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ARTICLE

Microwave Assisted a Highly Atom Economic, Chemo-, Regio- and Stereoselective Synthesis and Evaluation of Dispiro[1*H*-indene-2,3′-pyrrolidine -2′,3″-[3*H*]indole]-1,2″(1″H)diones as Antibacterial and Antifungal Agents

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Asian Journal of Organic & Medicinal Chemistry

Volume: 7 Year: 2022
Issue: 1 Month: January–March
pp: 23–30
DOI: https://doi.org/10.14233/ajomc.2022.AJOMC-P356

Received: 17 December 2021
Accepted: 19 February 2022
Published: 5 April 2022

ABSTRACT

1,3-Dipolar cycloaddition of *in situ* generated non-stabilized azomethine ylides through the decarboxylative condensation of sarcosine and substituted isatins with 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-ones in microwave produced dispiro[1H-indene-2,3′-pyrrolidine-2′,3″-[3H]indole]-1,2″(1″H)diones in a highly stereo- and regio-selective fashion. The synthesized compounds were subjected to antibacterial and antifungal studies. It was found that many compounds possess a considerable antibacterial and antifungal activity against all the tested organisms.

KEYWORDS

1,3-Dipolar cycloaddition,Azomethine, Ylides, Dispiro compounds, Antibacterial activity, Antifungal activity.

INTRODUCTION

Spiropyrrolidinyl-oxindole is the main component of natural alkaloids, including spirotryprostatine B and spirotryprostatine A [1,2], which are acquired from secondary *Aspergillus fugimatus* metabolites. *Aspergillus fugimatus* can inhibit the cycle of mammalian cells at the G2/M phase. Elacomine [3] and horsfiline [4-9] can be isolated from *Eleagnus commutata* and *Horsfieldia superba*, respectively. *Horsfieldia superba* is a small tree found in Malaysia, and its extracts are used in indigenous medicine. For human brain cancer cell lines, malignant glioma GAMG [10] and neuroblastoma SKN-BE (Fig. 1), a compound isolated from *Uncaria tomentosa* (cat's claw), mitraphylline, is an anti-tumour agent. Many oxindole derivatives behave as antitumour agents because of their inhibitory properties against tyrosine kinase [11-15].

Several methods have been established for the fabrication of enantiomeric and racemic spiro[pyrrolidin-3,3′-oxindole] frameworks of coerulescine and horsfiline. These methods involve oxidative rearrangements with following reagents: sodium tungstate [16], lead tetraacetate [17], *tert*-butyl *N*-bromo-succinimide [18] and hypochlorite [19]. Other means include ring expansion reactions [20], Mannich reaction [7], 1,3-dipolar cycloadditions [9,21,22], electrophilic cyclization [23], intramolecular radical cyclization [24-28], asymmetric

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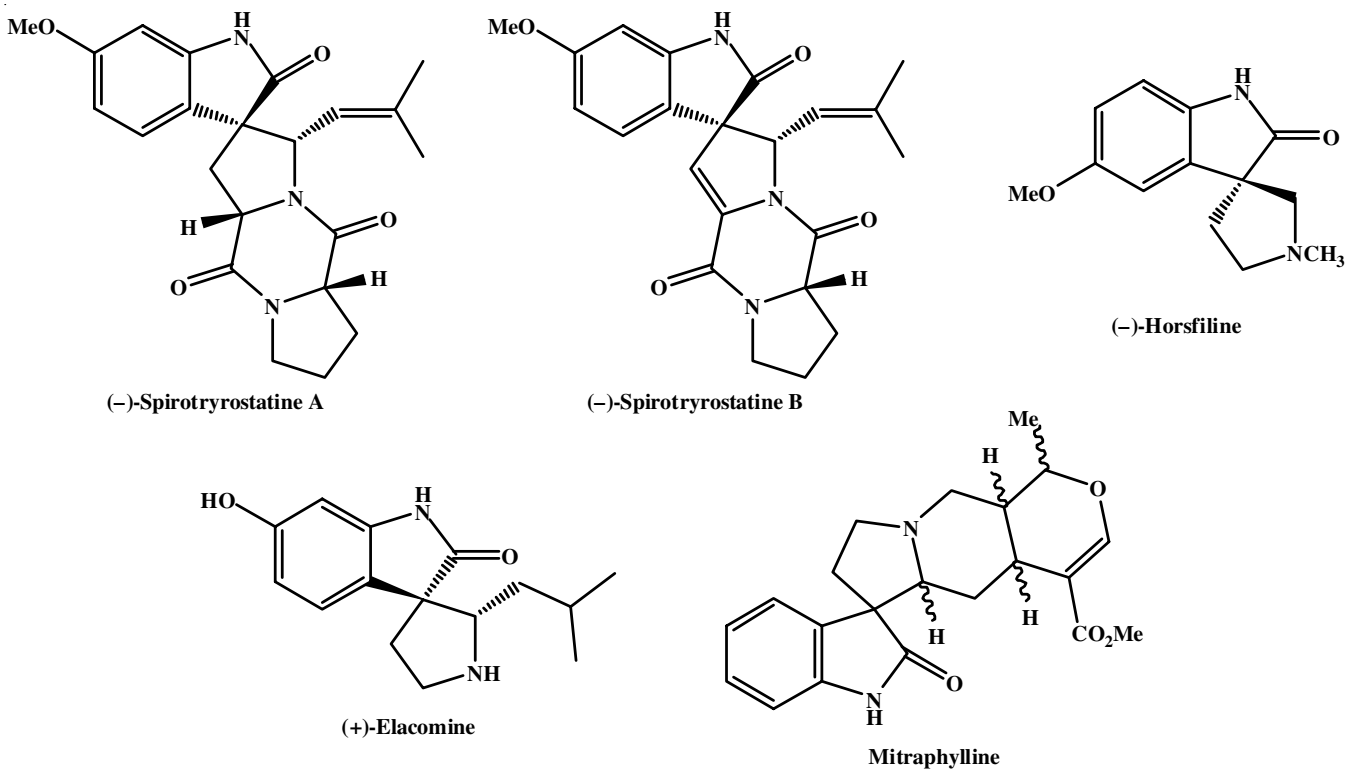


