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One-pot synthesis of carbazole based 3-hydroxy-4*H*-chromen-4-ones by a modified Algar–Flynn–Oyamada reaction and their antimicrobial activity

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Abstract: A new series of 2-(9-ethyl-9*H*-carbazol-3-yl)-3-hydroxy-4*H*-chromen-4-ones were synthesized from substituted 2-hydroxyacetophenones and 9-ethyl-9*H*-carbazole-3-carbaldehyde using NaOH and H_2O_2 by a modified Algar–Flynn–Oyamada reaction. In this method, the flavonols were synthesized in good yields (70–82 %) without isolating chalcones. The structures of the compounds were established based on ^{1}H-NMR, ^{13}C-NMR, FT-IR and mass spectral and analytical data. All the compounds were evaluated for their antimicrobial activity against bacteria, such as *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*, as well as fungi, such as *Aspergillus flavus* and *Fusarium oxysporum*.

Keywords: 2-(9-ethyl-9*H*-carbazol-3-yl)-3-hydroxy-4*H*-chromen-4-ones; modified Algar–Flynn–Oyamada reaction; antimicrobial activity.

INTRODUCTION

Flavonoids constitute an important class of secondary metabolites that are widely distributed in plants. Their widespread distribution in nature, their structural variability, relatively low toxicity and antioxidant activities have increased interest in flavonoids. Furthermore, flavonoids possess multimodal biological activities, such as anticancer,[1] antihypertensive,[2] anti-inflammatory,[3] antibacterial[4] and antifungal[5] activities. Chromones are interesting structural scaffolds and have been assigned as privileged structures for drug discovery. Substituted chromones were reported to show potential anticancer,[6] antihistamine[7] and antagonistic[8] activities against leukotriene D4. On the other hand, carbazole derivatives are an important class of heterocyclic compounds that are known to possess important biological properties, such as antibacterial, antifungal,[9] antitumor,[10] antioxidant[11] and antidiabetic[12] properties.

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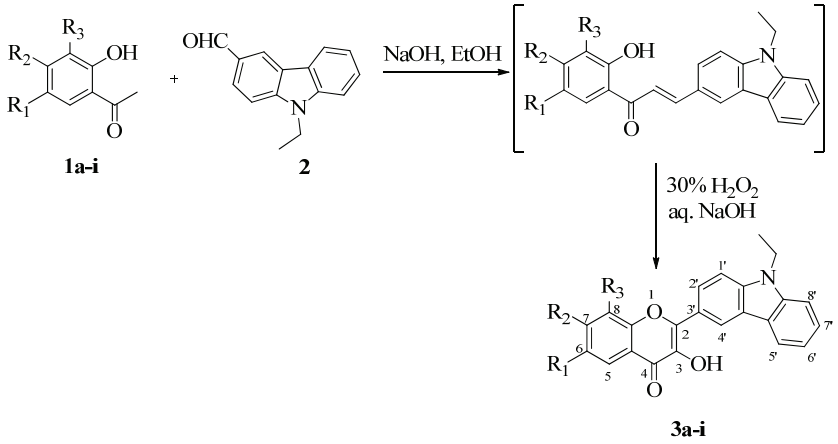
In the light of biological importance of the chromone and carbazole scaffolds and in continuation of the ongoing search for biologically active heterocyclic molecules,[13] herein, the one pot synthesis of 2-(9-ethyl-9*H*-carbazol-3-yl)-3-hydroxy-4*H*-chromen-4-ones using a modified Algar–Flynn–Oyamada reaction (Scheme 1) and the antimicrobial properties of the obtained derivatives are reported.



Scheme 1. Synthetic route for the preparation of carbazole based 3-hydroxy-4*H*-chromen-4-ones (**3a**–**i**).

RESULTS AND DISCUSSION

The original Algar–Flynn–Oyamada reaction[14] is a two step process for the synthesis of 3-hydroxy chromenones. In the first step, 2-hydroxy chalcones are formed, which on subsequent cyclisation in the second step in the presence of alkaline hydrogen peroxide yields the corresponding flavonols, whereas the modified Algar–Flynn–Oyamada reaction[15] is a one step process for the synthesis of flavonols from 2-hydroxyacetophenone and aromatic aldehydes in the presence of alkaline hydrogen peroxide (Table I). In this modified version there is no need to isolate the intermediate chalcones. As a model case, 2-hydroxyacetophenone **1a** was condensed with 9-ethyl-9*H*-carbazole-3-carbaldehyde **2** at room temperature using alkali and subsequently treated with alkaline hydrogen peroxide at room temperature to yield the flavonol derivative. It was identified as 2-(9-ethyl-9*H*-carbazol-3-yl)-3-hydroxy-4*H*-chromen-4-one **3a** by IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data, which ruled out the formation of corresponding aurones and some other benzofuran derivatives, which were reported to form as by-products in Algar–Flynn–Oyamada reaction. In the ^{1}H-NMR spectra of **3a**, the OH proton appeared at δ 7.08 ppm as broad singlet and H_4' proton appeared as a doublet at δ 9.03 ppm. In the ^{13}C-NMR spectra of **3a**, the carbonyl carbon appeared at δ 173.0 ppm and the N–CH_2 carbon resonated at δ 37.7 ppm. The ESI mass spectra of **3a** showed a molecular ion peak at m/z = 356 $[M+H]^+$.

