationship, it is indicated that the presence of substitution at the 5thposition of isatin ring and on the side chain influences the antioxidant potency of the molecule. Halogen substitution at position 5 of isatin ring exhibited good antioxidant activity and aromatic substitution at R_1 with electronegative atom in the ring or as a substituent also showed good activity. Compounds with H substitution on isatin ring were found to exhibit moderate antioxidant activity. Among the synthesized compounds 4c, 4d, 4i and 4j were found to be more potent. Results are given in fig. 1.

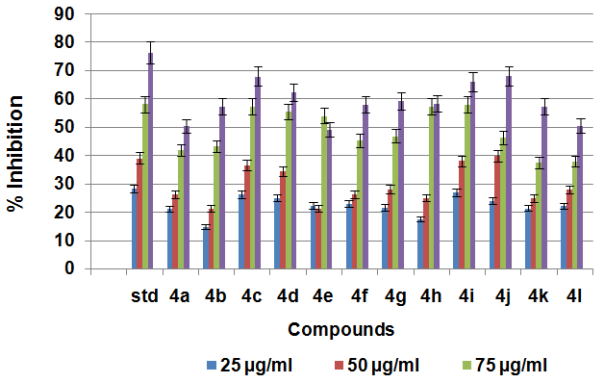


Fig. 1: DPPH radical scavenging activity of synthesized compounds at Conc. 25, 50, 75, 100 µg/ml, The graph represents the mean±SEM, (n=3), P<0.01-significant compared to the standard group

CONCLUSION

A series of twelve compounds novel 5-substituted *N*-phenylpropyl-3-substituted indoline-2-one derivatives 4(a-l) was prepared and characterized by TLC, M. P, spectral and analytical data. All the synthesized compounds were evaluated for *in vitro* antimicrobial activity and antioxidant activity against different bacterial and fungal strains. Compounds 4a, 4b, 4e and 4f were highly active against gram positive and gram negative bacteria, 4e is found to be more potent against all the bacterial strains. Compounds 4c, 4d, 4e, 4f, 4j and 4j exhibited potent antifungal activity and 4c, 4d, 4i and 4j exhibited good antioxidant activity. All the experiments were found in triplicate and the mean were calculated.

AUTHORS CONTRIBUTION

All the authors have contributed in various degrees to commencement, design, acquisition of data, analysis, interpretation of data and writing present article.

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CONFLICT OF INTERESTS

Declared none

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