Research Paper



Propylphosphonic acid anhydride-mediated amidation of Morita-Baylis-Hillman-derived indolizine-2-carboxylic acids

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Abstract

Propylphosphonic acid anhydride has been successfully used as a coupling agent in the synthesis of a series of indolizine-2-carboxamido derivatives from indolizine-2-carboxylic acid and its 3-acetylated analogue. The acid substrates were obtained by saponification of the corresponding methyl esters produced, in turn, selectively and efficiently, by timecontrolled cyclisation of a single Morita–Baylis–Hillman adduct. Various amino and hydrazino compounds with medicinal potential have been used to prepare indolizine-2-carboxamido and hydrazido derivatives.

Keywords

anti-mycobacterial, carboxamide, indolizine, Morita–Baylis–Hillman, propylphosphonic acid anhydride, synthesis

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Introduction

Although the indolizine {pyrrolo[1,2-*a*]pyridine} nucleus is isomeric with indole, the chemistry and biological potential of its derivatives are relatively unexplored. The indolizine nucleus is, however, present in polycyclic compounds exhibiting anti-cancer activity, notably camptothecin 1 (first reported in 1966) and its synthetic derivatives, irinotecan and topotecan (Figure 1).^{1,2} 1-Substituted indolizines, such as the hydrazido derivative 2, have been shown to exhibit anti-microbial and anti-tubercular activity,³ while others, such as the 1,2,3,7-tetrasubstituted derivative 3, exhibit central nervous system (CNS) depressant activity.3 Various methods of synthesising indolizine derivatives have been developed.^{4,5} These include transition metal-catalysed cyclo-isomerisation of readily available non-conjugated 2-propargylpyridines6; cycloaddition reactions of 2-cyclopropenylpyridine in the presence of $RhCl(PPh_{2})_{2}$ or CuI^{7} ; cyclisation of propargylic pyridines in the presence of iodine, Pd, Cu or other metals in a polar protic solvent⁸⁻¹⁰; and the Chichibabin indolizine synthesis,

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Figure 1. Biologically active indolizine derivatives.

involving base-mediated cyclisation of 1-substituted-2-methylpyridinium salts.¹¹ The application of Morita– Baylis–Hillman (MBH) methodology in the synthesis of indolizines was first reported by our group^{12,13} and, in this communication, we now report the use of this approach in the preparation of a range of indolizine-2-carboxamido derivatives and the evaluation of their anti-malarial, antitrypanosomal and anti-tuberculosis potential and their HIV-1 protease (PR) and integrase (IN) inhibition activity.

Results and discussion

In the synthesis of the critical indolizine-2-carboxylic acids 9 and 10 (Scheme 1), a step-wise approach was initially adopted, in which thermal cyclisation is facilitated by prior acetylation of the MBH adduct 5.14 Such acetylation has been achieved either by refluxing the adduct in acetic anhydride for 30 min or by treating the adduct with acetyl chloride in the presence of pyridine at room temperature. However, we found that prior acetylation and isolation of the acetylated adduct 7 is not necessary, and that the indolizine-2-carboxylate esters 7 and 8 may be generated efficiently by refluxing a mixture of the MBH adduct 5 in acetic anhydride in a one-pot approach.12,15 The complicating co-formation of both the desired product 7 and its 3-acetylated analogue 8 was simply avoided by controlling the reaction time. Thus, treatment of the MBH adduct 5 with refluxing Ac₂O for 2 days yielded methyl indolizine-2-carboxylate 7 in 93% yield,¹⁶ while extending the reaction time to 7 days afforded the 3-acetylated analogue 8, in 83% yield.

The indolizine-2-carboxylic acids 9 and 10, the critical precursors for the amidation studies, were obtained by base-catalysed hydrolysis of the respective esters 7 and 8. Indolizine-2-carboxylic acid 9 was obtained as yellow crystals in 86% yield, the 3-acetoxy analogue 10 in up to 93% yield, contaminated in some cases with the non-acetylated acid 9 – an observation attributed to competitive, sequential de-acylation and saponification steps.