Fig. 1. Naturally occurring alkaloids possessing spiropyrrolidinyl-oxindole skeleton

nitro-olefination [29], palladium-catalyzed domino Heck-cyanation [30], palladium asymmetric allylic alkylation [31], Pd-catalyzed intramolecular cyanoamidation [32], dimethyldioxirane-mediated oxidation [33], NHC-mediated O- to C-carboxyl transfer [34] and tandem intramolecular photocyclo-addition–retro-Mannich reaction [35].

Now-a-days microwave assisted synthesis is an efficient and cost-effective method of formation of novel biologically active heterocycles [36-40]. 1,3-Dipolar cycloaddition reactions for the synthesis of heterocyclic compounds under microwave irradiation have seem to be effective as a green synthetic protocol [41-46].

EXPERIMENTAL

The melting point was determined using an open capillary tube and is reported uncorrected. DEPT, ^{1}H NMR, H,H-COSY, ^{13}C NMR, HMBC and C,H-COSY spectra were measured using the Bruker (Avance) 300 MHz NMR equipment by using TMS and $CDCl_3$ as internal standard and solvent, respectively. Standard Bruker software was employed. The chemical shifts and coupling constants are presented in parts per million (δ scale) and Hertz, respectively. For TLC, silica gel-G plates (Merck) were utilized with an eluent of ethyl acetate and petroleum ether (60-80 ºC). A Perkin-Elmer 2400 Series II Elemental CHNS analyzer was employed for elemental analyses. The microwave reactions were conducted in a Biotage Microwave synthesizer, India.

Synthesis of dispiro[1*H*]-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-diones

Conventional method: A mixture of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-one (**1**) (1 mmol), isatin (**2**) (1 mmol) and sarcosine (**3**) (1.2 mmol) was refluxed in methanol for 3 h. After the completion of the reaction (TLC), the separated solid was filtered, washed with methanol and dried.

Microwave irradiation: In a 10 mL quartz vial, a mixture of isatin (**2**) (1 mmol), 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-one (**1**) (1 mmol) and sarcosine (**3**) (1.2 mmol) was placed and sealed. The vial was put in a Biotage microwave oven and irradiated with microwaves at 120 ºC, 2 bar pressure, 53 W and considerably high absorption levels for the time specified in Table-1. After 1 to 2 min, temperature stabilized at 120 ºC. Subsequently, gas jet cooled down to room temperature (3 min). After reaction completion, revealed by TLC, concentrated *in vacuo* to obtain a crude product. By employing ethyl acetate-petroleum ether (1:4 v/v) as an eluent, this crude product was purified through short column chromatography on silica gel to acquire the pure product **4a-o**.

