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RESEARCH ARTICLE

New Chalcone-Pyrrole Amide Hybrids: Synthesis and Screening of Caspase-7 Inhibitory Activity

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ABSTRACT:
Chalcone-pyrrole derivatives are unique structures and possess a wide range of biological activities. A total of eight new chalcone-pyrrole amide derivatives were successfully synthesized through the application of the Claisen-Schmidt condensation followed by an amidation reaction and characterized by the spectroscopic method. The synthesized compounds were investigated for their biological activities targeting caspase-7. Among eight compounds (10a-10d, 11a-11d), only compound 10c showed 8.13% inhibition against caspase-7.

KEYWORDS: Caspase-7, Chalcone, Claisen-Schmidt condensation, pyrrole.

INTRODUCTION:

Chalcones are precursors for flavonoids and isoflavonoids, containing two aromatic rings connected by an α,β-unsaturated carbonyl moiety. Chalcone is widely distributed in higher and lower plants and possesses numerous biological activities, such as anticancer[1], antibacterial[2], anti-inflammation[3], antioxidant[4], antiviral[5], antimalarial[6], and antifungal[7]. A commercially marketed chalcone-based drug is metochalcone (**1**) as a choleretic drug and sofalcone (**2**) as an antiulcer drug[8], as shown in Figure 1. Conventionally, chalcone can be synthesized using Claisen-Schmidt condensation with an acid or base as a catalyst[9].

The structural modification of chalcones has been reported by many researchers[10].

A common method of chalcone modification is by replacing the A- or B-ring with pharmacologically active heterocycles such as pyrrole[11], furan[12], thiophene[13], pyrazine[14], and so on[15-24]. As presented in Figure 1, naturally occurring modified chalcone is glycopentalone (**3**), isolated from *Glycosmis pentaphylla*. Glycopentalone is a chalcone derivative containing pyrrole as ring B, and this compound possesses anti-hepatocellular carcinoma[25].

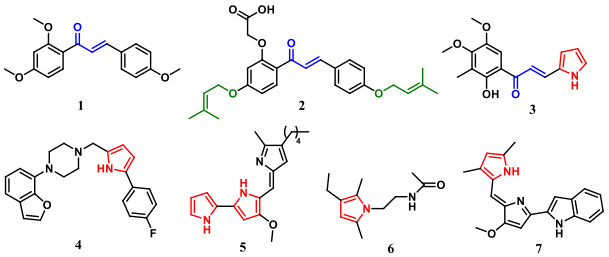

Figure 1. Marketed drugs of chalcone (1 and 2), natural chalcone (3), and pyrroles (4-7).

Pyrrole moiety (Figure 1) is known to exist in many available marketed drugs such as elopirazole (antipsychotic) (**4**), prodigiosin (antibacterial) (**5**), aloracetam (anti-Alzheimer) (**6**), and obatoclax (anticancer) (**7**)[26]. The pyrrole moiety in drugs can interact with numerous biomolecules through hydrogen bonding or π-π stacking[27]. Several studies have shown that chalcones combined with pyrrole moieties exhibit several biological activities, such as antibacterial,

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antiproliferative, anticancer, antifungal, and antioxidant[28-30].

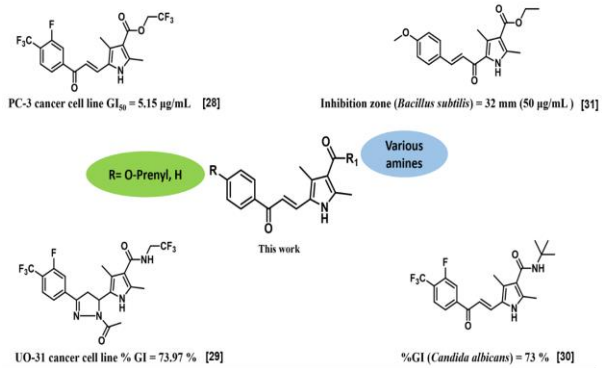


Figure 1. The current study of pyrrole-chalcone derivatives.