TABLE I. Physical data of the synthesized compounds **3a**–**i**

Compd.	M.p., °C	R_1	R_2	R_3	Time, h	Yield, %[a]
3a	134–136	H	H	H	6	72
3b	128–130	F	H	H	7	78
3c	178–180	Cl	H	H	5	82
3d	190–192	Br	H	H	8	76
3e	140–142	CH_3	H	H	6	80
3f	206–208	Cl	CH_3	H	6	82
3g	212–214	Cl	H	Cl	7	80
3h	148–150	H	OCH_3	H	7	76
3i	172–174	H	OC_2H_5	H	8	70

[a]Isolated yield

Antibacterial activity

The newly synthesized compounds **3a**–**i** were screened *in vitro* for their antibacterial activity against two Gram-positiveve bacterial strains (*Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633)) and two Gram-negative bacterial strains (*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883)) at two different concentrations 20 and 40 μg mL^{-1}. The zone of inhibition was measured in mm and ciprofloxacin was used as a standard antibacterial substance, under similar conditions for comparison. All the synthesized compounds showed good activity against the tested microorganisms (Table II). Among all, compounds **3a**, **3h** and **3i** showed maximal zones of inhibition against the tested bacterial strains. It could be concluded that 3-hydroxy chromenones with electron releasing groups, such as methoxy, ethoxy and unsubstituted compounds, showed the maximum activity. Furthermore, the antibacterial results observed for other substitutions on the phenyl ring were very similar

TABLE II. Antimicrobial activities (zones of inhibition in mm) of the synthesized compounds **3a**–**i**

	Concentration, μg mL^{-1}									
Compd.	*S. aureus*		*B. subtilis*		*E. coli*		*K. pneumoniae*		*A. flavus*	*F. oxysporum*
	20	40	20	40	20	40	20	40	50	50
3a	**16**	**28**	**14**	**26**	**14**	**30**	**13**	**26**	**10**	**12**
3b	11	22	6	14	05	12	7	15	5	10
3c	12	24	9	18	08	17	8	15	6	5
3d	11	20	9	17	10	17	9	19	6	4
3e	10	22	7	16	7	7	5	10	9	8
3f	11	21	9	18	9	10	7	15	11	4
3g	13	23	10	21	7	15	9	19	8	12
3h	**16**	**29**	**16**	**29**	**17**	**32**	**24**	**35**	**13**	**16**
3i	**18**	**30**	**17**	**30**	**19**	**33**	**25**	**36**	**15**	**19**
Ciprofloxacin	15	28	16	30	18	35	23	35	–	–
Amphotericin-B	–	–	–	–	–	–	–	–	12	15

to each other. It was also concluded that changing the halogen substituent from F to Cl and Br does not provide any significant changes in antibacterial activity.

Antifungal activity

The antifungal activities of the synthesized compounds **3a**–**i** were tested against two pathogenic fungi, *Aspergillus flavus* (ATCC-9643) and *Fusarium oxysporum* (ATCC-48112), at a concentration of 50 μg mL^{-1} and the results were compared with those of the standard, amphotericin-B. All the compounds showed good activity against the tested fungal strains (Table II). Among all the compounds, **3a**, **3h** and **3i** showed maximal zones of inhibition against the tested fungal strains. Thus, electron releasing groups on 3-hydroxy chromenone, *i.e.*, methoxy and ethoxy substitutions and the unsubstituted compound showed the highest antifungal activities, followed by the halogen substituted compounds.

EXPERIMENTAL

Materials

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

Equipment

The 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 mass spectra were recorded on a Shimadzu LCMS 2020 mass spectrometer. The elemental microanalysis was realised on a Perkin Elmer CHN-2400 analyzer.

The physical, analytical and spectral data of compounds **3a**–**i** are given in the Supplementary material to this paper.

