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Aniline-containing derivatives of parthenolide: Synthesis and anti-chronic lymphocytic leukaemia activity

Alex S. Quya a,1, Xingjian Li a,1, Louise Male b, Tatjana Stankovic c, Angelo Agathanggelou c,**, John S. Fossey a,**

a School of Chemistry, University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK
b X-Ray Crystallography Facility, School of Chemistry, University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK
c Institute for Cancer and Genomic Sciences, University of Birmingham, Edgbaston, Birmingham, West Midlands, UK

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ABSTRACT

Parthenolide exhibits anti-leukaemia activity, whilst its synthetic modification to impart improve drug-like properties, including 1,4-conjugate addition of primary and secondary amines, have previously been used, 1,4-addition of aniline derivatives to parthenolide has not been fully explored. A protocol for such additions to parthenolide is outlined herein. Reaction conditions were determined using tulipane as a model Michael acceptor. Subsequently, aniline-containing parthenolide derivatives were prepared under the optimised conditions and single crystal X-ray diffraction structures were resolved for three of the compounds synthesised. The synthesised derivatives, along with compounds resulting from a side reaction, were tested for their in vitro anti-leukaemia activity using the chronic lymphocytic leukaemia (CLL) MEC1 cell line. Computational studies with the 2RAM protein structure suggested that the activity of the derivatives was independent of their in silico ability to dock with the Cys38 residue of NF-kB.

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1. Introduction

Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in western countries [1–3]. Current treatments targeting the disease include ibrutinib which inhibits B cell receptor (BCR) signalling driving CLL cells out of the lymphoid tissues and the replication centres [4] and Venetoclax which inhibits anti-apoptotic signalling by BCL2 [5]. However, these treatments are not curative and disease with high genome instability due to TP53-deficiencies, eventually relapse necessitating alternative approaches [6].

Parthenolide (1) is a natural product readily extracted from varieties of the plant feverfew (Tanacetum parthenium) [7–15] and has been widely reported to display promising activity against various diseases including types of cancer [16–21]. Mechanistically, parthenolide targets leukaemia cells by depleting glutathione and by alkylating Cys38 of NF-kB to inhibit DNA-binding [22,23]. However, its drug-like properties [24,25] are suboptimal for development as an orally available drug. Great advances in improving the bioavailability of parthenolide have been achieved through relatively straight forward synthetic, one-step, addition to the Michael-acceptor unit of the molecule. The most widely employed manipulation to-date being 1,4-addition of primary and secondary amines, such as the addition of dimethyl amine that results in clean conversion to dimethylaminoparthenolide (DMAPT 2a, Fig. 1) [26–39]. Notably, the addition of amines leading to 2a and analogues thereof proceeds with excellent stereoselectivity (typically a single diastereoisomer results) without evidence of any additional by-products arising from epoxide ring-opening. Our team has previously utilised this approach to generate a library of parthenolide derivatives from which compound 2a (Fig. 1) is a promising lead in addressing CLL, was identified [9]. Among reported amine derivatives of parthenolide, to the best of our knowledge [40], there are no reports of analogous N-aryl (aniline) derivatives (3, Fig. 1). However, a crystal structure report detailing an aniline derivative of 9-α-hydroxy parthenolide that was obtained under zinc(II) chloride Lewis acid-catalysed conditions, indicates feasibility of aniline introduction to related structures [41].

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Cytarabine, an N-pyrimidine anti-leukaemia drug, has been successfully appended to PTL with a DBU catalyst [42].

Whilst the addition of primary and secondary amines to parthenolide generally proceeds smoothly to give compounds 2 (reactions between 1 and amine in a range of solvents) [9,27,28], it was not possible, in our hands, to so readily react parthenolide with primary or secondary aniline derivatives to form analogous products 3. As such the challenge to deliver and probe the anti-leukaemic activity of the under explored aniline derivatives of parthenolide (3) was embarked upon. This study aims to expand the scope and breadth of compounds in this area, probe new chemical space and test the activity of the compounds against CLL.