Rasal et al. successfully synthesized chalcone-pyrrole analogs containing fluorinated acetophenone as an A-ring and dimethyl pyrrole as a B-ring with several amide derivatives[28-30], as shown in Figure 2. The synthesized compounds exhibited anticancer activity against the PC-3 cancer cell line with the highest growth inhibition (GI_{50}) = 5.15 µg/mL, UO-31 cancer cell line with the highest %GI = 73.9%, and antifungal activity against *Candida albicans* with the highest %GI = 73%. Furthermore, Rawat et al. synthesized numerous chalcone-pyrrole analogs composed of pyrrole containing ethyl ester functional group as an A-ring and several derived acetophenone as a B-ring (Figure 2)[31]. The synthesized compound exhibited antimicrobial activity against *Aspergillus niger* and *Bacillus subtilis* (inhibition zone 32 mm). However, synthesis of chalcone-pyrrole analogs from acetophenone and *p-O*-prenylated acetophenone as an A-ring and pyrrole as a B-ring containing amide derivatives have not been reported and evaluated for their activities against caspase-7 enzyme. Caspases (cysteine-aspartic proteases) can be divided into two main groups based on their functions: inflammatory and apoptotic caspases. Apoptotic caspases involved in the apoptosis process can be further subdivided into executioner caspases (caspase-3, 6, 7) and initiator caspases (caspase- 8, 9, 10). The inhibition of caspase enzymes disturbs the apoptosis process in the cell[32]. Caspase-7 is involved in cell detachment during apoptosis and Reactive Oxygen Species (ROS) production[33]. Thus, caspase inhibitors could be useful for the treatment of diseases caused by inappropriate apoptosis, such as vascular diseases (stroke and heart attacks) and Alzheimer's disease. The study was to produce two sets of chalcone-pyrrole hybrids incorporating phenyl and 4-*O*-prenylacetophenone on the A-ring and pyrrole amide derivatives on the B-ring. Additionally, we evaluated the inhibitory efficacy of these compounds on caspase-7.

MATERIALS AND METHODS:

Materials:

All chemicals and solvents used in this research were purchased from Sigma Aldrich. NMR data were recorded on an Agilent DD2 system at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. High-resolution electrospray ionization mass spectrometry (ESI-TOF) data were recorded on Waters LCT Premier XE mass spectrometer. Thin Layer chromatography (TLC) analysis was done using precoated silica gel plates (Merck Kielselgel 60 GF254, 0.25 mm thickness). Spots on TLC were detected by UV irradiation.

Methods:

General procedure for synthesized 4-*O*-prenylacetophenone:

2.0 g (15 mmol) of 4-hydroxy acetophenone was added to a three-necked flask along with 4.06 g K_2CO_3, 2.41 g prenyl bromide, and acetone (20 mL). The reaction was carried out at 65 °C under reflux for 1 h. The mixture was cooled to room temperature and quenched with water until all K_2CO_3 dissolved and the product was extracted using DCM, dried using anhydrous sodium sulfate, and evaporated DCM using a rotary evaporator. Characterization data obtained for 4-*O*-prenyl acetophenone (2.85 g, brownish liquid, yield = 95 %): ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 1.72 (s, 3H), 1.77 (s, 3H), 2.51 (s, 3H), 4.54 (d, *J* = 6.7 Hz, 2H), 5.45 (t, *J* = 6.75 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 2H), and 7.89 (d, *J* = 8.9 Hz, 2H).

General procedure for the synthesis of compound 9:

Compound 9 was prepared as a previous work[34]. 1.17 g (5.99 mmol) of Ethyl 5-formyl-2,4-dimethyl-1H-pyrrole-3-carboxylate (8) was added into a three-necked flask along with 10 mL of 1 M sodium hydroxide and 6 mL methanol. The reaction was carried out at 78 °C under reflux for 4.5 h. After the reaction was complete (monitored by TLC), the mixture was cooled to room temperature and dumped into a 20 mL ice-water mixture. The mixture was washed with 30 mL of DCM (3 × 10 mL) and neutralized using 2 M HCl until pH = 2-3. The resulting precipitation was filtered using vacuum filtration, washed with cold distilled water, and dried in an oven at 40 °C. Characterization data obtained for compound 9 (0.991 g; brownish solid, yield = 98.9 %); ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 2.40 (s, 3H), 2.44 (s, 3H), 9.59 (s, 1H), 12.07 (s, 1H).

General procedure for the synthesis of compounds 10 and 11:

A mixture of compound 9 (1 g, 5.98 mmol) and substituted acetophenones (1 eq) in 20 mL of methanol was cooled to 0 °C using an ice bath. KOH (10 eq) was added to the mixture and stirred until all the KOH was dissolved. The resulting mixture was kept at 0 °C or 30

min and at room temperature for 72 h. After the reaction was complete, the mixture was poured into 100 mL of an ice-water mixture, and 2 M HCl was added until the pH = 6. The resulting precipitate was filtered, washed with cold water, and dried under vacuum. Impurities were removed by recrystallization in hot methanol.

