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Microwave assisted synthesis of substituted 4-chloro-8-methyl-2--(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,5-dioxa-2*H*-phenanthren-6--ones and their antimicrobial activity

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Abstract: Due to the potential antimicrobial activity of pyranochromenones and pyrazolines moieties, hybrid compounds containing both substituted 4-chloro--8-methyl-2-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,5-dioxa-2*H*-phenanthren-6-ones (**4a**–**g**), were synthesized from substituted (*E*)-1-(7-hydroxy-4-methyl-8--coumarinyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-propen-1-ones (**3a**–**g**) in good yield using the Vilsmeier reaction, by the microwave-assisted method. The structures of all the compounds were established based on their analytical and spectral data. All the synthesized compounds were tested *in vitro* for their antibacterial and antifungal activities. Some of the compounds showed very good activity compared to standard drugs against all the tested pathogenic bacteria and fungi.

Keywords: antimicrobial activity; chromene; coumarin; microwave irradiation; pyrazolines; Vilsmeier reagent.

INTRODUCTION

The coumarin and chromene core moieties are important six-membered oxygen heterocyclic motifs embedded in several natural products and drugs. These systems are widely distributed in nature, and their derivatives have been shown to exhibit significant pharmacological activities.[1] Chromenes and fused chromenes are biologically important compounds due to their antibacterial,[2] anti-fungal,[3] antitumor[4] and antiviral[5] activities. Coumarin derivatives were reported to exhibit anti-inflammatory,[6,7] antimicrobial,[8] antioxidant,[9] anticancer[10] and chemoprophylactic[11] activities. Hybrid compounds containing both coumarin and chromene moieties, called pyranochromenone, due to the combined effect,

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may exhibit better biological activity. The compounds embedded with pyranochromenone (Fig. 1), soulattrolide, inophyllum G-1, cordatolide A and oblongulide were reported by Patil *et al*. to have potential application for the treatment of HIV.[12]

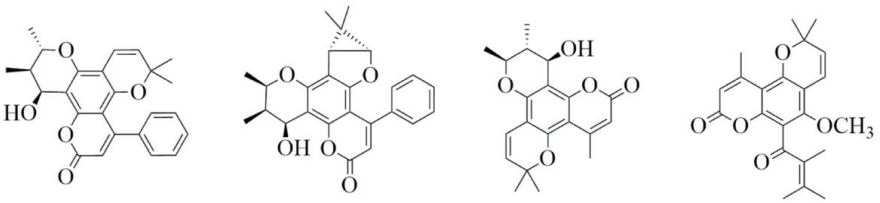


Fig. 1.Representative examples of pyranochromenones that exhibit anti-HIV activity.

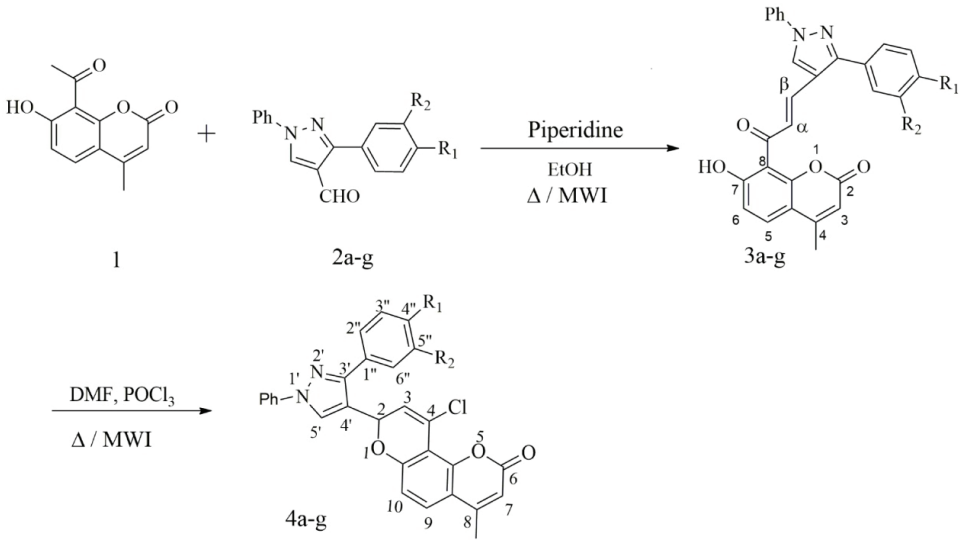
Compounds with the backbone of chalcones were reported to possess various biological activities, such as antimicrobial, anti-inflammatory, analgesic, antiplatelet, anti-ulcerative, antimalarial, anticancer,[13] antiviral, antileishmanial, antioxidant,[14] antitubercular,[15] antihyperglycemic, immunomodulatory, inhibiters of chemical mediators release,[16] inhibitors of leukotriene B4,[17] inhibiters of tyrosinase[18] and inhibitors of aldose reductase,[19] estrogenic activities.[20] Pyrazolines were found to possess antimicrobial,[21] antibacterial,[22] anti-amoebic,[23–24] antidepressant,[25] anticonvulsant,[26] anti–inflammatory[27–28] and antitumor activities. The recent literature is enriched with progressive findings concerning the synthesis and pharmacological properties of pyrazolines.[21–28]

