Proline-mediated formation of novel chroman-4-one tetrahydropyrimidines

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A R T I C L E   I N F O

Article history:
Received 4 April 2012
Received in revised form 6 June 2012
Accepted 20 June 2012
Available online 28 June 2012

Keywords:
Multi-component reactions
Cyclization
Chroman-4-one
Proline
β-Turn

A B S T R A C T

Novel tricyclic N-benzylated chroman-4-one tetrahydropyrimidine derivatives have been prepared through a multi-component reaction between various 2-substituted chroman-4-one derivatives, N-methylenebenzylamine and a catalytic amount of proline under mild reaction conditions. The tricyclic structure of 1a was determined by NMR spectroscopy and confirmed by X-ray crystallography. An additional product, 2a, was isolated from the reaction mixture and its structure and conformation were determined by a combination of theoretical (Monte Carlo conformational search) and NMR-based (NOE and 3JHH couplings) conformational analysis. The NMR analysis revealed one preferred geometry for 1a and 2a in CHCl3 solution.

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1. Introduction

Substituted chroman-4-ones are regarded as common structures for drug design.1 Along with related, higher order oxygen-containing ring systems, they are frequently found in plants and marine organisms and have been shown to possess antioxidant,3 antiviral4 and antibacterial5 activities. Protocols for the preparation of substituted chromones and chroman-4-ones have been developed by our group with the most recent progress being the incorporation of a carboxy functionality in the 6-position6,7 and an amino group in the 3-position (Fig. 1).7–9 Such chromone/chroman-4-ones have been designed as potential β-turn peptidomimetics.9

As an extension of these studies we explored the use of the Mannich reaction to introduce an aminomethyl group in the 3-position of the 2-alkyl-chroman-4-one scaffold. However, this reaction did not result in the desired product, instead tricyclic chroman-4-one derivatives were isolated. Herein, we report the synthesis and conformational analysis of these novel chroman-4-one tetrahydropyrimidines.

2. Results and discussion

As found in the present study a proline-mediated Mannich reaction resulted in the unexpected formation of two novel tricyclic derivatives 1 and 2 (Fig. 2). This fact indicates a yet unexplored potential of the amino acid-catalyzed Mannich reaction. The new compounds are expected to be of interest due to their high structural similarity to derivatives with significant pharmacological activities.10,11 Examples of such compounds are the amine containing tricyclic chroman-4-one analogues of 3, which exhibit anti-inflammatory12 and antiplatelet13 activities (Fig. 2) whereas the Biginelli analogue 414 belongs to a type of structures shown to act as calcium channel blockers.15

2.1. Synthesis

For the synthesis of the title compounds, the racemates of 8-bromo-6-chloro-2-alkyl substituted chroman-4-ones 5a–c (Scheme 1) were used. They were synthesized via a microwave promoted two-component reaction using commercially available...
3-bromo-5-chloro-2-hydroxyacetophenone and an aldehyde in the presence of \(N,N\)-diisopropylamine (DIPA) in ethanol. As l-proline is known to efficiently catalyze asymmetric Mannich reactions\(^{16-18}\) it was chosen for the corresponding reactions of derivatives 5a–c. Interestingly, reacting 5a with an excess of \(N\)-methylenbenzylamine\(^{19}\) (5 equiv) and a catalytic amount of l-proline (0.3 equiv) in DMSO at 50 °C for 48 h afforded instead the novel tricyclic derivative 1a in 52% yield (Scheme 1). The chroman-4-one 5b substituted with a phenethyl group in the 2-position and 5c with a considerably smaller 2-methyl substituent were also examined (Scheme 1). Applying the identical reaction conditions, an excess of \(N\)-methylenbenzylamine in DMSO in the presence of a catalytic amount of l-proline, the products 1b and 1c were formed but in lower yields (26% and 15%, respectively) as compared to 1a. An attempt to synthesize a derivative with a 2-phenyl substituent was unsuccessful.

![Scheme 1. The l-proline catalyzed formation of tricyclic derivatives 1a–c.](image)

The structure of 1a was determined by HMBC, HSQC and NOESY-based NMR spectroscopic investigation and was confirmed by X-ray crystallography (Fig. 3). Remarkably, the bulky substituent in the 5-position prefers to adopt an axial orientation.

