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SYNTHETIC APPROACHES TOWARDS QUINOLACTACIN DERIVATIVES VIA DIELS-ALDER, ACYL MIGRATION AND MULTICOMPONENT REACTIONS

(Pendekatan Sintesis Ke Arah Terbitan Quinolaktasin Melalui Tindak Balas Diels-Alder, Migrasi Asil dan Tindak Balas Pelbagai Komponen)

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Abstract

Quinolactacins are rare fungal alkaloids extracted from the culture broth of Penicillium species isolated from larvae mulberry pyralis (*Margonia pyloalis Welker*). The synthesis of the natural alkaloids has gained interests among many researchers due to its unique γ-lactam conjugated ring quinolone skeleton. Furthermore, the alkaloids are also proven to exhibited inhibitory activities against tumour necrosis factor (TNF) production. Synthesizing the alkaloids is initiated by forming the key pyrrolidine-2,4-dione intermediates *via* the acid-mediated Meldrum reaction and tetramic acid cyclisation of different amino acids. Subsequently, the diketo intermediates are reduced and eliminated to form hydroxy and enone analogues. The enone analogues are then reacted with an amine-substituted diene by aza Diels-Alder reaction to form the anticipated 4-pyridone-lactam moiety with different substitutions. Nonetheless, the availability of amine-substituted dienes is limited even though aza Diels-Alder reactions shorten the formation of the tricyclic compounds. Consequently, the present study synthesised quinolactacin derivatives through alternative routes, including acylating the key pyrrolidine-2,4-dione with 2-nitrobenzoyl chloride to obtain an acylated tetramic acid and hydrogenated and furnish the final quinolactacin derivatives. The derivatives were also procured through a multicomponent reaction of diethyl oxaloacetate salt with aldehydes and amines. The synthesised compounds were analysed and confirmed with proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and infrared (IR) spectroscopy.

Keywords: *Penicillium* sp., Diels-Alder, 2,4-pyrrolidinone, quinolactacin

Abstrak

Quinolaktasin adalah kulat alkaloid nadir yang terhasil daripada kultur kaldu Penicillium spesies, di ekstrak daripada larva piralis mulberi (*Marfonia pyloalis Welker*). Sintesis bahan alkaloid yang natural ini telah menarik minat ramai ahli penyelidik oleh kerana keunikan struktur quinolone di mana ianya konjugat bersama gegelang γ-lactam. Alkaloid ini telah dibuktikan bahawa ianya bersikap aktif terhadap aktiviti rencatan melawan penghasilan faktor tumor nekrosis (TNF). Sintesis ini bermula dengan bahantara pirolidina-2,4-dion melalui tindak balas asid Meldrum dan pengitaran asid tetramik dari variasi asid amino. Bahantara diketo pula akan melalui pengurangan berturutan dan tindak balas penyingkiran kumpulan hidroksi dan variasi enon. Variasi enon ini bertindak balas dengan diena amina gantian melalui reaksi aza Diels-Alder bagi menghasilkan sebatian 4-piridona-laktam dengan variasi gantian. Walaupun reaksi kimia aza Diels-Alder dapat membantu menghasilkan sebatian trisiklik dalam langkah yang pendek, tetapi variasi diena amina gentian juga adalah terhad. Oleh itu, sintesis bagi penghasilan variasi quinolaktasin dijalankan melalui cara alternatif di mana pirolidina-2,4-dion menjalani pengasilan dengan 2-nitrobenzoil klorida untuk menghasilkan tetramik asid terasilasi dan penghidrogenan bagi menghasilkan sebatian akhir quinolaktasin. Reaksi pelbagai komponen oleh garam dietil oxaloasetat bersama aldehid dan amina pula adalah alternatif lain bagi penghasilan variasi sebatian quinolaktasin. Semua sebatian di dalam kajian ini dianalisa dan disahkan menggunakan resonans magnet nucleus (^{1}H- dan ^{13}C-NMR) dan spektroskopi infra merah (IR).

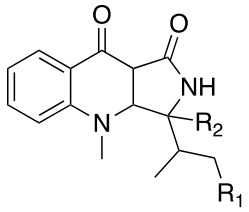
Kata kunci: *Penicillium* sp., diels-alder, 2,4-pirolidina, quinolaktasin

Introduction

The first quinolactacin was extracted from the fermented broth of Penicillium sp. EPF-6 extracted from mulberry pyralid larvae (*Margaronia pyloalis Welker*). Solvent extraction and chromatographic purification separated the quinolactacins from the culture medium. The substances were reported to behave as active inhibitors against tumour necrosis factor (TNF) produced by murine macrophages and macrophage-like J774.1 cells stimulated with LPS [1]. Consequently, numerous studies have attempted to synthesise the chemicals in the laboratory. Similarly, the current study described the synthesis of the quinolactacin derivatives.

