

EVALUATION OF NEWLY DESIGNED ISATIN DERIVATIVES AGAINST VARIOUS BACTERIAL SPECIES

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ABSTRACT

Objective: The main objective of the present research is to develop new isatin derivatives and evaluate their antibacterial properties.**Methods:** In this research article, some new spiro derivatives of isatin were synthesized by two different pathways. The chalcones were prepared by the reaction of different acetophenones and isatins through base-catalyzed condensation followed by the addition of acid. The compounds have been characterized by UV-Vis, FT-IR, ¹H- Nuclear magnetic resonance (NMR), ¹³C-NMR, and Mass spectra.**Results:** It was found that the compounds exhibited moderate to significant antibacterial action against four various bacterial strains, that is, Gram-negative *Klebsiella pneumoniae* and *Escherichia coli*, Gram-positive *Bacillus subtilis*, and *Staphylococcus aureus*. The action of all compounds was compared with standard drug, that is, Streptomycin.**Conclusion:** It was concluded that all synthesized isatin derivatives are useful for giving antibacterial responses for one or other bacterial strains. Their action was compared with the standard drug and found that ISP1 showed a response that was almost the same as streptomycin's effects against *Salmonella typhi*.**Keywords:** Isatin and its derivatives, Antibacterial action, Streptomycin, Zone of inhibition.© 2023 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2023v16i3.46609>. Journal homepage: <https://innovareacademics.in/journals/index.php/ajpcr>

INTRODUCTION

Isatin is one of the heterocyclic compounds and has atoms of a minimum of two different elements as members of its ring, making it a cyclic structure compound. Although many heterocyclic compounds are mostly inorganic substances and a few organic substances. Most inorganic substances contain one carbon atom, and one or more atoms other than carbon elements inside the ring structure, such as oxygen, sulfur, or nitrogen. Inorganic chemistry has many heteroatoms, that is, different from carbon and hydrogen [1].

Isatin (1H-indole-2,3-Dione) is a synthetically skilled substrate that can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis. Isatin has also been found in mammalian tissues, and its function as a modulator of biochemical processes has been the subject of several discussions [2]. The compound was first incurred by Erdman [3] and Auguste [4] in the year 1840 by oxidation of indigo dye by nitric acid and chromic acids.

It is a natural product that is found in various plants isatis [5] and humans, as a metabolic derivative of adrenaline [6]. It is proven that isatin derivatives are known to be associated with a broad spectrum of biological activity such as anti-inflammatory [7], antimicrobial, anticonvulsant activities [8], antioxidant [9], analgesic [10], and antianxiety [11]. The chemistry of various derivatives of isatin and isatin itself is having prospective applications in pharmaceutical chemistry. Isatins are very important compounds due to their antimicrobial properties. At millimolar concentration, isatin has been found to inhibit different enzymes, an effect that may contribute to its anti-infective actions [8].

It also suppressed the production of prostaglandin E2 (PGE2) and TNF- α . The suppression of nitric oxide (NO) and PGE2 production in LPS-INF- γ which is linked with the isatin effect on the manifestation

of inducible cyclooxygenase-2 and NO synthase, respectively, as it has been exhibited by a Western blot analysis. Isatins can cross the blood brain barrier. Isatin, a heterocyclic compound, identified in animals as a major component of the endogenous monoamine oxidase inhibitor [12]. It can be synthesized using various methods such as Sandmeyer methodology [13], Martinet isatin synthesis [14], and Stolle procedure [15]. The study's main aim is to synthesize various isatin derivatives and evaluate their antibacterial activities by comparing responses with the standard drug Streptomycin, with the zone of inhibition of all isatin derivatives and find which one will show maximum effects among all compounds.

MATERIALS AND METHODS

Chemical

Procurement of chemicals is from S. R. College of Pharmacy, Kakatiya University, Warangal, India. The chemicals used are acetophenone, dimethylamine, concentrated HCl, glacial acetic acid, hydrazine hydrate, ethanol, 5-bromoisatin, N-benzyl-isatin, streptomycin, p-chloroacetophenone, p-bromoacetophenone, p-bromoacetophenone, p-methoxy-acetophenone, DMSO, etc.

