3,6-Dihydro-5H-pyrazolo [4′,3′:5,6]pyrano[3,4-b]indol-5-one

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1. Introduction

Among the many different heterocycles used against various diseases, alpha-pyrone [1], coumarin [2,3] and pyrazole [4,5] derivatives have been extensively studied due to their promising activity. Furthermore, all three scaffolds, alpha-pyrone, coumarin and pyrazole, have been used as lead compounds for synthesizing various analogs and are endowed with many interesting, albeit diverse, biological activities.

In coumarin-containing heterocycles, indolo [2,3-c]coumarins have been extensively used for the development of potent anticancer and antiviral compounds [6]. Their natural precursors, i.e., marine alkaloids, lamellarins and ningalins display diverse biological functions [7–9]. They are potent topoisomerase I and non-selective kinase inhibitors in the sub-nanomolar range, and they exhibit strong cytotoxic activity against several cancer cell lines. In addition, pyrazole moieties form structural subunits in a wide variety of compounds, displaying a broad spectrum of pharmacological activities, such as anticancer, antifungal, antiviral, etc. [2,3]. Among the many different heterocycles used against various diseases, alpha-pyrone [1], coumarin [2,3] and pyrazole [4,5] derivatives have been extensively studied due to their promising activities. Furthermore, all three scaffolds, alpha-pyrone, coumarin and pyrazole, have been used as lead compounds for synthesizing various analogs, and are endowed with many interesting, albeit diverse, biological activities.

Based on these studies, it was speculated that combining the unique indolo-coumarin scaffold with the pyrazole moiety could have a substantial impact on the activity of the compounds. Prompted by the data mentioned above, we decided to further examine these two scaffolds and we present herein the synthesis of 3,6-dihydro-5H-pyrazolo [4′,3′:5,6]pyrano [3,4-b]indol-5-one (Figure 1). In this compound, the phenyl ring of indolo [2,3-c]coumarin is replaced by the pyrazole moiety to gain a better insight into the structure–activity relationship. The donor–acceptor group and the existence of tautomeric forms on the pyrazole ring are considered to contribute to the formation of essential interactions with several protein targets of high biological significance.
Figure 1. Pyrano- and chromeno-fused heterocycles of interest. Tautomerism of 2(3),6-dihydro-5H-pyrazolo [4′,3′:5,6]pyrano [3,4-b]indol-5-one.

2. Results and Discussion

The synthetic procedure is depicted in Scheme 1. Our efforts focused on developing a reliable and scalable procedure for the synthesis of 3,6-dihydro-5H-pyrazolo [4′,3′:5,6]pyrano [3,4-b]indol-5-one, using simple starting materials and classic chemistry reactions; thus, several analogs could be obtained.

Commercially available 2-ketoglutaric acid (1) was used as a starting material which, upon treatment with phenylhydrazine via classic Fischer indole synthesis, afforded diester 2 [10,11].

N,N-Dimethylformamide dimethyl acetal (DMF-DMA) treatment of diester 2 in anhydrous toluene led to a mixture of the E and Z isomers of compound 3. The formation of both E and Z isomers was evident by 1H NMR spectra in the reaction mixture; however, the two isomers could not be isolated by flash chromatography and used as a mixture for the next step of the synthetic procedure [12]. The reaction of 3 with a slight excess of hydrazine (1.5 eq.) afforded pyrazole 4 which, upon intramolecular esterification, could provide 3,6-dihydro-5H-pyrazolo [4′,3′:5,6]pyrano [3,4-b]indol-5-one (5). The aforementioned esterification could be accomplished by acidic catalysis. Nevertheless, the reaction of 3 with a high excess of hydrazine (17.5 eq.) provided the target compound 5 with 64% yield and in one step.
Pyrazolo compound 4 and pyrazolopyranoindole 5 were isolated in pure forms by column chromatography and unambiguously identified by $^1$H and $^{13}$C NMR spectra (see Supplementary Materials), using direct and long-range heteronuclear correlation experiments (HMBC and HMQC). In both compounds 4 and 5, the signals of the pyrazole group in the $^1$H NMR spectra were the primary indication that the pyrazole moiety was incorporated. More specifically, $^1$H NMR spectra showed a characteristic singlet at 7.60 and 7.69 ppm, respectively. The structural discrimination of the two compounds resulted from the absence of the characteristic chemical shifts of the ethyl ester group in compound 5 in the $^1$H NMR spectra (see Supplementary Materials). The HRMS of 4 and 5 were also obtained to further confirm the proposed structures determined by the NMR spectra.