Compounds 1a–c are formed via a Biginelli-type mechanism.\(^{15}\) Proline is suggested to catalyze the enolization of the chroman-4-one (Scheme 2) instead of mediating enamine formation, which was previously proposed for l-proline.\(^{16}\) This conclusion is based on experiments using additional secondary amine sources such as DIPA and pyrrolidine, which were shown to mainly react as nucleophiles leading to ring opening of the chroman-4-one ring (according to \(^1H\) NMR spectroscopic analysis of the crude reaction mixture). In addition, upon heating a mixture of l-proline and chroman-4-one 1a at 50 °C no enamine formation was observable by \(^1H\) NMR spectroscopy. Hence, in the proposed mechanism the enol of 5a–c attacks the preferred \(N\)-methylenbenzylamine providing the Mannich product as an intermediate. The subsequent nucleophilic attack of the newly formed amino function on a second \(N\)-methylenbenzylamine gives the aminal of which one amino group attacks the carbonyl functionality in the chroman-4-one. Subsequent dehydration provides the tetrahydropyrimidine ring and thus the final product.

![Scheme 2. Proposed mechanism for the synthesis of derivatives 1a–c.](image)
In an attempt to optimize the yield of the tricyclic derivatives 1a–c a series of reaction conditions were examined. The use of smaller amounts of N-methylenebenzylamine, shorter reaction times or higher temperatures (20 min or 2 h at 80, 120 or 150 °C under microwave irradiation) resulted in lower conversions. Similar observations were made upon variation of the chiral catalysts (sarcosine, L-pipolic acid), the use of achiral catalysts (glycine, DIPA, DIPEA or pyrrolidine), racemic catalyst (o/l-proline) or alteration of solvents (THF or DMF). Neither the change of substrate structure by removal of substituents or by introduction of electron donating (OMe) or electron withdrawing (NO2 or Cl) groups in the 6-position of the chroman-4-one resulted in improved yields.

Further attempts on reacting 5a with electrophiles such as N-methylene p-anisidine imine provided only traces of the Mannich product along with numerous impurities. Using dibenzyl imine as the electrophile resulted only in recovered starting material.

The isolated yields of derivatives 1a–c were moderate due to the competing formation of additional heterocyclic products. For example, the synthesis of 1a also yielded 2a in 7% isolated yield (Scheme 3). As expected the formation of analogous products was detected also in the synthesis of 1b and 1c (2b and 2c in 26% and 23% yields, respectively). Compound 2a was found to have identical molecular weight to 1a, but showed a different 1H NMR spectrum and chromatographic behaviour. Therefore additional HMBC and NOESY-based NMR spectroscopic investigations were performed, as described in detail below.

![Scheme 3](https://example.com/scheme3.png)

**Scheme 3.** The L-proline-mediated formation of tricyclic derivatives 1a–c and 2a–c.

The mechanism for the formation of compounds 2a–c is suggested to occur via a nucleophilic attack by benzylamine on the chroman-4-one ring system as shown in Scheme 4. Benzylamine is most likely formed by partial hydrolysis of N-methylenebenzylamine. However, using dry DMSO as the solvent and molecular sieves (4 Å) or MgSO4 as drying agents did not prevent the decomposition of N-methylenebenzylamine and hence the formation of the heterocyclic products 2a–c.

### 2.2. Conformational analysis of 1a and 2a

Small molecules encompassing flexible bonds commonly exist in solution as a mixture of rapidly interconverting conformers.

