

Synthesis, Characterisation and Anti-Microbial Activity Studies of Novel Dispiro-Oxindolopyrrolizidines

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Abstract: 2-arylidene-1,3-indanediones undergo a regioselective 1,3-dipolar cycloaddition reaction with the azomethine ylide derived from isatin and proline to give a rare class of novel complex dispirooxindolopyrrolizidines in better yield under microwave irradiation than classical heating. X-ray crystal structure analysis of one of the product confirms the structure and regiochemical outcome of the cycloaddition reaction. Anti-microbial activity studies were carried out with all the newly synthesized dispiro-oxindolopyrrolizidines.

Keywords: 1,3-dipole, azomethine ylide, oxindole, pyrrolizidines, antimicrobial activity.

INTRODUCTION

Multi component reactions (MCR's) have emerged as a powerful tool for delivering the molecular diversity needed in the combinatorial approaches for the syntheses of bioactive compounds [1]. MCR's leading to interesting heterocyclic scaffolds, are particularly useful for the creation of diverse chemical libraries of 'drug-like' molecules for biological screening [2].

Pyrrolizidine alkaloids have attracted a great deal of interest because of their wide distribution in nature and their varied biological activities [3, 4]. Besides a wide spectrum of interesting biological activities are associated with 2-indolinone derivatives [5] with C-3 as spiro atom. Oxindole derivatives are found to be potent aldose reductase inhibitors (ARI's), which help to treat and prevent diabetic complications arising from elevated level of sorbitol [6] and are known to possess anti-bacterial, anti-protozoal and anti-inflammatory activities [7]. Spiro oxindole ring system is a structural feature found in variety of oxindole alkaloids [8] and has been reported to behave as aldose reductase, poliovirus and rhinovirus 3C-proteinase inhibitors [9]. 1, 3-indanedione has also captured much attention due to their important pharmacological properties [10]. Bioactivity of some of the spiro-pyrrolizidine and spiro oxindolopyrrolizidines is reported by us recently [11].

RESULTS AND DISCUSSION

As a part of our endeavor [12-14] to synthesize novel dispirooxindole derivatives, we have examined 1,3-dipolar cycloaddition reactions of unusual dipolarophiles 2-arylidene-1,3-indanediones with the dipole generated from isatin and L-proline. The multicomponent reaction is carried out by addition of one equivalent of isatin to one equivalent of L-proline followed by addition of one equivalent of 2-arylidene-1, 3-indanedione and subjected to different reaction

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condition to afford novel dispirooxindolopyrrolizidine derivatives **4(a-k)**. The reaction proceeded through decarboxylative condensation of isatin and L-proline forming the azomethine ylide which underwent subsequent cycloaddition reaction with various derivatives of 2-arylidene-1,3-indanediones to afford a series of novel dispirooxindolopyrrolizidine derivatives which were characterized on the basis of spectroscopic data. The regio and stereochemical outcome of the cycloaddition was confirmed by single crystal X-ray analysis of **4h** (Fig. 1). The IR spectra of **4e** revealed the presence of a carbonyl peak at 1743.5 cm⁻¹ showing an increase of 13.5 cm⁻¹ from the normal value observed for 2-(nitrophenyl)-1,3-indanedione indicating the loss of conjugation. A peak at 1704.9 cm⁻¹ due to carbonyl group of oxindole is observed. The proton ¹H NMR spectrum of **4e** exhibited peaks at δ values 2.03-2.30 (m, 4H), 2.78-2.87(m, 2H, -NCH₂), 4.91-4.98 (m, 1H, H_b), 4.98-5.00 (d, J = 9.28 Hz, 1H, H_a), 6.67-7.97 (m, 12H, ArH). No trace of the other regioisomer **5(a-k)** was detected. If the other regioisomer **5(a-k)** were formed one would expect a singlet for benzylic proton which is not observed. ¹³C NMR spectra of **4(a-k)** add conclusive support to the proposed structure. ¹³C NMR spectra of **4e** exhibited the presence of benzylic carbon at δ 51.43, spiro carbons at δ 68.41 and δ 74.80, oxindole carbonyl at δ 177.69, indanedione carbonyl at δ 196.50 and δ 197.20 ppm respectively. These observed chemical shift value confirmed the proposed structure. The mass spectrum of **4e** showed a peak at *m/z* 479 (M⁺). The structure and the regiochemistry of the cycloadducts were finally confirmed by X-ray diffraction studies. Identical results were obtained with other derivatives of 2-arylidene-1,3-indanediones irrespective of the nature of the substituent present in the arylidene moiety of 2-arylidene-1, 3-indanedione. The reaction time and the chemical yield are indicated in the (Table 1).

In conclusion, the multicomponent 1,3-dipolar-cycloaddition reaction described here provides a simple and direct entry into a number of interesting novel dispirooxin-

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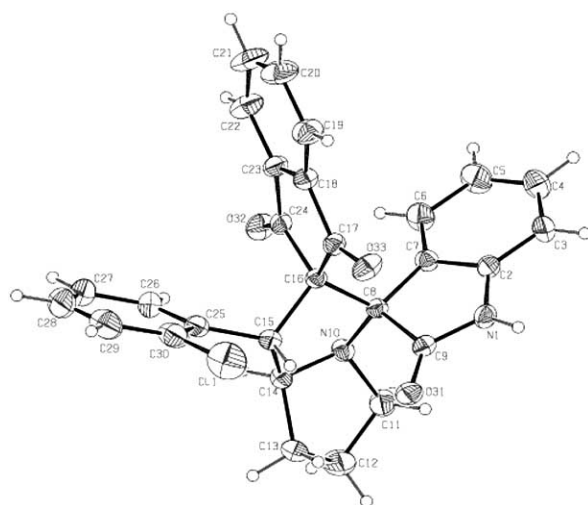


Fig. (1). ORTEP diagram of 4h.

dolopyrrolizidine derivatives possessing 1,3-indanedione moieties that may be of value in medicinal chemistry. From the results of a comparative study of the synthesis of dispirooxindolopyrrolizidines by the classical reflux method and the microwave irradiation, it is evident that the reaction time has been reduced from several hours to only a few minutes by using microwave irradiation indicating that microwave played a significant role in the rate enhancement. High regioselective nature of this type of methodology would be of potential interest in the construction of various alkaloids.

To the best of our knowledge to date there has been no report of the cycloaddition reaction of azomethine ylide derived from isatin and L-proline with 2-arylidene-1,3-indanediones as dipolarophiles.