General procedure for the synthesis of 2-(9-ethyl-9H-carbazol-3-yl)-3-hydroxy-4H-chromen--4-ones

To a well stirred solution of 2-hydroxyacetophenone **1a**–**i** (1 mmol) and 9-ethyl-9*H*--carbazole-3-carbaldehyde **2** (1 mmol) in EtOH (20 mL) was added NaOH (4 mmol in 10 mL of EtOH) at room temperature. The reaction mixture was further stirred for 4–5 h. After consumption of reactants (as indicated by TLC), the reaction mixture was dissolved in aqueous NaOH (5 mmol in 5 mL), 3 mL of 30 % H_2O_2 was added dropwise and the stirring was continued for 2–3 h. After completion of reaction (monitored by TLC), the resulting light yellow reaction mixture was poured onto crushed ice and neutralized with dilute HCl. The thus-obtained light yellow solid was filtered, washed with water and dried. The crude product was purified by column chromatography on silica gel using hexane:ethyl acetate (7:3) as eluent to afford the desired products **3a**–**i**. The respective yields are given in Table I.

Biological assay

Synthesized compounds were screened for their antibacterial activities against pathogenic bacteria, *i.e.*, *S. aureus*, *B. subtilis*, *E. coli* and *K. pneumoniae* and their antifungal activity against *A. flavus* and *F. oxysporum.*

The test organisms were cultured on agar slants, incubated for 24 h at 37±0.5 °C and 24––48 h at 27±0.2 °C for the bacteria and fungi, respectively, to obtain freshly prepared cultures. The synthesized compounds were evaluated for antibacterial activity and antifungal activity against these freshly prepared strains of test organisms by the agar diffusion method and the poison plate technique, respectively. Muller–Hinton agar (MHA) and potato dextrose agar (PDA) were used as nutrient media for bacterial and fungal strains, respectively. The broth cultures were diluted with sterilized saline to bring the final size of the inoculum to approximately 10^5–10^6 CFU mL^{-1}. The compounds were diluted in acetone, dimethyl sulphoxide (DMSO) and diethyl ether for biological assays. Of the three solvents, diethyl ether is found to be the best. The bacterial cultures were placed on the media and incubated at 37 °C for 24 h along with the diluted compounds introduced through discs (diameter 5 mm) dipped and placed over the nutrient media. The discs of ciprofloxacin (20–40 μg) and amphotericin-B (50 μg) were also incorporated into the medium for comparison. The same procedure was employed for determining the antifungal activity except that the culture strains of fungi were maintained on PDA and spores were transferred into PDA medium and the plates were incubated at 27±0.2 °C for 24–48 h. Inhibition of growth of the test organisms (bacterial and fungal) in presence of the test material and the standards was measured with the help of a standard scale. The values of the inhibition zones are reported in Table II.

CONCLUSIONS

In conclusion, an easy, facile and one-pot route for the synthesis of 2-(9--ethyl-9*H*-carbazol-3-yl)-3-hydroxy-4*H*-chromen-4-ones in good yields is reported. In this method, there is no need to isolate the intermediate chalcones, which tremendously reduces the man power, time and cost and also improves the overall yields. The antimicrobial assay of these compounds revealed that compounds **3a**, **3h** and **3i** showed maximal zones of inhibition against the tested microorganisms compared with the standards.

SUPPLEMENTARY MATERIAL

Physical, analytical and spectral data of compounds **3a**–**i** are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА КАРБАЗОЛСКИХ ДЕРИВАТА 3-ХИДРОКСИ-4*H*-ХРОМЕН-4-ОНА МОДИФИКОВАНОМ АЛГАР–ФЛИН–ОЈИМАДИНОМ РЕАКЦИЈОМ И ИСПИТИВАЊЕ ЊИХОВЕ АНТИМИКРОБНЕ АКТИВНОСТИ

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Синтетисана је серија деривата 2-(9-етил-9*H*-карбазол-3-ил)-3-хидрокси-4*H*-хромен-4-она полазећи од супституисаних 2-хидрокси-ацетофенона и 9-етил-9*H*-карбазол--3-карбалдехида помоћу NaOH и H_2O_2 у модификованој Алгар–Флин–Ојамадином реакцији. Овим поступком синтетисани су флавоноли, без изоловања халкона, у добром

приносу (70–82 %). Структуре једињења утврђене су ^{1}H-NMR и ^{13}C-NMR техникама, FT-IR спектроскопијом, масеном спектрометријом и елементалном анализом. Испитана је антимикробна активност добијених једињења према бактеријама *Staphylococus aureus*, *Bacillus subtilis*, *Escherichia coli* и *Klebsiella pneumoniae* као и према гљивицама *Aspergillus flavus* и *Fusarium oxysporum*.

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