Characterization data obtained for compound 10 (810 mg, yellow solid, yield = 50.3 %); ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 2.31 (s, 3H), 2.48 (s, 3H), 7.52 (d, J = 15.3 Hz, 1H), 7.56 (t, J = 7.2 Hz, 2 H), 7.62 (d, J = 15.4 Hz, 1 H), 7.63 (t, J = 7.8 Hz, 1 H), 8.02 (dd, J = 7.8 Hz, 2 H), 11.75 (s, 1H), 11.94 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 16.2, 18.9, 118.5, 118.9, 130.2, 133.0, 133.9, 134.6, 135.9, 137.7, 143.6, 171.3, 193.2. HRESITOF-MS calcd. For $[M+H]^+$ $C_{16}H_{16}NO_3^+$ *m/z*: 270.1125, found: 270.1125.

Characterization data obtained for compound 11 (775 mg, yellow solid, yield = 36.7 %); ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 1.72 (s, 3H), 1.75 (s, 3H), 2.30 (s, 3H), 2.48 (s, 3H), 4.63 (d, J = 6.5 Hz, 2H), 5.44 (tm, J = 6.1 Hz, 1H), 7.08 (d, J = 8.5 Hz, 2H), 7.52 (d, J = 15.2 Hz, 1H), 7.57 (d, J = 15.2 Hz, 1H), 8.00 (d, J= 8.6 Hz, 2H), 11.71 (s, 1H), 11.89 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 11.4, 14.2, 18.5, 25.8, 65.1, 113.6, 114.2, 115.0, 119.8, 125.5, 129.1, 130.3, 130.5, 131.4, 138.2, 141.0, 162.5, 166.6, 186.8. HRESITOF-MS calcd. $[M+H]^+$ for $C_{21}H_{24}NO_4^+$ *m/z*: 354.1700, found: 354.1701

General procedure for the synthesis of compounds 10a-d and 11a-d:

50 mg of compound 10 or 11 (0.186 and 0.141 mmol) and CDI (0.371 and 0.283 mmol) were added to the carousel tube, dissolved in 1.5 mL THF, and refluxed for 3 h. After the reaction was complete, three equivalents of the corresponding amines were added to the reaction mixture and heated at 50 °C overnight. The reaction mixture was cooled to room temperature, and 20 mL of DCM was added to the mixture. The mixture was washed using 3 × 10 mL of 0.5 M HCl and 3 × 10 mL of water, and the remaining water in the organic phase (10a, 10c, 10d,11a, 11c, and 11d) was removed using anhydrous sodium sulfate and dried under vacuum. The water phase of (10b, 11b) was neutralized using 0.5 M NaOH, and the resulting precipitate was dried under vacuum. All impurities were removed by recrystallization using hot ethyl acetate.

Characterization data obtained for compound 10a (42.0 mg, yellow solid, yield = 69.7 %); ^{1}H-NMR (500 MHz DMSO-d_6) δ ppm: 0.89 (d, J = 6.7 Hz, 6H), 1.78 (m, 1H), 2.23 (s, 3H), 2.37 (s, 3H), 3.02 (t, J = 6.3 Hz, 2H), 7.46 (d, J = 15.3 Hz, 1H), 7.49 (t, J= 5.8, 1H), 7.56 (t, J= 7.3 Hz, 2 H), 7.59 (d, J = 15.1 Hz, 1 H), 7.62 (t, J = 7.3 Hz, 1 H), 8.01 (d, J =7.3 Hz, 2 H), 11.50 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 10.6, 13.1, 20.7, 28.7, 46.6, 113.1, 120.5, 125.0, 127.0, 128.2, 129.1, 131.4, 132.8, 135.9, 139.0, 165.3, 188.3. HRESITOF-MS calcd. $[M+H]^+$ for $C_{20}H_{25}N_2O_2^+$ *m/z*: 325.1911, found: 325.1913.

