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Research Article

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.

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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 year1840 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 vmax are reported in cm^{-1}.

1H-NMR

HNMR spectra were recorded on DMM X - 200 MHz NMR, Bruken Daltonics, Karsruhe, 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/inch^2 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/inch^2 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/inch^2 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

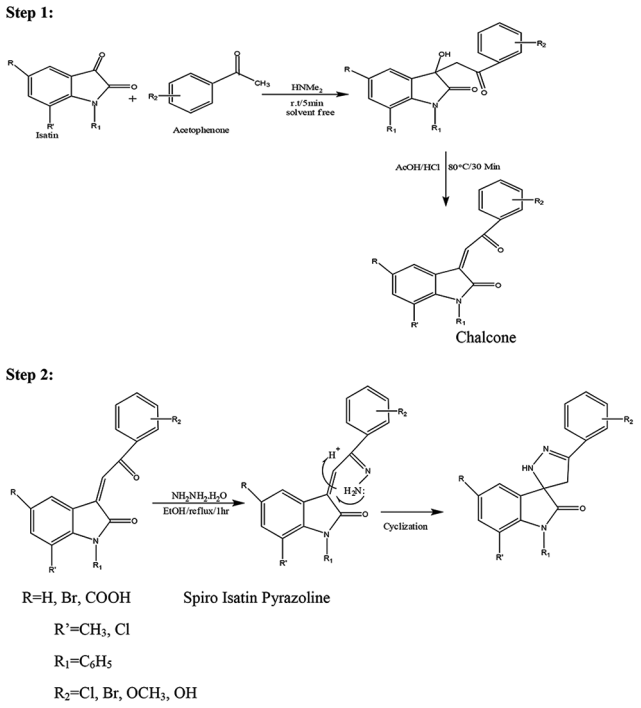
Physical data

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



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: $C_{16}H_{11}NO_2$

Molecular weight: 249

Solubility: Ethanol

Yield: 87%

Melting point: 177–180°C.

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

Molecular formula: $C_{16}H_{14}N_3O$

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)

^{1}H-NMR (δ): 3.43 (1H, d,CH_2), 3.72 (1H, d, CH_2), 6.20 (1H, s, N-H of pyrazoline)

6.92–8.00 (9H, m, Ar-H), 9.16 (1H, s, NH of isatin)

^{13}C-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: $C_{16}H_{12}ClN_3O$

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)

^{1}H-NMR (δ): 3.43 (1H, d, CH_2), 3.58 (1H, d, CH_2), 6.33 (1H, s, N-H pyrazoline)

6.92–7.75 (8H, m, Ar-H), 9.32 (1H, s, NH of isatin)

^{13}C-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: $C_{23}H_{19}N_3O$

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)

^{1}H-NMR (δ): 3.44 (1H, d, CH_2), 3.55 (1H, d, CH_2), 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, CH_2)

^{13}C-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: $C_{16}H_{13}N_3O_2$

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)

^{1}H-NMR (δ): 3.43 (1H, d, CH_2), 3.76 (1H, d, CH_2), 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,CH_2)

9.4 (1H, s, OH)

^{13}C-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: $C_{16}H_{12}BrN_3O$

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)

^{1}H-NMR (δ): 3.42 (1H, d, CH_2), 3.58(1H, d, CH_2),6.36 (1H, s, NH of pyrazoline)

6.92–7.68 (8H, m, Ar-H), 9.32 (1H, s, NH of isatin)

^{13}C-NMR (δ): 44.1,70.2 (2C, spiro carbons),51.7 (1C, CH_2), 110–141.8(13C,ArC),

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: $C_{17}H_{15}O_2N_3$

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 (OCH_3)

^{1}H-NMR (δ): 3.42 (1H, d, CH_2), 3.72 (1H, d, CH_2),3.8 (3H, s, OCH_3) 6(1H,s,NH of pyrazoline), 6–7.3 (8H, m, Ar-H), 8 (1H, s, NH of isatin)

^{13}C-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,OCH_3)

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)

^{1}H-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)

^{1}H-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)