2. Results and discussion

The 1,4-conjugate addition of primary and secondary aniline derivative to various Michael acceptor motifs has been widely reported [43–49], including related natural product derived lactones [38,41,43,44,50] that have been shown to be amenable to Lewis or Brønsted acid catalysed conjugate addition of amines. In order to extend the scope of such aniline derivative additions to parthenolide (1) a series of model, precedent-informed, reactions were investigated.

Parthenolide (1) was obtained by in-house extraction from feverfew and was deemed too valuable to be deployed in the initial development of aniline addition methodology and as such a model substrate was sought. Tulipane (4) was selected as a surrogate substrate to probe conditions suitable for conjugate addition of anilines (Table 1). Tulipane, like parthenolide, contains a five-membered lactone ring and bares a double bond that can function as a Michael acceptor.

Tulipane (4) and aniline (5a) in the presence of one equivalent of potassium carbonate or triethylamine failed to deliver desired product 6 (Table 1, entry 1 (water or methanol at reflux) and entry 2 (methanol at reflux) respectively). Triethyl ammonium acetate (TEAA) was prepared according to previously reported procedures [51–55]. At room temperature, use of TEAA as solvent gave only a trace amount of product 6 (Table 1, entry 3); increasing the temperature to 100 °C gave 6 in 18% isolated yield (Table 1, entry 4). The use of manganese(II) chloride as a catalyst [48] at 10 or 20 mol% loading in a water/methanol (1:1) solvent mixture at room temperature (Table 1, entries 5 and 6 respectively) resulted in the formation of 6 in approximately 48% isolated yield (49 and 47%). Increasing the temperature to 65 °C (reflux, 10 mol% loading, Table 1, entry 7) improved the yield to 66%. Under solvent-free conditions (neat) with lithium tetrafluoroborate (10 mol%) at room temperature for 24 h, compound 6 was formed in 79% yield (Table 1, entry 8) [45]. Conducting the reaction in methanol at room temperature resulted in 35% yield, whereas heating at reflux gave a promising 85% isolated yield (Table 1, entries 9 and 10 respectively). Yttrium(III) nitrate hexahydrate was used at 10 mol% under solvent-free conditions at room temperature giving compound 6 in 85% isolated yield (Table 1, entry 11) [56]. Azizi et al. showed that squaric acid was an excellent organocatalyst for the addition of aromatic amines (and thiols) to Michael acceptors under aqueous conditions [46]. The formation of 6 under control of squaric acid as catalyst (10 mol%) in water at room temperature proceeded in 39% yield (Table 1, entry 12). When a mixture of water and methanol (1:1) was used as solvent at room temperature the yield was 56% which was further improved to 95% upon heating the reaction mixture to 50 °C (Table 1, entry 13 (24 h) and entry 14 (20 h) respectively). In comparison, croconic acid as catalyst (10 mol%) under similar conditions at room temperature or 50 °C gave product 6 in 13 and 31% isolated yield respectively (Table 1, entries 15 and 16).

From model reactions of tulipane (4) and aniline (5a) detailed in Table 1, yttrium(III) nitrate hexahydrate and squaric acid were selected as catalysts for evaluation of their suitability to facilitate the addition of p-toluidine (4-methylaniline, 5d) to parthenolide (1). p-Toluidine was used in these test reactions as it allowed ready reaction monitoring by proton NMR spectroscopy. Use of these catalysts at 10 mol% at 50 °C in a water/methanol (1:1) solvent

![Fig. 1. Structures of parthenolide (1) and previously reported derivatives thereof DMAPT (2a) and compound 2b. Present study: N-Aryl parthenolide derivatives 3.](image-url)
system gave sluggish conversion to the desired product, parthenolide derivative 3d: after 48 h yttrium(III) nitrate hexahydrate gave 25% yield, squaric acid performed better with a 51% isolated yield of product 3d (Scheme 1). Hence, squaric acid as catalyst (10 mol%) at 50 °C for 48 h in a mixture of water and methanol (1:1) as solvent was chosen as the most suitable conditions for synthesis of a range of aniline derivatives of parthenolide.

