Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *87* (7–8) 813–827 (2022)
JSCS–5559
Original scientific paper
Published 4 May 2022

Microwave assisted synthesis of novel spiro diarylidenes and their antimicrobial assay

SABITA SHROFF[1], PRAJNA PARIMITA MOHANTA[1], ISWAR BAITHARU[2], BHAWANI PRASAD BAG[3] and AJAYA KUMAR BEHERA[1]*

[1]Organic Synthesis Lab, School of Chemistry, Sambalpur University, Jyoti Vihar Odisha--768019, India, [2]Toxicology Lab, P.G. Department of Environmental Sciences, Sambalpur University, Jyoti Vihar, Odisha-768019, India and [3]Department of Biotechnology and Bioinformatics , Sambalpur University, Jyoti Vihar, Odisha-768019, India

(Received 23 January 2021, revised 20 February, accepted 30 March 2022)

Abstract: A rapid and high yield microwave assisted synthesis of a series of novel 7,9-bis-(arylidene)-4-methyl-2,6,10-triphenyl-2,3-diazaspiro[4,5]dec-3--ene-1,8-dione has been explored. The spiro diarylidene derivatives having nitrogen atom and *α*,*β*-unsaturated ketone moiety were synthesized by aldol condensation between 4-methyl-2,6,10-triphenyl-2,3-diazaspiro[4,5]dec-3-ene-1,8--dione and corresponding aryl aldehydes followed by dehydration. The synthesized series of novel spirodiarylidene derivatives were characterized using IR, ^{1}H- and ^{13}C-NMR and mass spectra. Density functional theory (DFT) study was performed by Gaussian 09 software. The antimicrobial activities of the synthesized derivatives were evaluated against two pathogenic Gram-positive and Gram-negative bacterial strain and three pathogenic fungal species by disk diffusion method. The minimum inhibitory concentration was determined by the microbroth dilution technique. The results of the present study demonstrated that the examined compounds marked **5a** and **5c**, possessing 4-NO_2 and 5-Br-2-OH substituents, are found to be more active against Gram-positive bacterium *Staphylococcus aureus*, 8 and 16 μg mL^{-1}, respectively, and moderately active against Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, compared to other synthetic derivatives. However, none of the synthesized derivatives showed any activity against *Streptococcus pyrogenes*. Compound **5e**, possessing 2,4,6-$(OCH_3)_3C_6H_3$ moiety, exhibited broad spectrum activity against all fungal strains under study, but showed no antibacterial activity.

Keywords: pyrazolone; Michaelis addition; aldol condensation; antibacterial activity; irradiation; antifungal activity.

* Corresponding author. E-mail: ajayakumar.behera@suniv.ac.in
https://doi.org/10.2298/JSC210123031S

INTRODUCTION

The development of resistance among pathogenic microbial strains against existing antimicrobial agents is increasing day by day and has become a major concern for public health over the last few decades.[1] There is an utter necessity to develop effective antimicrobial agents with novel modes of action and a broad spectrum of activities to keep pace with evolving pathogenic microbial strains. Number of phytochemicals has been demonstrated to pose robust broad spectrum antimicrobial activity with minimal side effects.[2] Spirocyclic compounds that are found abundantly in plants and animals draw special attention because of their wide applications in medicinal chemistry.[3] Some of them are found to have remarkable biological activities and have been reported to act as antibacterial,[4] antifungal,[5] anti-inflammatory,[6] antimalarial[7] and anticancer agents. The cyclic structures in the spiro compounds, fused at a central carbon, play a very significant role in numerous biological activities of molecules which possess them.[9,10]

Pharmacological activities of several spiro compounds and their derivatives are widely investigated to develop therapeutics against microbial as well as numerous metabolic diseases. The presence of the reactive carbonyl functionalized heterocycles in spiro framework has been shown to exhibit anti-HIV[11] and anti-inflammatory[12] effect. Spiro [indolo-3,10'-indeno[1,2-*b*]quinolin]-2,4,11'-triones and their derivatives have been reported as a new class of antifungal and antimicrobial agents.[13] Besides their antimicrobial activities, the literature reveals that spiro compounds like 7-(3-pyridinyl)-1,7-diazaspiro[4.4]nonane analogue (I)[14] and spirocyclic pyrrolidines (II)[15], Fig. 1, are also used for the treatment of central and autonomic nervous systems dysfunctions and various neurological and psychiatric disorders, as potential drug candidates. Spiro compounds containing heteroatoms in the exo-olefinic scaffold (III) and (IV) are found to have significant antitumor[16] and antibacterial activities,[17] respectively. However, pharmacological activities of spirodiarylidenes are poorly examined so far.

Though spiro compounds and their derivatives possess promising therapeutic potentials, their synthesis using conventional methods are very time consuming and tedious.[18] Microwave irradiation dramatically reduces the reaction times by choosing lower energy pathways, minimizing the number of steps, and reducing energy usage by providing uniform heating. Further microwave assisted synthesis is a greener route of organic synthesis as it ensures minimum use of organic solvents and subsequent generation of waste.[19] Numerous studies have explored the synthesis of spiro compounds, such as novel dispiro-oxindolopyrrolizidines and pyrrolidines, in good yield using microwave assisted organic synthesis methods.[20,21] However, the synthesis of spirodiarylidene derivatives using microwave irradiation is not yet explored. In this paper, we highlight the application of microwave methodology in the synthesis of diarylidenes of spiro compounds from pyrazolone and dibenzalacetone precursors. A series of novel spiro deri-

vatives synthesized are further evaluated for their antibacterial and antifungal activities against a panel of bacterial strains and fungal species.