^{1}H-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

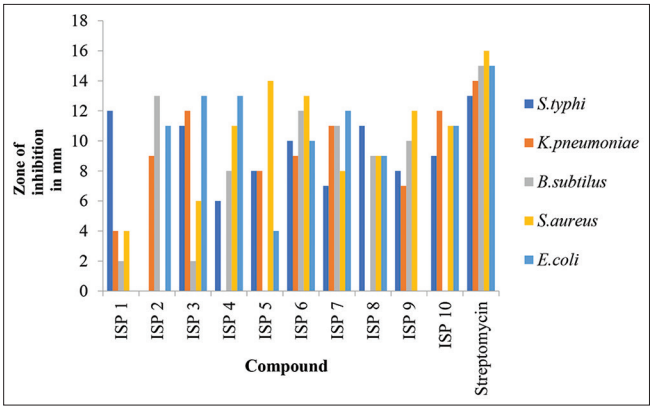


Fig. 1: Antibacterial activity by a zone of inhibition

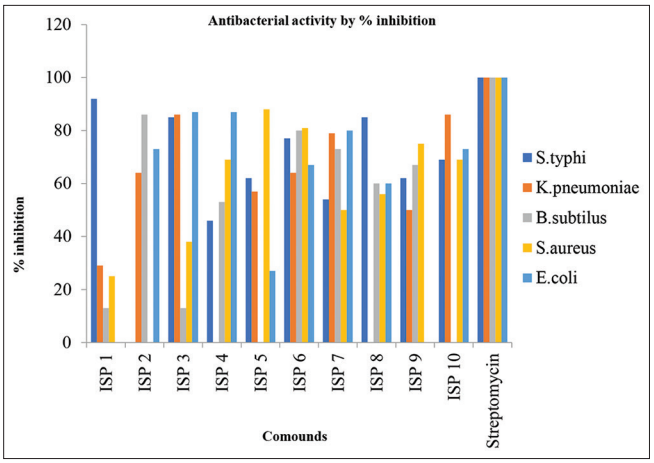


Fig. 2: Graph representing percentage zone of inhibition by taking readings of above Table 3

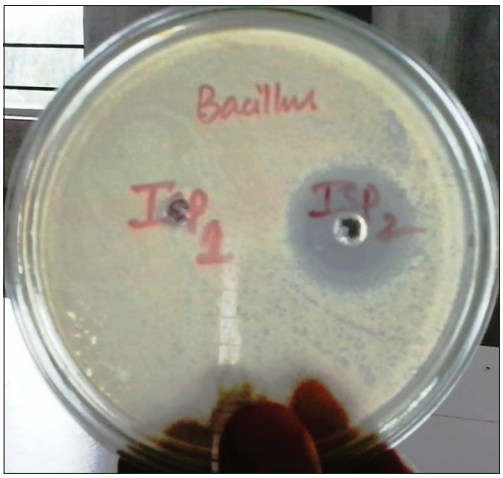


Fig. 3: Photograph of antibacterial activity by a zone of inhibition

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)

Table 1: Summary of important physical data of different isatin derivatives

S. No.	Comp code	Molecular formula	Mol. Wt	R_f value	Melting point	% Yield
1.	ISP1	$C_{16}H_{14}ON_3$	264	0.5	198–200°C	84
2	ISP2	$C_{16}H_{12}ON_3Cl$	295	0.62	223–225°C	87
3	ISP3	$C_{23}H_{19}ON_3$	353	0.55	152–155°C	63
4	ISP4	$C_{16}H_{13}O_2N_3$	279	0.52	240–243°C	71
5	ISP5	$C_{16}H_{12}ON_3Br$	341	0.59	232–234°C	83
6	ISP6	$C_{17}H_{15}O_2N_3$	293	0.69	204–206°C	86
7	ISP7	$C_{16}H_{11}ON_3Br$	340	0.58	235–238°C	88
8	ISP8	$C_{17}H_{15}ON_3$	277	0.56	222–225°C	73
9	ISP9	$C_{16}H_{12}ON_3Cl$	297	0.63	215–217°C	69
10	ISP10	$C_{17}H_{13}O_3N_3$	307	0.52	245–247°C	72