Under the squaric acid-catalysed aniline addition conditions described above, a range of primary and N-methylated secondary anilines (Table 2, entries 1 to 11 and 12 to 14 respectively) were tested. Based on moderate to good isolated yields, no readily discernible correlation of electronic properties of the aniline derivatives used as starting materials to reaction outcomes was observed. The best yields (>70%) were obtained for the use of aniline, p-hydroxy aniline and o-hydroxy aniline, resulting in products 3a-c in 72–77% isolated yield (Table 2, entries 1 to 3). Primary aniline derivatives bearing p-methyl or p-methoxy substituents gave products 3d and 3g in 51 and 54% isolated yield respectively (Table 2, entries, 4 and 7 respectively). The remaining six primary anilines tested all gave rise to the corresponding desired products, albeit in yields below 40%. Three N-methylated secondary anilines were tested as substrates for the same squaric acid-catalysed protocol (Table 2, entries 12 to 14). Unfortunately, the N-methylated congener of 3a (3l) was formed in only 8% isolated yield. p-Fluoro- and p-methoxy analogues did not fare much better giving rise to 3m and 3n in 12 and 36% isolated yield respectively. Since tertiary amine derivatives of parthenolide have shown superior pharmaceutical properties (e.g. 2a and 2b, Fig. 1) it was reasoned that alternative access to tertiary aniline products should be probed.

Since secondary aniline-containing derivatives of parthenolide

![Scheme 1](image1)

**Scheme 1.** Evaluation of yttrium(III) nitrate hexahydrate and squaric acid as catalysts for the synthesis of compound 3d from p-toluidine (5d) and parthenolide (1).

**Table 2**

Squaric acid-catalysed reaction of parthenolide (1) with aniline-containing substrates to give the corresponding 1,4-conjugate addition products (3a–n).

| Entry | R   | Ar           | Yield/% |
|-------|-----|--------------|---------|
| 1     | 3a  | H            | 72      |
| 2     | 3b  | H            | 77      |
| 3     | 3c  | H            | 75      |
| 4     | 3d  | H            | 51      |
| 5     | 3e  | H            | 35      |
| 6     | 3f  | H            | 9       |
| 7     | 3g  | H            | 54      |
| 8     | 3h  | H            | 26      |
| 9     | 3i  | H            | 28      |
| 10    | 3j  | H            | 24      |
| 11    | 3k  | H            | 18      |
| 12    | 3l  | Me           | 8       |
| 13    | 3m  | Me           | 12      |
| 14    | 3n  | Me           | 36      |
3a and 3b were available on a larger scale (due to their more ready synthetic access, Table 2) they were selected as substrates upon which to probe the suitability of reductive amination as a strategy to deliver N-alkylated tertiary aniline derivatives (Scheme 2). Upon reaction of 3a or 3b with formaldehyde with an excess of sodium acetox borohydride in 1,2-dichloromethane the corresponding desired products 3i and 3o were furnished both in 61% isolated yield. Whilst the yields over the two-steps required to generate them are in the region of 45% this represents a significant improvement over the one-step reaction of N-methylated anilines with parthenolide (compare the one-step yield of 3i of 8% to the two-step reductive amination route giving 3i in 44% yield overall). Reductive amination is therefore confirmed to be a compatible strategy for N-alkylation of secondary aniline-containing derivatives of parthenolide.

The structures and relative stereochemistries of 3b and 3d were confirmed by single crystal X-ray diffraction structure determination of the isolated major products (Fig. 2). Thus, the stereoselectivity of aniline addition was determined to be consistent with that previously reported for the more facile addition of primary and secondary amines, i.e. generation of (R) stereogenic carbon centres, alpha to the carbonyl in these cases. The absolute stereochemistry of the remaining highly diastereoselective (single isomer detected) squaric acid-catalysed aniline-derivative addition products of Table 2 were assigned by analogy.

Exposure of parthenolide 1 to acidic conditions in methanol as solvent have been reported to lead to epoxide ring-opening reactions. Although not detected in the reactions of Table 2 unidentified side-products were observed in very low quantities in earlier reactions (Scheme 1). In order to ascertain the nature of the unidentified side-products and to probe whether the squaric acid catalyst could lead to loss of starting material, a blank reaction in the absence of aniline (or derivative thereof) was performed where parthenolide was treated with squaric acid (10 mol%), Scheme 3.