Their solution structure usually cannot be correctly represented by a single averaged structure, but is preferably described as the probability-weighted ensemble of several conformations present in solution. The determination of such ensembles is possible, although the set of structures derived by the conformational analysis present in solution, yet allow their determination when utilized in combination with experimental data. Accordingly, the set of structures derived by the conformational analysis was evaluated in a subsequent NAMFIS (NMR analysis of molecular flexibility in solution) analysis. Distances were determined by acquisition of NOE-buildups with five mixing times (100, 150, 200, 250, and 400 ms) using the initial rate approximation, whereas scalar couplings were obtained from standard 1H and P.E. COSY spectra. Despite the few available protons on the tricyclic backbone of 1a and 2a, a sufficient number of NOEs were observed for description of the orientation of their flexible fragments (Fig. 4). As enantiomeric mixtures yield a single set of NMR signals, the conformational analysis of 1a and 2a was carried out without chiral separation. Assignment of the diastereotopic CH2 protons was based on relative NOE intensities and corresponded to the expected generated structures was performed using the OPLS-2005 all atom force field and the Born solvation model for chloroform as implemented in the MacroModel program (v. 9.7).
Fig. 4. NOE correlations observed in NMR spectra of 1a and 2a in chloroform. Two and six additional J-couplings, respectively, are described in Supplementary data.

Fig. 5. (a) The solution structure of the core of 1a (yellow) overlapped with its X-ray derived conformation (green). (b) The solution conformation of the tricyclic core of 2a, as identified by NAMFIS analysis.

Fig. 6. Alignment of a modified structure of the tricyclic derivative 6 and a type VIII β-turn (φ(1+1)=−60°, φ(1+2)=−30°, φ(2+1)=−120° and φ(1+2)=−120°).

distances, when starting the identification from the chiral centres (Fig. 4). The solution ensembles were determined by identification of the geometries truly present in solution from the theoretically predicted conformational pool using experimental selection criteria. Hence, 8 and 11 possibly time-averaged NMR-derived distances and dihedral angles were used to deconvolute the conformational pool of 1a and 2a using the NAMFIS protocol. Details of the analysis, including the comparison of the observed and the calculated distances are given in Supplementary data.

The analysis of 1a indicated one preferred conformation in solution. This geometry showed dihedral angles corresponding to those observed in the solid state by X-ray analysis including also the axially positioned 5-substituent (Fig. 5a).

Compound 2a was revealed to also prefer a single conformation (Fig. 5b) in which the large 4-substituent is equatorially oriented. Thus, the refinement revealed that only one of the computationally predicted conformational families exists in solution for 1a and 2a. It should be underlined that application of the NAMFIS protocol ensures that the identified geometries are the real solution structures and therefore are pharmacologically relevant.

Given our aim to use chroman-4-one/chromone scaffolds as novel mimetics of bioactive peptides it was especially interesting to find that the tricyclic cores of 1a and 2a efficiently mimic a native type VIII β-turn (Fig. 6; see the Experimental section for details on the calculations). Their Cl- and Br-substitutions provide possibilities for selective functionalization through Pd-mediated cross-coupling reactions,6 and thereby allow broad applicability. However, any further investigations in this direction are outside the scope of the present study.

4. Conclusions

A one-pot proline catalyzed synthetic route to novel chroman-4-one tetrahydropyrimidine derivatives 1a–c and 2a–c has been developed. The reactions are proposed to proceed through an L-proline-mediated enolization mechanism. Depending on the identity of the 2-substituent of the chroman-4-one products were obtained in varying yields. Combined NMR spectroscopic and theoretical conformational analysis of 1a and 2a revealed the presence of a single geometry in solution, which for 1a was revealed to be identical to its solid state (X-ray) structure. The obtained products are of considerable interest due to their potential pharmacological applicability. Their use as potential scaffolds for type VIII β-turn peptidomimetics will be further explored.

4. Experimental section

4.1. General

Commercially available chemicals were used without prior purification. The reactions were monitored by thin-layer chromatography (TLC) on silica plated aluminium sheets (Silica gel 60 F254, E. Merck). Flash chromatography was performed on silica gel 60 (0.040–0.063 mm, manually or using a Biotage SP4 Flash instrument). Microwave reactions were carried out using a Biotage Initiator™ Sixty with fixed hold time modus in 2–5 mL or 10–20 mL capped microwave vials. IR was recorded with a ChiralIR-2X™ from BioTools. Every compound was dissolved in 0.5 mL CDCl3. High-resolution mass spectral analysis (Q-TOF-MS) was performed at Stenhagen Analyslab AB, Gothenburg, Sweden. Elemental analyses were performed at Kolbe Mikroanalytisches Laboratorium, Mülheim and der Ruhr, Germany.