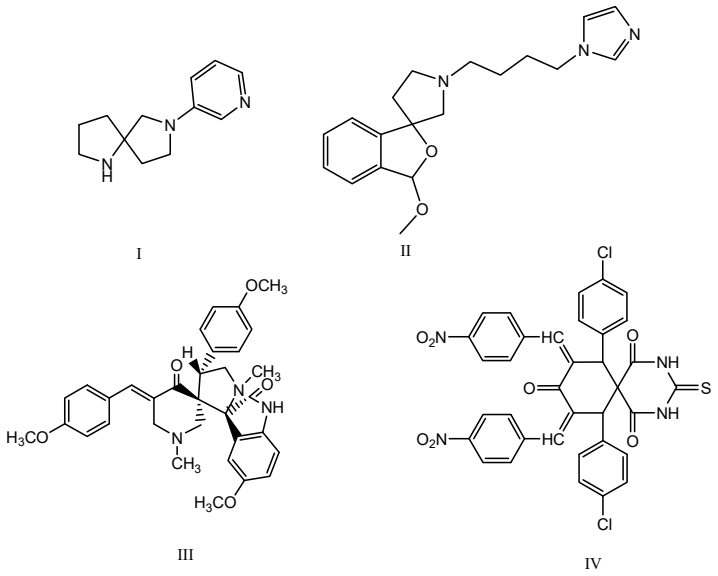


Fig. 1. Bioactive spiro compounds for the treatment of dysfunction of central and autonomic nervous systems (I),[14] neurological and psychiatric disorders (II),[15] as well as antitumor (III)[16] and antibacterial (IV)[17] agents.

EXPERIMENTAL

General procedure

All the reagents and solvents used were analytical grade and were purchased from Merck, Sigma Aldrich and Finar (India). The reactions were performed in Sineo Mas II microwave synthesizer in sealed reaction vessels, power voltage: AC 220 V (±10 %), 50 Hz, rated input power: 1360 W, rated high frequency output power: 1000 W, operating frequency: 2450 MHz. The reaction conditions are optimized by changing different temperatures, time, and power under solvent medium.

Melting points were taken in open capillaries using sulphuric acid-bath and were uncorrected. Reactions were monitored by thin-layer chromatography (TLC) using Merck silica gel 60 F254 precoated plates (0.25 mm) in an appropriate mixture of ethyl acetate and hexane and observed using UV-lamp of 365 nm. IR spectra were obtained on Bruker Alpha-II. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance III spectrometer (at 400 and 100 MHz, respectively). Chemical shifts were expressed in parts per million downfield from tetramethylsilane (TMS) dissolved in $CDCl_3$ as an internal standard.[22]

LC–MS analysis was performed with an Agilent 6110 LC–MS instrument with Mercury Luna 3 μ C18 column-attached mass spectrometer with ESC ionisation source in ESI mode. Water:acetonitrile (50:50) containing 0.1 % formic acid was used as the mobile phase. Sample preparation was made using acetonitrile as the diluent. Flow rate was maintained at 1.0 ml/min. The nebulizer pressure ESI mass spectrometer was maintained at 413.7 kPa, vaporizer temp

150 °C, drying gas flow 5 ml/min and drying gas temperature 300 °C. The voltage capacity of single ion polarity was 2000 V, polarity switching 1000v and charging electrode capacity was maintained at 2000 V.

Analytical and spectral data of the compounds are given in Supplementary material to this paper.

Density function theory (DFT) study was performed using Gaussian 09 programme and the calculation was done by applying DFT B3LYP/6-31++g(d,p).[23] Elemental analyses were performed by Flash2000 elemental analyzer and were in agreement with the calculated values within ±0.4 %. The physical, analytical and spectral data for the compounds are given in the Supplementary material to this paper.

Synthetic procedures

*Synthesis of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**)*. A mixture of ethyl acetoacetate (12.76 mL, 100 mmol) and freshly distilled phenylhydrazine (9.8 mL, 100 mmol) was heated at 120 °C in an oil bath for 1 h. The resulting red oil was cooled and stirred with diethyl ether until solidification occurred. The colourless solid thus obtained was filtered and finally recrystallized with ethanol. Yield: 78 %, m.p. 124 °C (lit. m.p. 127 °C).[24]

*Synthesis of dibenzalacetone (**2**)*. To a solution of 10 % NaOH and ethyl alcohol (80 mL), acetone (3.7 mL, 50 mmol) was added and stirred for 15 min. Then, benzaldehyde (10.19 mL, 100 mmol) was added in two phases and stirred for two hours at 25 °C. Yellow crystals thus separated were filtered, washed with water, and crystallized from ethyl acetate. Yield: 73.2 %, m.p. 112 °C (lit. m.p. 112 °C)[25].

Synthesis of 4-methyl-2,6,10-triphenyl-2,3-diaza-spiro[4.5]dec-3-ene-1,8-dione (3)

Conventional method. A mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (**1**, 0.87 g, 5 mmol) and dibenzalacetone (**2**, 1.17 g, 5 mmol) in 15 ml ethanol in presence of 5 drops of triethanolamine was refluxed for 16 h. The completion of the reaction was monitored by TLC. The reaction mixture was allowed to cool at room temperature. The solution was poured onto ice cold water. The brown solid formed was filtered, dried, and recrystallized from rectified spirit to obtain **3**.[17]

Microwave-assisted (MW) method. A mixture of 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)--one (**1**, 0.87 g, 5 mmol) and dibenzalacetone (**2**, 1.17 g, 5 mmol) in 15 mL ethanol in the presence of 5 drops of triethanolamine were irradiated in the microwave at 300 W and 80 °C for 10 min. The reaction mixture was allowed to cool at room temperature. The solution was poured onto ice cold water. The brown solid formed was filtered, dried, and recrystallized from rectified spirit to obtain **3.**

*General procedure for the formation of arylidene derivatives of 4-methyl-2,6,10-triphenyl-2,3--diaza-spiro[4.5]dec-3-ene-1, 8-dione (**5a**–**f**)*

Conventional method. A mixture of spiro compound **3** (0.2 g, 0.5 mmol) and substituted aryl aldehydes **4a**–**f** (1 mmol) was refluxed for 10 h in the presence of sodium ethoxide (1 mmol) in 10 mL ethanol. The completion of the reaction was monitored by TLC. The reaction mixture was allowed to cool at room temperature. The solution was poured onto ice cold water. The solid formed was filtered, dried, and recrystallized from the rectified spirit to obtain the compounds **5a**–**f**.