After 72 h, and no further development of product(s) as judged by TLC analysis, the reaction of parthenolide 1 in methanol with squaric acid revealed the formation of O-methylated transannular epoxide ring-open product 7 in 40% isolated yield. Compound 7 was not altogether an unexpected result; it has been previously reported to result from the reaction of parthenolide with methanol in acidic conditions and aspects of its biological activity have been probed [58–60]. Furthermore, with an authentic sample of 7 in-hand it was possible to confirm the small amounts of side-products formed in earlier experiments were indeed likely to be compound 7. Compound 7 was analysed by single crystal XRD structure determination which confirmed the identity and relative stereochemistry to be that depicted (Fig. 3). Since compound 7 retains the Michael acceptor motif of the parent compound and attempt to derivatise it with the well-understood dimethyl amine group, in order to furnish 8, an analogue of 2a, was made (Scheme 3). Treatment of 7 with dimethylamine and potassium carbonate in ethanol for 16 h resulted in the formation of the expected product 8.

Table 3  
Cytoxic activity against the MEC1 CLL cell line and in silico docking score to Cys38 NF-κB.

| Entry | Compound | Docking Score<sup>a</sup> | LC<sub>50</sub> μM<sup>b</sup> |
|-------|----------|--------------------------|--------------------------|
| 1     | 1        | -5.3                     | 4.6 ± 0.9                |
| 2     | 2a       | -4.5                     | 3.4 ± 1.0                |
| 3     | 2b       | -6.4                     | 5.3 ± 0.5                |
| 4     | 3a       | -5.2                     | >20.0                    |
| 5     | 3b       | -5.2                     | 12.5 ± 1.2               |
| 6     | 3c       | -5.3                     | 11.7 ± 1.3               |
| 7     | 3d       | -5.2                     | >100.0                   |
| 8     | 3e       | -5.2                     | >100.0                   |
| 9     | 3f       | -5.1                     | >100.0                   |
| 10    | 3g       | -5.1                     | >100.0                   |
| 11    | 3h       | -5.1                     | >100.0                   |
| 12    | 3i       | -5.3                     | >100.0                   |
| 13    | 3j       | -5.3                     | >100.0                   |
| 14    | 3k       | -5.4                     | >100.0                   |
| 15    | 3l       | -5                      | >100.0                   |
| 16    | 3m       | -5                      | >100.0                   |
| 17    | 3n       | -4.9                     | 76.2 ± 0.3               |
| 18    | 3o       | -5.1                     | 12.9 ± 1.9               |
| 19    | 7        | -5.6                     | 14.8                     |

<sup>a</sup> Best pose calculated from docking each molecule with the p65 subunit of NF-κB with structure code 2RAM centred on Cys38.

<sup>b</sup> LC<sub>50</sub> values (CalcuSyn) from dose-response curves generated using low (0–20 μM) or high (0–100 μM) concentrations.
in 89% isolated yield. In order to establish the potential applicability of this structural class as anti-leukaemic compounds 7 and 8 were retained and subjected to in vitro cell-based assays below (see Fig. 4).

Derivatisation of parthenolide 1 has been widely used to alter its biochemical and pharmacokinetic properties. Inspired by the work of Neelakantan et al. in identifying compound 2a as a promising NF-κB inhibitor [28], the co-authors of this report had previously identified compound 2b as a potential anti-CLL therapy [8,9], which showed improved drug metabolism and pharmacokinetics (DMPK) properties. To the best of our knowledge the anti-leukaemic activity of aniline-derivatisation on parthenolide has not previously been addressed.