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EXPERIMENTAL

General

All melting points are uncorrected. IR spectra were recorded on a SHIMADZU-FTIR 8300 instrument. Mass spectral data were recorded on a JEOL HFDX 303 spectrometer. ^1H NMR and ^{13}C NMR spectra were recorded in CDCl_3 -TMS with JEOL 400 MHz and 100 MHz respectively. Microwave irradiations were carried out in a Kenstar oven Model 5250 at 2450 MHz. The starting material **1(a-k)** were prepared as per the literature procedure [15].

Three-Component Regioselective Synthesis of Spiro-Oxindolopyrrolizidines: General Procedure for the Synthesis of (4a-k) Under Classical Heating

A mixture of isatin **2** (1mmol), L-proline **3** (1 mmol) and 2-arylidene-1,3-indanediones **1(a-k)** (1 mmol) in aqueous methanol (10ml) were refluxed for the time shown in Table **1**, till the completion of the reaction as evidenced by TLC. After the reaction was over, the solvent was removed under reduced pressure and the crude product was chromatographed on silica gel using hexane-ethyl acetate (8:2) as eluent to afford the cycloadducts **4(a-k)**.

General Procedure for the Synthesis of (4a-k) Under Microwave Irradiation

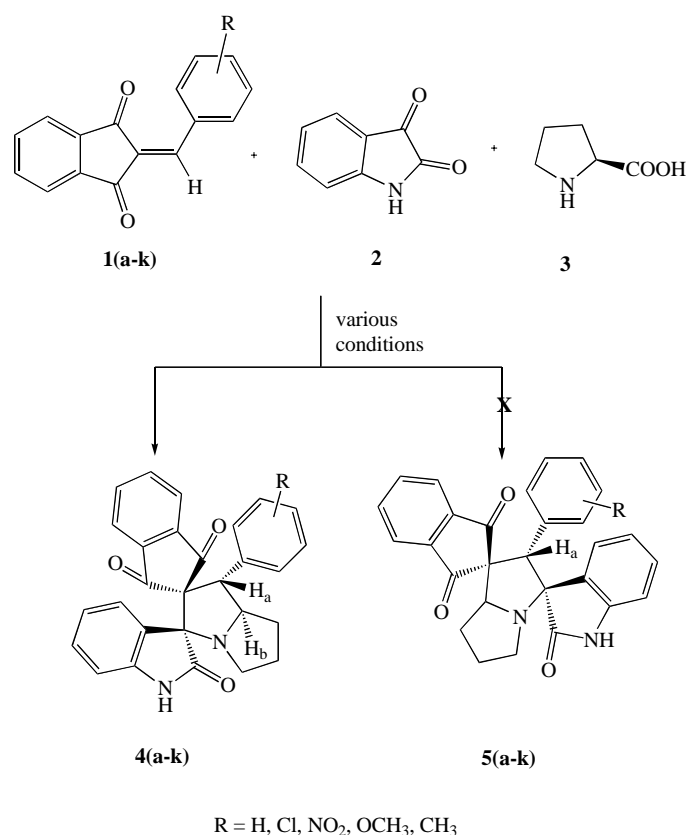
A mixture of 2-arylidene-1, 3-indanedione **1(a-k)** (1 mmol), isatin **2** (1 mmol) and L-proline **3** (1 mmol) in methanol (25ml) was placed in a tall beaker. The beaker was covered with a stemless funnel and then irradiated for 3-6 min in microwave oven. The resultant residues after cooling the mixture were recrystallised in methanol.

Crystal Data for Compound 4h [16]

Molecular formula $\text{C}_{28}\text{H}_{21}\text{N}_2\text{O}_3$. Molecular weight 468, Monoclinic, pale yellow crystals. Number of atoms = 55. A crystal with dimensions of $0.18 \times 0.17 \times 0.11$ mm was

Table 1. 1,3-Dipolar Cycloaddition Reaction of 2-Arylidene-1, 3-Indanediones **1(a-k)** with the Azomethine Ylide Generated From Isatin **2** and L-Proline **3**

Entry	Product	R	Reflux		Microwave	
			Time (h)	Yield (%)	Time (min)	Yield (%)
1	4a	H	2.8	67	5.0	73
2	4b	<i>p</i> -Cl	2.5	72	5.0	79
3	4c	<i>p</i> -Me	3.0	68	6.0	77
4	4d	<i>p</i> -OMe	3.0	70	6.0	81
5	4e	<i>p</i> -NO ₂	2.5	88	4.0	95
6	4f	<i>m</i> -Cl	3.0	70	5.0	76
7	4g	<i>m</i> -NO ₂	2.5	85	4.0	90
8	4h	<i>o</i> -Cl	3.0	70	5.0	77
9	4i	<i>o</i> -NO ₂	2.5	85	4.0	90
10	4j	3,4-OMe	3.0	60	6.0	72
11	4k	3,4,5-OMe	3.0	58	6.0	70

**Scheme 1.**

used for the X-ray data collections at 293K on a Bruker SMART APEXCCD area detector using molybdenum K α radiation and a graphite monochromator. θ Range for data collection was 2.3–24.3°. A total of 3882 reflections were measured.

In Vitro Antimicrobial Sensitivity Determination Test

The *in vitro* antimicrobial sensitivity of the antibiotics and the test compounds synthesized were determined by the disc diffusion [17] and well diffusion method [18] as recommended by the *National Committee for Clinical Laboratory Standards* (NCCLS) [19].

Antimicrobial Drugs and Test Compounds

Antibacterial and Antifungal Drugs

Thirteen antibacterial agents and two antifungal agents were used as standard drug for the comparative studies. The disc concentration levels were as per guidelines of National Committee for Clinical Laboratory Standards (NCCLS) column (See Tables 5 and 6). The antimicrobial discs were obtained from HiMedia Laboratories Pvt. Limited, Mumbai '86, India.)

Stock Solution Preparation of Test Compounds

Stock solutions of newly synthesized compounds were diluted in 100% Dimethyl sulfoxide (DMSO). The stock solutions were prepared at different concentration. Stock (A) 7.5mg /12 mL, (B) 15mg /12 mL, (C) 15mg /6 mL and (D) 15mg /3 mL were prepared in various concentrations. From the diluted stock solution were taken 100 μ L containing A-62.5 μ g, B-125 μ g, C-250 μ g and D-500 μ g concentration of

the test compounds. They were immediately dispensed into each agar wells of culture inoculated (MHA) plates using sterilized micropipette.