MW-assisted method. A mixture of spiro compound **3** (0.2 g, 0.5 mmol) and substituted aryl aldehydes **4a**–**f** (1 mmol) in the presence of sodium ethoxide (1mmol) in 10 mL ethanol was irradiated in microwave at 300 W and 80 °C for 7 min. The reaction mixture was allowed

to cool at room temperature. The solution was poured onto ice cold water. The solid formed was filtered, dried, and recrystallized from the rectified spirit to obtain the compounds **5a**–**f**.

Assessment of antimicrobial activity

Test microorganisms. The selection of seven different microbial species was done based on their pathogenic activities and clinical importance to human health. Two Gram-positive bacteria (*Staphylococcus aureus (*MTCC 3615) and *Streptococcus pyrogenes (*MTCC 442), two Gram-negative bacteria (*Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 424) and three fungi *Candida albicans (*MTCC 227), *Aspergillus niger (*MTCC 282), and *Aspergillus clavatus (*MTCC 1327) were chosen for assessing the antibacterial and antifungal activity of the synthesized compounds. All the microbial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. While the bacteria were further sub cultured on Nutrient agar, fungi were grown on Sabourauds dextrose agar.

Assessment of antibacterial activity using agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^8 CFU mL^{-1}. 20 mL of agar medium, was poured into each petri plate and plates were swabbed with 100 mL inocula of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates, and these were loaded with a 100 mL volume with a concentration of 8.0 mg mL^{-1} of each compound reconstituted in the dimethyl sulphoxide. All the plates were incubated at 37 °C for 24 h. The antimicrobial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms. DMSO was used as a negative control, whereas Ciprofloxacin was used as a positive control for bacteria and amphotericin-B for fungi. This procedure was performed in three replicate plates for each organism.[13]

*Determination of minimum inhibitory concentration (*MIC*) of the spiro compounds*

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. The antimicrobial potential of the synthesized compounds was evaluated by determining the *MIC* values by the microdilution method. The *MIC*s were determined by measuring the absorbance of microtiter plates at 570 nm for bacteria and 530 nm for fungi. The optical density from each well was compared with the optical density from the positive control wells, the lowest concentration with optical density <0.1 signifies inhibition and considered as *MIC* value.[4]

Antibacterial susceptibility testing by microbroth dilution method

The pure bacterial culture of each microorganism was adjusted to 0.5 in Mueller–Hinton broth (MHB). To avoid bacterial duplication, the suspension of bacterial cells was used immediately within 30 min of turbidity adjustment. All the spiro diarylidene derivatives were dissolved in 1 mL of dimethyl sulfoxide (DMSO) to prepare a stock solution of 1 mg. In this method, a two-fold serial dilution of each chemically synthesized compound was prepared by reconstituting the compound in DMSO first, followed by dilution in sterile distilled water to achieve a decreasing concentration range of 512–1 μg mL^{-1}. Ten different concentrations were prepared from each stock with the medium (1, 2, 4, 8, 16, 32, 64, 128, 256, 512 μg mL^{-1}). A total of 50 μL of each compound concentration was added to sterile 96-well microtiter plates. After that, 50 μL of diluted bacterial inoculums were added to each well including the negative control lane, and 100 μL of broth was added to the positive control lane. The plates were then incubated at 37 °C for 18–24 h. The inhibition of growth was determined by measuring

the absorbance at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader. Ciprofloxacin and all the spirodiarylidene derivatives were dissolved in 1 mL of dimethyl sulfoxide (DMSO) to prepare a stock solution of 1 mg. Amphotericin-B was used as a positive control while DMSO as a negative control.[26]

Antifungal susceptibility testing by microbroth dilution method

The Sabouraud's dextrose agar slant was used for subculturing the fungal strains. Freshly grown pure fungal culture was obtained after incubating the slant for 24–48 h at 35 °C. The fungal suspension was further adjusted to make it equivalent to 0.5 McFarland standards unit of turbidity and the size of the fungal population maintained to 0.5×10^5 or 2.5×10^5. The Sabor and dextrose agar slant culture was incubated for 5 days at 35 °C to obtain the suspension of conidia of the mold. The colonies were covered with 5 mL of sterile distilled water supplemented with Tween 20. The number of conidia in the suspension was counted using a hemocytometer and diluted at a ratio of 1:10 with RPMI to obtain final inoculums of $2–5\times10^5$ conidia mL^{-1}. A total of 50 µL of each compound concentration and 50 µL of fungal suspension were added to each well for the negative control lane and 100 µL of broth was added to the positive control lane. The plate was sealed with aluminum foil and incubated at 35 °C for 24–48 h in a humid atmosphere. The absorbance was determined using an ELISA reader at 530 nm for the yeast species and visually for mold species after 48 hours of incubation.[27,4]