Instruments

The various techniques utilized for the characterization of the synthesized compounds were Mass spectra, IR spectra, or ¹H- nuclear magnetic resonance (NMR) spectra.

Infrared spectra

The IR spectra of the synthesized compounds were recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400–4000 using KBr pellets and the values of ν_{max} are reported in cm^{-1} .

1H-NMR

¹H-NMR spectra were recorded on DMM X - 200 MHz NMR, Bruker Daltonics, Karlsruhe, Germany using DMSO, and chemical shifts were

reported in parts per million (d ppm) with tetramethylsilane as an internal standard.

Inoculation requirements

Materials

The Petri dishes, syringes, cork borers, pipettes, and conical flasks, etc., were cleaned by a suitable cleansing agent and sterilized at 121°C and 15 lb/inch² for 15 min.

Microorganisms used

The standard cultures of Gram-negative *Klebsiella pneumoniae* and *Escherichia coli*, Gram-positive *Bacillus subtilis*, and *Staphylococcus aureus* species were obtained from the Department of Microbiology, S. R. College of pharmacy.

Stock solution

The stock solutions of newly synthesized test compounds and standard streptomycin were prepared in dimethyl sulfoxide at a concentration of 100 µg/0.1 mL.

Culture medium

Beef extract (10 g), Peptone (10 g), Agar (M-173) (20 g), Distilled water (1 L), pH (7.0–7.5). The above-mentioned quantities of different ingredients were accurately weighed, dissolved in the appropriate amounts of distilled water, and sterilized at 121°C and 15 lb/inch² for 45 min.

Stock culture

The hot solution of the culture medium was transferred into test tubes in 10 mL portions. The tubes were plugged with cotton and sterilized at 121°C and 15 lb/inch² for 15 min. The tubes were cooled in a slant position and incubated at 37°C for 2 days. Afterward, they were observed: if the tubes were contaminated with microorganisms, the tubes were rejected, and the experiment was repeated until there was no contamination.

The stab culture was made in three tubes, incubated at 30–34°C for 18–24 h, and stored in the refrigerator. One tube was set aside as stock culture and the others were used for inoculation.

Inoculums

A volume of 3 mL sterile water was added aseptically into stab culture, shaken for 10 s, and the liquid was decanted aseptically into another sterile test tube. The resulting cell suspension was used as inoculums.

Method of testing

A volume of 25 mL of sterile hot agar medium was poured into each plate and allowed to harden on a level surface. The agar plates were inoculated with 24 h test cultures by spreading uniformly with sterile cotton swabs. The compounds were screened for antibacterial activity against *S. aureus*, *K. pneumoniae*, *E. coli*, and *B. subtilis* in a nutrient agar medium. The plates were then allowed to dry, then placed in the inverted position in an incubator for 30 min. Afterward, they were removed and the bore was made on the medium using a sterile borer of 5 mm diameter. A volume of 0.1 mL of test solution was added to respective bores. Streptomycin at 100 µg/0.1 mL concentration was taken as the standard reference. A control having only DMSO in the cup was maintained on each plate. The Petri plates were kept in the refrigerator at 4°C for 15 min for diffusion to take place. Afterward, they were incubated at 37°C for 24 h, and zones of inhibition were observed and measured using a scale. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded [16].

Methods

Physical data

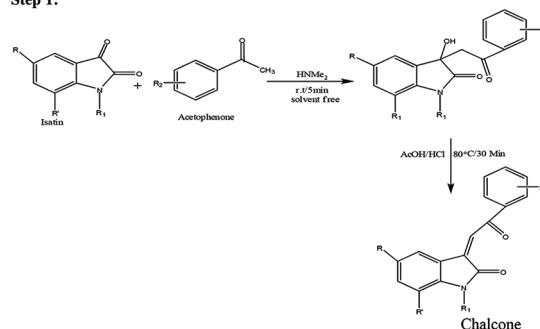
Melting points of the synthesized compounds were taken in open capillaries on the thermionic melting point apparatus and are corrected.