2,3-Dihydro-1′,1″-dimethyl-4′-(4-isopropylphenyl)-dispiro[1*H*]indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-dione (4c): Pale yellow solid; yield: 94%; m.p.: 221-222 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.25 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 2.72 (d, 1H, J = 18.0 Hz), 2.87 (d, 1H, J = 18.0 Hz), 2.96-3.00 (m, 1H, CH), 3.60 (t, 1H, J = 8.4 Hz,), 4.15 (t. 1H, J = 9.9 Hz), 4.96 (t, 1H, J = 8.7 Hz), 7.12 (d, 2H, J = 8.1 Hz, Ar-H), 7.25-7.45 (m, 8H, Ar-H), 7.64 (d, 2H, J = 8.1 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 21.5, 23.9, 33.7, 35.8, 36.4, 45.5, 58.3, 65.6, 78.8, 108.5, 122.2, 124.6, 125.5, 125.6, 126.4, 127.1, 128.6, 128.9, 131.6, 134.1, 135.7, 136.3, 137.6, 139.5, 151.4, 178.1, 205.8. Anal. calcd. (found) % of $C_{29}H_{28}N_2O_2$: C, 79.79 (79.82); H, 6.46 (6.41); N, 6.42 (6.45).

4′-(2-Chlorophenyl)-2,3-dihydro-1′-methyl-dispiro-[1*H*]indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″H)-dione (4d): Pale yellow solid; yield: 88%; m.p.: 158-159 ºC,

TABLE-1
SYNTHESIS OF COMPOUND **4**: COMPARISON OF YIELD AND REACTION TIME BETWEEN CONVENTIONAL AND MICROWAVE-ASSISTED REACTIONS

Compd.	R^1	R^2	Reaction time		Yield (%)		m.p. (°C)
			Reflux[a] (h)	MW[b] (min)	Reflux[c]	MW	
4a	*p*-BrC_6H_4	H	3	15	95	96	181-182
4b	*o,p*-$Cl_2C_6H_4$	H	3	15	94	92	185-186
4c	*p*-$Pr^iC_6H_4$	H	3	15	90	94	221-222
4d	*o*-ClC_6H_4	H	3	15	90	88	158-159
4e	*o*-BrC_6H_4	H	3	15	88	92	175-176
4f	*p*-$MeOC_6H_4$	5-NO_2	3	15	92	95	221-222
4g	*p*-MeC_6H_4	5-NO_2	3	15	94	96	195-196
4h	*o*-BrC_6H_4	5-NO_2	3	15	92	96	185-186
4i	*p*-$Pr^iC_6H_4$	5-NO_2	3	15	94	96	188-189
4j	*p*-$CH_3C_6H_4$	5-Cl	3	15	90	95	201-202
4k	*p*-$NO_2C_6H_4$	5-Cl	3	15	85	94	209-210
4l	*p*-FC_6H_4	5-Cl	3	15	92	95	168-169
4m	*o,p*-$Cl_2C_6H_3$	5-Cl	3	15	90	94	189-190
4n	*p*-$Pr^iC_6H_4$	5-Cl	3	15	86	90	216-217
4o	*p*-ClC_6H_4	5-Cl	3	15	78	92	206-207

[a]Refluxed in methanol; [b]Irradiation was programmed at 120 °C, 53 W, 2 bar with High absorption level; [c]Isolated yield after purification by column chromatography.

^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 2.25 (s, 3H, CH_3), 2.61 (d, 1H, *J* = 18.0 Hz), 2.87 (d, 1H, *J* = 18.0 Hz), 3.64 (t, 1H, *J* = 8.4 Hz,), 4.16 (t. 1H, *J* = 9.9 Hz), 4.91 (t, 1H, *J* = 8.7 Hz,), 6.56 (d, 1H, *J* = 7.5 Hz, Ar-H), 6.86-7.35 (m, 9H, Ar-H), 7.54 (d, 1H, 7.8 Hz, Ar-H), 8.01 (d, 1H, *J* = 7.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 35.2, 35.4, 45.1, 58.2, 65.4, 78.4, 109.1, 122.9, 124.2, 125.1, 125.4, 126.9, 127.2, 128.0, 129.2, 131.3, 132.5, 134.4, 135.6, 136.0, 137.3, 141.2, 151.3, 178.4, 205.6. Anal. calcd. (found) % of $C_{26}H_{21}N_2O_2Cl$: C, 72.81 (72.77); H, 4.94 (4.90); N, 6.53 (6.49).