To date, three different quinolactacins have been discovered by scientists, quinolactacins A, B, and C, differentiated by the alkyl groups at the C-5 position (see Figure 1). The quinolactacins were demonstrated to be weak antifungals against *Aspergillus niger* ATCC 9642 and inactive towards a range of bacteria, filamentous fungi, and yeasts [2]. Nonetheless, the substances are still gaining interest from researchers as they exhibit several biological activities.

The current study found other derivatives types, including quinolactacin H and quinolactacide. The current study also investigated pyrrolidine synthesis, as previous reports demonstrated that numerous pyrrolidine-type molecules were active antibacterial, antifungal, and anti-inflammatory agents [3]. Furthermore, pyrrolidine-2,4-dione or tetramic acid is the essential ring moiety required in the synthesis of quinolactacin.



Quinolactacin A : $R_1 = CH_3$, $R_2 = H$; Quinolactacin B : $R_1 = R_2 = H$; Quinolactacin C : $R_1 = CH_3$, $R_2 = OH$

Figure 1. The chemical structure of quinolactacins A, B, and C

Materials and Methods

The current study utilized Boc-Val-OH, *D*-valine, 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid), 4-dimethylaminopyridine (DMAP), *N,N'*-dicyclohexylcarbodiimide (DCC), 2-nitrobenzoylchloride, tetra-n-butylammonium fluoride (TBAF) in THF, sodium hydride (NaH), sodium borohydride ($NaBH_4$), acetic acid, sodium hydroxide (NaOH), di-*tert*-butyl dicarbonate (BOC_2O), aluminium chloride, *N*-benzylideneaniline, *trans*-1-methoxy-3-trimethylsilyloxy-buta-1,3-diene, 2,4-hexadiene, diphenyl ether, methanol, potassium bisulfate ($KHSO_4$), hexane, diethyl ether, ethyl acetate, tetrahydrofuran (THF), hydrochloric acid (HCl), potassium cyanonitrile

(KCN), *iso*butylraldehyde, formaldehyde, ammonia, aniline, 40% methylamine in water, formic acid diethyl oxaloacetate sodium salt, chloroform, dichloromethane, anhydrous magnesium sulphate, sodium chloride (NaCl), dioxane.

Synthesis of (*tert*-butoxycarbonyl)valine (1)

A solution of *D*-valine, (1.00 g, 8.51 mmol) in dioxane/water (2:1) (40 ml) and 1 M NaOH (8 ml) was stirred. Upon the formation of a homogenous mixture, the reaction mixture was cooled in an iced-water bath before adding the BOC_2O (3.73 g, 17.07 mmol). Then, the reaction mixture was left stirring overnight at room temperature. Progress of the reaction was monitored using TLC analysis. After the reaction completed, the undissolved materials were filtered and the filtrate was partially evaporated. The residue was cooled in an iced-water bath and diluted with ethyl acetate. The mixture was acidified to pH 2-3 with 1 M $KHSO_4$. The aqueous layer was extracted with ethyl acetate and the organic layer was washed with water and brine, dried with anhydrous magnesium sulphate and evaporated under reduced pressure to furnish the compound (*tert*-butoxycarbonyl)valine (**1)** 66 %. White solid, m.p 164-165ºC; 1H-NMR (400 MHz, CD_3OD) δ 3.97 (d, J = 5.5 Hz, 1H), 2.13-2.04 (m, 1H), 1.42 (s, 9H), 0.94 (d, J = 8.9 Hz, 6H); 13C-NMR (100 MHz, CH_3OD) δ 174.26, 157.20, 79.14, 59.02, 30.40, 27.37, 18.26, 16.85.

Synthesis of *tert*-butyl 2-isopropyl-3,5-dioxopyrrolidine-1-carboxylate (2)

To a solution of (*tert*-butoxycarbonyl)valine **(1)** (1.00 g, 4.60 mmol) Meldrum acid (0.66 g, 4.60 mmol), DMAP (0.79 g, 6.44 mmol) in dichloromethane (25 ml), DCC (1.14 g, 5.52 mmol) was added at room temperature. The resulting mixture was stirred for three hours at room temperature. Then the mixture was filtered and washed with ethyl acetate. The combined filtrates were diluted with cold ethyl acetate and were washed with 5% citric acid, water and brine, respectively. The organic layer was dried using anhydrous magnesium sulphate and evaporated under reduced pressure to give a yellow liquid which was dissolved in ethyl acetate and refluxed for one hour. The solvent was removed to furnish *tert*-butyl 2-isopropyl-3,5-dioxopyrrolidine-1-carboxylate, **(2)** 76 %. 1H-NMR (400 MHz, $CDCl_3$) δ 4.26 (d, J= 3.7 Hz, 1H), 3.10 (s, 2H), 2.38-2.28 (m, 1H), 1.52 (s, 9H), 1.09 (d, J= 7.3 Hz, 3H), 0.90 (d, J= 6.9 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 203.70, 168.08, 149.08, 84.34, 71.92, 44.2, 31.23, 28.33, 19.14,17.55.