RESULTS AND DISCUSSION

Chemistry

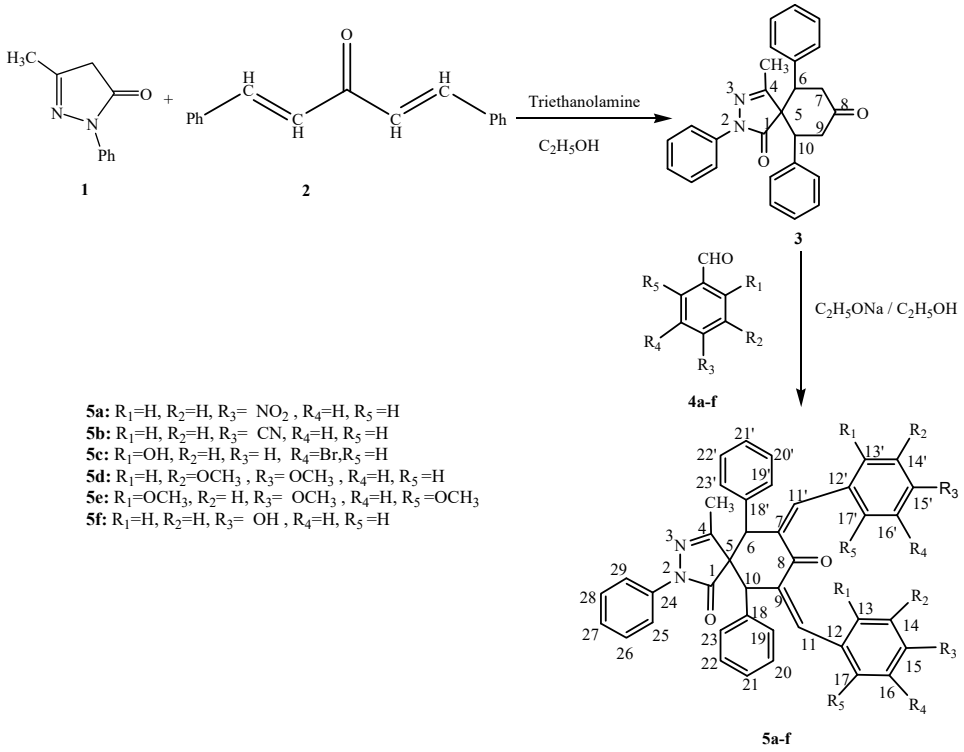
4-methyl-2,6,10-triphenyl-2,3-diaza-spiro[4,5]dec-3-ene-1,8-dione (**3**) and their diarylidene derivatives **5a**–**f** were synthesized by both conventional and microwave-mediated method from the synthetic precursors pyrazol-5-one (**1**) and dibenzalacetone (**2**). The synthetic pathway of 4-methyl-7,9-bis-(arylidene)--2,6,10-triphenyl-2,3-diazaspiro[4,5]dec-3-ene-1,8-diones (**5a**–**f**) is described in Scheme 1.

The equimolar mixture of **1** and **2** was refluxed in an ethanolic medium to furnish **3** in good yield. Alternatively, the same reaction was carried out under microwave (MW) irradiation at 300 W and 80 °C for 10 min to get the same desired compound **3** in better yield. The experimental results in the present study revealed that in the case of microwave heating, better yield (82 *vs*. 70 %) of the desired products was achieved in shorter reaction time (7 min *vs*. 10 h) compared to the conventional method. The MW heating is known to promote rapid molecular mixing of the reactants which increases the intimate contact between the reactant molecules and accelerates the reaction kinetics to afford higher yields in lesser reaction time.[28] The comparison of reaction time and yields between conventional and microwave assisted synthetic routes of the synthesized compounds has been summarized in (Table I).

From the experimental results, it is observed that electron withdrawing substituents on the phenyl ring enhance the yield of the products whereas electron donating groups comparatively reduce it. In the present study, the mixture of **3** and different substituted aryl aldehydes **4a**–**f** was refluxed for 10 h in ethanol to

produced **5a**–**f** (68–76 % yield) and enhancement in the yield of the products **5a**–**f** (80–87 % yield) was achieved by irradiating the same under MW radiation at 300 W and 80 °C for 7 min. The final yield percentage of the target products were calculated by:[29]

$$\text{Yield} = 100\frac{\text{Content of the product yielded}}{\text{Content of the product expected}} \quad (1)$$



Scheme 1. Synthetic pathway of **5a**–**f**.

TABLE I. Comparison between conventional and microwave assisted synthetic protocols for compounds **5a**–**f** (reaction time: conventional, 10 h, MW, 7 min)

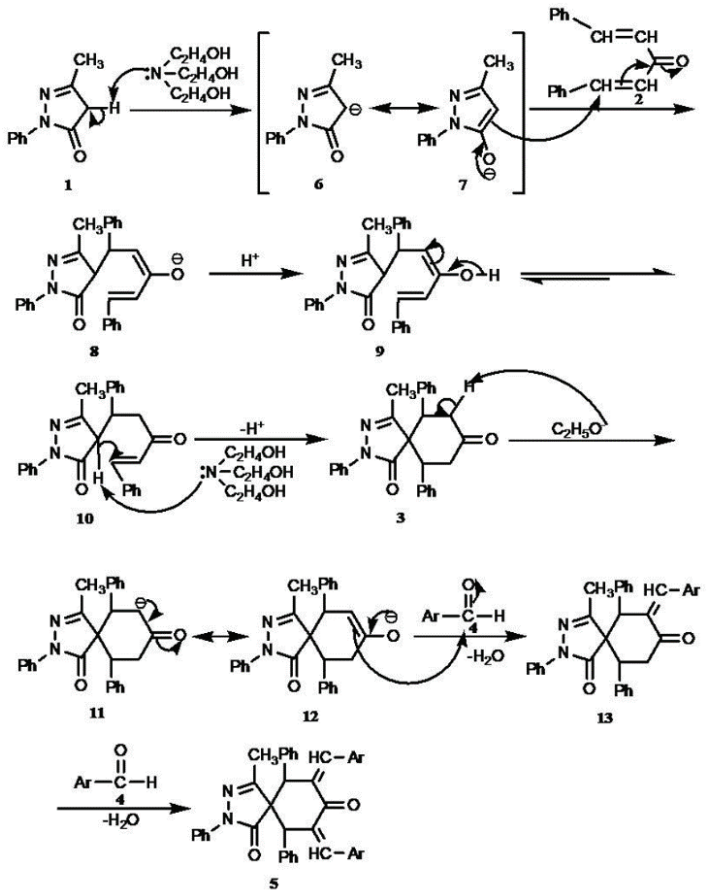
Compd.	R_1	R_2	R_3	R_4	R_5	Yield, %	
						Conventional	MW
5a	H	H	NO_2	H	H	76	84
5b	H	H	CN	H	H	75	87
5c	OH	H	H	Br	H	72	85
5d	H	OCH_3	OCH_3	H	H	68	81
5e	OCH_3	H	OCH_3	H	OCH_3	70	82
5f	H	H	OH	H	H	69	80

IR spectrum of **5a** ($R_1 = R_2 = R_4 = R_5 = H$, $R_3 = p\text{-}NO_2$) spectrum reveals two characteristic peaks at 1733 and 1691 cm^{-1} corresponding to amidic carbonyl function of pyrazolone moiety and carbonyl function of cyclohexanone scaffold, which is in conjugation with two exocyclic double bonds. The azomethine linkage C=N streching vibrations appears at 1533 cm^{-1} whereas the olefinic stretching vibrations are observed at 1525 cm^{-1}. The band appearing at 1380 cm^{-1} is attributed to symmetric stretching of $ArNO_2$ group.