Thin layer chromatography (TLC)

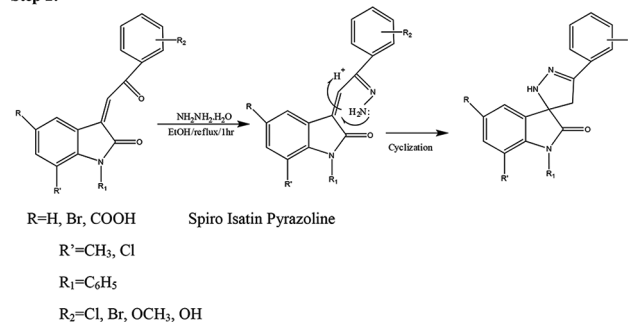
The purity of the compounds was checked by TLC using silica gel G as the stationary phase and various combinations of Chloroform: Ethyl acetate as the mobile phase. The spots resolved were visualized using iodine and ultra-violet chamber.

Scheme of works

Step 1:



Step 2:



Procedure for the synthesis of isatin chalcone

To a solid homogenous mixture of 10 mmol isatin and 10 mmol acetophenones, 10 drops of dimethylamine were added, stirred for 15–30 min, and a colorless solid formed. 20 mL glacial acetic acid and five drops of concentrated HCl were added to this precipitate and the mixture was warmed at 80°C for 30 min after dehydration, and chalcones were produced [17].

Physical data

Molecular formula: C₁₆H₁₁NO₂

Molecular weight: 249

Solubility: Ethanol

Yield: 87%

Melting point: 177–180°C.

Synthesis of 5'-Phenyl-2',4'-dihydrospiro[indol-3,3'-pyrazol]-2(1H)-one (ISP1)

The chalcones were refluxed with 11 mmol hydrazine hydrate in 20 mL absolute ethanol for 1 h to give corresponding spiro derivatives 5a-h (Scheme 1). In the second way or one-pot procedure, the chalcones did not separate and 11 mmol of hydrazine hydrate was added to the acidic solution of the chalcone and reaction continued at 70–80°C for 1 h and spiro compounds were formed in high yields [17].

Physical data of 5'-Phenyl-2',4'-dihydrospiro[indol-3,3'-pyrazol]-2(1H)-one

Molecular formula: C₁₆H₁₄N₃O

Molecular weight: 264.3

Solubility: Ethanol

Yield: 84%

Melting point: 198–200°C

Mobile phase: Ethyl acetate: Chloroform (1:1)
 Rf value: 0.5
 I.R Spectrum: 3479 (N-H of isatin), 3272 (N-H of pyrazoline), 1701 (C=O), 1613 (C=N of pyrazoline ring)
¹H-NMR (δ): 3.43 (1H, d, CH₂), 3.72 (1H, d, CH₂), 6.20 (1H, s, N-H of pyrazoline)
 6.92–8.00 (9H, m, Ar-H), 9.16 (1H, s, NH of isatin)
¹³C-NMR (δ): 45.1, 70.3 (2C, spiro carbons), 111.0–140 (13C, Ar-C) 151.1 (C=N of pyrazoline ring), 180.3 (1C, C=O).
 Mass Spectra (m/z): 265 (M+1).

General procedure for synthesizing isatin derivatives

To a solid homogenous mixture of 10 mmol isatins, namely, N-benzyl isatin, 5-Bromo isatin, 7-methyl isatin, 7-chloroisatin, 5-carboxyl isatin, and 10 mmol acetophenones, namely, P-chloroacetophenone, P-hydroxy-acetophenone, P-methoxy-acetophenone, 10 drops of dimethylamine, and P-bromoacetophenone were added, and then, the mixture was stirred for 15–30 min which formed colorless solid. 20 mL glacial acetic acid and five drops of concentrated HCl were added to this precipitate and the mixture was warmed at 80°C for 30 min, after dehydration, respective chalcones were produced. These chalcones were refluxed with 11 mmol hydrazine hydrate in 20 mL absolute ethanol for 1 h to give corresponding spiro derivatives [18-27].