Synthesis of *tert*-butyl-3-hydroxy-2-*iso*propyl-5-oxopyrrolidine-1-carboxylate (3)

In 10 ml chloroform, *tert*-butyl 2-isopropyl-3,5-dioxopyrrolidine-1-carboxylate **(2)** (0.35 g, 1.66 mmol) was dissolved. Then, acetic acid (0.10 ml, 1.66 mmol) and sodium borohydride (0.07 g, 1.82 mmol) were added into the reaction mixture. The resulting mixture was stirred at 0°C for one hour. After one hour, the mixture was continued to stir at room temperature for 8 hours. The progress of the reaction was monitored with TLC analysis. As the reaction completed, the solvent was removed under reduced pressure before being partitioned with ethyl acetate and sodium hydrogen carbonate for extraction. The organic layer was collected and dried over anhydrous magnesium sulphate and evaporated under reduced pressure to furnish the compound, *tert*-butyl-3-hydroxy-2-isopropyl-5-oxopyrrolidine-1-carboxylate, **(3)** 70 %. Yellow oil, 1H-NMR (400 MHz, CH_3OD) δ 4.65 (q, J = 8.5 Hz, 1H, CH), 4.08 (dd, J = 4.8 Hz, 1H), 2.19-2.37 (m, 1H), 1.49 (s, 9H), 1.05 (d, J = 7.3 Hz, 3H), 1.00 (d, J = 7.3 Hz, 3H); 13C-NMR (100 MHz, CH_3OD) δ 172.47, 150.30, 83.28, 66.82, 65.12, 40.66, 28.33, 28.01, 20.64, 18.82.

Synthesis of *tert*-butyl 2-*iso*propyl-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate (4)

In a stirred solution of *tert*-butyl-3-hydroxy-2-*iso*propyl-5-oxopyrrolidine-1-carboxylate **(3)** (1.61 g, 6.64 mmol) in anhydrous THF (35 ml), DMAP (2.43 g, 19.91 mmol) and BOC_2O (4.34 g, 19.91 mmol) were added. The resulting mixture was continued to stir for 48 hours at room temperature. After the reaction completed, THF was removed under reduced pressure. Then, the residue was dissolved in 30 ml ethyl acetate. The mixture was then extracted twice with 1 N HCl, twice with $KHCO_3$ and brine. The organic layer was collected and dried over anhydrous magnesium sulphate and the solvent was removed under reduced pressure to furnish compound, *tert*-butyl 2-*iso*propyl-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate, **(4)** 9 %. Yellow solid, m. p. 79-82ºC; 1H-NMR (400 MHz, $CDCl_3$) δ

7.20 (td, J = 2.1 Hz, 1H), 6.17 (td, J = 2.1 Hz, 1H), 4.36 (t, J = 1.8 Hz, 2H), 1.57 (s, 9H); 13-C NMR (100 MHz, $CDCl_3$) δ 169.64, 149.63, 147.91, 127.82, 82.90, 67.34, 28.93, 28.17, 19.56, 14.91.

Synthesis of *tert*-butyl 2-*iso*propyl-3-((2-nitrobenzoyl)oxy)-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate (9)

To a stirred solution of *tert*-butyl 2-isopropyl-3,5-dioxopyrrolidine-1-carboxylate **(4)** (0.22 g, 0.93 mmol) and sodium hydride (0.08 g, 1.20 mmol) in anhydrous THF (10 ml), 2-nitrobenzoyl chloride (0.23 ml, 1.87 mmol) was added. The reaction mixture was stirred at room temperature for one hour. The solvent was removed, and the crude product was then purified by silica gel column chromatography with ethyl acetate/ petroleum ether, 1/1 to furnish compound *tert*-butyl 2-isopropyl-3-((2-nitrobenzoyl)oxy)-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate, **(9)** 26 %. Brown oil, 1H-NMR (400 MHz, $CDCl_3$) δ 8.09-8.07 (m, 1H), 7.87-7.64 (m, 4H), 6.32 (d, J = 0.9 Hz, 1H), 4.53 (q, J = 1.2 Hz, 1H), 2.50-2.43 (m, 1H), 1.57-1.50 (m, 9H), 0.98 (d, J = 7.3 Hz, 3H), 0.87-0.83 (m, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 168.3, 165.6, 161.0, 149.3, 133.9, 133.1, 132.2, 130.0, 124.7, 109.2, 83.4, 77.4, 77.1, 76.8, 64.8, 29.6, 28.2, 18.6, 16.0.