Clinical Bacterial Isolates

Clinical isolates of all pathogenic bacterial isolates were obtained from Department of Clinical Medical Microbiology, Apollo Hospitals, greams road, Chennai – 06, Tamil Nadu, India. All the clinical isolates were identified by standard methods. From this departmental culture collection unit, ten clinically important bacterial pathogenic isolates were obtained of which there were two-gram positive and eight gram negative bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi 'A'*, *Salmonella paratyphi 'H'*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris* and *Escherichia coli* strains were used. All the isolates were stored frozen in skim milk 50% glycerol at -70°C.

Clinical Yeast Isolates

Clinical isolates of *Candida* spp. were obtained from Department of Clinical Microbiology, Apollo Hospitals, 21 Greams Road, Chennai -'06, Tamil Nadu, India. All the clinical isolates were identified by standard methods. *Candida albicans* isolate was obtained from blood sample and *Candida tropicalis* isolate was obtained from wound site. Additional reference including one American Type Culture Collection strain *Candida albicans* (ATCC 90028) was used. Isolates were stored as glycerol cell suspensions stocks frozen at -70°C until they were used. Stored clinical isolate cultures were revived in the Sabouraud dextrose broth or

Brain heart infusion broth incubated at 35°C for 24 hours and then subcultured onto Sabouraud dextrose agar medium.

Inoculum Preparation

Bacterial Inoculum

The inoculum was prepared using gram positive and gram-negative bacterial pathogens from a 24 hours old culture on Brain heart infusion agar. With a sterile loop, the tops of four to five colonies were transferred to a tube containing 5mL of Mueller Hinton broth or Brain Heart infusion broth. The tube was incubated at 35°C for 24 hours. The turbidity of the culture suspension was adjusted with broth or a sterile saline solution (0.85-0.9%). The density of this culture was adjusted with 0.5 McFarland standard and finally inoculum size approximately 5×10^5 CFU/mL.

Fungal Inoculum

The inoculum used was prepared using the yeasts from a 24 hours old culture on Sabouraud dextrose agar. With a sterile loop, the tops of four to five colonies were transferred to a tube containing 5mL of Mueller Hinton broth or Brain Heart infusion broth. The tube was incubated at 35°C for 24 hours. The turbidity of the culture suspension was adjusted with broth or a sterile saline solution (0.85-0.9%). The culture density was adjusted with 0.5 McFarland standard and finally inoculum size approximately $(0.5 - 2.5 \times 10^3)$ CFU/mL).

Test Medium

The disk diffusion and well diffusion methods were performed using two different agar mediums. For antibacterial test assay Mueller Hinton Agar (MHA) medium: Casein acid hydrolysate: 17.5g; Beef Heart infusion: 2g; Starch soluble 1.5g; ($p^H = 7.3 \pm 0.2$) Agar 17g; H₂O 1000mL. For antifungal test assay Casitone agar medium: (Bacto-casitone: 9g; yeast extract: 5g; tri-sodium citrate: 10g; glucose: 20g; bactoagar: 15g; phosphate buffer: KH₂PO₄: 1g; Na₂HPO₄: 1g (p^H 6.6); H₂O 1000mL) was used.

Antimicrobial Susceptibility Testing

Disk Diffusion Method

The disk diffusion test as described by Bauer *et al.* was performed using Mueller Hinton Agar (MHA) for bacteria and Casitone agar (CA) medium was used for fungal. The medium was mixed well, heated in boiling water bath to dissolve the medium completely and autoclaved at 15lbs pressure (121°C) for 15minutes. After removal from the autoclave the sterilized medium was immediately cooled to 50-55° in a water bath. The cooled medium was poured into sterile petri plates to a uniform depth of 4mm; this is equivalent to approximately 25mL in a 90mm plate. Once the medium had solidified then the culture was inoculated on the medium. Within 15minutes of adjusting the density of the inoculum, a sterile cotton swab was dipped into the standardized bacterial and yeast suspension or inoculated with 1mL of the organism suspension. The sterile swab was used to streak on the surface of the MHA and CA medium to ensure an even distribution of the inoculum. The plates were allowed undisturbed for 3 to 5 minutes to absorption of excess moisture. The selected antibiotic disks for bacteria (Table 1) and antifungal agents namely fluconazole (10µg) and Itra-

conazole (10µg) disks were placed on the inoculated plates and pressed firmly into the agar with the sterile forceps to ensure complete contact with the agar. The plates were then incubated at 35-37°C for 24 hours. However NCCLS disc diffusion and MIC standard breakpoints were used for the interpretative results.

Well Diffusion Method

The well diffusion test was performed using MHA and CA medium, as per the procedure described by Magaldi *et al.* 2004 and the medium was autoclaved at 15lbs pressure (121°C) for 15minutes then was immediately cooled to 50-55°C in a water bath after removing it from the autoclave. The cooled medium was poured into sterile petri plates to a uniform depth of 4mm; this is equivalent to approximately 25mL in a 90mm plate. Once the medium had solidified then the culture was inoculated on the medium. Within 15minutes of adjusting the density of the inoculums, a sterile cotton swab was dipped into the standardized bacterial and yeast suspension or inoculated with 1mL of the organism suspension. The sterile swab was used on the surface of the MHA and CA medium to ensure an even distribution of the inoculums. The plates were undisturbed for 3 to 5 minutes to ensure absorption of excess moisture. Sterilized 9mm cork borer was used to make agar wells, 100 µL of the diluted test compound stock solutions were placed into each wells and 100% DMSO as a control. The plates were incubated at 35-37 °C for 24 hours. However NCCLS disc diffusion and MIC standard breakpoints were used for the interpretative results.

