

Article

Synthesis and Antimicrobial Evaluation of Some Heterocyclic Chalcone Derivatives

Nagwa Mohamed Mahrous Hamada¹ and Essam Mohamed Sharshira^{2,*}

¹ Department of Chemistry, Faculty of Education, Alexandria University, Alexandria, Egypt; E-Mail: nagwahamada2002@yahoo.com (N.M.M.H.)

² Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt

* Author to whom correspondence should be addressed; E-Mail: dressamsharshira@yahoo.com.

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Abstract: Some new heterocyclic compounds containing isoxazole, pyrazole and oxadiazole ring systems were prepared from various chalcones. The synthesized compounds have been characterized by elemental analysis and spectral methods. These compounds were screened for their antimicrobial activities.

Keywords: chalcones; isoxazoles; pyrazoles; oxadiazoles; antimicrobial

1. Introduction

Chalcones are synthesized by condensing ketones with aromatic aldehydes in the presence of suitable bases. They are very useful intermediates for the synthesis of five- [1,2], six- [1,3] and seven-membered [4] heterocyclic compounds. Chalcone derivatives exhibit diverse pharmacological activities [5-14]. It is therefore, not surprising that many synthetic methods have been developed for the preparation of heterocycles starting from chalcone precursors that have been tested for their antimicrobial activities.

2. Results and Discussion

All of our results are shown in **Scheme 1**. The starting chalcones **1a–c** were obtained in good yields by a base catalyzed condensation [15-16] of appropriately substituted benzaldehydes and cyclopropylmethyl ketone [17]. The method is attractive since it specifically generates the (*E*) isomer [18].

characteristic for the olefinic =C–CH=CH proton. The cyclopropyl ring protons appeared as two multiplets in the range $\delta = 1.56$ – 2.64 ppm (CH) and $\delta = 0.70$ – 1.46 ppm (2CH₂), respectively.

The pyrazole derivatives **3a–f** were obtained by treatment of hydrazones **2a–f** with 30% hydrochloric acid. The IR of **3a–f** showed the characteristic bands for C=C–Ar at 1519–1596, C=N at 1623–1644 and amide carbonyl band at 1660–1686 cm⁻¹, while the ¹H-NMR spectra showed a singlet at $\delta = 6.64$ – 7.12 ppm for the pyrazole–C₄–H. On the other hand, refluxing of hydrazones **2a–f** with acetic anhydride gave the corresponding dihydro-1,3,4-oxadiazole derivatives **4a–f**. The mechanism of cyclization reaction has been well studied [19–20]. The IR spectra of the dihydro-oxadiazoles **4a–f** lacked the NH, but showed a carbonyl absorption at 1667–1677 cm⁻¹ for the acetyl group. Their structures were further confirmed from the ¹H-NMR spectra which does not reveal the presence of NH signal present in the starting hydrazone **2**, moreover, the ¹H-NMR of **4** exhibited a singlet of three protons intensity at $\delta = 2.10$ – 2.16 ppm for the COCH₃. Finally, treatment of chalcones **1a–c** with hydroxylamine hydrochloride in presence of sodium acetate produced isoxazoles **5a–c** in moderate yield. The structure of **5** was fully confirmed by spectral method. For example, the IR of **5** does not show the presence of carbonyl band characteristic for the starting chalcone **1**. The ¹H-NMR of **5** exhibited a singlet of one proton intensity at $\delta = 5.42$ – 5.69 ppm characteristic for the isoxazole–C₄–H. Melting points, elemental analysis and spectral methods are outlined in **Tables 1** and **2**.

2.1. Antimicrobial Activity

All the synthesized heterocyclic derivatives, pyrazoles **3a–f** oxadiazoles **4a–f** and isoxazoles **5a–c** were assayed for their antimicrobial activity against four test organisms: *Staphylococcus aureus* ATCC6538P, *Escherichia coli* ATCC8739, *Pseudomonas aeruginosa* ATCC9027 and *Candida albicans* ATCC2091 using rifampicin (5 µg/disc) and ampicillin (10 µg/disc) as standard drugs following agar well-diffusion method [21].

The tested heterocyclic compounds showed no significant effect against *Pseudomonas aeruginosa* and *Candida albicans*, whereas they showed a potent activity against *Staphylococcus aureus* and *Escherichia coli*. The maximum activity (+ + +) (MIC = 25 µg/mL) was indicated for compounds **3a**, **3b**, **4a**, **4b** and **5a**. These results suggest that the electron-withdrawing nitro group plays a crucial role in enhancing the observed activity.