^{1}H-NMR spectrum of compound **5a** recorded in $CDCl_3$ exhibited a singlet for methyl protons at δ = 1.8 ppm, and another singlet for two proton methine protons (H-6 and H-10) at δ = 3.06 ppm. The disappearance of the complex pattern of ^{1}H-NMR spectrum in the target molecule and the appearance of a sharp singlet for two olefinic protons (H-11 and H-11') at δ = 7.45 ppm ensure the incorporation of the double bond at C-7 and C-9 position. In addition, two separate doublets at δ = 8.27 (J = 8.0 Hz) and δ = 7.79 (J = 8.0 Hz) for the protons of the phenyl group attached to olefinic carbon C-11 and C-11' confirm the formation of diarylidene derivative. A doublet of doublet at δ = 7.47 (J = 4.0 Hz, 1.2 Hz) attributed to the ortho protons of the N-phenyl ring of amidic nitrogen of pyrazolone scaffold.

^{13}C-NMR spectrum of compound **5a** gives signals resonating at δ = 17.8, 158.7 and 189.9 ppm, attributed to the respective methyl carbon, azomethine carbon, and amidic carbonyl carbon of pyrazolone ring, whereas the signals at δ 48.5, 63.6, 141.2, 142.9 and 179.9 ppm correspond to the respective methine carbons at C-6 and C-10, spiro carbon C-5, exo-olefinic carbons C-11 and C-11', olefinic carbons in conjugation with carbonyl function and carbonyl carbon of cyclohexanone ring. The signals at δ 111.8 and 121.7 ppm are attributed to C-14, C-14', C-16, C-16' and C-13, C-13', C-17, C-17', signals at δ 137.9 and 143.9 ppm correspond to C-12, C-12' and C-15, C-15' of the phenyl rings attached to exo- olefinic carbons respecttively. The signals at δ = 127.3 ppm corresponds to C-21, C-21', δ = 127.8 ppm to C-19, C-19', C-23, C-23', δ 128.4 to C-20, C-20', C-22, C-22' and δ = 133.8 ppm to C-18, C-18' of the phenyl rings attached to methine carbons at C-6 and C-10. Further, the signals at δ = 124.8 ppm are attributed to C-25, C-29, δ = 125.9 ppm to C-27, δ = 129.9 ppm to C-26, C-28, δ = = 137.5 ppm to C-24 of the phenyl ring attached to pyrazolone ring.

The pyrazol-3-one anion **6** was formed by deprotonation of the active methylene group of pyrazol-3-one **2** in the presence of triethanolamine which generates the enolate intermediate **7**, Scheme 2. Then, the enolate intermediate **7** was added to dibenzalacetone **2** *via* Michael addition. The first Michael adduct thus formed underwent second intramolecular Michael addition under a similar reaction path to obtain the spiro intermediate **3**. Thereafter, the isolated spiro intermediate **3** reacted with two equivalents of aryl aldehydes, in the presence of sodium ethoxide, through Aldol condensation to produce the diarylidene derivative **5**.



Scheme 2. Plausible mechanism of the synthesized compounds.

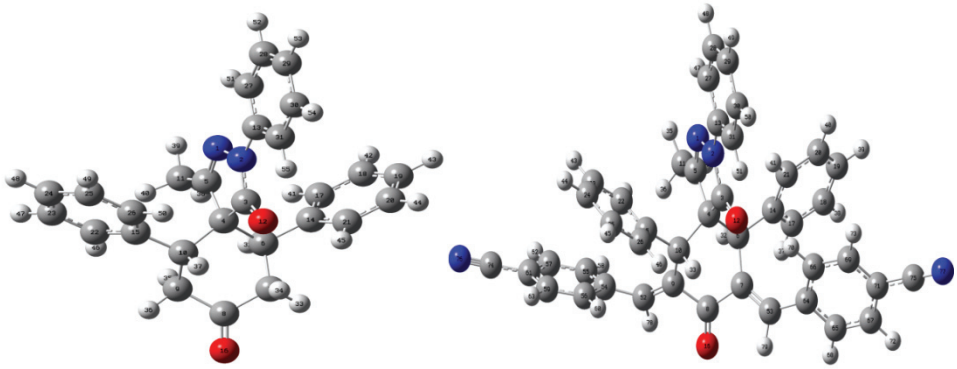
DFT study was performed for compound **3** and **5b** using DFT B3LYP/6--31++g(d,p). The optimized structures of compound **3** and **5b** are given. The optimized parameters of structure **3** and **5b** are provided in the Supplementary material.

The optimized structure of compound **3** based on DFT study reveals that N--phenyl, C-6 phenyl and C-10 phenyl rings are in propeller shape (Fig. 2).

In compound **5b**, the bond angles A (2,1,5), A (1,2,3),A (2,3,12), A (2,3,4), A (5,4,6), A (5,4,10), A (7,8,16), A (7,8,9), A (7,53,64), A (9,52,54), A (4,10,15), A (10,9,52) and A (6,7,53) are 108.7814, 112.0698,127.6833, 105.8661, 110.69, 116.1912, 123.1589, 114.7374, 132.4845, 133.5168, 113.1059, 133.2194 and 133.0574°, respectively (provided in Supplementary material).