2,3-Dihydro-1′,1″-dimethyl-4′-(4-methylphenyl)-dispiro[1*H*]indene-2,3′-pyrrolidine-2′,3″-(5-nitro)[3*H*]-indole]-1,2″(1″*H*)-dione (4g): Pale yellow solid; yield: 96%; m.p.: 195-196 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 2.24 (s, 3H, CH_3), 2.30 (s, 3H, -CH_3), 2.65 (d, 1H, *J* = 18.0 Hz), 2.88 (d, 1H, *J* = 18.0 Hz), 3.60 (t, 1H, *J* = 8.7 Hz,), 4.17 (t. 1H, *J* = 9.9 Hz), 4.88 (t, 1H, *J* = 8.4 Hz,), 6.86 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.06-7.38 (m, 7H, Ar-H), 7.62 (d, 2H, 8.1 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 21.6, 35.3, 35.7, 45.8, 58.3, 65.6, 78.6, 108.7, 122.4, 124.3, 125.8, 126.1, 126.7, 127.6, 128.4, 129.6, 131.5, 134.6, 135.7, 136.6, 137.4, 141.7, 151.6, 178.8, 205.2. Anal. calcd. (found) % of $C_{27}H_{23}N_3O_4$: C, 71.51 (71.55); H, 5.11 (5.14); N, 9.27 (9.31).

2,3-Dihydro-1′,1″-dimethyl-4′-(4-isopropylphenyl)-dispiro[1*H*]indene-2,3′-pyrrolidine-2′,3″-(5-nitro-[3*H*]-indole]-1,2″(1″*H*)-dione (4i) Pale yellow solid; yield: 96%; m.p.: 188-189 ºC, ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.28 (s, 3H, CH_3), 1.33 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.70 (d, 1H, *J* = 18.0 Hz), 2.88 (d, 1H, *J* = 18.0 Hz), 2.92-2.98 (m. 1H, CH), 3.62 (t, 1H, *J* = 8.4 Hz,), 4.10 (t. 1H, *J* = 9.6 Hz), 4.86 (t, 1H, *J* = 8.7 Hz,), 7.06 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.18-7.42 (m, 7H, Ar-H), 7.60 (d, 2H, *J* = 7.8 Hz, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 21.4, 23.5, 33.4, 35.2, 35.6, 45.5, 58.3, 65.6, 78.8, 109.5, 122.3, 124.6, 125.5, 125.6, 126.4, 127.1, 128.6, 128.8, 131.6, 134.1, 135.7, 136.1, 137.5, 139.4, 151.6, 176.1, 207.5. Anal. calcd. (found) % for $C_{29}H_{27}N_3O_4$: C, 72.33 (72.38); H, 5.65 (5.69); N, 8.73 (8.70).

2,3-Dihydro-1′,1″-dimethyl-4′-(4-methylphenyl)-dispiro[1*H*]indene-2,3′-pyrrolidine-2′,3″-(5-chloro)-[3*H*]-indole]-1,2''(1''*H*)-dione (4j) Pale yellow solid; yield: 95%; m.p.: 201-202 ºC, ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 2.24 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.77 (d, 1H, *J* = 17.7 Hz), 3.26 (d, 1H, *J* = 17.7 Hz), 3.59 (t, 1H, *J* = 8.7 Hz,), 4.04 (t. 1H, *J* = 9.3 Hz), 4.33 (t, 1H, *J* = 8.7 Hz,), 6.69 (d, 1H, *J* = 7.1 Hz, Ar-H), 6.97 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.03-7.31 (m, 7H, Ar-H), 7.55 (d, 2H, *J* = 7.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 20.9, 35.0, 35.3, 49.7, 58.9, 66.7, 78.4, 110.2, 123.9, 125.4, 127.4, 127.9, 128.1, 128.3, 129.1, 129.8, 134.7, 135.7, 136.7, 139.6, 148.1, 151.7, 159.8, 178.3, 206.3. Anal. calcd. (found) % for $C_{27}H_{23}N_2O_2Cl$: C, 73.21 (73.25); H, 5.23 (5.126); N, 6.32 (6.30).

4′-(2,4-Dichlorophenyl)-2,3-dihydro-1′-methyl-dispiro-[1*H*]indene-2,3′-pyrrolidine-2′,3″-(5-chloro)[3*H*]indole]-1,2″(1″*H*)-dione (4m): Pale yellow solid; yield: 94%; m.p.: 189-190 ºC. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 2.20 (s, 3H, CH_3), 2.42 (d, 1H, *J* = 17.7 Hz), 2.80 (d, 1H, *J* = 17.7 Hz), 3.60 (t, 1H, *J* = 8.4 Hz,), 4.22 (t. 1H, *J* = 9.6 Hz), 4.95 (t, 1H, *J* = 8.7 Hz), 6.15 (d, 1H, *J* = 8.4 Hz, Ar-H), 6.92 (dd, 1H, *J* = 8.4 Hz, 2.1 Hz, Ar-H), 7.20 (d, 1H, *J* = 2.1 Hz), 7.34-7.60 (m, 7H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 35.1, 35.4, 47.3, 58.6, 65.6, 79.2, 108.6, 122.4, 124.5, 125.2, 125.6, 126.1, 127.3, 127.4, 128.4, 128.9, 131.6, 131.4 134.5, 135.3, 136.1, 137.2, 141.6, 151.2, 178.1, 206.4. Anal. calcd. (found) % of $C_{26}H_{19}N_2O_2Cl_3$: C, 62.73 (62.76); H, 3.85 (3.81); N, 5.63 (5.67).