Microwave irradiation has gained popularity in the past decade as a powerful tool for rapid and efficient synthesis of a variety of compounds because of selective absorption of microwave energy by polar molecules.[29] The application of microwave irradiation provides enhanced reaction rates and improved products in the field organic synthesis and is quite successful in the formation of a variety of carbon–heteroatom bonds. Our research group has been making considerable efforts in the design and realization of innovative synthetic protocols in organic synthesis adopting a more eco-sustainable approach.[30–32]

RESULTS AND DISCUSSION

The synthetic route to compounds **4a**–**g** is shown in Scheme 1. Compounds **3a**–**g** were synthesized according to a previous work.[33] The condensation of 8-acetyl-7-hydroxy-4-methyl coumarin (**1**) with 1-aryl-3-phenyl-1*H*-pyrazole-4--carbaldehydes (**2a**–**g**) in the presence of piperdine under microwave irradiation gave (*E*)-1-(7-hydroxy-4-methyl-8-coumarinyl)-3-(1,3-diphenyl-1*H*-pyrazol-4--yl)-2-propen-1-ones (**3a**–**g**). Subsequently these chalcones **3a**–**g** on reaction with Vilsmeier reagent (DMF/$POCl_3$) yielded substituted 4-chloro-8-methyl-2-(1,3--diphenyl-1*H*-pyrazol-4-yl)-1,5-dioxa-2*H*-phenanthren-6-ones (**4a**–**g**). However,

as the Vilsmeier reagent (DMF/$POCl_3$) serves for formylation of electron-rich aromatic rings, it was highly efficient for intramolecular cyclization of 2′-hydroxychalcones to 4-chloro-2*H*-chromenes.[34-36] Initially, the reaction was performed at room temperature but no product was formed. Optimum results were obtained when the temperature was maintained at 90–100 °C by taking 6 equivalents of $POCl_3$. In case of the microwave irradiation method, optimum results were obtained by irradiating at 160 W for 4–5 min. The crude products were purified using column chromatography to afford the pure products.



Scheme 1. Synthetic route to substituted 4-chloro-8-methyl-2-(1,3-diphenyl-1*H*-pyrazol-4-yl)--1,5-dioxa-2*H*-phenanthren-6-ones; a, R_1=H, R_2=H; b, R_1=OCH_3, R_2=H; c, R_1=OCH_3, R_2= OCH_3; d, R_1=CH_3, R_2=H; e, R_1=F, R_2=H; f, R_1=Cl, R_2=H; g, R_1=Br, R_2=H.

It was observed that the yields were better when the microwave irradiation method was used rather than the conventional heating method, Table I. It is known that microwave irradiation is used for a variety of organic reactions due to

TABLE I. Comparisons of the yields of the synthesized compounds **4a–g**

Compd.	Conventional method		MWI	
	t / h	Yield, %	*t* / min	Yield, %
4a	5	54	4	81
4b	5	61	4.5	89
4c	6	64	4	87
4d	6	57	5	88
4e	5.5	58	4.5	88
4f	5.5	61	4	90
4g	3	54	2	86

short reaction times, cleaner reactions, easier work-up and good yield.