NMR spectra were recorded on JEOL GX-270 (400 MHz) or Varian Unity Innova (800 MHz) spectrometer. Assignments of signals of derivatives 1a and 2a were made using HMBC, HSQC and NOESY spectra. The samples were dissolved in CDCl3. Chemical shifts are reported in parts per million with the solvent residual peak as internal standard: CDCl3 [CHCl3 δH 7.26, CDCl3 δC 77.0]. The NOE buildup studies were performed on 0.5 mmol/dm3 solutions at mixing times of 100, 150, 200 and 250 and 400 ms. Distances were calculated with a reference distance of 1.78 Å for geminal protons. NOE peak intensities were calculated using normalization of both cross-peaks with both diagonal peaks according to [(xpeak1 × xpeak2)/[(diagpeak1 × diagpeak2)]0.5. Five mixing times yielding
a linear ($r^2 > 0.95$) initial NOE rate were used to estimate the $\sigma_{ij}$ buildup rates according to the equation $r_3 = r_2 \sigma_{ij}^2/\rho_{ij}$, where $r_3$ is the distance between protons $i$ and $j$ and $\sigma_{ij}$ is the normalized intensity obtained from NOE experiments. The $^3J_{HH}$ couplings were derived from E.COSY experiments.

The computer based studies were performed using the Macro-Model program (v. 9.7) as implemented in Maestro (v. 9.0). The conformational search for 1a and 2a was performed using the OPLS-2005 force field and the Born solvent model for chloroform. The number of torsional rotations were restricted to $3^\circ$ and the cut off was set to 0.5 Å. The conformational search was performed using the mixed torsions/low mode method with 5000 steps. The minimization method used was PRCG (Polak-Ribiere Conjugate Gradient) with a maximum of 500 iterations. Conformations within 21 kJ/mol from the global minimum were retained. This resulted in 2000 (1a) and 1351 (2a) conformations, respectively. The conformations were subsequently re-minimized using TNCG (truncated Newton conjugate gradient) using the same criteria as described above. The repeated minimization gave 162 (1a) and 349 (2a) conformations, respectively, with conformations within 21 kJ/mol from the global minimum were retained.

4.2. Synthesis of chroman-4-ones 5a–c

4.2.1. 8-Bromo-6-chloro-2-(2-(1-tosyl-1H-indol-3-yl)-ethyl)-chroman-4-one (5a). The chroman-4-one was synthesized according to the procedure reported by Friden-Saxin et al.8 To an ethanolic solution (2.5 mL) of 3′-bromo-5′-chloro-2′-hydroxycacetophene (0.250 g, 1.002 mmol, 1 equiv) 3′-(1-tosyl-1H-indol-3-yl) propanol (0.146 mL, 1.10 mmol, 1.1 equiv) and DIPA (0.154 mL, 1.10 mmol, 1.1 equiv) were added. The reaction was run in a microwave cavity for 1 h at 170 °C. The reaction mixture was diluted with EtOAc and the phases were separated. The organic phase was washed with NaOH (aq, 1%), HCl (aq, 0.1 M), water and brine. The organic phase was dried over anhydrous MgSO4, filtered and concentrated under vacuum. The obtained crude product was purified by flash chromatography using EtOAc/heptane (5% 2a) yielding 5a as an orange oil (0.31 g, 88%) as previously reported.8

4.2.2. 8-Bromo-6-chloro-2-phenethylchroman-4-one (5b). 3′-Bromo-5′-chloro-2′-hydroxycacetophene (1.38 g, 5.54 mmol) was reacted with 3-phenylpropanol (2.00 g, 6.10 mmol) and DIPA (0.856 mL, 6.10 mmol) in ethanol (15 mL) following the general procedure. Purification by flash chromatography using toluene/ heptane (50%) afforded 5b as a yellow solid (2.28 g, 74%) as previously reported.8