Antibacterial assay: Through agar diffusion, the antibacterial activities of the synthesized compounds against four human pathogens, namely *Staphylococcus*, *Streptococcus*, *Salmonella* and *Pseudomonas* were analyzed. Each pathogen inoculum was collected using a sterile cotton bud by dipping completely in a test tube containing bacterial cultures grown for 12 h. Then, with cotton buds containing bacterial inoculums, human bacterial pathogens were swabbed on the surface of a solidified NA medium in Petri dishes. Subsequently, five 5 mm wells were prepared in all the plates by employing a sterile cork borer. All the compounds were dissolved in DMSO to

acquire different concentrations and by using a 0.25 µm filter paper. The resulting solution was sterilized through filtration. Each well received the solution (50 µL) of each compound. At room temperature, the plates were incubated. DMSO was added to each plate into the well centre, which was utilized as the control. After 48 h, an inhibition zone appeared around the well.

Antifungal assay: A medium of glucose-nitrate necessary for fungal growth was synthesised by dissolving 2.5 g of KNO_3, 10 g of glucose, 0.5 g of $MgSO_4 \cdot 7H_2O$ and 1 g of KH_2PO_4 in 1 L of sterile distilled water. In the medium, KNO_3 and glucose provide the nitrogen and carbohydrate sources, respectively. $MgSO_4 \cdot 7H_2O$ and KH_2PO_4 behave as growth activators. The fungal cultures were purified through single spore isolation. For fungal growth, the glucose-nitrate medium was employed. Mycelial biomass and the filter paper were dried in an oven at 65 ± 5 ºC to acquire a constant weight and cooled. Finally, on semi-microanalytical balance, the biomass and filter paper were weighed. The mycelial dry weight (MDW) was determined by subtracting the mycelium-free filter paper weight from the final dry weight. The treatments were performed in triplicates at each concentration. Each time under similar experimental conditions, MDW was corrected by subtracting the dry weight acquired from the incubated flask. For each case, the percentage decrease in the mycelia dry weight caused by the test compound was computed and tabulated for average percentage inhibition.

RESULTS AND DISCUSSION

In present investigation 1,3-dipolar cycloaddition of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-ones (**1**) with non-stabilized azomethine ylides, generated *in situ via* decarboxylative condensation of substituted isatins (**2**) and sarcosine (**3**), under microwave irradiation condition in solvent free condition afforded dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]-indole]-1,2″(1″H)-diones (**4**) in a highly regioselective manner (**Scheme-I**). This reaction assumes importance from the view point of green chemistry. The efficacy of this approach is compared with conventional thermal method.

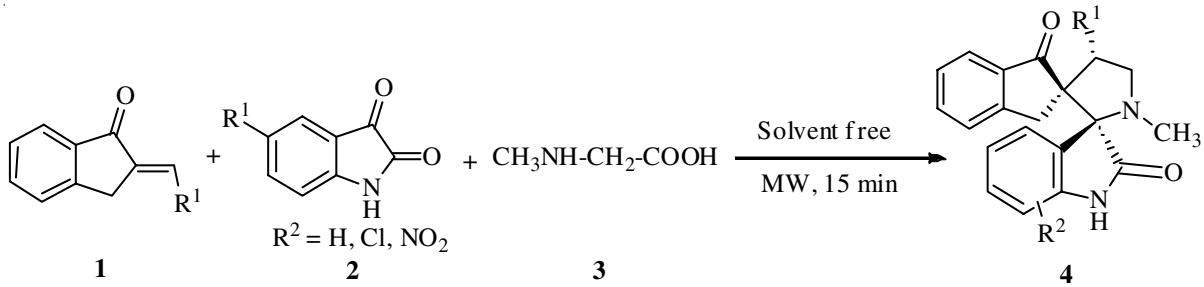
An equimolar mixture of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-one (**1**), substituted isatin (**2**) and sarcosine (**3**) was subjected to under microwave irradiation affording **4** was carried out at temperatures ranging from 80 to 120 ºC with an increment of 10 ºC. The results show that the yield of **4** reached a peak at 120 ºC and that the reaction is completed in 15 min. Consequently, all subsequent reactions under microwave-irradiation were performed under solvent free conditions at 120 ºC. After completion of the reaction as indicated by the TLC, the reaction mixture was separated by column chromatography to furnish pure dispiro[1*H*]-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole-1,2″(1″H)-diones (**4**).