Physical data of 5'-(4-chlorophenyl)-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-2)

Molecular formula: C₁₆H₁₂ClN₃O
 Molecular weight: 297
 Solubility: Ethanol
 Yield: 87.4%
 Melting point: 223–225°C
 Mobile phase: Ethylacetate: Chloroform (1:1)
 Rf value: 0.62
 I.R Spectrum: 3461 (N-H of isatin), 3279 (N-H of pyrazoline), 1712 (C=O), 1609 (C=N of pyrazoline ring)
¹H-NMR (δ): 3.43 (1H, d, CH₂), 3.58 (1H, d, CH₂), 6.33 (1H, s, N-H pyrazoline)
 6.92–7.75 (8H, m, Ar-H), 9.32 (1H, s, NH of isatin)
¹³C-NMR (δ): 44.1, 70.1 (2C, spiro carbons), 110.05–141.7 (13C, Ar-C), 147.5 (C=N of Pyrazoline), 178.55 (C=O)
 Mass Spectra (m/z): 298 (M+1).

Physical data of 1-benzyl-5'-phenyl-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-3)

Molecular formula: C₂₃H₁₉N₃O
 Molecular weight: 353
 Solubility: Ethanol
 Yield: 63%
 Melting point: 152–155°C
 Mobile phase: Ethylacetate: Chloroform (1:1)
 Rf value: 0.55
 I.R Spectrum: 3476 (N-H of isatin), 3276 (N-H of pyrazoline), 1706 (C=O), 1603 (C=N of pyrazoline ring)
¹H-NMR (δ): 3.44 (1H, d, CH₂), 3.55 (1H, d, CH₂), 7 (1H, s, N-H of pyrazoline)
 6.9–7.6 (8H, m, Ar-H), 10.6 (1H, s, NH of isatin), 4.6 (2H, s, CH₂)
¹³C-NMR (δ): 44, 62.4 (2C, spiro carbons), 51.7 (1C, CH₂), 117–136.1 (13C, Ar-C),

151.7 (1C, C=N of Pyrazoline ring), 171.2 (1C, C=O).
 Mass Spectra (m/z): 354 (M+1).

Physical data of 5'-(4-hydroxyphenyl)-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-4)

Molecular formula: C₁₆H₁₃N₃O₂
 Molecular weight: 279
 Solubility: Ethanol
 Yield: 71%
 Melting point: 240–243°C
 Mobile phase: Ethylacetate: Chloroform (1:1)
 Rf value: 0.52
 I.R Spectrum: 3436 (N-H of isatin), 3386 (N-H of pyrazoline), 1704 (C=O), 1615 (C=N of pyrazoline ring), 3456 (OH stretch)
¹H-NMR (δ): 3.43 (1H, d, CH₂), 3.76 (1H, d, CH₂), 7 (1H, s, N-H of pyrazoline)
 6.8–7.8 (8H, m, Ar-H), 10.6 (1H, s, NH of isatin), 4.6 (2H, s, CH₂)
 9.4 (1H, s, OH)
¹³C-NMR (δ): 43.7, 64.6 (2C, spiro carbons), 116–149.2 (13C, Ar-C), 151.7 (1C, C=N of Pyrazoline ring), 172.7 (1C, C=O)
 Mass Spectra (m/z): 280 (M+1).

Physical data of 5-bromo-5'-phenyl-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-5)

Molecular formula: C₁₆H₁₂BrN₃O
 Molecular weight: 341
 Solubility: Ethanol
 Yield: 83%
 Melting point: 232–234°C
 Mobile phase: Ethylacetate: Chloroform (1:1)
 Rf value: 0.59
 I.R Spectrum: 3467 (N-H of isatin), 3292 (N-H of pyrazoline), 1704 (C=O), 1619 (C=N of pyrazoline ring), 3456 (OH stretch)
¹H-NMR (δ): 3.42 (1H, d, CH₂), 3.58 (1H, d, CH₂), 6.36 (1H, s, NH of pyrazoline)
 6.92–7.68 (8H, m, Ar-H), 9.32 (1H, s, NH of isatin)
¹³C-NMR (δ): 44.1, 70.2 (2C, spiro carbons), 51.7 (1C, CH₂), 110–141.8 (13C, Ar-C), 147.5 (1C, C=N of Pyrazoline ring), 179.4 (1C, C=O)
 Mass Spectra (m/z): 342 (M+1).