Compounds **3e**, **4c**, **4e** and **4f** showed a moderate activity (+ +) (MIC = 50 µg /mL) against *Staphylococcus*, while these compounds exhibited slight activity (+) (MIC = 75 µg/mL) against *Escherichia coli*. All other compounds were inactive towards the different strains of bacteria. The results are summarized in **Table 3**.

Table 1. Physical and analytical data of compounds, **2a–f**, **3a–f**, **4a–f** and **5a–c**.

Compound	X	Y	Yield (%)	Mp (°C)	Molecular Formula	Calculated %			Found %		
						C	H	N	C	H	N
2a	NO ₂	Me	72	181	C ₂₀ H ₁₉ N ₃ O ₃	68.77	5.44	12.03	68.71	5.39	12.09
2b	NO ₂	NO ₂	79	201	C ₁₉ H ₁₆ N ₄ O ₅	60.00	4.21	14.74	60.06	4.19	14.77
2c	H	H	66	166	C ₁₉ H ₁₈ N ₂ O	78.62	6.21	9.66	78.59	6.19	9.62
2d	H	Me	65	180	C ₂₀ H ₂₀ O N ₂	78.95	6.58	9.21	79.01	6.49	9.28
2e	OMe	Me	69	190	C ₂₁ H ₂₂ O ₂ N ₂	75.45	6.59	8.38	75.49	6.62	8.44
2f	OMe	H	74	160	C ₂₀ H ₂₀ O ₂ N ₂	75.00	6.25	8.75	74.97	6.26	8.77
3a	NO ₂	Me	81	183	C ₂₀ H ₁₇ O ₃ N ₃	69.16	4.90	12.10	69.22	4.87	12.08
3b	NO ₂	NO ₂	92	197	C ₁₉ H ₁₄ O ₅ N ₄	60.32	3.70	14.81	60.33	3.69	14.77
3c	H	H	62	159	C ₁₉ H ₁₆ O N ₂	79.17	5.56	9.72	79.21	5.53	9.71
3d	H	Me	66	164	C ₂₀ H ₁₈ O N ₂	79.47	5.96	9.27	79.51	5.91	9.27
3e	OMe	Me	64	171	C ₂₁ H ₂₀ O ₂ N ₂	75.90	6.02	8.43	75.93	6.06	8.51
3f	OMe	H	59	180	C ₂₀ H ₁₈ O ₂ N ₂	75.47	5.66	8.81	75.52	5.60	8.86
4a	NO ₂	Me	83	201	C ₂₂ H ₂₁ O ₄ N ₃	67.52	5.37	10.74	67.52	5.43	10.80
4b	NO ₂	NO ₂	97	210	C ₂₁ H ₁₈ O ₆ N ₄	59.72	4.27	13.27	59.69	4.26	13.22
4c	H	H	59	177	C ₂₁ H ₂₀ O ₂ N ₂	57.90	6.02	8.43	57.84	5.99	8.44
4d	H	Me	58	179	C ₂₂ H ₂₂ O ₂ N ₂	76.30	6.36	8.09	76.28	6.31	8.12
4e	OMe	Me	67	189	C ₂₃ H ₂₄ O ₃ N ₂	73.40	6.38	7.45	73.44	6.39	7.49
4f	OMe	H	80	162	C ₂₂ H ₂₂ O ₃ N ₂	72.93	6.08	7.73	72.91	6.01	7.76
5a	NO ₂	—	54	165	C ₁₂ H ₁₀ O ₃ N ₂	62.61	4.35	12.17	62.66	4.29	12.21
5b	H	—	49	159	C ₁₂ H ₁₁ O N	77.84	5.95	7.57	77.90	5.99	7.62
5c	OMe	—	74	161	C ₁₃ H ₁₃ O ₂ N	72.56	6.05	6.51	72.60	6.01	6.48

Table 2. IR and ¹H-NMR spectral data of compounds 2a–f, 3a–f, 4a–f and 5a–c.