Table 2: Zone of inhibition of different isatin derivatives

Micro-organisms	Zone of inhibition in mm at 50 μg/mL										
	ISP 1 (mm)	ISP 2 (mm)	ISP 3 (mm)	ISP 4 (mm)	ISP 5 (mm)	ISP 6 (mm)	ISP 7 (mm)	ISP 8 (mm)	ISP 9 (mm)	ISP 10 (mm)	Strepto-mycin (mm)
Salmonella typhi	12#	NA	11*	06^	08^	10*	07^	11*	08^	09^	13
Klebsiella pneumoniae	04^	09^	12#	NA	08^	09^	11*	NA	07^	12#	14
Bacillus subtilis	02^	13#	02^	08^	NA	12#	11*	09^	10*	NA	15
Staphylococcus aureus	04^	NA	06^	11*	14#	13#	08^	09^	12#	11*	16
Escherichia coli	NA	11*	13#	13#	04^	10*	12#	09^	NA	11*	15

#Compounds showing strong activity, *Compounds showing moderate activity, ^Compounds showing poor activity, NA: No activity

Table 3: Percentage zone of inhibition of different isatin derivatives

Micro-organisms	% Zone of inhibition in mm at 50 μg/mL										
	ISP 1 (mm)	ISP 2 (mm)	ISP 3 (mm)	ISP 4 (mm)	ISP 5 (mm)	ISP 6 (mm)	ISP 7 (mm)	ISP 8 (mm)	ISP 9 (mm)	ISP 10 (mm)	Strepto-mycin (mm)
Salmonella typhi	92#	NA	85*	46^	62^	77*	54^	85*	62^	69^	100
Klebsiella pneumoniae	29^	64^	86#	NA	57^	64^	79*	NA	50^	86#	100
Bacillus subtilis	13^	86#	13^	53^	NA	80#	73*	60^	67*	NA	100
Staphylococcus aureus	25^	NA	38^	69*	88#	81#	50^	56^	75#	69*	100
Escherichia coli	NA	73*	87#	87#	27^	67*	80#	60^	NA	73*	100

#Compounds showing strong activity, *Compounds showing moderate activity, ^Compounds showing poor activity, NA: No activity. *Standard drug (streptomycin) taken as 100 % inhibited zone and other derivatives compared with standard drug

^{1}H-NMR (δ): 3.43 (1H, d, CH_2), 3.77 (1H, d, CH_2), 12.7 (1H, s, OH) 7 (1H, s, NH of pyrazoline), 7.5–7.9 (8H, m, Ar-H),10.6 (1H, s, NH of isatin)

^{13}C-NMR (δ): 43.7, 64.6 (2C,spiro carbons),109–149.1(13C,Ar-C),151.7(1C,C=N of Pyrazoline ring), 172.7 (1C, C=O), 169.3 (1C, COOH)

Mass Spectra (m/z): 308 (M+1).

RESULTS

From the above table and graph, it was proved that the newly synthesized isatin derivatives were having antibacterial activity for one or another bacterial strain. When all derived Isatin compounds were compared with the standard drug Streptomycin, it was found that ISP1 showed an almost equal response to that streptomycin effects, especially against *Salmonella* typhi bacterial species. From above Figure 3, we can clearly see that ISP2 inhibited more zone in Diameter against the Bacillus strain when we compare it to ISP1. ISP5 is almost effective as a standard drug against S. Aureus. From the obtained results, it was proven that the above-all derived Isatin compounds are effective as well as ineffective for one or more chosen bacterial strains, which were the same as in the case of antibiotics.

DISCUSSION

Isatin is a natural product found in plants isatis and humans, as a metabolic derivative of adrenaline, there is a likely probability of less harmful action in the human body. By taking this as an advantage, it will be a novelty to develop isatin derivatives molding them into nanocarriers to get more precise action against bacterial strains.