Synthesis of *tert*-butyl 2-*iso*propyl-4-(2-nitrobenzoyl)-3,5-dioxopyrrolidine-1-carboxylate (10)

To a stirred solution of *tert*-butyl 2-*iso*propyl-3-((2-nitrobenzoyl)oxy)-5-oxo-2,5-dihydro-1H-pyrrole-1-carboxylate **(9)** (0.06 g, 0.17 mmol) and potassium cyanonitrile (0.01 g,0.19 mmol) in anhydrous acetonitrile (10 ml), trimethylamine (0.02 g, 0.19 mmol) was added. The reaction mixture was let to stir for 12 hours at room temperature. Then, the reaction mixture was acidified with 1 N HCl and extracted with diethyl ether. After washing the ether layer with aqueous sodium bicarbonate, the resulting aqueous phase was neutralized to pH 7, extracted with ether, dried, and concentrated to furnish the compound *tert*-butyl 2-*iso*propyl-4-(2-nitrobenzoyl)-3,5-dioxopyrrolidine-1-carboxylate, **(10)** 20%. Yellow oil, 1H-NMR (400 MHz, $CDCl_3$) δ 8.20-8.26 (1H), 7.47-7.83 (4H), 6.91-7.01 (1H), 4.50-4.54 (1H), 2.50-2.59 (1H), 1.48 (s, 9H), 1.13 (d, J = 6.9 Hz, 3H), 0.89 (d, J = 6.9 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 207.1, 193.8, 168.9, 149.3, 144.7, 135.4, 134.7, 134.0, 126.3, 123.8, 82.5, 75.2, 68.9, 28.4, 25.9, 19.0.

General synthesis of compounds 11a-c

A mixture of equimolar amount of diethyl oxaloacetate salt (10.00 g, 47.62 mmol), *iso*butyraldehyde (4.34 ml, 47.62 mmol) and 40% methylamine in water (4.12 ml, 47.62 mmol) in ethanol (100 ml) was refluxed for one hour. After cooling, the mixture was poured into ice water and acidified using concentrated hydrochloric acid. The precipitate formed was filtered and washed with water and diethyl ether to furnish compounds **11a-c**.

Ethyl 4-hydroxy-1-methyl-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate, **11a**, 20 %. White solid, m.p 152-155ºC; 1H-NMR (400 MHz, $CDCl_3$) δ 8.95 (s, 1H), 4.30 (q, J = 7.2 Hz, 2H), 3.97 (s, 2H), 3.08 (s, 3H), 1.32 (t, J = 7.3 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 157.3, 107.6, 77.4, 77.1, 76.8, 61.2, 48.1, 30.1, 14.3.

Ethyl 4-hydroxy-2-isopropyl-1-methyl-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate, **11b**, 54 %. Yellow solid, m.p 165-169ºC; 1H-NMR (400 MHz, $CDCl_3$) δ 4.36-4.26 (m, 2H), 4.08 (d, J = 1.8 Hz, 1H), 3.03 (s, 3H), 2.44-2.37 (m, 1H), 1.34-1.30 (m, 3H), 0.97 (d, J = 6.9 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 165.8, 164.3, 158.6, 110.8, 82.4, 77.5, 77.1, 76.8, 64.1, 62.0, 61.3, 30.2, 30.1, 29.3, 19.6, 18.9, 16.5, 14.3, 13.9.

Ethyl 4-hydroxy-2-isopropyl-5-oxo-2,5-dihydro-1H-pyrrole-3-carboxylate, **11c**, 42 %. Yellow solid, m.p 161-164ºC; 1H-NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 4.37-4.28 (m, 2H), 4.24 (q, J = 1.4 Hz, 1H), 2.43-2.34 (m, 1H), 1.34 (td, J = 7.3, 4.1 Hz, 3H), 1.10 (d, J = 7.3 Hz, 3H), 0.63 (d, J = 6.9 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 166.9, 166.0, 158.3, 113.2, 82.5, 77.4, 77.1, 76.8, 62.0, 61.3, 58.8, 30.2, 29.1, 20.5, 19.6, 14.3, 14.2, 14.1, 13.9

General synthesis of compounds 12a-c

To a solution of ethyl 4-hydroxy-1-methyl-5-oxo-2,5-dihydro-1H-pyrrole-3- carboxylate (2.00 g, 10.80 mmol) in ethanol (10 ml) was added formic acid (0.65 ml, 17.28 mmol) and aniline (1.28 ml, 12.96 mmol). The resulting mixture was heated to reflux for 12 hours. The mixture was cooled to room temperature and the product was separated as solid which was collected by filtration to furnish compounds **12a-c**.