Compound	IR cm ⁻¹ (KBr)				¹ H NMR (δ / ppm) ^a						
	C=C	C=N	C=O	NH	Ar–H's and =C–CH=CH (m)	=C–CH=CH (d), J=12 Hz	Pyrazole- C ₄ –H (s) or isoxazole- C ₄ –H (s)	NH (s)	Cyclopropyl ring H's		Ar–CH ₃ (s) Ar–OCH ₃ and CH ₃ CO–
									CH (m)	2 (CH ₂) (m)	
2a	1592	1629	1678	3420	7.36–7.91	6.72	—	10.66	1.91–2.55	0.75–1.37	2.21
2b	1617	1647	1698	3390	7.23–8.45	6.93	—	10.45	1.80–2.64	0.71–1.40	—
2c	1582	1616	1664	3330	7.19–7.63	6.60	—	9.97	1.82–2.49	0.78–1.29	—
2d	1587	1636	1669	3336	7.29–7.82	6.71	—	10.82	1.71–2.36	0.70–1.40	2.10
2e	1601	1628	1687	3411	7.20–7.71	6.75	—	10.73	1.63–2.40	0.77–1.46	2.19, 3.49
2f	1617	1644	1681	3332	7.17–7.49	6.95	—	9.99	1.56–2.59	0.78–1.16	3.42
3a	1519	1629	1670	—	7.26–7.92 ^b	—	6.85	—	1.90–2.48	0.69–1.21	2.13
3b	1586	1633	1684	—	7.24–8.33 ^b	—	7.12	—	1.76–2.66	0.80–1.36	—
3c	1556	1641	1660	—	7.22–7.71 ^b	—	6.78	—	1.73–2.40	0.71–1.28	—
3d	1590	1644	1678	—	7.24–7.77 ^b	—	6.64	—	1.75–2.41	0.77–1.33	2.11
3e	1571	1623	1686	—	7.21–7.68 ^b	—	6.69	—	1.66–2.39	0.71–1.33	2.10, 3.44
3f	1596	1633	1664	—	7.11–7.62 ^b	—	6.74	—	1.49–2.55	0.77–1.19	3.39
4a	1587	1645	1669	—	7.18–7.79 ^b	—	—	—	1.92–2.41	0.67–1.32	2.01, 2.16
4b	1610	1646	1667	—	7.22–8.22 ^b	—	—	—	1.70–2.66	0.71–1.40	2.13
4c	1602	1626	1669	—	7.16–7.75 ^b	—	—	—	1.68–2.39	0.75–1.26	2.11
4d	1594	1630	1670	—	7.33–7.76 ^b	—	—	—	1.73–2.44	0.71–1.38	1.99, 2.13
4e	1600	1633	1677	—	7.26–7.72 ^b	—	—	—	1.60–2.47	0.71–1.26	2.13, 3.32, 2.15
4f	1615	1646	1671	—	7.25–7.51 ^b	—	—	—	1.47–2.61	0.76–1.33	3.34, 2.10
5a	1580	1627	—	—	7.19–7.99 ^b	—	5.69	—	1.82–2.51	0.71–1.36	—
5b	1571	1629	—	—	7.22–7.62 ^b	—	5.42	—	1.43–2.45	0.69–1.27	—
5c	1569	1633	—	—	7.26–7.70 ^b	—	5.46	—	1.57–2.41	0.67–1.29	3.30

^a Solution in DMSO-d₆; ^b The chemical shift only indicates Ar–H's.

Table 3. Antibacterial activities of newly synthesized compounds 3–5.

Compound	X	Y	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
3a	NO ₂	Me	+++	+++
3b	NO ₂	NO ₂	+++	+++
4a	NO ₂	Me	+++	+++
4b	NO ₂	NO ₂	+++	+++
5a	NO ₂	—	+++	+++
3e	OMe	Me	++	+
4c	H	H	++	+
4e	OMe	Me	++	+
4f	OMe	H	++	+
3c	H	H	—	—
3d	H	Me	—	—
3f	OMe	H	—	—
4d	H	Me	—	—
5b	H	—	—	—
5c	OMe	—	—	—

+++ for maximum activity, MIC = 25 µg/mL; ++ for moderate activity, MIC = 50 µg/mL; + for slight activity, MIC = 75 µg/mL and – for inactive.

3. Experimental

3.1. General

Melting points were taken in open capillary tubes using Electrothermal apparatus 9100 (UK) and are uncorrected. Microanalyses were performed at Faculty of Science, Cairo University, Cairo, Egypt, using a Elementary Vario el III C, H, N, S Analyzer (Germany). IR spectra were recorded using potassium bromide disks on a Perkin-Elmer 1650 spectrophotometer (Faculty of Science, Alexandria University, Alex, Egypt). ¹H-NMR spectra were determined on a Varian EM-390 MHz spectrophotometer, using TMS as internal standard.