Physical data of 5'-(4-methoxyphenyl)-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-6)

Molecular formula: C₁₇H₁₅O₂N₃
 Molecular weight: 292
 Solubility: Ethanol
 Yield: 86%
 Melting point: 204–206°C
 Mobile phase: Ethylacetate: Chloroform (1:1)
 Rf value: 0.69
 I.R Spectrum: 3438 (N-H of isatin), 3391 (N-H of pyrazoline), 1710 (C=O), 1617 (C=N of pyrazoline ring), 1301 (OCH₃)
¹H-NMR (δ): 3.42 (1H, d, CH₂), 3.72 (1H, d, CH₂), 3.8 (3H, s, OCH₃)
 6 (1H, s, NH of pyrazoline), 6–7.3 (8H, m, Ar-H), 8 (1H, s, NH of isatin)
¹³C-NMR (δ): 43.9, 69.4 (2C, spiro carbons), 51.7 (1C, CH₂), 109–147.4 (13C, Ar-C)
 159 (1C, C=N of Pyrazoline ring), 178.7 (1C, C=O), 55.1 (1C, OCH₃)
 Mass Spectra (m/z): 293 (M+1).

Physical data of 5'-(4-bromophenyl)-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-7)Molecular formula: $C_{16}H_{12}BrN_3O$

Molecular weight: 341

Solubility: Ethanol

Yield: 88%

Melting point: 235–238°C

Mobile phase: Ethylacetate: Chloroform (1:1)

Rf value: 0.58

I.R Spectrum: 3439 (N-H of isatin), 3296 (N-H of pyrazoline), 1705 (C=O), 1615

(C=N of pyrazoline ring)

 1H -NMR (δ): 3.43 (1H, d, CH_2), 3.75 (1H, d, CH_2), 7 (1H, s, N-H of pyrazoline),

7.1–7.7 (8H, m, Ar-H), 10.6 (1H, s, NH of isatin)

 ^{13}C -NMR (δ): 43.7, 64.6 (2C, spiro carbons), 115–149.2 (13C, Ar-C) 151.7 (1C,

C=N of Pyrazoline ring), 172.7 (1C, C=O)

Mass Spectra (m/z): 342 (M+1).

Physical data of 7-methyl-5'-phenyl-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-8)Molecular formula: $C_{17}H_{15}N_3O$

Molecular weight: 277

Solubility: Ethanol

Yield: 73%

Melting point: 222–225°C

Mobile phase: Ethylacetate: Chloroform (1:1)

Rf value: 0.56

I.R Spectrum: 3436 (N-H of isatin), 3283 (N-H of pyrazoline), 1710 (C=O), 1618

(C=N of pyrazoline ring), 2865 (CH stretch in CH_3) 1H -NMR (δ): 3.43 (1H, d, CH_2), 3.76 (1H, d, CH_2), 3.44 (3H, s, CH_3) 7 (1H, s, NH of pyrazoline), 7–7.6 (8H, m, Ar-H), 10.6 (1H, s, NH of isatin) ^{13}C -NMR (δ): 43.7, 69.4 (2C, spiro carbons), 17.3 (1C, CH_3), 120–136.4 (13C, ArC),

151.7 (1C, C=N of Pyrazoline ring), 172.7 (1C, C=O)

Mass Spectra (m/z): 278 (M+1).