To investigate the anti-leukaemic activity of aniline derivatise 3a-o, the MEC1 chronic lymphocytic leukaemia (CLL) cell line was used. MEC1 is p53-deficient and represents a treatment-refractory model of CLL [61]. The alamarBlue® assay was employed to measure their in vitro anti-leukaemic and LC50 values were determined using CalcuSyn (Biosoft) software, Table 3. The LC50 values of parthenolide (1), 2a and 2b are consistent with previously published reports (Table 3, 4.6, 3.4 and 5.3 μM, entries 1, 2 and 3 respectively) [9]. The most active aniline derivatives were phenol-containing 3b, 3c and 30. LC50 values of 12.5, 11.7 and 12.9 μM respectively (Table 3, entries 5, 6 and 18), whilst a control experiment confirmed that p-aminophenol were in themselves inactive against CLL, the significance of the phenol functionality to the observed activity in these derivatives remains to be elucidated. Compounds 3a, 3d-m and 3n did not show appreciable activity (with LC50 determined as >20, >100 and 76.2 μM, Table 3, entry 4, entries 7–16 and 17 respectively). The non-aniline-bearing analogues of 1 and 2a, compounds 7 and 8 showed reduced cytotoxic activity (Table 3, entries 1 and 2 versus entries 19 and 20). LC50 values of 4.6 and 3.4 μM versus 14.8 and 14.0 μM respectively).

One biological mechanism by which the active compounds may act is through inhibition of the binding DNA to the p65 domain of NF-κB [22,23] through binding with the Cys38 residue on this protein [59,60]. A series of docking scores were determined using software Chimera and the structure code 2RAM from the Protein Data Bank. The 3D representations of the parthenolide derivatives were first minimised using MM2 minimisation calculations. Using default settings of Autodock Vina code, and a binding site centred on the Cys38 residue with an 18 Å box of enclosure, all the molecules were analysed with the lowest scoring pose confirmed as a potential anti-CLL candidate centred on the Cys38 residue with an 18 Å box of enclosure, all the molecules were analysed with the lowest scoring pose confirmed as a potential anti-CLL candidate.

3. Conclusion

Successful protocols for the synthesis of aniline-derivatives of parthenolide were developed. The use of squaric acid as an organocatalyst facilitated 1,4-conjugate addition of aniline and aniline derivatives to parthenolide and will permit exploration of hitherto uncharted chemical space in the study of parthenolide, its activity and its drug-like derivatives and analogues.

4. Experimental

4.1. General procedures

4.1.1. Synthetic chemistry

All commercially available solvents, catalysts and reagents were purchased and used from suppliers without any further purification. Proton NMR spectra were recorded at 400 MHz on a Bruker AVIII400 NMR spectrometer. Carbon NMR spectra are proton decoupled and were recorded at 101 MHz on a Bruker AVIII400 NMR spectrometer at room temperature. Fluorine NMR spectra are proton decoupled and were recorded at 377 MHz on a Bruker AVIII400 NMR spectrometer at room temperature. Chemical shifts (δ) were reported in ppm relative to TMS (δ 0.00) for 1H NMR and to chloroform (δ 7.76) for 13C NMR spectroscopy; coupling constants (J) are expressed in Hertz (Hz). The following abbreviations are used for multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, p = pentet, h = hexet, and br = broad. Mass spectra were recorded on an electrospray MS Waters LCT Time of Flight Mass Spectrometer and with EI (GC/MS) Waters GCT Premier Time of Flight Mass Spectrometer. Infrared Spectra Varian 660-IR FT-IR spectrometer at room temperature using an ATR attachment. Melting points were measured using a StuartTM digital melting point apparatus (SMP10) and reported as a range. Specific optical rotations were recorded on an Optical PolAar 2001 automatic polarimeter at room temperature. The X-ray crystal structures were determined using an Agilent SuperNova X-ray diffractometer with an Atlas detector (wavelength 1.5418 Å). Column chromatography was carried out using standard flash column chromatography and a Combiflash RF 200i (stationary phase silica), chromatograms were recorded by evaporative light scattering detector (ELSD) and absorbance at two wavelengths (254 nm and 280 nm). Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F254 plates. TLC plates were visualised by either UV light with 254 nm/365 nm, a methanolic solution of ninhydrin or with potassium permanganate.