Besides, the reactions for synthesizing **4** were also performed under classical thermal method in methanol under refluxing condition. The results revealed that the yield of the reaction under microwave irradiation and conventional method is almost equal. But the reaction under microwave irradiation was completed more rapidly in minutes than under traditional heating conditions (Table-1).

Each reaction proceeded chemoselectively because cycloaddition involved only the C=C bond of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-one (**1**), which exclusively furnished mono spiro-cycloadduct **4**. For the incorporation of the electron-rich carbon of dipole into the α-carbon of α,β-unsaturated carbonyl system of **1**, this reaction is regioselective. The stereoselective reaction affords excellent yields of only one diastereomer; however, four stereocentres are available in the cycloadducts. Furthermore, the atom economy of this reaction is considerably high, 88-96%, because only carbon dioxide and water are produced as byproducts.

The structure of dispiropyrrolidines **4j** was characterized by ^1H & ^{13}C NMR spectroscopic data and elemental analysis. For example, in ^1H NMR spectrum of **4j** (Fig. 4), the methine hydrogen, H-4′ appears as a triplet at 4.33 ppm (J = 8.7 Hz), which shows H,H-COSY correlations with the methylene hydrogens, H-5′ appearing as triplets at 3.59 ppm (J = 8.7 Hz) and 4.04 ppm (J = 9.3 Hz). This excludes the formation of the other regioisomeric form **4′j** (Fig. 2). The methine hydrogen H-4′ also shows HMBCs with C-5′, spiro carbon (C-2,3′), C-1‴*ipso*, C-2‴ *ortho* carbons and C-1 carbonyl at 58.9, 66.7, 135.7, 129.8 and 206.3 ppm, respectively (Fig. 3). The H-5′ hydrogens show HMBCs with N-CH_3, C-4′, C-2,3′, C-2,3″ and C-1‴*ipso* at 35.3, 49.7, 66.7, 78.4 and 135.7 ppm, respectively (Fig. 4). The methylene protons, H-3 appearing as doublets at 2.74 ppm (J = 17.7 Hz) and 3.26 ppm (J = 17.7 Hz) show HMBCs with the C-2,3′, C-2,3‴ and C-1 carbonyl at 66.7, 78.4 and 206.3 ppm, respectively. The singlet appearing at 8.26 ppm is assigned to indole NH proton as evident from its disappearance upon D_2O wash. The assignment of signals of carbons bearing hydrogens has been done from the chemical shifts of hydrogens and C,H-COSY correlations.

Mass spectrum (EI) of **4j** reveals the presence of (M + 1) peak at *m/e* 443.67 in considerable intensity. A plausible mechanism for the formation of **4** is described in **Scheme-II**. In the first step, azomethine ylide is formed from the decarboxylative condensation of sarcosine with isatin. Then the reactive intermediate undergoes 1,3-dipolar cycloaddition reaction with the



Scheme-I: Synthesis of dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″H)-diones