Physical data of 7-chloro-5'-phenyl-2',4'-dihydrospiro[indoline-3,3'-pyrazol]-2-one (ISP-9)Molecular formula: $C_{16}H_{12}ClN_3O$

Molecular weight: 297

Solubility: Ethanol

Yield: 69%

Melting point: 215–217°C

Mobile phase: Ethylacetate: Chloroform (1:1)

Rf value: 0.63

I.R Spectrum: 3435 (N-H of isatin), 3253 (N-H of pyrazoline), 1708 (C=O), 1618

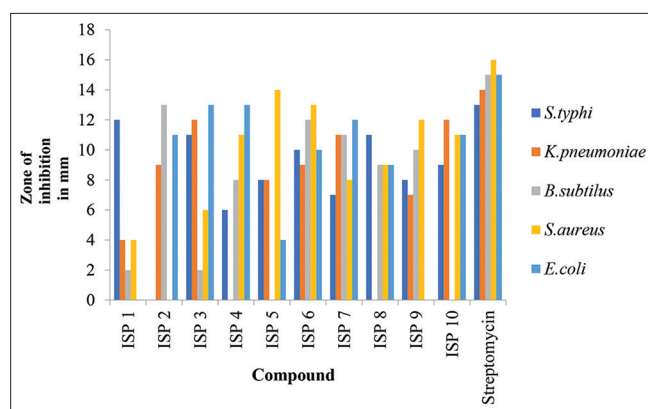
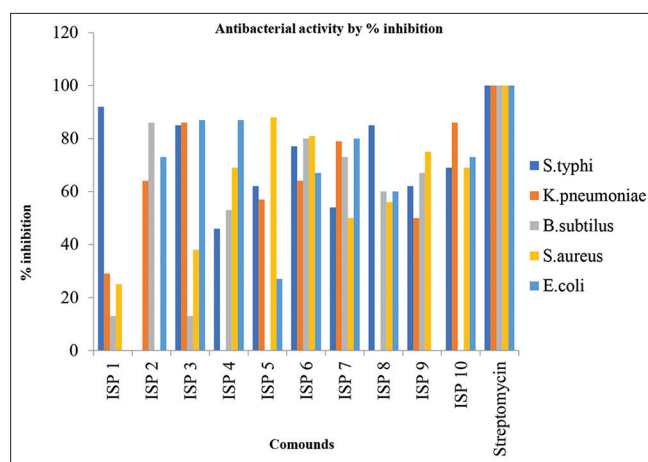
(C=N of pyrazoline ring)

 1H -NMR (δ): 3.44 (1H, d, CH_2), 3.76 (1H, d, CH_2), 7(1H,s,N-H of pyrazoline),7–7.6 (8H, m, Ar-H), 10.6 (1H, s, NH of isatin) ^{13}C -NMR (δ):43.7, 64.1 (2C, spiro carbons), 121–150 (13C, Ar-C), 151.7 (1C, C=N of pyrazoline ring), 172.7 (1C, C=O)

Mass Spectra (m/z): 298 (M+1).

Physical data of 2-oxo-5'-phenyl-2',4'-dihydrospiro[indoline-3,3'-pyrazole]-5-carboxylic acid (ISP-10)Molecular formula: $C_{17}H_{13}N_3O_3$

Molecular weight: 307

**Fig. 1: Antibacterial activity by a zone of inhibition****Fig. 2: Graph representing percentage zone of inhibition by taking readings of above Table 3****Fig. 3: Photograph of antibacterial activity by a zone of inhibition**

Solubility: Ethanol

Yield: 72%

Melting point: 245–247°C

Mobile phase: Ethylacetate: Chloroform (1:1)

Rf value: 0.52

I.R Spectrum: 3438(N-H of isatin), 3256(N-H of pyrazoline),1706 (C=O), 1619

(C=N of pyrazoline ring), 1710(C=O of COOH), 3456(OH)

development and can trap into a novel drug delivery system that may show more bioavailability compared to various other antibacterial drugs with lesser side effects. From the above figure, we can clearly observe that between ISP1 and ISP2 against *Bacillus*, ISP2 inhibited more zone in diameter. As various marketed, antibacterial drugs show serious side or adverse effects on the human body which is sometimes irreversible also.

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Author Queries???

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