3.2. General Procedure for Preparation of *E-l-Cyclopropyl-3-(p-substituted-phenyl)-2-propenenones 1a–c*

To a cold solution of sodium hydroxide (3 g) in aqueous ethanol (50 mL, 60%), cyclopropylmethyl ketone (10 mmol), was added dropwise (30 min), while rapidly stirring and the temperature kept below 20 °C, then the desired *p*-substituted benzaldehyde (10 mmol) was added dropwise (30 min). After five hours, the mixture was left overnight in refrigerator. The separated solid was filtered, washed with water and dried, then recrystallized from ethanol as colorless needles. The physical properties and all the spectral data were as reported in the literature [17].

3.3. General Procedure for Preparation of 1-Cyclopropyl-3-(p-substituted-phenyl)-2-propene-1-aryl hydrazones **2a–e**

A solution of chalcones **1a–c** (10 mmol) in ethanol (10 mL) was refluxed with the appropriate aryl hydrazines (10 mmol) in glacial acetic acid (2 mL) for about six hours, then the reaction mixture was poured onto crushed ice and was kept overnight at room temperature, the separated solid was filtered off, washed successively with water and dried, then recrystallized from methanol. IR and NMR data: see **Tables 1** and **2**.

3.4. General Procedure for Preparation of 1-Aroyl-3-cyclopropyl-5-(p-substituted-phenyl)-pyrazoles **3a–e**

A solution of the appropriate hydrazone **3a–e** (10 mmol) in 30% hydrochloric acid (15 mL) was refluxed for about two hours, the reaction mixture was concentrated, separated solid was filtered off, washed with water, dried and recrystallized from methanol. IR and NMR data: see **Tables 1** and **2**.

3.5. General Procedure for Preparation of 3-Acetyl-2-cyclopropyl-2-(p-substituted styryl)-5-(p-substituted phenyl)-1,3,4-oxadiazoles **4a–e**

A mixture of the appropriate hydrazone **2a–e** (10 mmol) and acetic anhydride (15 mL) was heated under reflux for three hours. After the reaction mixture attained room temperature, it was poured into crushed ice and the oily product deposited was decanted from water and extracted with ether. The ether layer was washed with sodium bicarbonate, followed by water, dried over anhydrous sodium sulphate and evaporated to give the corresponding oxadiazoles **4a–e** as needles. IR and NMR data: see **Tables 1** and **2**.

3.6. General Procedure for Preparation of 3-Cyclopropyl-5-(p-substituted Phenyl)isoxazole **5a–c**

A mixture of chalcone **1a–c** (20 mmol), hydroxylamine hydrochloride (20 mmol) and sodium acetate (20 mmol) in ethanol (25 mL) was refluxed for six hours. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice-water. The precipitate obtained was filtered, washed and recrystallized from ethanol to give isoxazole **5** as needles. IR and NMR data: see **Tables 1** and **2**.

3.7. Determination of Antimicrobial Activity

All the synthesized heterocyclic compounds **3a–f**, **4a–f** and **5a–f** were tested against four different microorganisms: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The agar well-diffusion method was applied for the determination of inhibition zone and minimum inhibitory concentration (MIC). Briefly, 0.75 mL of broth culture containing *ca.* 10⁶ colon-forming units (CFU) per mL of the test strain was added to 75 mL of nutrient agar medium at 45 °C, mixed well, and then poured into a 15 cm sterile metallic Petri plate. The medium was allowed to solidify, and 8 mm wells were dug with a sterile metallic borer. Then, a DMSO solution of the test sample (1 mL) at 1 mg/mL was added to the respective wells. DMSO served as negative control, and the standard antimicrobial drugs rifampicin (5 µg/disc) and ampicillin (10 µg/disc) were used as

positive controls. Triplicate plates of each microorganism strain were prepared and were incubated aerobically at 37 °C for 24 h. The activity was determined by measuring the diameter of zone showing complete inhibition (mm), thereby, the zones were precisely measured with the aid of a Vernier Caliper (precision 0.1 mm). The growth inhibition was calculated with reference to the positive control.

4. Conclusions

In summary, this work demonstrates a rapid, efficient method for synthesis of new heterocyclic compounds of pharmacological interest.

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Sample Availability: Samples of all the compounds are available from the authors.

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