Ethyl 1-methyl-5-oxo-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate, **12a**, 72 %. Yellow solid, m.p 239-242^{o}C; 1H-NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.28-7.07 (m, 5H), 4.15 (q, J = 7.0 Hz, 2H), 4.04 (s, 2H), 3.07 (s, 3H), 1.16 (t, J = 7.1 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 165.2, 164.8, 143.3, 138.8, 128.4, 124.4, 122.5, 103.7, 77.5, 77.2, 76.8, 60.2, 49.9, 30.0, 14.3.

Ethyl 2-isopropyl-1-methyl-5-oxo-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate, **12b**, 51 %. Yellow solid, m.p 252-255^{o}C; 1H-NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.28-7.24 (m, 2H), 7.10-7.06 (m, 3H), 4.16 (d, J = 1.8 Hz, 1H), 4.14-4.08 (m, 2H), 3.03 (s, 3H), 2.51-2.45 (m, 1H), 1.13 (t, J = 7.1 Hz, 3H), 1.06 (d, J = 7.3 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H); 13C-NMR (100 MHz, $CDCl_3$) δ 165.1, 143.5, 139.2, 128.5, 124.3, 122.4, 107.8, 77.4, 77.1, 76.8, 65.5, 60.2, 30.3, 29.8, 19.6, 16.0, 14.1.

Ethyl 2-isopropyl-5-oxo-4-(phenylamino)-2,5-dihydro-1H-pyrrole-3-carboxylate, **12c**, 45 %. Yellow solid, m.p 245-249^{o}C; 1H-NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 0H), 7.28-7.06 (m, 1H), 4.28 (t, J = 1.5 Hz, 0H), 4.15 (qd, J = 7.2, 3.7 Hz, 0H), 2.49 (td, J = 7.0, 2.9 Hz, 0H), 1.57-1.80 (0H), 1.16 (t, J = 7.1 Hz, 1H), 1.07 (d, J = 7.3 Hz, 1H), 0.68 (d, J = 6.9 Hz, 1H); 13C-NMR (100 MHz, $CDCl_3$) δ 167.8, 165.2, 143.4, 139.1, 128.5, 124.2, 122.1, 110.5, 77.4, 77.1, 76.8, 60.3, 59.9, 29.4, 20.6, 14.2, 14.0.

General synthesis of compounds 13a-c

A solution of ethyl 1-methyl-5-oxo-4-(phenylamino)-2,5-dihydro-1H-pyrrole- 3-carboxylate (1.00 g, 3.84 mmol) in diphenyl ether (100 ml) was heated to reflux at 250°C under nitrogen for one hour. The resulting clear solution was cooled at room temperature and the product was separated as solid which was collected by filtration. The solid product was washed with petroleum ether to furnish compounds **13a-c**.

2-Methyl-1,4-dihydro-3H-pyrrolo[3,4-b]quinoline-3,9(2H)-dione, **13a**, 62 %. Yellow solid, m.p >320^{o}c; 1H-NMR (400 MHz, DMSO-D6) δ 8.14 (d, J = 8.2 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 4.31 (s, 2H), 3.08 (s, 3H); 13C-NMR (100 MHz, DMSO-D6) δ 132.7, 125.5, 124.1, 119.9, 49.1, 40.4, 40.2, 40.0, 39.8, 39.6, 39.4, 30.1.

1-*iso*propyl-2-methyl-1,4-dihydro-3H-pyrrolo[3,4-b]quinoline-3,9(2H)-dione, **13b**, 63 %. Yellow solid, m.p 278-283^{o}C; 1H-NMR (400 MHz, DMSO-D6) δ 8.12 (d, J = 8.2 Hz, 1H), 7.75-7.63 (m, 2H), 7.33 (t, J = 7.3 Hz, 1H), 4.52 (s, 1H), 3.03 (s, 3H), 2.62 (t, J = 6.9 Hz, 1H), 1.01-0.82 (m, 6H); 13C-NMR (100 MHz, DMSO-D6) δ 173.5, 141.6, 140.5, 132.7, 127.1, 125.8, 124.1, 122.3, 119.8, 65.7, 40.6, 40.4, 40.2, 40.0, 39.8, 39.6, 39.4, 29.8, 28.5, 18.7, 17.2.