All the newly synthesized compounds were characterized using spectral analysis. The results are given in the Supplementary material to this paper.

The IR spectra of intermediate compounds **3a**–**g** showed characteristic peaks of functional group C=N stretching between 1576–1598 cm^{-1}, C=O stretching (of chalcone) between 1633–1654 cm^{-1} and OH stretching between 3436–3448 cm^{-1}. For the final derivatives **4a**–**g**, characteristic bands were present in each spectra in wave number (cm^{-1}) ranges: 703–721, 1078–1086, 1654–1665 and 1725–1736, which correspond to C–Cl, C–O–C, C=N and C=O stretching, respectively. The ^{1}H-NMR spectra of chalcones **3a**–**g** showed characteristic signals in the δ (ppm) ranges: 8.03–8.05, 8.15–8.18 and 8.60–8.64, corresponding to H_{α}, H_{β} and the triazole H, respectively. In case of compounds **4a**–**g**, the protons, H2, H7, H3 and H10 appeared as doublets in the δ ranges 5.96–5.96, 6.11–6.20, 6.18–6.21 and 6.83–6.89, respectively. In the ^{13}C-NMR spectra, the carbonyl carbon of the chalcones **3a**–**g** appeared in the δ 192.4–196.1. In the case of the final compounds **4a**–**g**, C2 appeared in δ range 75.5–78.0. The rest of the carbons appeared in their usual regions, which supports the formation of compounds **3a**–**g** and **4a**–**g**. In the mass spectra of **3a**–**g** and **4a**–**g**, the molecular ion peaks, observed as [M+1] peaks, confirmed the molecular weights of the compounds.

Antibacterial activity

All the compounds were screened for their antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* using ampicillin as the standard drug (Table II). The activity was determined using the cup plate agar diffusion method by measuring the zone of inhibition in mm. The compounds were screened at a concentration of 50 μg mL^{-1} in DMSO. From the screening studies, it was evident that the synthesized compounds **4b** and **4c** showed good antibacterial activity against all the tested organisms. It was further observed that the electron rich compound **4c**, with both aryl rings possessing a methoxy substituent showed the best activity, closely followed by **4b**,

TABLE II. Antibacterial activity (zone of inhibition, mm) of compounds **4a**–**g**

Compd.	Gram-positive bacteria		Gram-negative bacteria	
	S aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
4a	20	7	5	22
4b	30	15	12	30
4c	32	16	13	33
4d	18	10	6	18
4e	22	11	8	25
4f	10	8	2	23
4g	28	11	9	28
Ampicillin	30	12	10	30

which has only one methoxy substituent. This led to the conclusion that electron rich chalcones showed higher activity. Furthermore, changing in the halogens from F to Cl or Br did not provide any significant change in the levels of activity against the bacteria.

Antifungal activity

All the compounds were screened for their antifungal activity against *Aspergillus nigerzeae*, *Penicillium italicum* and *Fusarium oxysporum* using grieseofulvin as the standard drug (Table III). The activity was determined using the cup plate agar diffusion method by measuring the zone of inhibition in mm. The compounds were screened at a concentration of 50 μg mL^{-1} in DMSO. From the screening studies, it is evident that the synthesized compounds **4a, 4c** and **4g** showed good antifungal activity against all the tested organisms. The unsubstitiuted compound **4a** showed the highest activity against the fungi. The highly electron rich compound **4c** showed activity which was comparable to that of **4a,** but in general, substituents on the pyrazole were detrimental to the observed activity. Among the halogen derivates, the bromo substituent (**4g**) showed significantly higher activity than the chloro (**4f**) and fluoro (**4e**) substituted compounds.