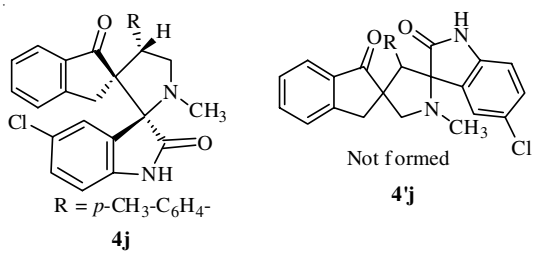


Fig. 2. Regioisomers of **4j**

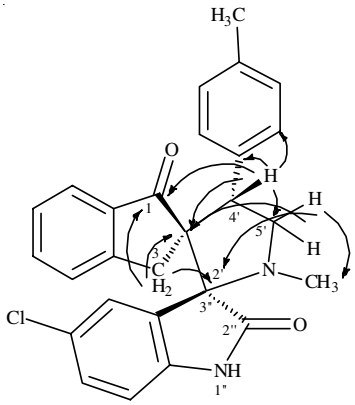


Fig. 3. Selected HMBC correlation of **4j**

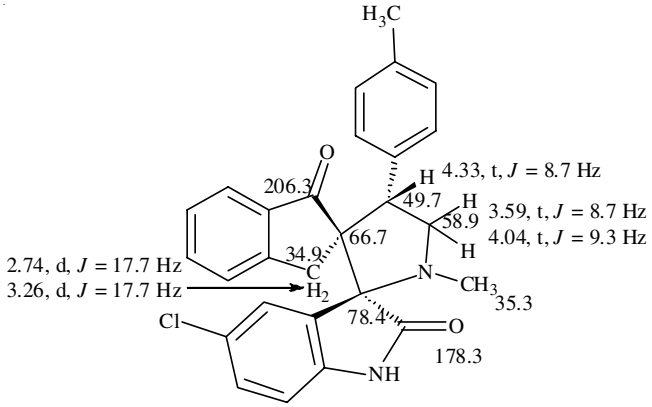
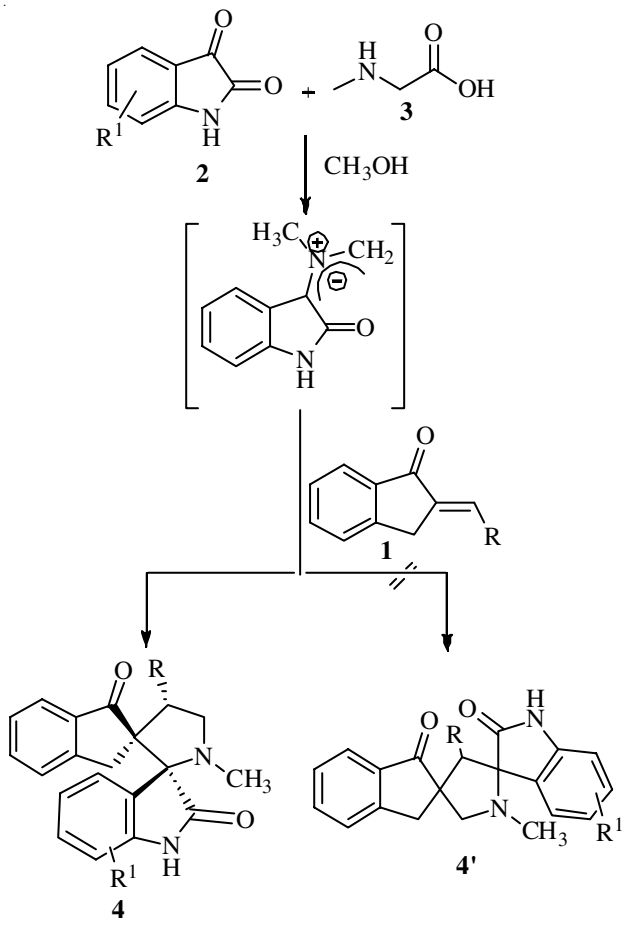


Fig. 4. NMR data of **4j**



Scheme-II: Mechanism for the formation of dispiro[1*H*-indene-2,3′-pyrrolidine-2′,3″-[3*H*]indole]-1,2″(1″*H*)-diones (**4**)

exocyclic olefinic bond derived from 1-indanone, which results in the formation of **4** in a regio- and stereoselective manner.

Antimicrobial studies: The antimicrobial activity of the compounds (**4a-o**) were investigated *in vitro* against bacteria such as *Streptococcus*, *Staphylococcus*, *Pseudomonas aeruginosa* and *Salmonella typhi* by paper disc plate method [47] with

TABLE-2
ANTIBACTERIAL ACTIVITY OF DISPIRO[1*H*-INDENE-2,3′-PYRROLIDINE-2′,3″-[3*H*]INDOLE]-1,2″(1″H)-DIONES (**4**)

Compd.	*Strepto coccus*				*Stephylo coccus*				*Pseudomanas aeruginosa*				*Salmonella typhi*			
	25 mM	50 mM	75 mM	100 mM	25 mM	50 mM	75 mM	100 mM	25 mM	50 mM	75 mM	100 mM	25 mM	50 mM	75 mM	100 mM
4a	+	++	+++	++++	+	++	++	+++	–	++	++	+++	++	+++	+++	+++
4b	++	++++	+++++	++++	–	++	++	++++	+	++	++	++++	+	++	+++	+++
4c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4d	+	++	+++	+++	–	–	–	–	+	++	++	++	+	++	++	++
4e	+	+++	+	+	+	++	+	+	–	–	–	–	++	++	+++	++++
4f	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4g	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4h	–	–	–	–	+	–	–	–	+	++	+	+	+	+	++	+++
4i	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4j	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
4k	–	+	+	+	–	–	–	–	+	+	+	+	+	+	++	+++
4l	+	++	++	+++	–	++	++	++	++	++	+++	+++	–	++	+++	++++
4m	+	++	++	++	+	+	+	+	+	++	++	++	–	++	++	++
4n	–	–	–	–	+	+	+	+	+	+	+	+	–	–	–	–
4o	+	++	++	++	–	–	–	–	–	++	++	++	–	–	+	+
Tetracycline	+	++	+++	++++	++	++	+++	++++	+	+	++	++++	++	++	+++	++++

+ Mild activity, ++ Moderate activity, +++ High activity, ++++ Very high activity, – No activity.

nutient agar media. The compounds were tested using 25, 50, 75 and 100 mmol solutions in DMSO and compared with that of known antibiotics *viz*. tetracyclin (Table-2). For fungicidal activity, compounds were screened *in vitro* against *Aspergillus niger*, *Trichoderma* and *Candida albicans* by mycelia dry weight method [48] with glucose nitrate media. The activity of the synthesized compounds measured at 25, 50, 75 and 100 mmol concentrations in DMSO were compared with that of standard drug, kanamycin.