Characterization data obtained for compound 10b (43.5 mg; yellow solid, yield = 63.8 %); ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 0.96 (t, J= 7.15 Hz, 6H), 2.23 (s, 3H), 2.38 (s, 3H), 2.49 (q, J = 7.1 Hz, 4H), 2.51 (t, J = 6.5 Hz, 2H), 3.25 (m, 2H), 7.23 (t, J = 5.4 Hz, 1H), 7.47 (d, J = 15.2 Hz, 1H), 7.56 (t, J = 7.25 Hz, 2H), 7.59 (d, J = 15.4 Hz, 1H), 7.62 (t, J = 7.3 Hz, 1H), 8.00 (dd, J = 7.8 Hz; 1.2 Hz, 2H), 11.52 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 10.7, 12.3, 13.2, 37.3, 46.9, 52.1, 113.2, 119.8, 125.1, 126.9, 128.2, 129.1, 131.4, 132.9, 136.3, 139.0, 165.1, 188.3. HRESITOF-MS calcd. $[M+H]^+$ for $C_{22}H_{30}N_3O_2^+$ *m/z*: 368.2333, found: 368.2333.

Characterization data obtained for compound 10c (27.0 mg; yellow solid, yield = 27.1 %) : ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 2.09 (s, 3H), 2.23 (s, 3H), 3.47 (brs, 4H), 3.55 (brs, 4H), 7.46 (d, J = 15.2 Hz, 1H), 7.56 (t, J= 7.1 Hz, 2H), 7.57 (d, J = 15.0 Hz, 1H), 7.62 (t, J = 7.4 Hz, 1H), 8.00 (d, J = 7.3 Hz, 2H), 11.56 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 10.2, 12.4, 66.9, 66.9, 113.3, 118.9, 125.4, 126.1, 128.2, 129.1, 131.4, 132.9, 134.0, 138.9, 166.1, 188.3. HRESITOF-MS calcd. $[M+H]^+$ for $C_{20}H_{23}N_2O_3^+$ m/z: 339.1703 found: 339.1700.

Characterization data obtained for compound 10d (46.4 mg; yellow solid, yield = 64.3 %): ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 2.24 (s, 3H), 2.38 (s, 3H), 3.72 (s, 3H), 4.34 (d J = 6.0 Hz, 2H), 6.88 (d J = 8.7 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 7.47 (d, J = 15.2 Hz, 1H), 7.56 (t, J = 7.7 Hz, 2H), 7.59 (d, J = 15.2 Hz, 1H), 7.62 (t, J = 7.3 Hz, 1H), 7.94 (t, J = 6.0 Hz, 1H), 8.01 (dd, J = 7.9 Hz, 1.4 Hz, 2H), 11.53 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm : 10.7, 13.2, 42.13, 55.49, 113.26, 114.1, 119.9, 125.1, 127.1, 128.2, 128.9, 129.1, 131.4, 132.5, 132.9, 136.3, 139.0, 158.5, 165.3, 188.3. HRESITOF-MS calcd. $[M+H]^+$ for $C_{24}H_{25}N_2O_3^+$ *m/z*: 389.1860, found: 389.1865.

Characterization data obtained for compound 11a (41.0 mg; yellow solid, yield = 70.9 %) ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 0.89 (d, J = 6.7 Hz, 3H), 1.72 (s, 3H), 1.75 (s, 3H), 1.78 (m, 1H), 2.22 (s, 3H), 2.36 (s, 3H), 3.02 (t, J = 6.4 Hz, 2H), 4.63 (d, J = 6.7 Hz, 2H), 5.44 (t, J = 6.7 Hz, 1H), 7.08 (d, J = 8.9 Hz, 2H), 7.46 (d, J = 15.1 Hz, 1H), 7.47 (t, J = 6.3 Hz, 1H), 7.55 (d, J = 15.2 Hz, 1H), 7.99 (d, J = 8.8 Hz, 2H), 11.44 (s, 1H); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 10.6, 13.1, 18.5,

20.7, 25.8, 28.7, 46.6, 65.1, 113.2, 114.9, 119.8, 120.3, 125.0, 126.3, 130.4, 130.6, 131.6, 135.4, 138.2, 162.4, 165.4, 186.7. HRESITOF-MS calcd. $[M+H]^+$ for $C_{25}H_{33}N_2O_3^+$ *m/z*: 409.2486, found: 409.2491.