3. Materials and Methods

3.1. General

All commercially available reagents and solvents were purchased from Alfa Aesar and used without any further purification. Melting points were determined on a Büchi apparatus and were uncorrected. All NMR spectra were recorded on 400 or 600 MHz Bruker spectrometers with Avance™ DRX and III instruments (Bruker BioSpin GmbH—Rheinstetten, Germany). $^1$H NMR (400 and 600 MHz) and $^{13}$C NMR (101 and 151 MHz, recorded with complete proton decoupling) spectra were obtained with samples dissolved in CDCl$_3$ or DMSO-d$_6$ with the residual solvent signals used as internal references: 7.26 ppm for CHCl$_3$, and 2.50 ppm for (CD$_3$)(CD$_2$H)S(O) regarding the $^1$H NMR experiments; 77.2 ppm for CDCl$_3$ and 39.4 ppm for (CD$_3$)$_2$S(O) concerning the $^{13}$C NMR experiments. Chemical shifts ($\delta$) are given in ppm to the nearest 0.01 ($^1$H) or 0.1 ppm ($^{13}$C). The coupling constants ($J$) are given in Hertz (Hz). The signals are reported as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Assignments of $^1$H and $^{13}$C NMR signals were unambiguously achieved with the help of D/H exchange and 2D techniques: COSY, NOESY, HMQC, and HMBC experiments. Systematic indole and pyrazole nomenclatures were used below for the assignment of each spectrum. Flash chromatography was performed on Merck silica gel (40–63 µm) with the indicated solvent system using gradients of increasing polarity in most cases (Merck KGaA—Darmstadt, Germany). The reactions were monitored by analytical thin-layer chromatography (Merck pre-coated silica gel 60 F254 TLC plates, 0.25 mm layer thickness). Compounds were visualized on TLC plates by UV radiation (254 and 365 nm). All solvents for absorption and fluorescence experiments were of spectroscopic grade. Mass spectra were recorded on a UPLC triple TOF-MS (UPLC: Acquity of Waters (Milford, MA, USA), SCIEX Triple TOF-MS 5600+ (Framingham, MA, USA).

3.1.1. Ethyl 3-(2-ethoxy-2-oxoethyl)-1H-indole-2-carboxylate (2)

A solution of 2-ketogluartic acid (10 g, 0.7 mol, 1) and phenylhydrazine (6.8 mL, 0.7 mol) in EtOH abs. (100 mL) was refluxed for 1 h. Methanesulfonic acid (40 mL) was added dropwise, and reflux was continued for 20 more hours. After completion of the reaction, the mixture was left to cool down to room temperature and was poured into water. The precipitate was filtered off and dried under vacuum over P$_2$O$_5$ to afford 10 g (53%) of the title compound 2, which was practically pure, and was used for the next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.03 (s, 1H), 7.65 (dq, $J$ = 8.1, 0.9 Hz, 1H), 7.38 (dt, $J$ = 8.3, 1.0 Hz, 1H), 7.32 (ddd, $J$ = 8.3, 6.8, 1.1 Hz, 1H), 7.16 (ddd, $J$ = 8.0, 6.8, 1.1 Hz, 1H), 4.39 (q, $J$ = 7.1 Hz, 2H), 4.21-4.12 (m, 4H), 1.40 (t, $J$ = 7.2 Hz, 3H), 1.25 (t, $J$ = 7.2 Hz, 3H) [10].

3.1.2. Ethyl 3-(3-ethoxy-3-oxo-1-(pyrrolidin-1-yl)prop-1-en-2-yl)-1H-indole-2-carboxylate (3)

To a suspension of diester 2 (300 mg, 1.09 mmol) in toluene dry (1 mL), DMF-DMA (234 µL, 1.7 mmol) and pyrrolidine (200 µL, 2.4 mmol) were added and the resulting mixture was heated at 110 °C for 2.5 h. After the completion of the reaction, volatiles were removed under reduced pressure and water (100 mL) was added to the crude product. The aqueous mixture was washed with EtOAc (3 × 100 mL) and the organic layers were collected and
dried over anh. Na$_2$SO$_4$ was concentrated under reduced pressure to afford 360 mg (93%) of the title compound, and was used for the next step without any further purification.