In the arylidene derivative 4-methyl-(7*E*,9*E*)-7,9-bis-(4-cyano-benzylidene)--2,6,10-triphenyl-2,3-diazaspiro[4,5]dec-3-ene-1,8-dione (**5b**) with the minimum

energy of –55133.6 eV, both *p*-CN phenyl groups and the carbonyl function are *trans* to each other across the double bonds (Fig. 2).



Energy = –35465.9 eV Energy = –55133.6 eV

Fig. 2. Optimizedstructure of compound **3** and **5b**.

Microbial test

All the synthesized compounds **5a**–**f** were analyzed for their antibacterial and antifungal activity. All compounds showed variable antibacterial activity against the Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli)* and Gram-positive bacterium (*Staphylococcus aureus*). However, none of the compounds showed activity against *Streptococcus pyrogene*s. Two compounds, **5a** and **5c**, were found to be most effective against *S. aureus* with a zone of inhibition of 25.2 and 24.1 mm, respectively. Compounds **5a** and **5c** showed higher inhibitory effect with the respective zone of inhibition of 23.7 and 22.1 mm against Gram-negative bacteria *P. aeruginosa* and 22.8 and 20.4 mm, respectively, against *E. coli* (Table II).

TABLE II. Antibacterial activity of novel siro derivatives through agar well diffusion method

Compound	Diameter of the zone of inhibition[a], mm			
	Gram-positive		Gram-negative	
	S. aureus MTCC (3615)	*S. pyrogenes* MTCC (442)	*P. aeruginosa* MTCC (424)	*E. coli* MTCC (443)
5a	**25.2**	–[b]	**23.7**	**22.8**
5b	18.6	–	14.1	13.4
5c	**24.1**	–	**22.1**	**20.4**
5d	16.2	–	16.1	14.9
5e	13.2	–	15.3	12.3
5f	14.7	–	**22.9**	17.5
Ciprofloxacin	**26.7**	**24.3**	**25.0**	**26.3**

[a]Values including diameter of the well (8 mm) and are mean of the three replicates; [b]no activity

However, in the case of fungi, two compounds, **5c** and **5e**, were found to be very effective against *Candida albicans* with the zone of inhibition of 15.8 and 15.1 mm, respectively. Two compounds, **5d** and **5e**, were found to be most effective against *Aspergillus niger and Aspergillus clavatus* with the zone of inhibition of 18.6 and 18.8, and 16.9 and 17.1 mm, respectively (Table III).

TABLE III. Antifungal activity of spiro derivatives through agar well diffusion method

Compound	Diameter of the zone of inhibition[a], mm		
	C. albicans MTCC (227)	*A. niger* MTCC (282)	*A. clavatus* MTCC (1327)
5a	13.1	10.8	14.3
5b	–[b]	–	–
5c	15.9	10.1	11.3
5d	11.6	18.6	16.9
5e	15.1	18.8	17.1
5f	12.3	12.8	13.1
Amphotericin B	16.6	19.3	17.2

[a]Values including diameter of the well (8 mm) and are mean of the three replicates; [b]no activity

The *MIC* of tested compounds ranged between 8 and 512 μg mL^{-1} against bacteria and ranged between 16 and 512 μg mL^{-1} against fungal strains. Compound **5a** was found to be the most effective synthetic derivative having the lowest *MIC* value and the widest spectrum of antibacterial activity as compared to the other tested compounds. The *MIC* value for compound **5a** was 8 μg mL^{-1} against *S. aureus*, 16 μg mL^{-1} against *P. aeruginosa* and 32 μg mL^{-1} aagainst *E. coli*. Compound **5c** also showed remarkable broad spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria except *S. pyrogene*s. The *MIC* value for compound **5c** was 16 μg mL^{-1} against *S. aureus*, 32 μg mL^{-1} against *P. aeruginosa*, and 64 μg mL^{-1} against *E. coli*. Besides, compound **5f** showed strong antibacterial effect against *P. aeruginos*a with the *MIC* value of 32 μg mL^{-1} but showed poor and non-detectable antibacterial activity against *E. coli* and *S. aureus* (Table IV).

Based on the *MIC* calculated for pathogenic fungi, compound **5e** displayed broad spectrum antifungal activity with the *MIC* value of 16 μg mL^{-1} against *C. albicans*, 16 μg mL^{-1} against *A. niger* and 16 μg mL^{-1} against *A. clavatus.* Compound **5c** showed promising antifungal activity against *C. albicans* with the *MIC* of 16 μg mL^{-1} but was observed to be ineffective against *A. clavatus* and *A. niger.* Compound **5d** showed strong antifungal activity against *A. clavatus* and *A. niger* with the *MIC* of 16 and 32 μg mL^{-1}, respectively, but showed no activity against *C. albicans* (Table V).

The results obtained in the present study are in line with our previous report showing the promising antimicrobial activity of diazaspiroundecanetetraone derivatives against *E. coli*, *B. cirroflagellosus*, *A. niger* and *C. albicans*.[17] Several

studies show that the antifungal activity of compounds depends on the presence of electron donating or withdrawing substituent in para position in aromatic scaffold.[30] The antibacterial activity of the compounds **5a** and **5c** could therefore be attributed to the present electron withdrawing group such as the bromo group and nitro group, surrounded by high electron density.[4] However, the cellular mechanism underlying the bactericidal and antifungal action of nitro, bromide, and methyl substituted spiro diarylidene derivatives need further investigation.