Characterization data obtained for compound 11b (37.0 mg; yellow solid, yield = 61.7 %) ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 0.96 (t, *J*= 7.05, 6H), 1.72 (s, 3H), 1.75 (s, 3H), 2.22 (s, 3H), 2.38 (s, 3H), δ 2.49 (q, *J* = 7,0 Hz, 4H), 2.51 (t, *J* = 6.8 Hz, 2H), 3.25 (m, 2H), 4.63 (d, *J* = 6.7 Hz, 2H), 5.44 (t, *J* = 6.9 Hz, 1H), 7.07 (d, *J* = 8.7 Hz, 2H), 7.21 (t, *J* = 5.5 Hz, 1H), 7.46 (d, *J* = 15.3 Hz, 1H), 7.55 (d, *J* = 15.2 Hz, 1H), 7.99 (d, *J* = 8.7 Hz, 2H), 11.46 (s, 1H); ^{13}C-NMR (125 MHz DMSO-d_6) δ ppm : 10.7, 12.3, 13.2, 18.5, 25.8, 37.3, 46.9, 52.1, 65.1, 113.4, 114.9, 119.6, 119.8, 125.1, 126.2, 130.4, 130.6, 131.5, 135.9, 138.2, 162.4, 165.2, 186.7. HRESITOF-MS calcd. $[M+H]^+$ for $C_{27}H_{38}N_3O_3^+$ *m/z*: 452.2908 found: 452.2905.

Characterization data obtained for compound 11c (21.0 mg; yellow solid; yield = 35.1 %) ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 1.72 (s, 3H), 1.75 (s, 3H), 2.08 (s, 3H), 2.22 (s, 3H), 3.44 (br s, 4H), 3.55 (brs, 4H), 4.63 (d, *J* = 6.9 Hz, 2H), 5.44 (t, *J* = 7.4 Hz, 1H), 7.07 (d, *J* = 8.8 Hz, 2H), 7.45 (d, *J* = 15.3 Hz, 1H), 7.52 (d, *J* = 15.2 Hz, 1H), 7.99 (d, *J* = 8.8 Hz, 2H), 11.50 (s, 1H); ^{13}C-NMR (125 MHz DMSO-d_6) δ ppm: 10.2, 12.3, 18.5, 25.8, 65.1, 66.8, 66.9, 113.4, 114.9, 118.6, 119.8, 125.4, 125.5, 130.4, 130.6, 131.5, 133.5, 138.2, 162.4, 166.3, 186.8. HRESITOF-MS calcd. $[M+H]^+$ for $C_{25}H_{31}N_2O_4^+$ *m/z*: 423.2278 found: 423.2280.

Characterization data obtained for compound 11d (40.1 mg; yellow solid; yield = 60.9 %) ^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 1.72 (s, 3H), 1.75 (s, 3H), 2.22 (s, 3H), 2.37 (s, 3H), 3.72 (s, 3H), 4.34 (d, *J* = 5.9 Hz, 2H), 4.63 (d, *J* = 6.8 Hz, 2H), 5.44 (t, *J* = 6.8 Hz, 1H), 6.88 (d, *J* = 8.6 Hz, 2H), 7.07 (d, *J* = 8.9, 2H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.47 (d, *J* = 15.2 Hz, 1H), 7.55 (d, *J* = 15.2 Hz, 1H), 7.91 (t, *J* = 6.1, 1H), 7.99 (d, *J*= 8.85 Hz, 2H), 11.47 (s, 1H); ^{13}C-NMR (125 MHz DMSO-d_6) δ ppm: 10.7, 13.2, 18.5, 25.8, 42.1, 55.4, 65.1, 113.3, 114.0, 114.9, 119.7, 119.8, 125.1, 126.4, 128.9, 130.4, 130.6, 131.5, 132.5, 135.8, 138.2, 158.5, 162.4, 165.3, 186.7. HRESITOF-MS calcd. $[M+H]^+$ for $C_{29}H_{33}N_2O_4^+$ *m/z*: 473.2435 found: 473.2436.

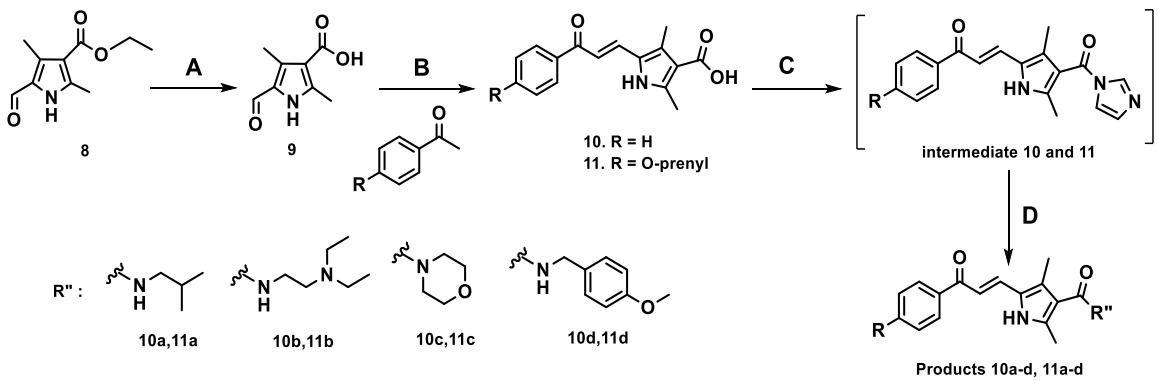
Inhibition Activity Assay against Caspase-7:

The procedure for the inhibition activity assay against caspase-7 was adopted from Abcam (catalog number: ab102495) and stored at -80 °C. All samples and controls (Z-VAD-FMK) were dissolved in distilled water/DMSO (10% DMSO), and active caspase-7, buffer solution, and substrate (DEVD-AFC) were added to the mixture. The mixture was incubated at 37 °C for 0.5-1 hour and the fluorescence in the enzyme assay was measured with a microplate reader of Glomax Explorer equipped with a 400 nm excitation filter and 505 nm emission filter[35]. The measurement of inhibition activity involved a comparison of the fluorescence intensity of the caspase activity positive control, which allowed for the determination of the efficiency of the testing inhibitors in inhibiting the activity.

RESULTS AND DISCUSSION:

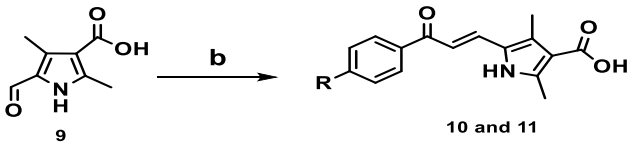
Chemistry:

Eight new chalcone-pyrrole amides were synthesized according to previous work with CDI as a coupling agent for amide formation[28-30]. Chalcone-pyrroles (10 and 11) were synthesized from acetophenone and pyrrole (9) using Claisen-Schmidt condensation at room temperature, as presented in Table 1, with yields of approximately 50.3 and 36.7%, respectively. Amide formation was carried out using carbonyldiimidazole (CDI) as a coupling agent in THF under reflux conditions at 66 °C[36], as shown in Scheme 1 and Table 2.



Scheme 1. Reagents and conditions: A. (1) 1 M NaOH, MeOH reflux (2) HCl 2M pH 2. B. (1) 10 eq KOH, MeOH, 72 h, rt; (2) HCl 2 M, pH 6. C. 2 eq CDI, THF, reflux 66 °C. D. 2-5 eq of various amines, 12-48 h, 50 °C

Table 1: Synthesis of compounds 10 and 11



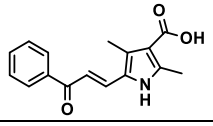
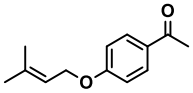
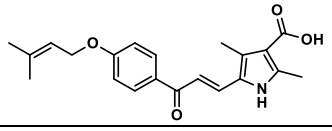
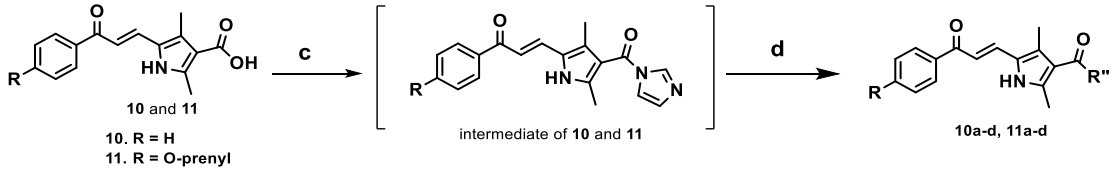
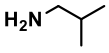
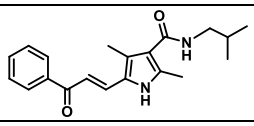
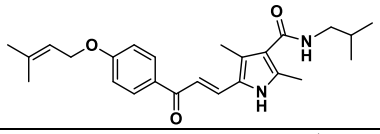
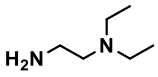
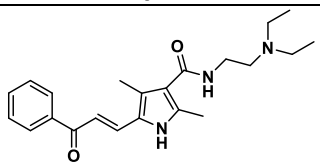
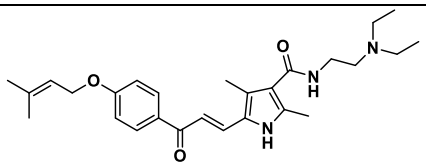
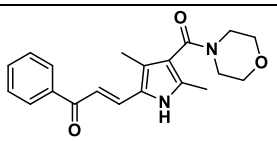
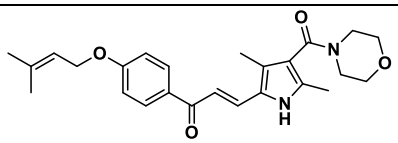
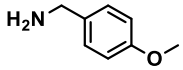
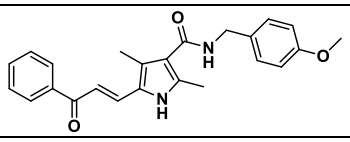
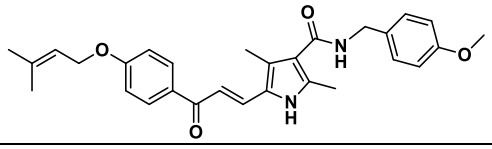
No.	Acetophenone (b)	Time	Eq	Product	Yields (%)
10		72 h	1.1		50.3
11		72 h	1.1		36.7