TABLE III. Antifungal activity (zone of inhibition, mm) of compounds **4a**–**g**

Cmpd.	Fungi		
	A. nigerzeae	*P. italicum*	*F. oxysporum*
4a	17	24	26
4b	8	14	19
4c	14	20	26
4d	7	15	18
4e	10	12	12
4f	8	11	12
4g	13	21	24
Grieseofulvin	12	20	25

EXPERIMENTAL

Materials

All the used materials were obtained commercially, mostly from Sigma–Aldrich, and were used without further purification.

Equipment

Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel 60 F_{254} (Merck). The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance II 400 spectrometer using TMS as an internal standard. The IR spectra were recorded in KBr on a Shimadzu FTIR 8400S spectrophotometer. The high-resolution electron spray ionization mass spectra (ESI-HRMS) were recorded

on a Micromass Q-Tof (ESI-HR-MS) mass spectrometer. The microwave reactions were performed in the milestone multiSYNTH microwave system.

*General procedure for the synthesis of substituted of (*E*)-1-(7-hydroxy-4-methyl-8-coumarinyl)-3-(1-phenyl-3-phenyl-1*H*-pyrazol-4-yl)-2-propen-1-one analogues **3a**–**g***

A mixture of 8-acetyl-7-hydroxy-4-methyl coumarin **1** (1 mmol), substituted 1-(phenyl)--3-phenyl-1*H*-pyrazole-4-carbaldehyde (**2a**–**g**, 1 mmol) in ethanol (2 ml) and few drops of piperidine was taken in a glass vial equipped with a cap and then subjected to microwave irradiation at 100 watts, by maintaining 80 °C for 10 to 15 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with cold water and acidified with dil. HCl. The precipitate that formed was filtered, dried and recrystallized from ethanol to afford pure chalcone.

*General procedure for the synthesis of substituted 4-chloro-8-methyl-2-(1,3-diphenyl-1*H*-pyrazol-4-yl)-1,5-dioxa-2*H*-phenanthren-6-ones **4a**–**g***

a) Conventional method A. DMF (5 mL) was taken in a round-bottomed flask and cooled to 0–5 °C. $POCl_3$ (0.006 mol) was added drop wise under stirring. The mixture was stirred at 0–5 °C for 15 min and then a substituted (*E*)-1-(7-hydroxy-4-methyl-8-coumarinyl)-3-(1,3--diphenyl-1*H*-pyrazol-4-yl)-2-propen-1-one (**3a**–**g**, 0.001 mol) solution in 3 mL of DMF was added at 0–5 °C and the temperature was maintained at 0–5 °C for 30 min. The reaction mixture was then heated in a water bath at 90 °C for 6–8 h. After completion of the reaction (monitored by TLC, EtOAc:hexane in 1:4 volume ratio), the reaction mixture was poured into ice–water and neutralized with 10 % NaOH solution and extracted with chloroform (2×20 mL). The combined organic layer was washed with 10 mL water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was purified by silica gel column chromatography to afford pure product **4a**–**g**.

b) Microwave method B. DMF (5 mL) was taken in a round-bottomed flask and cooled to 0–5 °C. $POCl_3$ (0.006 mol) was added drop wise under stirring. The stirring at 0–5 °C was continued for 15 min and then a substituted (*E*)-1-(7-hydroxy-4-methyl-8-coumarinyl)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-propen-1-one (**3a**–**g**, 0.001 mol) solution in 3 mL of DMF was added at 0–5 °C. The temperature was maintained at 0–5 °C for 30 min. The reaction mixture was placed in a microwave oven and subjected to microwave irradiation at 160 W by maintaining 90 °C for 4–5 min. The progress of reaction was monitored by TLC (EtOAc:hexane in 1:4 volume ratio). After completion of the reaction, the mixture was poured into ice water, neutralized with 10 % NaOH solution and extracted with chloroform (2×20 mL). The combined organic layer was washed with 10 mL water and dried over anhydrous magnesium sulfate. The solvent was evaporated and the residue was purified by silica gel column chromatography to afford pure product **4a**–**g**.

Biological assay

Antibacterial activity. The synthesized novel compounds **3a**–**g** and **4a**–**g** were screened for their antibacterial activity against different types of bacterial strains, *i.e.*, the Gram-negative bacterial strains *P. aeruginosa* and *E. coli* and the Gram-positive bacterial strains *B. subtilis* and *S. aureus* at a concentration of 50 μ mL^{-1}.