TABLE IV. Minimum inhibitory concentration (μg mL^{-1}) of spiro diarylidene derivatives against bacterial species using microbroth dilution method

Compound	Bacteria			
	Gram positive		Gram negative	
	S. aureus MTCC (3615)	*S. pyrogenes* MTCC (442)	*P. aeruginosa* MTCC (424)	*E. coli* MTCC (443)
5a	8	16	32	128
5b	256	256	512	–
5c	16	32	64	16
5d	256	128	128	256
5e	512	512	512	16
5f	512	32	128	128
Ciprofloxacin	8	8	16	Nt

TABLE V. Minimum inhibitory concentration (μg mL^{-1}) of spiro diarylidene derivatives against fungal species using microbroth dilution method

Compound	Fungi		
	C. albicans MTCC (227)	*A. niger* MTCC (282)	*A. clavatus* MTCC (1327)
5a	13.1	10.8	14.3
5b	–	–	–
5c	15.9	10.1	11.3
5d	11.6	18.6	16.9
5e	15.1	18.8	17.1
5f	12.3	12.8	13.1
Amphotericin B	16.6	19.3	17.2

CONCLUSION

The present study demonstrates a rapid and novel microwave assisted method for synthesis of a series of pharmacologically important spiro diarylidene compounds from 4-methyl-2,6,10-triphenyl-2,3-diaza-spiro[4,5]dec-3-ene-1,8--dione in high yield in comparison to conventional methods. The study further establishes antimicrobial and antifungal behaviour of some of the synthetic derivatives which could have significant clinical application in developing better antibiotics against infectious organisms. The obtained results indicate that 4-NO_2 derivative (compound **5a**) and 2,4,6-$(OCH_3)_3$ derivative (compound **5e**) may

have considerable potential for therapeutic application as a novel wide spectrum drug candidate against bacterial and fungal infections respectively**.** However, further studies are needed to evaluate their efficacy as drug candidates.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/10319, or from the corresponding author on request.

Acknowledgments. This work was supported by the UGC (DRS-SAP), New Delhi, under Grant No. F.540/14/DRS/2013/SAP-I. The authors thank FIST-DST under Grant No. SR/FST/CSII-021/2012(C) for providing infrastructural research facility. The author, Sabita Shroff is thankful to UGC-BSR for providing fellowship under Grant (F. NO. 25-1/2014-15 (BSR)/7-166/2007/(BSR)) for smooth conduction of research work. The authors acknowledge National Institute of Technology, Rourkela, Odisha, for recording NMR and Mass spectra.

ИЗВОД

МИКРОТАЛАСНА СИНТЕЗА НОВИХ СПИРО ДИАРИЛЕДЕНА И ИСПИТИВАЊЕ ЊИХОВЕ АНТИМИКРОБНЕ АКТИВНОСТИ

SABITA SHROFF[1], PRAJNA P. MOHANTA[1], ISWAR BAITHARU[2], BHAWANI P. BAG[3] и AJAYA K. BEHERA[1]

[1]*Organic Synthesis Lab, School of Chemistry, Sambalpur University, Jyoti Vihar, Odisha-768019, India,* [2]*Toxicology Lab, P.G. Dept. of Environmental Sciences, Sambalpur University, Jyoti Vihar, Odisha-768019, India и* [3]*Biotechology and Bioinformatics Lab, Sambalpur University, Jyoti Vihar, Odisha-768019, India*

Испитана је брза синтеза серије нових 7,9-бис-(арилиден)-4-метил-2,6,10-трифенил-2,3-диазаспиро[4,5]дец-3-ен-1,8-диона, помогнута микроталасима, са високим приносом. Диспиро диарилиден деривати који садрже азот и *α*,*β*-незасићене кето-групе, синтетисани су реакцијама алдолне кондензације, праћене дехидратацијом, између 4-метил-2,6,10-трифенил-2,3-диазаспиро[4,5]дец-3-ен-1,8-диона и одговарајућих арилалдехида. Добијени спироарилиденски деривати окарактерисани су IC, ^{1}H- ^{13}C-NMR и масеним спектрима. DFT (density functional theory) прорачуни извршени су помоћу програма Gaussian 09. Испитана је антимикробна активност синтетисаних деривата према две Грам-позитивне и две Грам-негативне бактерије и према три врсте гљивица диск-дифузионом методом. Минимална инхибиторна концентација (*MIC*) одређена је микроброт техником разблаживања. На основу добијених резултата показано је да једињења **5a** и **5c** која поседују 4-NO_2 и 5-Br-2-OH супституенте имају већу активност према Грам-позитивној бактерији *Staphylococcus aureus*, са *MIC* вредностима 8 и 16 μg mL^{-1}, редом, и да су умерено активни према Грам-негативним бактеријама *Pseudomonas aeruginosa* and *Escherichia coli* у поређењу са другим тестираним једињењима. Ипак, ни једно од тестираних једињења не показује активност према *Streptococcus pyrogenes*. Једињење **5e** са 2,4,6-$(OCH_3)_3C_6H_3$ структурним делом показује шири спектар активности према свим сојевима гљивица, али не показује антибактеријску активност.

(Примљено 23. јануара 2021, ревидирано 22. фебруара, прихваћено 30. марта 2022)

REFERENCES

1. M. Grare, M. Mourer, S. Fontanay, J. B. Regnouf-de-Vains, C. Finance, R. E. Duval, *J. Antimicrob. Chemother.* **60** (2007) 575 (https://dx.doi.org/10.1093/jac/dkm244)
2. P. Dahiya, S. Purkayastha, *Indian J. Pharm. Sci.* **74** (2012) 443 (https://dx.doi.org/10.4103/0250-474X.108420)