3.1.3. Ethyl 3-(3-hydroxy-1H-pyrazol-4-yl)-1H-indole-2-carboxylate (4)

A solution of diester 3 (300 mg, 0.87 mmol) and hydrazine monohydrate (63 µL, 1.3 mmol) in EtOH abs. (10 mL) was refluxed for 4.4 h. After the completion of the reaction, the solution was vacuum evaporated to 1/3 and the obtained precipitate was filtered off and further purified by column chromatography (silica gel) using Chex/EtOAc 3/1—0/1 and EtOAc/MeOH 10/1 as the eluent to afford 150 mg (64.3%) of the title compound 4.

M.p.: >200 °C (THF/ n-pentane); $^1$H NMR (600 MHz, DMSO-d$_6$) $\delta$ 11.59 (s, 1H, H-1' or H-2'), 9.43 (s, 1H, H-1), 7.60 (s, 1H, H-5'), 7.57 (d, $J$ = 8.3 Hz, 1H, H-4), 7.43 (d, $J$ = 8.3 Hz, 1H, H-7), 7.25 (t, $J$ = 8.2 Hz, 1H, H-6), 7.03 (t, $J$ = 8.1 Hz, 1H, H-5), 4.25 (q, $J$ = 7.1 Hz, 2H, CH$_2$CH$_3$), 1.26 (t, $J$ = 7.1 Hz, 3H, CH$_2$C); $^{13}$C NMR (151 MHz, DMSO-d$_6$) $\delta$ 162.17 (COO), 161.78 (C-3'), 136.20 (C-7a), 128.80 (C-5'), 127.17 (C-2), 124.64 (C-6), 122.91 (C-3a), 121.89 (C-4), 119.42 (C-5), 113.40 (C-3), 112.14 (C-7), 96.05 (C-4'), 60.03 (CH$_2$CH$_3$), 14.04 (CH$_2$C); HRMS (ESI) calculated for C$_{14}$H$_{13}$N$_3$O$_3$ + H$^+$ [M + H]$^+$: 272.1030. Found: 272.1022.

3.1.4. 3,6-Dihydro-5H-pyrazolo [4',3':5,6]pyrano [3,4-b]indol-5-one (5)

A solution of diester 3 (65 mg, 0.18 mmol) and hydrazine monohydrate (150 µL, 30.8 mmol) in EtOH abs. (2 mL) was refluxed for 1.5 h. After the completion of the reaction, the precipitate was filtered off and further purified by column chromatography (silica gel) using Chex/EtOAc 2/1—1/1 and EtOAc/MeOH 10/1 as the eluent to afford 25 mg (64%) of the title compound 5.

M.p.: >200 °C (EtOAc); $^1$H NMR (600 MHz, DMSO-d$_6$) $\delta$ 11.45 (s, D$_2$O exchang., 1H), 7.69 (s, 1H, H-1), 7.50 (d, $J$ = 8.1 Hz, 1H, H-10), 7.42 (d, $J$ = 8.2 Hz, 1H, H-7), 7.21 (t, $J$ = 7.6 Hz, 1H, H-8), 7.04 (t, $J$ = 7.5 Hz, 1H, H-9); $^{13}$C NMR (151 MHz, DMSO) $\delta$ 162.49 (CO), 158.38 (C-3a), 135.64 (C-6a), 131.12 (C-1), 127.63 (C-10a), 126.45 (C-5a), 123.70 (C-8), 120.63 (C-10), 116.12 (C-9), 111.92 (C-7), 108.03 (C-10b), 96.89 (C-10c); HRMS (ESI) calculated for C$_{12}$H$_{13}$N$_3$O$_2$ + H$^+$ [M + H]$^+$: 226.0611. Found: 226.0611.

4. Conclusions

3,6-dihydro-5H-pyrazolo [4',3':5,6]pyrano [3,4-b]indol-5-one, a pyrazolo-fused analog of indolo [2,3-c]coumarin, was synthesized as an attempt to study the influence of pyrazole moieties on biological activity and gain a better insight into the structure–activity relationship. The methodology described herein is straightforward and scalable; thus, it could be used for the synthesis of several analogs of this scaffold. The compound is currently under biological evaluation.

Supplementary Materials: The following material is available online. Figure S1: $^1$H NMR spectrum of compound 4; Figure S2: $^1$H NMR spectrum of compound 5; Figure S3: $^{13}$C NMR spectrum of compound 4; Figure S4: $^{13}$C NMR spectrum of compound 5.

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References