4.1.2. Biological assays

Tissue culture, MEC1 cells were obtained from the American Type Culture Collection (Manassas, VA 20110 USA) and were cultured in RPMI 1640 medium (Sigma-Aldrich, Irvine, UK) with 10% fetal bovine serum (Sigma-Aldrich). The alamarBlue® cytotoxicity assay was conducted by seeding MEC1 cells in triplicate at density of 25000 cells/well in a 96 well plate, final volume of 200 μL [67]. Following treatment with test compound, viability was determined by measuring the reduction of resazurin. Resazurin solution was added to each well at a final concentration of 50 μg/mL and incubated for 3 h at 37 °C with 5% CO2. Reduction of resazurin was determined by measuring absorbance at 590 nm using a PheraSTAR FS plate reader (BMG Labtech). Cell viability was calculated as a fraction of the untreated cells after subtracting background fluorescence of resazurin in media only. Data is presented as the mean of five independent experiments and significance was determined by Student’s t-test.

4.1.3. General procedure A: synthesis of aryl aminoparthenolide derivatives

To a 1:1 water-methanol (0.2 M) solution of 3,4-unsaturated ester (1 equiv.), the corresponding aniline (1.2 equiv.) and squaric acid (10 mol%) were added. The resulting solution was stirred at 50 °C for 48 h. The solution was cooled to room temperature and...
the solvent was removed in vacuo. The residual was extracted with dichloromethane (3 × 25 ml), the combined organic layers were washed with brine and then dried over anhydrous magnesium sulphate, filtered, and solvent removed in vacuo. The product was isolated by flash chromatography over silica gel.

4.1.4. General procedure B: synthesis of tertiary aniline parthenolide derivatives from secondary aniline parthenolide To a solution of aniline secondary derivative (1 equiv.) in 1,2-dichloroethane (10 ml), formaldehyde (2 equiv.) and sodium tri-acetoxoborohydride (3 equiv.) were added. The reaction mixture was stirred under nitrogen protection at room temperature for 48 h and quenched with saturated aqueous sodium bicarbonate. The solvent was removed in vacuo and the residual was extracted with ethyl acetate. The combined organic solution was dried over anhydrous magnesium sulphate, filtered, and concentrated in vacuo. Further purification was carried out by flash chromatography over silica gel.

4.2. Chemical synthesis

4.2.1. Synthesis of 3a (3R,3aS,9aR,10aR,10bS)-6,9a-Dimethyl-3-((phenylamino)methyl)-3a,4,5,8,9,9a,10a,10b-octahydroxireno[2',3',4:9,10]cyclodeca[1,2-b]furan-2(3H)-one Following general procedure A, a mixture of parthenolide (150 mg, 0.605 mmol), aniline (75 mg, 0.80 mmol) and squaric acid (7 mg, 0.06 mmol), was stirred at 50 °C in 1:1 water-methanol (20 ml) for 48 h. The title compound was obtained as a pale-yellow oil in 72% yield (148 mg, 0.434 mmol). 

4.2.4. Synthesis of 3d (3R,3aS,9aR,10aR,10bS)-6,9a-Dimethyl-3-((p-tolylamino)methyl)-3a,4,5,8,9,9a,10a,10b-octahydroxireno[2',3',9:10]cyclodeca[1,2-b]furan-2(3H)-one Following general procedure A, a mixture of parthenolide (100 mg, 0.403 mmol), 2-aminophenol (88 mg, 0.40 mmol) and squaric acid (5 mg, 0.04 mmol), was stirred at 50 °C in 1:1 water-methanol (20 ml) for 48 h. The title compound was obtained as a colourless solid in 75% yield (107 mg, 0.300 mmol). 