Isatin (1H-indole-2,3-Dione) can easily obtain a synthetically that can be used for the synthesis of a large variety of heterocyclic compounds. It is also proven that isatin derivatives are known to be associated with a broad spectrum of biological activity such as anti-inflammatory, antimicrobial, anticonvulsant activities, antioxidant, analgesic, and antianxiety. It is also proven that the chemistry of various derivatives of isatin and isatin itself is having a promising future in chemistry, especially in pharmaceutical industries due to its various pharmacological responses in microbes, enzymes, oxidative stress, convulsions, etc.

In the present work, the newly synthesized isatin derivatives were tested for their antibacterial activity. It was visible in the graph that proves all the compounds which showed actions against one or the other various bacterial species; simultaneously, it was also compared with the standard drug, that is, Streptomycin.

SUMMARY AND CONCLUSION

The main aim of the research is to design, synthesize, and evaluate isatin derivatives for their possible antibacterial activities by comparing their activity with the standard drug Streptomycin. 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 effects against *Salmonella* typhi bacterial species. By considering the activity of isatin derivatives ISP1, it is evident that these are having good antibacterial activity and can take it for further

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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REFERENCES

1. Guzman-Perez A, Wester RT, Allen MC, Brown JA, Buchholz AR, Cook ER, *et al*. Discovery of zoniporide: A potent and selective sodium-hydrogen exchanger Type 1 (NHE-1) inhibitor with high aqueous solubility. Bioorg Med Chem Lett 2001;11:803-7. doi: 10.1016/s0960-894x(01)00059-2, PMID 11277524
2. Amr Ael-G, Abdel-Latif NA, Abdalla MM. Synthesis and antiandrogenic activity of some new 3-substituted androstano[17,16-c]-5'-aryl-pyrazoline and their derivatives. Bioorg Med Chem 2006;14:373-84. doi: 10.1016/j.bmc.2005.08.024, PMID 16182532
3. Erdmann OL. Untersuchungen uber den Indigo. J Prakt Chem 1840;19:321-62. doi: 10.1002/prac.18400190161
4. Auguste L. Recherche sur l Indigo. Ann Chim Phys 1840;3:393-434.
5. Silva JF, Garden SJ, Pinto AC. The chemistry of isatins: A review from 1975 to 1999. J Braz Chem Soc 2001;12:273-324. doi: 10.1590/S0103-50532001000300002
6. Chiyanzu I, Hansell E, Gut J, Rosenthal PJ, McKerrow JH, Chibale K. Synthesis and evaluation of isatins and thiosemicarbazone derivatives against cruzain, falcipain-2 and rhodesain. Bioorg Med Chem Lett 2003;13:3527-30. doi: 10.1016/s0960-894x(03)00756-x, PMID 14505663
7. Sharma PK, Balwani S, Mathur D, Malhotra S, Singh BK, Prasad AK, *et al*. Synthesis and anti-inflammatory activity evaluation of novel triazolyl-isatin hybrids. J Enzyme Inhib Med Chem 2016;31:1520-6. doi: 10.3109/14756366.2016.1151015, PMID 27146339
8. Medvedev A, Buneeva O, Gnedenko O, Fedchenko V, Medvedeva M, Ivanov Y, *et al*. Isatin interaction with glyceraldehyde-3-phosphate dehydrogenase, a putative target of neuroprotective drugs: Partial agonism with deprenyl. J Neural Transm Suppl 2006;71:97-103. doi: 10.1007/978-3-211-33328-0_11, PMID 17447420
9. Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, *et al*. New isatin derivatives and its antioxidant properties. Eur J Med Chem 2010;45:1374-8. doi: 10.1016/j.ejmech.2009.12.035, PMID 20060202