It was found that compounds, **4a**, **4b**, **4l** and **4m** possess a pronounced antibacterial activity against all the tested organisms (Tables 2 & 3). Compounds **4a**, **4b**, **4l** and **4n** have shown growth inhibitory action almost equal to the reference drug tetracyclin against *Streptococcus*, *Staphylococcus*, *Pseudomonas aeruginosa* and *Salmonella typhi*, indicating that the presence of halogens enhances the inhibitory activity. Other compounds that lack halogens show moderate to mild activity or showed no activity (**4c**, **4f**, **4g** and **4i**).

TABLE-3
COMPARISON OF ANTIBACTERIAL ACTIVITY OF DISPIRO[1*H*-INDENE-2,3′-PYRROLIDINE-2′,3″-[3*H*]INDOLE]-1,2″(1″H)-DIONES (**4**)

Compd.	*Strepto-coccus*	*Stephylo-coccus*	*Pseudomanas*	*Salmonella*
4a	25	25	50	25
4b	25	50	25	25
4c	–	–	–	–
4d	25	–	25	25
4e	25	25	–	25
4f	–	–	–	–
4g	–	–	–	–
4h	–	25	25	25
4i	–	–	–	–
4j	–	25	–	–
4k	50	–	25	25
4l	25	50	25	50
4m	25	25	25	50
4n	–	25	25	–
4o	25	–	50	75

The value indicates the minimum inhibition concentration in mM.

Compounds which showed higher antibacterial activity were investigated for antifungal studies. Most of the compounds show considerable activity against all the organisms tested. Compounds **4a**, **4b**, **4h** and **4m** were found to be almost equipotent to that of reference drug kanamycin (Table-4).

Conclusion

In conclusion, 1,3-dipolar cycloaddition of azomethine ylide generated in situ from substituted isatin and sarcosine to a series of 2-(arylmethylene)-2,3-dihydro-1*H*-inden-1-ones furnishing 15 dispiro[1*H*]-indene-2,3′-pyrrolidine-2′,3″-[3*H*]-indole]-1,2″(1″H)-diones in near quantitative yields in a regio- and stereoselective manner under microwave condition was investigated. The reaction condition and the yield is compared with conventional thermal reflux method. These spiro heterocycles displayed good *in vitro* antibacterial and antifungal activities. The antimicrobial potency of these spiro heterocycles renders them valid leads for synthesizing new heterocycles endowed with enhanced biological activity.

ACKNOWLEDGEMENTS

The authors are grateful to UGC, New Delhi, India for their financial support (F.No.43-244/2014 (SR), Madurai Kamaraj University, Madurai, and Indian Institute of Chemical Technology, Hyderabad, India for their support in the spectral analysis and biological studies.

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TABLE-4
ANTIFUNGAL ACTIVITY OF DISPIRO[1*H*-INDENE-2,3′-PYRROLIDINE-2′,3″-[3*H*]INDOLE]-1,2″(1″H)-DIONES (**4**)

Compd.	*Aspergillus niger*				*Trichoderma*				*Candida albicans*			
	25 mmol	50 mmol	75 mmol	100 mmol	25 mmol	50 mmol	75 mmol	100 mmol	25 mmol	50 mmol	75 mmol	100 mmol
4a	++	++	+++	++++	+	+	++	++	++	++	+++	+++
4b	–	–	++	++	+	++	+++	+++	–	+	+	++
4d	+	+	++	++	–	–	–	–	–	–	–	–
4e	++	++	++	+++	–	–	–	++	+	+	++	++
4h	++	+++	+++	++++	+	+	++	++	–	–	+	++
4k	–	–	–	–	+	++	++	+++	–	–	–	–
4l	+	++	++	+++	–	–	–	–	–	–	–	–
4m	–	–	–	++	+	++	+++	+++	+	+	++	+++
4o	–	+	+	++	–	–	+	+	–	–	–	–
Kanamycin	++	++	+++	+++	++	+++	+++	++++	+	++	+++	+++

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