The percentage (%) of inhibition was calculated by using the formula.

$$\% \text{ of inhibition} = \frac{I \text{ (Diameter of the inhibition zone in mm)}}{90 \text{ (Diameter of the petri-plates in mm)}} \times 100$$

Antibacterial Activity Results

The results of the antimicrobial screening of the nine compounds were given in the (Table 2). From this data, it's evident that most of the compounds showed significant inhibitory activity against ten clinical human pathogenic isolates of bacterial species namely *S. aureus*, *B. subtilis*, *P. aeruginosa*, *S. typhi*, *S. paratyphi* A, *S. paratyphi* H, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *E. coli*. The antibacterial activities of the tested compounds were found to be dose dependent. Most of compounds significantly inhibited bacterial growth at the concentration between 125 µg / mL to 500 µg / mL and activities were found to be good at concentrations ≥ 250 µg/mL. It was interesting to observe that all the nine compounds **4(a-i)** exhibited good inhibitory activity against *S. typhi*, *S. paratyphi* A and *S. paratyphi* H and *P. aeruginosa*. Among the compounds tested, **4a** exhibited good inhibitory activity and **4(b-i)** showed less to moderate activity against *S. aureus*. The results also showed that the two isolates *B. subtilis* and *P. mirabilis* were resistant to the most of the compounds screened except compound **4g** and **4i** which demonstrated moderate inhibitory activity against *B. subtilis*. Compound **4b** showed good inhibitory activity against both the *Proteus* species. Compound **4a** showed less activity against *P. mirabilis* while other five compounds (**4c**, **4d**, **4e**, **4f**, and **4h**) were found to be resistant. Except **4b**, **4c**,

Table 2. Antibacterial Activity Results of Compounds 4(a-i)

Clinical Bacterial Test Isolates	Compound 4a (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g / 100 μ L		250 μ g / 100 μ L		500 μ g / 100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	15	16.7	19	21.1	22.5	25
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	15	16.7
<i>Pseudomonas aeruginosa</i>	-	-	14	15.6	18.4	20.4	22	24.4	25.8	28.7
<i>Salmonella typhi</i>	-	-	-	-	16	17.8	18	20	19	21.1
<i>Salmonella paratyphi 'A'</i>	-	-	17	18.9	21.4	23.8	25	27.8	28.7	31.9
<i>Salmonella paratyphi 'H'</i>	-	-	14	15.6	18	20	20.5	22.2	22	24.4
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	13	14.4	16.5	18.3
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	14	15.6
<i>Proteus vulgaris</i>	-	-	-	-	-	-	14	15.6	14.7	16.3
<i>Escherichia coli</i>	-	-	-	-	13	14.4	17.5	19.4	21	23.3
Clinical Bacterial Test Isolates	Compound 4b (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g / 100 μ L		250 μ g / 100 μ L		500 μ g / 100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	16	17.8	18.6	20.7
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	16	17.8
<i>Pseudomonas aeruginosa</i>	-	-	15.3	17	18	20	21	23.3	24.8	27.6
<i>Salmonella typhi</i>	-	-	-	-	14.5	16.1	19	21.1	22	24.4
<i>Salmonella paratyphi 'A'</i>	-	-	14.5	16.1	18.4	20.4	22	24.4	26	28.9
<i>Salmonella paratyphi 'H'</i>	-	-	13	14.4	16.7	18.6	18	20	24.5	27.2
<i>Klebsiella pneumoniae</i>	-	-	-	-	13.5	15	15.8	17.6	18	20
<i>Proteus mirabilis</i>	-	-	-	-	12.6	14	16	17.8	20	22.2
<i>Proteus vulgaris</i>	-	-	13	14.4	17	18.9	19.4	21.6	22.7	25.2
<i>Escherichia coli</i>	-	-	12	13.3	15.7	17.4	18	20	21.4	23.8
Clinical Bacterial Test Isolates	Compound 4c (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g / 100 μ L		250 μ g / 100 μ L		500 μ g / 100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	12	13.3	16	17.8
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	17	18.9	23.6	26.2	27	30	29.6	32.9
<i>Salmonella typhi</i>	-	-	-	-	15	16.7	18	20	22.4	24.9
<i>Salmonella paratyphi 'A'</i>	-	-	18	20	22.5	25	26	28.9	29.5	32.8
<i>Salmonella paratyphi 'H'</i>	-	-	15	16.7	19.8	22	24.3	27	27.5	30.6
<i>Klebsiella pneumoniae</i>	-	-	-	-	12	13.3	18	20	18.7	20.8
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	14	15.6	17.8	19.8	21	23.3

Table 2. Contd....

Clinical Bacterial Test Isolates	Compound 4d (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 µL DMSO		62.5µg / 100 µL		125µg /100 µL		250µg /100 µL		500µg /100 µL	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	12	13.3	16	17.8
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	15	16.7	18	20	22.8	25.3	26	28.9
<i>Salmonella typhi</i>	-	-	18	20	20.4	22.7	25.3	28.1	28	31.1
<i>Salmonella paratyphi 'A'</i>	-	-	-	-	-	-	12	13.3	15.8	17.6
<i>Salmonella paratyphi 'H'</i>	-	-	-	-	-	-	16	17.8	19	21.1
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	13.5	15	17.6	19.6
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	15.8	17.6	19	21.1
<i>Escherichia coli</i>	-	-	-	-	15	16.7	15	16.7	17	18.9
Clinical Bacterial Test Isolates	Compound 4e (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 µL DMSO		62.5µg / 100 µL		125µg /100 µL		250µg /100 µL		500µg /100 µL	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	13.5	15	15	16.7	17.8	19.8
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	14.7	16.3	17.3	19.2	21	23.3
<i>Salmonella typhi</i>	-	-	-	-	16	17.8	19.4	21.6	23	25.6
<i>Salmonella paratyphi 'A'</i>	-	-	-	-	-	-	15	16.7	18.3	20.3
<i>Salmonella paratyphi 'H'</i>	-	-	-	-	16	17.8	19	21.1	24	26.7
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	13.5	15	17.6	19.6
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	-	17.4	19.3	19	21.1	23.8	26.4
Clinical Bacterial Test Isolates	Compound 4f (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 µL DMSO		62.5µg / 100 µL		125µg /100 µL		250µg /100 µL		500µg /100 µL	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	12.6	14	15.6	17.3
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	13	14.4
<i>Pseudomonas aeruginosa</i>	-	-	-	-	16	17.8	18.6	20.7	22	24.7
<i>Salmonella typhi</i>	-	-	-	-	13	14.4	17.4	19.3	23.5	26.1
<i>Salmonella paratyphi 'A'</i>	-	-	14.7	16.3	18	20	24	26.7	26.8	29.8
<i>Salmonella paratyphi 'H'</i>	-	-	-	-	-	-	14.8	16.4	18.3	20.3
<i>Klebsiella pneumoniae</i>	-	-	11	12.2	14.3	15.9	17	18.9	19.6	21.8
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	13	14.4	15.7	17.4	18	20
<i>Escherichia coli</i>	-	-	-	-	-	-	17	18.9	21	23.3

Table 2. Contd....