In earlier studies in our group, 12,13,17 the inability to generate the acid chloride of indolizine-2-carboxylic acid prompted the use of 1,1'-carbonyldiimidazole (CDI) as a coupling agent, which permitted the formation of indolizine-2-carboxamides from the acid **9** and aliphatic amines in good yields.^{18,19} However, attempted application of this

method to access indolizine-2-carboxamides using more complex and medicinally interesting amines proved unsuccessful. Following further exploratory, but unsuccessful studies, involving the use of organoboron catalysts (boric acid, trimethyl borate, phenylboronic acid and tris(2,2,2trifluoroethyl) borate), attention was turned to the use of propylphosphonic acid anhydride (T3P) - a coupling agent that has found use in a range of multi-component transformations, including the amidation of carboxylic acids.²⁰ Examination of the efficacy of this reagent, by varying the base, the solvent, the temperature and the work-up protocol, finally permitted the synthesis, albeit in no more than modest yields, of novel carboxamides from reactions of the indolizine-2-carboxylic acids 9 and 10 with a series of amines as outlined in Scheme 2. These amines included: phenylhydrazine and hydrazine to afford compounds 11a,b and 13b as analogues of isoniazid, an anti-tuberculosis drug to which resistance has emerged;²¹ compounds containing the medicinally significant pyridinyl,²² furfuryl²³ and thiazolyl²⁴ moieties, namely, 2-(2-pyridinyl)ethylamine, furfurylamine, 2-aminothiazole and 2-aminopyridine to afford the N-substituted carboxamides 11c, 11e, 12f and 12g, respectively; and benzylamine, leading to N-benzylindolizine-2-carboxamide 11d. It should be noted that the indolizine-2-carboxamides 11d and 11e were obtained from 3-acetylindolizine-2-carboxylic acid 10, the reactions proceeding with a de-acetylation step. While the yields were variable and the products subject to decomposition, nuclear magnetic resonance (NMR) and high-resolution mass spectra (HRMS) analyses permitted their unambiguous characterisation, with infrared (IR) data confirming the presence of the required NH and the amide C=O absorption bands. Other researchers have observed inherent instability and lack of reactivity in certain indolizine derivatives.25,26

Indolizines have been reported to exhibit a wide range of biological activities, including anti-mycobacterial²⁷ and anti-HIV-1 activity.^{28,29} Preliminary assessments of the biological activity of indolizine-2-carboxamide derivatives prepared in this study against HIV-1 IN and HIV-1 PR, malaria, trypanosomiasis and tuberculosis were undertaken. While none of these compounds showed toxicity against Human Embryonic Kidney 293 (HEK 293) cells at a concentration of 20 µM, their activity in the other assays proved to be generally disappointing. The carbohydrazide



Scheme I. Synthetic access to the indolizine-2-carboxylic acids **9** and **10** as critical precursors for the formation of the targeted indolizine-2-carboxamides **11** and **12**. 3-HQ = 3-hydroxyquinuclidine.

(11a) and thiazole (12f) derivatives, however, exhibited some anti-mycobacterial activity (11a: $MIC_{90} = 31.3 \ \mu g \ mL^{-1}$; 12f: $MIC_{90} = 15.6 \ \mu g \ mL^{-1}$ (both after 14 days in the 7H9 GLU Tx medium).

In conclusion, the indolizine-2-carboxylic acids **9** and **10** were obtained efficiently *via* time-controlled, chemoselective cyclisation of the MBH adduct **5**. Furthermore, use of T3P has provided access to a range of indolizines-2-carboxamide derivatives, reflecting the substrate scope of this coupling agent. Although, in some cases, the amides were obtained in rather low yields, sufficient quantities were isolated to permit their full characterisation and preliminary biological evaluation.

Experimental

General

The reagents used in this project were supplied by Merck[®] and used without further purification. Thin layer chromatography was carried out using Merck silica gel 60 PF₂₅₄ plates and was viewed under UV light, while column and

preparative layer chromatography was carried out using Merck silica gel 60 and Kieselgel 60 PF₂₅₄, respectively. Where required, solvents were purified according to methods prescribed by Vogel et al.³⁰ Melting points were measured using a Reichert hot-stage apparatus and are uncorrected. The IR data were obtained using a Perkin-Elmer Fourier-transform infrared (FTIR) spectrophotometer. NMR spectra were recorded on Bruker Fourier 300 MHz, Bruker Avance III 400 MHz or Bruker Avance II 600 MHz spectrometers in $CDCl_3$, methanol- d_4 or DMSO- d_6 and calibrated using solvent signals ($\delta_{\text{H}}\!\!:$ 7.26 ppm for CDCl₃, 2.50 ppm for DMSO-d₆ and 3.31 ppm (quintuplet) for methanol- d_4 ; δ_C : 77.0 ppm for CDCl₃, 39.4 ppm for DMSO- d_6 and 49.1 for methanol- d_4). The spectra were processed using Bruker Topspin® 3.5 software. High-resolution electrospray ionisation mass spectra (ESI-HRMS) were recorded on a Waters Synapt G2 spectrometer (University of Stellenbosch, Stellenbosch, South Africa).