1-*iso*propyl-1,4-dihydro-3H-pyrrolo[3,4-b]quinoline-3,9(2H)-dione, **13c**, 82 %. Yellow solid, m.p 286-296^{o}C; 1H-NMR (400 MHz, DMSO-D6) δ 9.22 (s, 1H), 8.12 (dd, J = 8.2, 0.9 Hz, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.68-7.64 (m, 1H), 7.35-7.31 (m, 1H), 4.50 (q, J = 1.4 Hz, 1H), 2.66-2.59 (m, 1H), 1.05 (d, J = 7.3 Hz, 3H), 0.46 (d, J = 6.9 Hz, 3H); 13C-NMR (100 MHz, DMSO-D6) δ 174.1, 166.4, 141.8, 140.7, 132.6, 126.9, 125.6, 124.2, 124.0, 119.9, 60.2, 28.4, 20.7, 14.8.

Results and Discussion

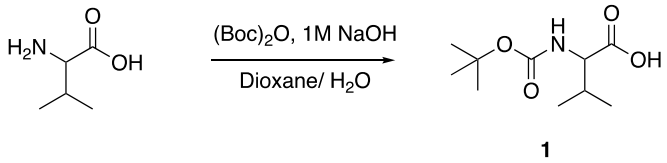
In the present study, the nitrogen atoms in amino acids were protected before synthesising the desired quinolactacins derivates. The step was performed as amino acids are good nucleophiles and bases, making the protection of the nitrogen atoms crucial. Moreover, protecting the atoms prevented any possible side reactions from occurring. The current study protected the nitrogen atoms with *tert*-butyloxycarbonyl (Boc), later deprotected by adding an acid or heating. Besides Boc anhydrides, several other protecting groups could also be employed, such as carbobenzyloxy (Cbz) and benzyl (Bn) groups.

The Boc anhydride was chosen as the protecting agent for the nitrogen atoms in the present investigation as it was commercially available. Moreover, Boc anhydride could be easily introduced under basic conditions and is easily removed under acidic conditions. Caplar and co-workers (2003) [4] protected the nitrogen atoms in the amino acid *D*-valine with Boc anhydride to synthesise amino alcohols. The *D*-valine was treated with 1 M NaOH as the base and dioxane/water as the solvent. Subsequently, Boc anhydride was added to produce the *N*-protected amino acid in a moderate yield of 66%. The protected *D*-valine was successfully synthesised following Caplar's procedure in the present study, as illustrated in Scheme 1.

Tetramic acid or 2,4-pyrrolinedione ring was synthesised post-*N*-protected amino acids production as the acid was the key ring moiety in the present study. According to the method reported by Ma, the tetramic acid was synthesised with Meldrum's acid, DMAP as the base, and DCC as the peptide coupling agent, in dichloromethane, the solvent (Scheme 2). The coupling agent, DCC, was added to activate the carbonyl group on the amino acid, making it more electrophilic for nucleophilic attacks that led to the amide bonds formation. Cyclisation occurred upon reflux in ethyl acetate, producing C-5 isopropyl substituted tetramic acid, compound 2.

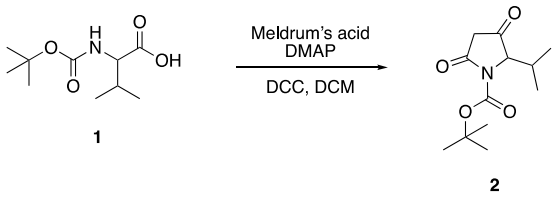
Spectral studies proved that the tetramic acid, compound 2, could tautomerise to its enol form depending on the solvents used. The observation was supported by the data from the 1H-NMR spectroscopy of the tetramic acid that employed deuterated solvents of different polarities, namely $CDCl_3$ as the less-polar solvent and CH_3OD as the more-polar solvent. The acid existed in both forms, keto and enol tautomers, when analysed with the less-polar solvent, $CDCl_3$. A total of 18 protons from the *tert*-butyloxycarbonyl substituents that belonged to the tautomers of the Boc groups and enol olefin protons were identified. Conversely, the tetramic acid was more stable in its keto form in the more-polar solvent as only one CH_2 at the C-3 position of the tetramic acid was present.

Compound 2 was converted to an enone to obtain the appropriate dienophile for the subsequent Diels-Alder reaction to form the desired quinolactacin derivatives. First, the carbonyl functionality of the pyrrolidine-2,4-dione at the C-4 position was reduced to a hydroxyl group and eliminated (Scheme 3). Subsequently, the pyrrolidine-2,4-dione was treated with $NaBH_4$ as the reducing agent and acetic acid in dichloromethane as the solvent to reduce the carbonyl group at the C-4 position. The $NaBH_4$ was selected as the reducing agent due to its selectivity, thus reducing only carbonyl-keto in the presence of other active functional groups. Finally, the hydroxyl pyrrolidinone was treated with Boc anhydride and NaOH to remove the hydroxyl group. The Boc group was introduced again to make the hydroxyl group a good leaving group during the elimination reaction, deriving enone, compound **4**, at a 9% yield.