Clinical Bacterial Test Isolates	Compound 4g (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g / 100 μ L		250 μ g / 100 μ L		500 μ g / 100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	12	13.3	14.8	16.4	16.2	18
<i>Bacillus subtilis</i>	-	-	-	-	-	-	14	15.6	15.4	17.1
<i>Pseudomonas aeruginosa</i>	-	-	12	13.3	17.3	19.2	22	24.4	25.8	28.7
<i>Salmonella typhi</i>	-	-	-	-	-	-	15.9	17.7	17.5	19.4
<i>Salmonella paratyphi 'A'</i>	-	-	12	13.3	16.4	18.2	18	20	22.6	25.1
<i>Salmonella paratyphi 'H'</i>	-	-	-	-	14	15.6	18.5	20.6	23	25.6
<i>Klebsiella pneumoniae</i>	-	-	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	16	17.8
<i>Escherichia coli</i>	-	-	18	20	21.7	24.1	25.4	28.2	28.5	31.7
Clinical Bacterial Test Isolates	Compound 4h (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g / 100 μ L		250 μ g / 100 μ L		500 μ g / 100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	12	13.3	14.8	16.4
<i>Bacillus subtilis</i>	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	15	16.7	18	20
<i>Salmonella typhi</i>	-	-	16.5	18.3	18.6	20.7	21.4	23.8	25.7	28.6
<i>Salmonella paratyphi 'A'</i>	-	-	-	-	12	13.3	14.9	16.6	18.5	20.6
<i>Salmonella paratyphi 'H'</i>	-	-	-	-	-	-	-	-	15.6	17.3
<i>Klebsiella pneumoniae</i>	-	-	13	14.4	15.8	17.6	18	20	19.5	21.7
<i>Proteus mirabilis</i>	-	-	-	-	12	13.3	13.9	15.4	16.5	18.3
<i>Proteus vulgaris</i>	-	-	-	-	-	-	14	15.6	17	18.9
<i>Escherichia coli</i>	-	-	-	-	15	16.7	18.7	20.8	23.5	26.1
Clinical Bacterial Test Isolates	Compound 4i (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g / 100 μ L		250 μ g / 100 μ L		500 μ g / 100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Staphylococcus aureus</i>	-	-	-	-	14.3	15.9	17	18.9	20.4	22.7
<i>Bacillus subtilis</i>	-	-	-	-	12	13.3	14.6	16.2	15.8	17.6
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-
<i>Salmonella typhi</i>	-	-	-	-	-	-	14.3	15.9	16.4	18.2
<i>Salmonella paratyphi 'A'</i>	-	-	-	-	-	-	-	-	15	16.7
<i>Salmonella paratyphi 'H'</i>	-	-	-	-	-	-	14	15.6	17.5	19.4
<i>Klebsiella pneumoniae</i>	-	-	14	15.6	17.5	19.4	21	23.3	25.7	28.6
<i>Proteus mirabilis</i>	-	-	-	-	-	-	15	16.7	17.5	19.4
<i>Proteus vulgaris</i>	-	-	-	-	12	13.3	15.8	17.6	18.5	20.6
<i>Escherichia coli</i>	-	-	-	-	16	17.8	20.4	23.8	23.6	26.2

4e and **4g**, all the remaining compounds **4a**, **4d**, **4f**, **4h** and **4i** exhibited moderate activity against *P. vulgaris*.

Antifungal Activity Results

Table 3 summarizes the *invitro* antifungal activity of compounds **4(a-i)** against the two important clinical human pathogenic yeast isolates of *Candida* species using well diffusion method. Fluconazole and itraconazole were used as standard drug as determined by disc diffusion method. *Candida albicans* was found to be resistant strain to both the drug whereas fluconazole showed good inhibitory activity against *Candida tropicalis* but Itraconazole showed moderate activity. Most of the synthesized compounds were found to inhibit fungal growth (16-30 %) at the concentration between 125 µg / mL to 500 µg / mL significantly. Compound **4f**, **4g**, **4h** & **4i** were found to be very good antifungal agent whereas compound **4c**, **4d** & **4e** showed moderate activity. **4a** & **4b** showed less activity against *C. albicans*. Though compound **4a**, **4f**, **4i** exhibited moderate activity and **4b** showed low activity against *C. tropicalis*, yet the compounds **4c**, **4e** and **4h** were found to be very good antifungal agents. Compound **4d** was found to be resistant against *C. tropicalis*. Two compounds **4g** and **4h** exhibited very good activity against clinical isolates of *Candida* Species.

Table 4 depicts the consolidated anti-microbial activity results of the dispirooxindolopyrrolizidines **4(a-i)**.

Physical and Spectroscopic Details of the Dispirooxindolopyrrolizidines 4(a-k)

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-phenyl-pyrrolizidine 4a. 0.20g, 67 %, pale yellow crystal, mp; 166-168 °C; IR (KBr): 1701, 1732, 3345.1 cm⁻¹; ¹H NMR: δ 1.95–2.29 (m, 4H), 2.76–2.86 (m, 2H, -NCH₂), 4.83–4.87 (m, 1H, H_b), 5.09 (d, J = 9.24 Hz, 1H, H_a), 6.67–7.68 (m, 13H, ArH), 8.40 (s, 1H, -NH); ¹³C NMR: δ 30.47, 31.42, 47.65, 51.97, 52.69, 68.54, 74.51, 110.00, 122.45, 122.93, 122.95, 127.38, 128.37, 128.91, 135.37, 135.79, 140.70, 177.99, 197.29, 197.81; MS *m/z*: 434 (M⁺); Anal. Calcd for C₂₈H₂₂N₂O₃: C, 77.40; H, 5.10; N, 6.44. Found: C, 77.52; H, 5.15; N, 6.50.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-(p-chlorophenyl)-pyrrolizidine 4b. 0.21 g, 72 %, pale yellow crystal, mp; 173-175 °C; IR (KBr): 1705, 1739, 3398.3 cm⁻¹; ¹H NMR: δ 1.99-2.29 (m, 4H), 2.74-2.88 (m, 2H, -NCH₂), 4.82-4.86 (m, 2H, H_a, H_b), 6.67-7.85 (m, 2H, ArH), 8.25 (s, 1H, -NH); ¹³C NMR: 30.35, 31.28, 47.51, 51.62, 52.32, 68.53, 74.88, 109.87, 109.98, 122.91, 122.95, 123.45, 126.04, 128.47, 129.50, 130.25, 133.10, 133.25, 135.51, 135.95, 140.44, 177.72, 196.99, 197.61; MS *m/z*: 468 (M⁺); Anal. Calcd for C₂₈H₂₁N₂O₃Cl: C, 71.71, H, 4.51, N, 5.97. Found: C, 71.62, H, 4.60, N, 5.75.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-(p-methylphenyl)-pyrrolizidine 4c 0.20 g, 68 %, yellow solid, mp; 180-182 °C; IR (KBr): 1704, 1730, 3341.7 cm⁻¹; ¹H NMR: δ 2.02-2.10 (m, 4H), 2.46 (s, 3H), 2.28-2.91 (m, 2H, -NCH₂), 4.84-4.86 (m, 1H, H_b), 5.02 (d, J = 9.25 Hz, 1H, H_a), 6.68-7.70 (m, 12H, ArH), 8.56 (s, 1H, -NH); ¹³C NMR: 20.97, 30.37, 31.31, 47.82, 51.06, 52.30, 68.82, 74.76, 110.11, 122.51, 122.96, 123.01, 128.76, 129.12, 135.42, 135.83, 140.70, 141.76, 177.63, 197.19, 197.83; MS *m/z*:

448 (M⁺); Anal. Calcd for C₂₉H₂₄N₂O₃: C, 77.65, H, 5.39, N, 6.24. Found: C, 77.73, H, 5.52, N, 6.32.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-(p-methoxyphenyl)-pyrrolizidine 4d. 0.20 g, 70 %, yellow solid, mp; 179-181 °C; IR (KBr): 1704, 1731, 3352.3 cm⁻¹; ¹H NMR: δ 2.02-2.12 (m, 4H), 2.84-2.92 (m, 2H, -NCH₂), 3.72 (s, 3H), 4.86-4.88 (m, 1H, H_b), 5.03 (d, J = 9.25 Hz, 1H, H_a), 6.70-7.72 (m, 12H, ArH), 8.62 (s, 1H, -NH); ¹³C NMR: 30.39, 31.33, 47.87, 51.20, 52.11, 55.42, 68.84, 74.39, 110.08, 110.24, 122.99, 122.97, 123.23, 128.79, 129.32, 130.21, 133.07, 134.03, 135.51, 135.81, 140.67, 141.77, 177.81, 197.23, 197.87; MS *m/z*: 464 (M⁺); Anal. Calcd for C₂₉H₂₄N₂O₄: C, 74.98; H, 5.20; N, 6.03. Found: C, 75.07; H, 5.30; N, 6.12.

Spiro [2.3']oxindole-spiro[3.2'']indane-1'',3''-dione-4-(p-nitrophenyl)-pyrrolizidine 4e. 0.24 g, 90 %, yellow solid, mp; 179-181 °C; IR (KBr): 1346.2, 1523.7, 1704.9, 1743.5, 3352.1 cm⁻¹; ¹H NMR: δ 2.03-2.30 (m, 4H), 2.78-2.87 (m, 2H, -NCH₂), 4.91-4.98 (m, 1H, H_b), 4.99 (d, J = 9.28 Hz, 1H, H_a), 6.67-7.97 (m, 12H, ArH), 8.56 (s, 1H, -NH); ¹³C NMR: 30.35, 31.35, 47.49, 50.72, 51.43, 68.41, 74.80, 110.03, 122.49, 123.01, 123.04, 123.45, 125.63, 126.02, 129.68, 129.88, 135.76, 136.20, 140.51, 141.39, 142.18, 143.57, 147.08, 177.69, 196.50, 197.20; MS *m/z*: 479 (M⁺); Anal. Calcd for C₂₈H₂₁N₃O₅: C, 70.06; H, 4.51, N, 8.75. Found: C, 70.20; H, 4.60; N, 8.86.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-(m-chlorophenyl)-pyrrolizidine 4f. 0.20 g, 70 %, yellow solid mp; 188-189 °C; IR (KBr): 1704.1, 1738.2, 3396.7 cm⁻¹; ¹H NMR: δ 2.02-2.27 (m, 4H), 2.79-2.87 (m, 2H, -NCH₂), 4.84-5.02 (m, 2H, H_a, H_b), 6.76-7.70 (m, 12H, ArH), 8.48, (s, 1H, -NH); ¹³C NMR: 30.38, 31.35, 47.69, 47.76, 51.86, 68.61, 74.82, 110.15, 122.56, 123.06, 123.36, 127.12, 127.68, 129.17, 129.67, 134.20, 135.61, 136.04, 140.68, 141.64, 142.64, 142.35, 177.81, 196.79, 197.41; MS *m/z*: 468 (M⁺); Anal. Calcd for C₂₈H₂₁N₂O₃Cl: C, 71.71; H, 4.51; N, 5.97. Found: C, 71.81; H, 4.59; N, 6.05.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-(m-nitrophenyl)-pyrrolizidine 4g. 0.22 g, 85%, yellow solid, mp; 178-179 °C; IR (KBr): 1346.1, 1523.4, 1703.7, 1742.8, 3351.9 cm⁻¹; ¹H NMR: δ 2.04-2.30 (m, 4H), 2.79-2.89 (m, 2H, -NCH₂), 4.91-4.98 (m, 1H, H_b), 5.00 (d, J = 9.29 Hz, 1H, H_a), 6.71-7.91 (m, 12H, ArH), 8.60 (s, 1H, -NH); ¹³C NMR: 30.34, 31.37, 47.53, 50.77, 51.14, 68.49, 74.72, 110.09, 122.42, 122.47, 123.04, 123.91, 125.67, 126.01, 129.43, 129.70, 135.11, 135.67, 136.10, 138.07, 140.51, 141.43, 142.25, 148.06, 177.79, 196.50, 197.19; MS *m/z*: 479; Anal. Calcd for C₂₈H₂₁N₃O₅: C, 70.06; H, 4.51, N, 8.75. Found: C, 70.22; H, 4.65; N, 8.85.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3''-dione-4-(o-chlorophenyl)-pyrrolizidine 4h. 0.20 g, 70 %, yellow crystal, mp; 182-183 °C; IR (KBr): 1704, 1738, 3396.3 cm⁻¹; ¹H NMR: δ 1.88-2.24 (m, 4H), 2.81-2.88 (m, 2H, -NCH₂), 4.67-4.80 (m, 1H, H_b), 5.68-5.70 (d, J = 9.26 Hz, 1H, H_a), 6.61-6.61-7.83 (m, 12H, ArH), 9.86 (s, 1H, -NH); ¹³C NMR: 29.87, 30.64, 47.76, 51.41, 67.76, 74.59, 109.80, 121.57, 122.59, 122.63, 126.35, 126.47, 128.26, 129.38, 129.52, 129.56, 135.11, 135.42, 135.49, 141.40, 141.69, 141.87, 177.64, 196.64, 196.97; MS *m/z*: 468 (M⁺);