HRMS (ES+ m/z): [M+Na]+ calc. for C21H28NO4Na+: 356.2225, found 356.2224; IR (neat, cm⁻¹) 3363 (NH), 1747 (C=O); HRMS (TOF MS ES+) m/z: [M+H]+ calc. for C22H29NO4: 358.2224, found 358.2226; mp: 195–198 °C; [α]D₂₀ = −1.08° (c 2.3, CHCl₃).
4.2.6. Synthesis of 3f (3R,3aS,9aR,10aR,10bS,E)-3-(((4-Bromophenyl)amino)methyl)-6,9a-dimethyl-3a,4,5,8,9,9a,10a,10b-octahydrooxireno[2',3',9,10]cyclodec[a,1,2-b]furan-2(3H)-one

Following general procedure A, a mixture of parthenolide (100 mg, 0.403 mmol), 4-bromoaniline (83 mg, 0.48 mmol) and squaric acid (5 mg, 0.04 mmol) was stirred at 50 °C in 1:1 water-methanol (2 mL) for 48 h. The title compound was obtained as a white solid in 28% yield (42 mg). IR = 0.62 (hexane/ethyl acetate, 50:50%); HRMS (TOF MS ES−) m/z: [M−H]+ calcd. for C23H32NO3 372.2175, found 372.2177; mp 154−156 °C; [α]D = −17.31° (c 1.0, CH2Cl2).

4.2.7. Synthesis of 3g (3R,3aS,9aR,10aR,10bS,E)-3-(((4-Methoxyphenyl)amino)methyl)-6,9a-dimethyl-3a,4,5,8,9,9a,10a,10b-octahydrooxireno[2',3',9,10]cyclodec[a,1,2-b]furan-2(3H)-one

Following general procedure A, a mixture of parthenolide (100 mg, 0.403 mmol), 4-methoxyaniline (60 mg, 0.48 mmol) and squaric acid (5 mg, 0.04 mmol) was stirred at 50 °C in 1:1 water-methanol (2 mL) for 48 h. The title compound was obtained as a white solid in 54% yield (81 mg). IR = 0.61 (hexane/ethyl acetate, 50:50%); HRMS (TOF MS ES−) m/z: [M−H]+ calcd. for C23H32NO3 370.2383, found 370.2382; mp 191−197 °C; [α]D = 11.08° (c 0.5, CH2Cl2).

4.2.10. Synthesis of 3j (3R,3aS,9aR,10aR,10bS,E)-3-(((3-Methoxyphenyl)amino)methyl)-6,9a-dimethyl-3a,4,5,8,9,9a,10a,10b-octahydrooxireno[2',3',9,10]cyclodec[a,1,2-b]furan-2(3H)-one

Following general procedure A, a mixture of parthenolide (80 mg, 0.32 mmol), 3-methoxyaniline (48 mg, 0.38 mmol) and squaric acid (4 mg, 0.03 mmol) was stirred at 50 °C in 1:1 water-methanol (2 mL) for 48 h. The title compound was obtained as a white solid in 24% yield (28 mg). IR = 0.52 (hexane/ethyl acetate, 50:50%); HRMS (TOF MS ES−) m/z: [M−H]+ calcd. for C23H32NO3 372.2175, found 372.2177; mp 154−156 °C; [α]D = −17.31° (c 1.0, CH2Cl2).

4.2.11. Synthesis of 3k (3R,3aS,9aR,10aR,10bS,E)-6,9a-dimethyl-3-(3-tolylamino)methyl)-3a,4,5,8,9,9a,10a,10b-octahydrooxireno[2',3',9,10]cyclodec[a,1,2-b]furan-2(3H)-one

Following general procedure A, a mixture of parthenolide (80 mg, 0.32 mmol), m-toluidine (42 mg, 0.38 mmol) and squaric acid (4 mg, 0.03 mmol) was stirred at 50 °C in 1:1 water-methanol (2 mL) for 48 h. The title compound was obtained as a white solid in 18% yield (20 mg). IR = 0.64 (hexane/ethyl acetate, 50:50%); HRMS (TOF MS ES−) m/z: [M−H]+ calcd. for C23H32NO3 372.2175, found 372.2177; mp 154−156 °C; [α]D = −17.31° (c 1.0, CH2Cl2).