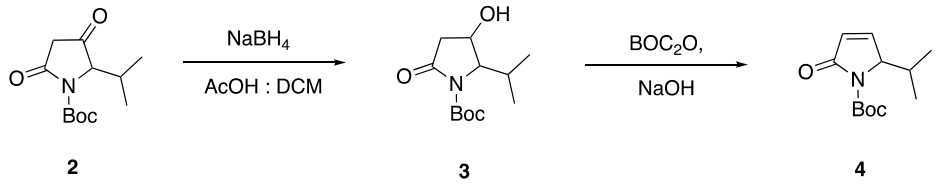


1

Scheme 1. The amine protection of *D*-valine



Scheme 2. The synthesis of tetramic acid, 2

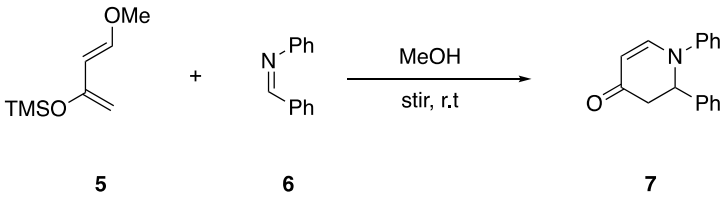


Scheme 3. The reduction and elimination reaction of the functional group at the C-4 position of compound 2

The Diels-Alder reaction was performed to synthesise the derivatives of quinolactacin. Studies on Diels-Alder reactions by Yuan et al. [5] and Jurcik [6], demonstrated that an aza Diels-Alder reaction was successfully performed between *N*-benzylidiene and Danishefsky's diene, as described in Scheme 4. The present study repeated a similar reaction, but the desired compound 8 was not obtained (Scheme 5). The unsuccessful attempt might be due to the different nature of the functional groups in the starting materials utilised in the current study.

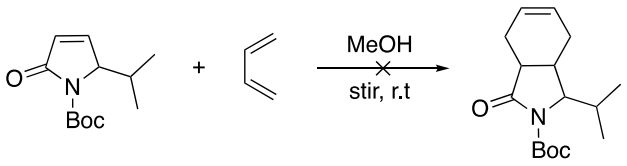
Enone, compound 4, was employed as the dienophile and was reacted with 2,4-hexadiene. Nonetheless, the reaction did not successfully yield the desired final product. The observation was due to the amide group in the enone, an electron-withdrawing group, and 2,4-hexadiene had a methyl group, an electron-donating group. Furthermore, Yuan and Jurcik demonstrated that Danishefsky's diene was rich in electrons with a trimethylsilyl group as the activating group. Consequently, future studies on the configuration, temperature, catalyst, and reaction conditions involving the diene should be done prior to reaction attempts.

Having failed to synthesise the derivatives of quinolactacin via the aza Diels-Alder reactions, an alternative route that involved acylation and hydrogenation reactions was attempted. The key substance, pyrrolidine-2,4-dione, was treated with 2-nitrobenzoyl chloride and sodium hydride as the base in anhydrous THF, as depicted in Scheme 6. The reaction was performed in an inert condition, a nitrogen (N_2) atmosphere, hence producing either the *O*-acylated or the *C*-acylated compounds. The observation could be explained based on the stability of the intermediate enolate formed during the reaction. The *O*-acylation reaction was favoured since the enol was likely to be more stable due to electron conjugation within the ring system.



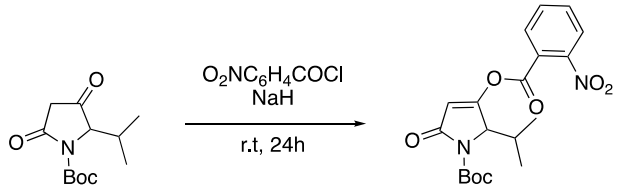
5 **6** **7**

Scheme 4. Aza Diels-Alder reaction [5, 6]