Table 3. Antifungal Activity Results of Compounds 4(a-i)

Clinical Yeast Test Isolates	Compound 4a (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g /100 μ L		250 μ g /100 μ L		500 μ g /100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<u>Candida albicans</u>	-	-	-	-	-	-	12	13.3	16.4	18.2
<i>Candida tropicalis</i>	-	-	-	-	-	-	14.5	16.1	17.4	19.3
Clinical Yeast Test Isolates	Compound 4b (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g /100 μ L		250 μ g /100 μ L		500 μ g /100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<u>Candida albicans</u>	-	-			10	11.1	13	14.4	16.5	18.3
<i>Candida tropicalis</i>	-	-					11	12.2	14.2	15.8
Clinical Yeast Test Isolates	Compound 4c (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g /100 μ L		250 μ g /100 μ L		500 μ g /100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<u>Candida albicans</u>	-	-	-	-	13.4	14.9	15.6	17.3	17	18.9
<i>Candida tropicalis</i>	-	-	10	11.1	12.4	13.8	14	15.6	16.8	18.7
Clinical Yeast Test Isolates	Compound 4d (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g /100 μ L		250 μ g /100 μ L		500 μ g /100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<u>Candida albicans</u>	-	-	-	-	-	-	14	15.6	16.5	18.3
<i>Candida tropicalis</i>	-	-	-	-	-	-	-	-	13	14.4
Clinical Yeast Test Isolates	Compound 4e (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g /100 μ L		250 μ g /100 μ L		500 μ g /100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<u>Candida albicans</u>	-	-	-	-	-	-	12	13.3	15.8	17.6
<i>Candida tropicalis</i>	-	-	-	-	13.4	14.9	15.2	16.9	17	18.9
Clinical Yeast Test Isolates	Compound 4f (Concentration mg / mL)									
	Zone of Inhibition (diameter in mm)									
	100 μ L DMSO		62.5 μ g / 100 μ L		125 μ g /100 μ L		250 μ g /100 μ L		500 μ g /100 μ L	
	I	% I	I	% I	I	% I	I	% I	I	% I
<u>Candida albicans</u>	-	-	12	13.3	15.6	17.3	17.8	19.8	20.4	22.7
<i>Candida tropicalis</i>	-	-	-	-	11.5	12.8	13	14.4	15	16.7

Table 3. Contd....

Clinical Yeast Test Isolates	Compound 4g (Concentration mg / mL) Zone of Inhibition (diameter in mm)									
	100 µL DMSO		62.5µg / 100 µL		125µg /100 µL		250µg /100 µL		500µg /100 µL	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Candida albicans</i>	-	-	12.3	13.7	15.8	17.6	17	18.9	20.7	23
<i>Candida tropicalis</i>	-	-	14	15.6	17.4	19.3	19	21.1	21	23.3
Clinical Yeast Test Isolates	Compound 4h (Concentration mg / mL) Zone of Inhibition (diameter in mm)									
	100 µL DMSO		62.5µg / 100 µL		125µg /100 µL		250µg /100 µL		500µg /100 µL	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Candida albicans</i>	-	-	10	11.1	13	14.4	17.5	19.4	19	21.1
<i>Candida tropicalis</i>	-	-	15	16.7	18.2	20.2	21.8	24.2	23	26.4
Clinical Yeast Test Isolates	Compound 4i (Concentration mg / mL) Zone of Inhibition (diameter in mm)									
	100 µL DMSO		62.5µg / 100 µL		125µg /100 µL		250µg /100 µL		500µg /100 µL	
	I	% I	I	% I	I	% I	I	% I	I	% I
<i>Candida albicans</i>	-	-	14	15.6	18.5	20.6	23	25.6	26.8	29.8
<i>Candida tropicalis</i>	-	-	-	-	12	13.3	16.4	18.2	18	20

Table 4. Consolidated Anti-Microbial Results of Compounds 4(a-i)

Antibacterial Activity	Compounds Tested Using Well Diffusion Met								
	4a	4b	4c	4d	4e	4f	4g	4h	4i
<i>Staphylococcus aureus</i>	+++	++	++	++	+	+	++	+	++
<i>Bacillus subtilis</i>	-	-	-	-	-	-	++	-	++
<i>Pseudomonas aeruginosa</i>	+++	+++	+++	+++	+++	+++	+++	++	-
<i>Salmonella typhi</i>	+++	+++	+++	+++	+++	+++	++	+++	++
<i>Salmonella paratyphi 'A'</i>	+++	+++	+++	++	++	+++	+++	++	-
<i>Salmonella paratyphi 'H'</i>	+++	+++	+++	++	+++	++	+++	-	++
<i>Klebsiella pneumoniae</i>	++	++	++	++	+	+++	-	+++	+++
<i>Proteus mirabilis</i>	-	+++	-	-	-	-	-	++	+
<i>Proteus vulgaris</i>	++	+++	-	++	-	++	-	++	++
<i>Escherichia coli</i>	+++	+++	+++	+++	+++	+++	+++	+++	+++
Antifungal activity									
<i>Candida albicans</i>	+	+	++	++	++	+++	+++	+++	+++
<i>Candida tropicalis</i>	++	+	+++	-	+++	++	+++	+++	++

+++ = Go od activity or Sensitive; ++ = Moderate or Intermediate activity; + = Low or Mild activity; - = Resistant

Table 5. Screening Antibacterial Activity Using Standard Antibiotic Disk Against Various Pathogens by Disk Diffusion Method

Antibacterial Activity	Disk Diffusion Method												
	Diameter of Zone of Inhibition in mm for Various Antibiotics												
	Ce	T	Cf	C	Ak	Cm	Va	G	M	Ck	Of	S	Pf
<i>Staphylococcus aureus</i>	13.0	10.0	-	32.0	21.5	-	14.5	26.0	31.5	30.5	18.0	22.0	-
<i>Bacillus subtilis</i>	13.0	11.5	17.0	18.0	16.0	17.0	14.0	15.0	12.5	16.0	17.5	10.0	13.0
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	8.0	-	-	6.0	-	-
<i>Klebsiella pneumoniae</i>	7.0	12.0	8.0	15.0	7.0	13.0	-	7.0	-	-	12.0	-	9.0
<i>Salmonella typhi</i>	9.5	-	11.5	-	11.5	-	-	12.5	-	20.5	11.0	-	9.0
<i>Salmonella paratyphi 'A'</i>	9.0	-	10.0	10.0	10.0	10.0	-	-	-	12.5	12.0	7.0	9.0
<i>Salmonella paratyphi 'H'</i>	11.0	-	16.0	-	-	11.0	-	11.0	-	17.0	16.0	9.0	14.0
<i>Proteus mirabilis</i>	12.5	-	20.0	-	13.5	-	-	9.0	-	17.0	17.5	-	15.0
<i>Proteus vulgaris</i>	-	-	-	23.5	14.0	-	-	11.0	-	17.0	19.0	-	-
<i>Escherichia coli</i>	19.0	11.0	-	23.5	19.0	-	-	21.0	-	18.5	13.5	-	-
Antifungal Activity	Disk Diffusion Method												
	Diameter of Zone of Inhibition in mm for Various Antibiotics												
	Fluconazole (10 µg)						Itraconazole (10 µg)						
<i>Candida albicans</i>	-						10.0						
<i>Candida tropicalis</i>	35.0						18.0						