The cultures were diluted with 5 % autoclaved saline and the final volume was made with a concentration of approximately 10^5–10^6 CFU mL^{-1}. The synthesized compounds were diluted in acetone for antibacterial biological assays. For the agar disc diffusion method,[37] the liquid form of the test compound was soaked on to the disc and then allowed to air dry, so that

the disc became completely saturated with test compound. The saturated chemical discs were introduced onto the upper layer of the medium evenly loaded with the bacteria.

The discs dipped in different chemical samples were placed over the evenly spread bacterial nutrient media and incubated at 37 °C for 24 to 48 h for better inhibition of bacteria. The zones of inhibition were measured after 24 to 48 h. All the experiments were carried out in triplicates and the results were expressed as zone of Inhibition in mm. The zones of inhibition of synthesized compounds **4a**–**g** were compared with the zone of inhibition of standard antibiotic concentration of Ampicillin (50 μg/mL). The Antibacterial activity was evaluated and the results are presented in Table I.

Antifungal activity. The antifungal activity of synthesized compounds **4a**–**g** was tested against three pathogenic fungi, namely *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium italicum*, by the poison plate technique at a concentration of 50 μg mL^{-1}. The three kinds of fungi were incubated in potato dextrose agar (PDA) at 25±1 °C for 5 days to obtain new mycelium for the antifungal assay, then a mycelia as disks of approximately 0.45 cm diameter cut from the culture medium were picked up with a sterilized inoculation needle and inoculated in the center of the PDA plate. Test compounds were dissolved in acetone (10 mL) then added to the medium PDA (90 mL). The final concentration of compounds in the medium was adjusted to 50 μg mL^{-1}. The inoculated plates were incubated at 25±1 °C for 5 days. Acetone was diluted with sterilized distilled water and used as control, while griseofulvin (50 μg mL^{-1}) was used as standard control for each treatment. Three replicates of the experiments were performed. The radial growth of the fungal colonies was measured on the sixth day.

CONCLUSIONS

A new series of compounds (**4a**–**g**) were synthesized under conventional and microwave irradiation conditions. Using the microwave irradiation method, the reactions were completed in a shorter time with better yields as compared to the conventional method. All the new compounds were screened for their antimicrobial activity. It was observed that compounds **4b** and **4c** exhibited a broad spectrum of antibacterial activity, and compounds **4a, 4c** and **4g** showed a broader spectrum of antifungal activity against all the tested strains as compared to the standard drugs with their respective concentrations.

SUPPLEMENTARY MATERIAL

Analytical and spectral data of the compounds are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА ОЗРАЧИВАЊЕМ МИКРОТАЛАСИМА СУПСТИТУИСАНИХ 4-ХЛОР-8-МЕТИЛ-2-(1,3-ДИФЕНИЛ-1*H*-ПИРАЗОЛ-4-ИЛ)-1,5-ДИОКСА-2*H*--ФЕНАНТРЕН-6-ОНА И ЊИХОВА АНТИМИКРОБНА АКТИВНОСТ

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Због потенијалне антимикробне активности пиранохроменонских и пиразолинских једињења, Вилсмајеровом реакцијом су синтетисани хибридни деривати супституисаних 4-хлор-8-метил-2-(1,3-дифенил-1*H*-пиразол-4-ил)-1,5-диокса-2*H*-фенантрен-6-она (**4a**–**g**), полазећи од супституисаних (*E*)-1-(7-хидрокси-4-метил-8-кумаринил)-3-(1,3-дифенил-1*H*-пиразол-4-ил)-2-пропен-1-она (**3a**–**g**), у добром приносу, применом микроталаса. Структура свих једињења утврђена је на основу аналитичких и спектралних података. Одређена је *in vitro* антибактеријска и антифунгална активност свих синтетисаних једињења. Поједини деривати показују веома добру активност према свим испитиваним бактеријама и гљивицама, у поређењу са стандардним лековима.

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