4 **8**

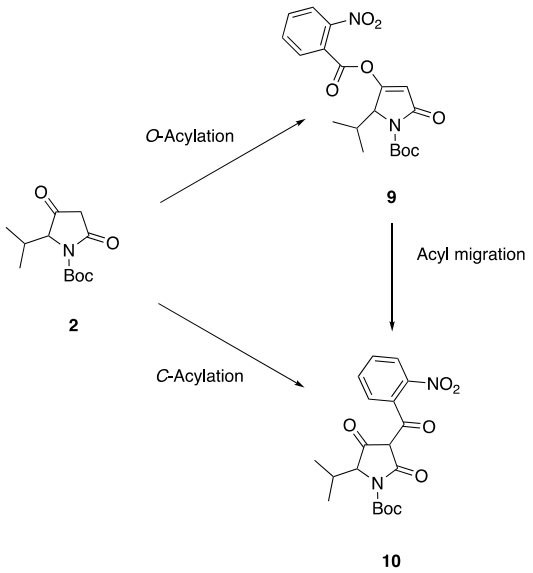
Scheme 5. The Diel-Alder of enone, 4



2 **9**

Scheme 6. The acylation of tetramic acid, 2

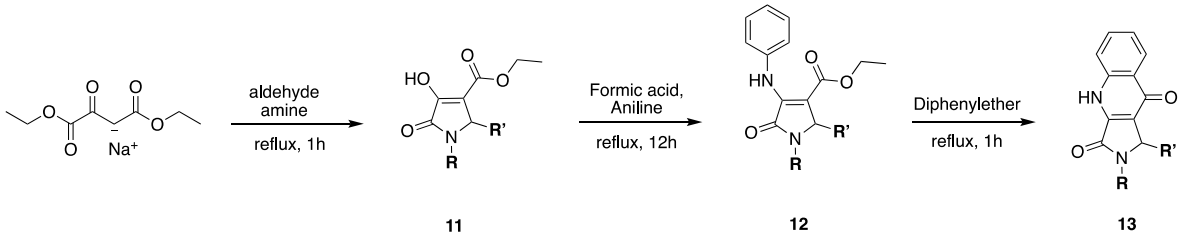
During the synthesis of quinolactacin derivatives, a hydrogenation reaction to convert the nitro functionality in the acylated compound to an amine is required to react the nucleophilic amine with the C-4 carbonyl ketone, yielding the quinolone-lactam ring moiety. Nevertheless, the acyl group must be at C-3 to produce the fused tricyclic system. Consequently, repositioning the acyl group from the *O*-enol position to the C-3 position was mandatory in the current study (Scheme 7).



Scheme 7. The *O*- and *C*-acylations of tetramic acid, 2

The *O*-acylated pyrrolidinone, compound 9, was treated with potassium cyanide for almost 12 hours to furnish the *C*-acylated pyrrolidinedione, compound 10, but in a low yield. Consequently, the present study conducted the hydrogenation reaction following the procedure described by Masaki (2006) [7]. Resultantly, the nitro functional group was reduced to an amine that spontaneously attacked the C-4 carbonyl ketone to form the quinolactacin ring moiety.

Studies on the synthesis of such derivatives via alternative ways have allowed the employment of a multicomponent reaction or a one-pot reaction, as illustrated in Scheme 8. The present study synthesised derivatives of quinolactacin through multicomponent reactions of diethyl oxaloacetate sodium salt, aldehydes, and amines in good yields. The C-5 substituted pyrrolidine-2,3-diones were reacted with formic acid and aniline and heated in diphenyl ether to yield derivatives of quinolactacin (Tables 1, 2, and 3). The synthetic route was focused on utilising pyrrolidine-2,3-dione as the key intermediate, which is different from those in Diels-Alder and synthetic acylation routes abovementioned.



Scheme 8. The synthesis of quinolactacin derivatives *via* a multicomponent reaction

Table 1. The yield percentage of compounds 11 a–c from the multicomponent reactions of diethyl oxaloacetate sodium salt, aldehydes, and amines

Entry	Aldehyde	Amine	Yield, %
11a	Formaldehyde	Methylamine	20
11b	Isobutyraldehyde	Methylamine	54
11c	Isobutyraldehyde	Ammonia	42

Table 2. The yield percentage of compounds 12 a–c from the insertion of aniline in ethanol

Entry	Yield, %
12a	72
12b	51
12c	45

Table 3. The yield percentage of compounds 13 a–c from cyclisation by refluxing in diphenyl ether

Entry	Yield, %
13a	62
13b	63
13c	82

Conclusion

The current study explored and attempted synthesising quinolactacin and its derivatives through several synthetic routes. The routes employed during the investigation involved numerous chemistry concepts, including intramolecular cyclisation, acylation, Diels-Alder, and hydrogenation reactions. Currently, the synthesis of quinolactacin via aza Diels-Alder reaction is being further studied. Consequently, the present study might be an essential reference for other researchers in procuring similar target compounds in the future.

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