The synthesis and characterisation of compounds **5** and **6** have been reported previously.^{12,19} In this study, however, methyl indolizine-2-carboxylate **6** was generated directly from the MBH adduct **5**. Full experimental details and ¹H



Scheme 2. Synthesis of indolizine-2-carboxamide derivatives. *Reagents and conditions*: (i) $CHCl_3$: DIPEA (2:1), (ii) T3P, -20 to -10 °C then rt and (iii) T3P, -20 to -10 °C then reflux.

and ¹³C NMR spectra for new compounds are provided in the Supplementary file. The bioassay protocols follow those reported previously,¹⁵ and the mycobacterial assay and results are also provided in the Supplementary file.

General procedure for the synthesis of the indolizine-2-carboxylate esters **7** and **8**

A stirred solution of methyl 3-hydroxy-2-methylene-3-(2pyridinyl)propanoate **5** (2.00 g, 10.4 mmol) in acetic anhydride (15 mL) was refluxed for 2 days. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and the residue poured with stirring into a mixture of CH_2Cl_2 and satd. aq. NaHCO₃. The organic phase was washed with 10% aq. NaHCO₃ and dried over anhydr. MgSO₄. The crude product was purified by column chromatography on silica gel (elution with hexane: EtOAc (3:2)) to afford methyl indolizine-2-carboxylate **7** as light green crystals (1.69 g, 93%), m.p. 99–101 °C (lit.¹² 99–101 °C).

General procedure for the synthesis of the indolizine-2-carboxylic acids **9** and **10**

To a solution of methyl indolizine-2-carboxylate 7 (1.18 g, 6.70 mmol) in a mixture of methanol: water (1:1; 10 mL) was added NaOH (1.61 g, 40.2 mmol) and the reaction mixture was stirred at room temperature for 2 days. Sufficient water was added to solubilize the solid material present in the reaction mixture and the pH was adjusted with dilute HCl to *ca.* pH 2–3. The aqueous layer was extracted with

EtOAc (2 × 100 mL) and the combined organic extracts were dried over MgSO₄ and concentrated *in vacuo* to afford, as yellow crystals, indolizine-2-carboxylic acid **9** (0.93 g, 86%); m.p. 216–218 °C (lit.¹² 219–222 °C).

General procedure for the amidation reactions of indolizine-2-carboxylic acids **9** and **10**

A stirred solution of indolizine-2-carboxylic acid 9 (0.10 g, 0.62 mmol), phenylhydrazine (73.6 mg, 0.68 mmol) in a mixture of DIPEA:CHCl₃ (12 mL; 2:1) in a stoppered flask was cooled to between -20 and -10 °C. Propylphosphonic anhydride solution (0.59 g, 1.9 mmol) was slowly added to maintain the internal temperature below 0 °C. The stirred mixture was allowed to warm to room temperature and stirred for 2 days. The reaction mixture was diluted with CHCl₂ (10 mL), filtered and the filtrate was concentrated in vacuo. The crude product was purified by preparative layer chromatography on silica gel (elution with hexane: EtOAc (5:2)) to afford, as red crystals, N²-phenylindolizine-2carbohydrazide 11a (25.8 mg, 17%), m.p. 199-201 °C (Found MH⁺: 252.1135. Calc. for C₁₅H₁₄N₃O: 252.1137); v_{max} (ATR)/cm⁻¹ 3339, 3223 (NH) and 1643 (C=O); δ_{H} (600 MHz; DMSO- d_6) 6.62 (1H, t, J = 7.3 Hz, ArH), 6.70 (1H, t, J = 7.3 Hz, ArH), 6.73-6.76 (3H, m, ArH), 6.78(1H, s, ArH), 6.88 (1H, s, 3H), 7.14 (2H, t, J = 7.9 Hz, ArH), 7.45 (1H, d, J = 9.1 Hz, ArH), 7.85 (1H, d, J = 2.7 Hz, NH), 8.05 (1H, s, ArH), 8.28 (1H, d, *J* = 7.0 Hz, ArH) and 10.10 (1H, d, J = 2.7 Hz, NH); $\delta_{\rm C}$ (150 MHz;

DMSO-*d*₆) 98.0, 111.6, 112.2, 114.4, 118.3, 118.4, 119.6, 121.8, 126.2, 128.7, 132.0, 149.8 (ArC) and 163.8 (C=O).

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Supplemental material

Supplemental material for this article is available online.

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