Table 2 Synthesis of compound 10a-d and 11a-d



No.	Amine (d)	Time	Eq	Product	Yields (%)
10a		12 h	2		69.7
11a					70.9
10b		12h	2		63.8
11b					61.7
10c		48h	5		27.1
11c					35.1

10d		48h	2		64.3
11d					60.9

The yield of amide formation varied from 27.1% to 70.9%. The synthesis of showed high yields using primary amine groups, such as isobutylamine, *N*,*N*-diethylethylenediamine, and p-O-methyl benzylamine, as shown in Table 2. However, the use of secondary amine of morpholine decreased the yield of amide products by approximately 27.1% (10c) and 35.1 % (11c). These results show that CDI-mediated amidation coupling is more reactive with primary amines than with secondary amines because of steric hindrance.

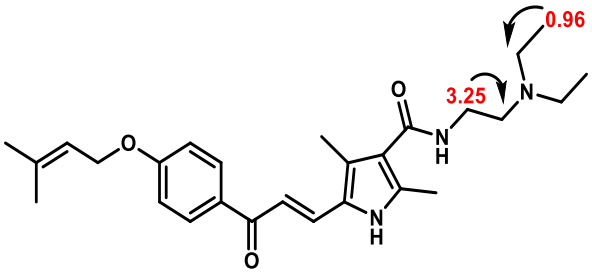


Figure 3. Selected ^{1}H-NMR compound 11b for 1D-TOCSY experiment to determine the overlapping methylene signals.

All synthesized chalcone-pyrrole derivatives were confirmed using ^{1}H-NMR, 1D-TOCSY, ^{13}C-NMR, and HRMS. Compound 11b was used as a representative compound for structural elucidation. The ^{1}H-NMR spectra of 11b showed δ 0.96 (t, *J* = 7.1, 6H) (*N*,*N*-diethyl), 1.72 and 1.75 (s, 6H) (CH_3-Prenyl), 2.22 (s, 3H) (CH_3-Pyrrole), and 2.38 (s, 3H) (CH_3-Pyrrole). However, the proton signals of the methylene group (-CH_2-) overlapped with each other and with the solvent signal (DMSO-d_6) at approximately δ 2.46-2.54 ppm (Figure 3 and 4C). Thus, 1D-TOCSY was performed to distinguish these proton signals, as shown in Fig. 4A and 4B. Based on Figure 4A, the quartet proton belongs to methylene δ 3.25 (m, 2H) attached to triplet δ 2.51 (t, *J* = 6.8 Hz, 2H). Meanwhile, Fig. 4B triplet at δ 0.96 ppm (t, *J* = 7.1, 6H), have a correlation with δ 2.49 (q, *J* = 7.0 Hz, 4H). The ^{1}H-NMR spectrum exhibited two signals indicating para-substitution on the phenyl ring at chemical shifts of δ 7.07 (d, *J* = 8.7 Hz, 2H) and 7.99 (d, *J* = 8.7 Hz, 2H). Additionally, the spectrum revealed the presence of a trans-alkene originating from an α,β-unsaturated ketone, observed at δ 7.46 (d, *J*= 15.3 Hz, 1H) and δ 7.55 (d, *J*= 15.2 Hz, 1H).