3. M. Benabdallah, O. Talhi, F. Nouali, N. Choukchou-Braham, K. Bachari, A. M. S. Silva, *Curr. Med. Chem.* **25** (2018) 3748 (https://doi.org/10.2174/0929867325666180309124821)
4. M. A. K. Shakhatreh, M. L. Al-Smadi, O. F. Khabour, F. A. Shuaibu, E. I. Hussein, K. H. Alzoubi, *Drug Des. Devel. Ther.* **10** (2016) 3653 (https://doi.org/10.2147/DDDT.S116312)
5. S. Bag, S. Ramar, M. S. Degani, *Med. Chem. Res.* **18** (2009)309 (https://doi.org/10.1007/s00044-008-9128-x)
6. M. Funakoshi-Tago, K. Nakamura, R. Tsuruya, M. Hatanaka, T. Mashino, Y. Sonoda, T. Kasahara, *Int. Immunopharmacol.* **10** (2010) 562 (https://doi.org/10.1016/j.intimp.2010.02.003)
7. S. J. Yeo, D. X. Liu, H. S. Kim, H. Park, *Malar. J.* **16** (2017)80 (https://doi.org/10.1186/s12936-017-1725-z)
8. W. M. El-Husseiny, M. A. A. El-Sayed, N. I. Abdel-Aziz, A. S. El-Azab, E. R. Ahmed, A. A. M. Abdel-Aziz, *J. Enzyme Inhib. Med. Chem.* **33** (2018) 507 (https://doi.org/10.1080/14756366.2018.1434519)
9. R. Pradhan, M. Patra, A. K. Behera, B. K. Mishra, R. K. Behera, *Tetrahedron* **62** (2006) 779 (https://doi.org/10.1016/j.tet.2005.09.039)
10. P. Saraswat, G. Jeyabalan, M. Z. Hassan, M. U. Rahman, N. K. Nyola, *Synth. Commun.* **46** (2016) 1643 (https://doi.org/10.1080/00397911.2016.1211704)
11. M. Palomba, L. Rossi, L. Sancineto, E. Tramontano, A. Corona, L. Bagnoli, C. Santi, C. Pannecouque, O. Tabarrini, F. Marini, *Org. Biomol. Chem.* **14** (2016)2015 (https://doi.org/10.1039/c5ob02451j)
12. K. Chakraborty, T. Antony, *Nat. Prod. Res.* **35** (2021)1 (https://doi.org/10.1080/14786419.2019.1608545)
13. K. Meena, S. Kumari, J. M. Khurana, A. Malik, C. Sharma, H. Panwar, *Chin. Chem. Lett.* **28** (2017) 136 (https://doi.org/10.1016/j.cclet.2016.06.025)
14. J. P. Strachan, J. J. Farias, J. Zhang, W. S. Caldwell, B. S. Bhatti, *Bioorg. Med. Chem. Lett.* **22** (2012) 5089 (https://doi.org/10.1016/j.bmcl.2012.05.108)
15. A. Jasper, D. Schepmann, K. Lehmkuhl, J. M. Vela, H. Buschmann, J. Holenz, B. Wünsch, *Eur. J. Med. Chem.* **53** (2012) 327 (https://doi.org/10.1016/j.ejmech.2012.04.018)
16. A. S. Girgis, S. S. Panda, I. S. A. Farag, A. M. El-Shabiny, A. M. Moustafa, N. S. M. Ismail, G. G. Pillai, C. S. Panda, C. D. Hall, A. R. Katritzky, *Org. Biomol. Chem.* **13** (2015) 1741 (https://doi.org/10.1039/c4ob02149e)
17. R. K. Behera, A. K. Behera, R. Pradhan, A. Pati, & M. Patra, *Synth. Commun.* **36** (2006) 3729 (https://doi.org/10.1080/00397910600946231)
18. P. Prasanna, K. Balamurugan, S. Perumal, P. Yogeeswari, D. Sriram, *Eur. J. Med. Chem.* **45** (2010) 5663 (https://doi.org/10.1016/j.ejmech.2010.09.019)
19. R. Sakhuja, K. Bajaj, S. M. Abdul Shakoor, & A. Kumar, *Mini. Rev. Org. Chem.* **11** (2014) 55 (https://doi.org/10.2174/1570193x1101140402101513)
20. A. R. Suresh Babu, R. Raghunathan, *Tetrahedron Lett.* **48** (2007) 305 (https://doi.org/10.1016/j.tetlet.2006.11.012)
21. G. Sridhar, T. Gunasundari, R. Raghunathan, *Tetrahedron Lett.* **48** (2007) 319 (https://doi.org/10.1016/j.tetlet.2006.11.002)
22. E. D. Becker, *High resolution NMR Theory and Chemical Applications,* 3rd ed., Academic Press, London, 2000, p. 83 (https://doi.org/10.1016/B978-0-12-084662-7.X5044-3)

23. *Gaussian 16, Rev. C. 01*, Gaussian, Inc., Wallingford, CT, 2016 (https://gaussian.com/)
24. B. S. Furniss, A. J. Hannaford, P. W. Smith, A. R. Tatchell, in *Vogel's Textbook of Practical Organic Chemistry*, 4th ed., ELBS and Longman, London, 1990, p. 143
25. R. Adams, O*rganic Synthesis,* John Wiley, London, 1946, p. 22 (https://library.sciencemadness.org/library/books/organic_reactions_v2.pdf)
26. I. Wiegand, K. Hilpert, R. E. W. Hancock, *Nat. Protoc.* **3** (2008) 163 (https://doi.org/10.1038/nprot.2007.521)
27. J. L. Rodriguez-Tudela, *Clin. Microbiol. Infect.* **14** (2008) (https://doi.org/10.1111/j.1469-0691.2007.01935.x)
28. W. S. Bremner, M. G. Organ, *J. Comb. Chem.* **9** (2007) 14 (https://doi.org/10.1021/cc060130p)
29. J. Isac-Garcia, J. A. Dobado, F. G. Calvo-Flores, H. Martinez-Garcia, *Chemistry: Laboratory Manual,* Academic Press, London, 2016, p. 239 (https://doi.org/10.1016/C2015-0-00644-X)
30. S. N. López, M. V. Castelli, S. A. Zacchino, J. N. Domínguez, G. Lobo, J. Charris--Charris, J. C. G. Cortés, J. C. Ribas, C. Devia, A. M. Rodríguez, R. D. Enriz, *Bioorganic Med. Chem.* **9** (2001)1999 (https://doi.org/10.1016/S0968-0896(01)00116-X).