Table 6. NCCLS M2-A7- Disk Diffusion Method Zone Diameter Interpretive Standards and Equivalent Minimal Inhibitory Concentration (MIC) NCCLS Breakpoints

Antimicrobial Agents	Symbol	Disc Content	Zone Diameter Nearest Whole mm			Equivalent MIC Breakpoints (µL/mL)	
			Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)	Resistant	Sensitive
Amikacin	Ak	30 µg	14	15-16	17	≥ 32	≤ 16
Ceftizoxime	Ck	30 µg	14	15-19	20	≥ 32	≤ 8
Cefotaxime	Ce	30 µg	14	15-22	23	≥ 64	≤ 8
Chloramphenical	C	30 µg	12	13-17	18	≥ 32	≤ 8
Ciprofloxacin	Cf	5 µg	15	16-20	21	≥ 4	≤ 1
Gentamycin	G	10 µg	12	13-14	15	≥ 8	≤ 4
Methicillin	M	30 µg	9	10-13	14	≥ 16	≤ 8
Ofloxacin	Of	5 µg	12	13-15	16	≥ 8	≤ 2
Peflozacin	Pf	30 µg				-	-
Streptomycin	S	10 µg	11	12-14	15	-	-
Tetracycline	T	30 µg	14	15-18	19	≥ 16	≤ 4
Trimazine	Cm-Co	25 µg				-	-
Vancomycin	Va	30 µg			15	≥ 32	≤ 4
Antifungal Agents							
Fuloconozole	Fu	10µg	12	13-18	19	≥ 64	≤ 0.8
Itraconozole	It	10µg	12	13-18	19	≥ 1	≤ 0.125

Anal. Calcd for $C_{28}H_{21}N_2O_3Cl$: C, 71.71; H, 4.51; N, 5.97. Found: C, 71.84; H, 4.62; N, 6.03.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3'''-dione-4- (o-nitroophenyl)-pyrrolizidine 4i. 0.23 g, 85 %, pale yellow crystal, mp; 184-185 °C; IR (KBr): 1346, 1523, 1703.4, 1742.6, 3352 cm^{-1} ; 1H NMR: δ 2.08-2.35 (m, 4H), 2.73-3.03 (m, 2H, -NCH₂), 4.71-4.79 (m, 1H, H_b), 5.48 (d, J = 9.27 Hz, 1H, H_a), 6.52-7.82 (m, 12H, ArH), 9.91 (s, 1H, -NH); ^{13}C NMR: 29.29, 29.68, 30.21, 43.91, 57.12, 69.71, 74.62, 109.74, 121.34, 122.57, 123.63, 125.74, 127.96, 129.35, 129.99, 131.45, 132.22, 135.51, 135.63, 135.72, 140.97, 141.54, 141.64, 151.60, 177.63, 196.81, 197.42; MS m/z : 479 (M⁺); Anal. Calcd for $C_{28}H_{21}N_3O_5$: C, 70.06; H, 4.51; N, 8.75. Found: C, 70.18; H, 4.62; N, 8.84.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3'''-dione-4- (3, 4-dimethoxyphenyl)-pyrrolizidine 4j. 0.17 g, 60 %, yellow solid, mp; 177-178 °C; IR (KBr): 1704.3, 1730, 3352.1 cm^{-1} ; 1H NMR: δ 1.94-2.15 (m, 4H), 2.74-2.85 (m, 2H, -NCH₂), 3.98 (s, 3H), 4.09 (s, 3H), 4.79-4.82 (m, 1H, H_b), 5.74 (d, J = 9.28 Hz, 1H, H_a), 7.52-7.73 (m, 11H, ArH), 8.85 (s, 1H, NH); ^{13}C NMR: 29.63, 29.96, 31.27, 45.54, 52.02, 54.60, 55.18, 66.13, 70.64, 121.45, 122.38, 122.80, 123.11, 124.46, 128.56, 129.95, 13.38, 132.21, 133.60, 133.90, 135.25, 141.67, 148.24, 154.48, 166.16, 171.72, 189.76, 189.97; MS m/z : 494 (M⁺); Anal. Calcd for $C_{30}H_{26}N_2O_5$: C, 72.86; H, 5.29; N, 5.66. Found: C, 72.90; H, 5.36; N, 5.72.

Spiro [2.3'] oxindole-spiro [3.2''] indane-1'', 3'''-dione-4- (3, 4, 5-trimethoxyphenyl)-pyrrolizidine 4k. 0.15 g, 57 %, yellow solid, mp; 176-178 °C; IR (KBr): 1704, 1730, 3352.1 cm^{-1} ; 1H NMR: δ 1.92-2.14 (m, 4H), 2.72-2.82 (m, 2H, -NCH₂), 3.96 (s, 3H), 3.98 (s, 3H), 4.06 (s, 3H), 4.74-4.78 (m, 1H, H_b), 5.69 (d, J = 9.25 Hz, 1H, H_a), 7.48-7.72 (m, 10H, ArH), 8.88 (s, 1H, -NH); ^{13}C NMR: 27.79, 28.04, 30.33, 44.87, 51.98, 54.01, 54.22, 55.18, 66.04, 70.60, 121.40, 122.32, 123.10, 124.42, 127.27, 129.37, 129.58, 13.30, 132.18, 133.52, 135.20, 141.65, 148.52, 155.41, 166.04, 171.68, 189.70, 189.89; MS m/z : 524 (M⁺); Anal. Calcd for $C_{31}H_{28}N_2O_6$: C, 70.98; H, 5.37; N, 5.34. Found: C, 70.86; H, 5.46; N, 5.24.

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