Furthermore, the prenyl group was indicated by the presence of two methyl signals at a chemical shift of δ 1.72 (s, 3H) (CH_3-prenyl) and 1.75 (s, 3H) (CH_3-prenyl), as well as a methene signal at δ 5.44 (tm, *J*= 6.9 Hz, 1H) close to a methylene signal at a chemical shift of δ 4.63 (d, *J* = 6.7 Hz, 2H). A single signal was observed at δ 7.21 ppm (t, *J* = 5.5 Hz, 1H) corresponding to the N-H amide bond. Additionally, another signal was observed at δ 11.46 ppm (s, 1H), indicating the presence of a single N-H pyrrole. The ^{13}C-NMR spectra of 11b showed twenty-three signals, including nine signals from C-sp^3 at δ 10.7, 12.3, 13.2, 18.5, 25.8, 37.3, 46.9, 52.1, 65.1, twelve signals from C-sp^2 at δ 113.4, 114.9, 119.6, 119.8, 125.1, 126.2, 130.4, 130.6, 131.5, 135.9, 138.2, 162.4, and one signal from amide carbonyl at δ 165.2, and unsaturated ketone at δ 186.7. The structure of 11b was also confirmed by ^{13}C-NMR which showed twenty-three signals consistent with the structure of 11b. The HR-ESI-TOF-MS spectrum of 11b showed a molecular formula of $C_{27}H_{37}N_3O_3$ for this compound ([M+H]$^+$ $C_{27}H_{38}N_3O_3^+$ m/z: 452.2908 found: 452.2905).

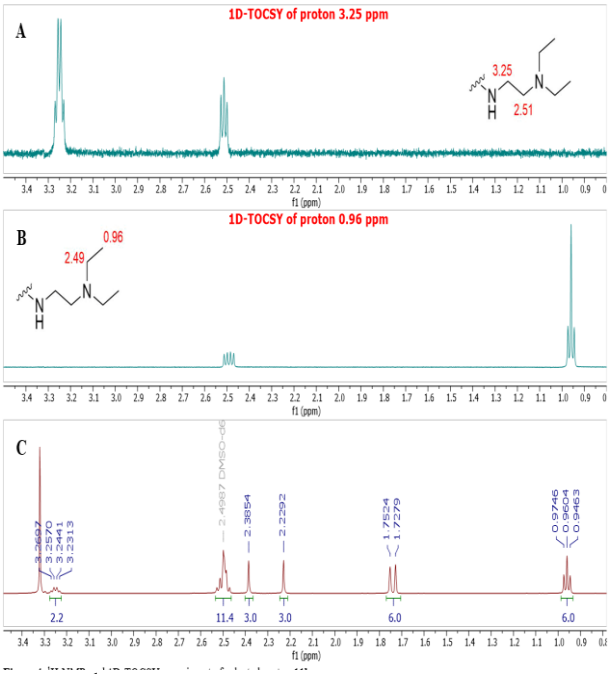


Figure 4. ^{1}H-NMR and 1D-TOCSY experiment of selected proton 11b.

Biological Activity

Eight synthesized compounds were evaluated against caspase-7 using the fluorescence method with Z-VAD-FMK as a positive control. Among eight chalcone-pyrrole amide derivatives, only 10c showed 8.13% inhibition against caspase-7 as presented in Table 3.

Table 1 Inhibition of eight chalcone-pyrrole derivatives

Compounds	% Inhibition (10 µM)[a]	Compounds	% Inhibition (10 µM)[a]
10a	NI[b]	11a	NI
10b	NI	11b	NI
10c	8.13%	11c	NI
10d	NI	11d	NI

[a]The data were obtained in triplicate experiments. [b]NI= no inhibition. [c]Inhibition of positive control (Z-VAD-FMK) = 98.53% (10 µM).

The synthesized compounds against caspase-7 containing primary amines (10a, 10b, 10d, 11a, 11b, and 11d) showed no activity. The presence of aromatic rings with methoxy (10d, 11d), nitrogen heteroatoms (10b, 11b), or branched alkyl (10d, 11d) groups in primary amines did not increase the inhibitory activity against caspase-7. Moreover, compound 10c exhibited inhibitory activity against caspase-7 with a value of 8.13% at 10 µM, whereas compound 11c containing prenyl on ring A was inactive. This result suggests that chalcone-pyrrole amides containing morpholine could be a potential candidate for future studies

CONCLUSION

Eight new chalcone-pyrrole amide derivatives were successfully synthesized via the Claisen-Schmidt condensation reaction, followed by amidation using CDI. Among the eight newly synthesized derivatives (10a-d and 11a-d), only compound 10c exhibited activity as a caspase-7 inhibitor with an inhibition rate of 8.13 %. This result suggests that chalcone-pyrrole amide hybrids have potential for further study.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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