10. Prakash CR, Raja S, Saravanan G. Design, and synthesis of 4-(1-(4-chlorobenzyl)-2,3-dioxoindolin-5-yl)-1-(4-substituted/unsubstituted benzylidene) semicarbazide: Novel agents with analgesic, anti-inflammatory and ulcerogenic properties. Chem Lett 2012;23:541-4. doi: 10.1016/j.cclet.2012.03.014
11. Medvedev A, Igosheva N, Crumeyrolle-Arias M, Glover V. Isatin-role in stress and anxiety. Stress 2005;8:175-83. doi: 10.1080/10253890500342321, PMID 16236622
12. Justo LA, Durán R, Alfonso M, Fajardo D, Faro LRF. Effects and mechanism of action of isatin, an MAO inhibitor, on *in vivo* striatal dopamine release. Neurochem Int 2016;99:147-57. doi: 10.1016/j.neuint.2016.06.012, PMID 27374845
13. Hewawasam PM. A general method for the synthesis of isatin: Preparation of region specifically functionalized isatin from anilines. Tetrahedron Lett 1994;35:7295-306.
14. Gassman PG, Cue BW Jr., Luh TY. A general method for the synthesis of isatins. J Org Chem 1977;42:1344-8. doi: 10.1021/jo00428a016
15. Magnus NA, Diseroad WD, Nevill RC, Wepsiec JP. Synthesis of imidazole based p38 MAP (mitogen-activated protein) kinase inhibitors under buffered conditions. Org Proc Res Dev 2006;10:545-56.
16. Berry A. Procedure and theoretical considerations for testing antimicrobial agent in agar media. In: Corian V, editor. Antibiotics in Laboratory Medicine. 5th ed. United States: Williams and Wilkins; 1991. p. 1-16.
17. Suresh S, Priyanka B, Maharaj P, Srikanth C, Karthik K, Sammaiah G. Synthesis of benzimidazole-isatin derivatives for analgesic activity. Asian J Pharm Clin Res 2013;6:65-7.
18. Shibinskaya MO, Lyakhov SA, Mazepa AV, Andronati SA, Turov AV, Zholobak NM, *et al*. Synthesis, cytotoxicity, antiviral activity, and interferon inducing ability of 6-(2-aminomethyl)-6H-indolo [2, 3-b] quinoxalines. Eur J Med Chem 2010;45:1237-43. doi: 10.1016/j.ejmech.2009.12.014
19. Chen LR, Wang YC, Lin YW, Chou SY, Chen SF, Liu LT, *et al*. Synthesis and evaluation of isatin derivatives as effective SARS coronavirus 3CL protease inhibitors. Bioorg Med Chem Lett 2005;15:3058-62. doi: 10.1016/j.bmcl.2005.04.027, PMID 15896959
20. Andreani A, Burnelli S, Granaiola M, Leoni A, Locatelli A, Morigi R, *et al*. New isatin derivatives with antioxidant activity. Eur J Med Chem 2010;4:1374-8. doi: 10.1016/j.ejmech.2009.12.035, PMID 20060202
21. Singh A, et al. Synthesis of some novel ethoxyphthalimide derivatives of pyrazolo[3,4-c] pyrazoles. Orient J Chem 2000;16:315.
22. Azarifar D, Shaebanzadeh M. Microwave-assisted synthesis of some 3,5-arylated 2-pyrazolines. Molecules 2002;7:885-8.
23. Firdhouse J, Nalini D. Corrosion inhibition of mild steel in acidic media by 5'-phenyl-2',4'-dihydrospiro[indole-3,3'-pyrazol]-2(1H)-oneA. J Chem 2013;9:1-9.
24. Von Auwers K, Muller. One-pot rapid and efficient synthesis of new spiro derivatives of 11H-indeno[1,2-b] quinoxalin-11-one, 6H-indeno[1,2-b] pyrido [3,2-e]pyrazin-6-one and isatin-based 2-pyrazolines. Ber Dt Chem Ges 1908;41:4230.
25. Levai A, Jek J. Synthesis of carboxylic acid derivatives of 2-pyrazolines. Arkivoc 2005;9:344.
26. Patel VM, Desai KR. Eco-friendly synthesis of fluorine-containing pyrazoline derivatives over potassium carbonate. Arkivoc 2004;1:123-9.
27. Lévai A, Jeko J. Synthesis of hydroxylated 3,5-diaryl-2-pyrazolines by the reaction of hydroxychalcones with hydrazines. Arkivoc 2005;10:199-205. doi: 10.3998/ark.5550190.0006.a17