4.2.3. 8-Bromo-6-chloro-2-methylchroman-4-one (5c). 3′-Bromo-5′-chloro-2′-hydroxycacetophene (1.51 g, 6.05 mmol), acetaldehyde (0.373 mL, 6.61 mmol) and DIPA (0.932 mL, 6.61 mmol) were reacted following the general procedure. Purification by flash chromatography using EtOAc/heptane (1:9) gave 5c (0.47 g, 28%) as a yellow oil. $R_f = 0.77$ (5% EtOAc/heptane); IR 3403, 2930, 1661, 1457, 1377 cm$^{-1}$; $^1$H NMR (400 MHz) δ 7.79–7.15 (m, 13 H), 4.03–3.92 (m, 2 H), 3.55–3.48 (m, 3 H), 3.26–3.12 (m, 2 H), 3.00–2.91 (m, 2 H), 2.83–2.75 (m, 1 H), 2.09–2.00 (m, 1 H), 1.82–1.74 (m, 1 H); $^13$C NMR (100 MHz) δ 148.4, 141.2, 138.3, 137.7, 143.6, 131.2, 128.8, 128.5, 128.4, 128.3, 128.1, 127.2, 126.0, 126.0, 122.1, 119.4, 119.3, 117.0, 113.8, 75.7, 67.4, 59.1, 54.5, 52.8, 35.2, 31.7; HRMS (Q-TOF-MS) [M$^+$H]$^+$ calc for C$_{19}$H$_{16}$Br$_4$O$_4$: 585.1380, found: 585.1320.

4.3. Synthesis of the tricyclic derivatives 1a–c and 2a–c

4.3.1. 1,3-Dibenzyl-7-bromo-9-chloro-5-(2-(1-tosyl-1H-indol-3-yl)ethyl)-2,3,4,5-tetrahydro-1H-chromeno[4,3-d]pyrimidine (1a) and 1,3-dibenzyl-7-bromo-9-chloro-4-(2-(1-tosyl-1H-indol-3-yl)ethyl)-2,3,4,5-tetrahydro-1H-chromeno[4,3-d]pyrimidine (2a). Chroman-4-one 5a (0.100 g, 0.179 mmol, 1 equiv) was dissolved in DMSO (2.5 mL). N-Methylbenzylamine (0.106 g, 0.895 mmol, 5 equiv) and 1-proline (6.18 mg, 0.054 mmol, 0.3 equiv) were added. The reaction mixture was stirred at 50 °C for 48 h. The reaction was quenched with NH$_4$Cl (satd, aq) followed by the addition of EtOAc. The phases were separated and the aqueous phase was extracted with EtOAc. The combined organic phases were washed three times with brine, dried over anhydrous MgSO$_4$, filtered and the solvent was removed under vacuum. Purification by flash chromatography using a gradient of heptane/toluene (20%) gave 1a (72 mg, 52%) and 2a (10 mg, 7%) as orange oils.
(Q-TOF-MS) [M+H]+, calcld for C32H32BrClN2O: 585.1308, found: 585.1309.

4.3. 1,3-Dibenzy1-7-bromo-9-chloro-5-methyl-2,3,4,5-tetrahydro-1H-chromено(4,3-d)pyrimidine (1c) and 1,3-dibenzy1-7-bromo-9-chloro-4-methyl-2,3,4,5-tetrahydro-1H-chromено(4,3-d)pyrimidine (2c). Chroman-4-one 5c (0.010 g, 0.363 mmol) was reacted with N-methylenbenzylamine19 (0.216 g, 1.82 mmol) and L-proline with methylamidated. A low energy conformation of the novel ring obtained from the NAMFIS calculations was aligned with the crystal data and experimental parameters are summarized in Fig. 6.12

Supplementary data. Crystallographic data (excluding structure factors) for the structure 1a in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 838242. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Acknowledgements

We thank the Swedish Research Council (Project # 2010-4868 and #2007-4407) and the Department of Chemistry and Molecular Biology, University of Nijmegen, for financial support.

Supplementary data

NMR spectra of compounds 1a–c, 2a–c and 5c; results from NAMFIS calculations of 1a and 2a; crystal data and refinement of 1a. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1021/jt201206777.

References and notes