4.2.12. Synthesis of 3l (3R,3aS,9aR,10aR,10bS,E)-6,9a-dimethyl-3-((3,5-Dimethoxyphenyl)amino)methyl)-6,9a-dimethyl-3a,4,5,8,9,9a,10a,10b-octahydrooxireno[2',3',9,10]cyclodec[a,1,2-b]furan-2(3H)-one

Following general procedure A, a mixture of parthenolide (100 mg, 0.403 mmol), 3,5-dimethoxyaniline (52 mg. 0.48 mmol) and squaric acid (5 mg, 0.04 mmol) was stirred at 50 °C in 1:1 water-methanol (2 mL) for 48 h. The title compound was obtained as a white solid in 8% yield (9 mg). IR = 0.67 (hexane/ethyl acetate, 50:50%); HRMS (TOF MS ES−) m/z: [M−H]+ calcd. for C23H32NO3 372.2175, found 372.2177; mp 154−156 °C; [α]D = −7.62° (c 0.5, CH2Cl2).
6.79–6.70 (3H, m), 5.05 (1H, dd, J 11.8 & 2.3), 4.01 (1H, dd, J 15.4 & 4.9), 3.79 (1H, t, J 9.0), 3.65 (1H, dd, J 15.5 & 6.1), 3.01 (3H, s), 2.74–2.62 (2H, m), 2.41–2.28 (1H, m), 2.19–2.03 (4H, m), 1.96–1.86 (1H, m), 1.79–1.68 (1H, m), 1.64 (3H, s), 1.65–1.53 (1H, m), 1.26 (3H, s), 1.19 (1H, td, J 13.0 & 5.9); 1H NMR (100 MHz, CDCl3) δ 176.0, 149.0, 134.4, 129.5, 125.0, 117.2, 112.5, 82.3, 66.5, 61.5, 52.1, 48.1, 46.4, 40.9, 39.6, 36.6, 30.4, 24.1, 172, 169; IR (neat, cm⁻¹) 1760 (C=O); HRMS (TOF MS ES⁺) m/z: [M+H⁺] calc. for C22H29FNO3: 356.2224, found 356.2232; mp 153–156 °C; [α]D²⁰ = −124.65° (c 1.0, CH2Cl2).

4.2.12.2. Method (ii). Following general procedure B, a mixture of aminoparlenolide derivative 3a (109 mg, 0.320 mmol), formaldehyde (30 mg, 0.96 mmol) and sodium triacetoxyborohydride (112 mg, 0.528 mmol), was stirred at room temperature in dichloroethane (10 mL) for 16 h. The title compound was obtained as a colourless solid in 61% yield (72 mg, 0.20 mmol). IR (neat) 3421 (OH), 1751 (C=O), 1523 (C=C), 1374 (C=C), 1243 (CH3), 1156 (CH2), 84.2 (CH2), 66.7 (epoxide moiety), 61.8 (Cq), 52.9 (CH2), 46.1 (CH), 40.5 (Cq), 39.3 (CH2), 36.2 (CH2), 29.4 (CH3), 23.5 (CH2), 16.0 (CH3), 15.7 (CH2); IR (neat, cm⁻¹) 3349 (OH), 1761 (C=O); HRMS (ES⁻) m/z: [M−H]⁻ calc. for C22H28FNO3: 372.2175, found: 372.2172; mp: 192–194 °C; [α]D²⁰ = −65.8° (c 4, CHCl3).

4.2.13. Synthesis of 3m (3R,3aS,9aR,10aR,10bS,E)-3-((4-Fluoroophenyl)(methyl)amino)methyl)-6,9a-dimethyl-3a,4,5,8,9,9a,10a,10b-octahydroxyirenol[2’,3’,9:10]cycloeda[1,2-b]furan-2(3H)-one

Following general procedure A, a mixture of parlenolide (80 mg, 0.32 mmol), 4-fluoro-N-methylaniline (48 mg, 0.38 mmol) and squaric acid (4 mg, 0.03 mmol) was stirred at 50 °C in 1:1 water-methanol (2 mL) for 48 h. The title compound was obtained as a white solid in 8% yield (9 mg); IR (neat) 3421 (OH), 1764 (C=O), 1523 (C=C), 1374 (C=C), 1243 (CH3), 1156 (CH2), 84.2 (CH2), 66.7 (epoxide moiety), 61.8 (Cq), 52.9 (CH2), 46.1 (CH), 40.5 (Cq), 39.3 (CH2), 36.2 (CH2), 29.4 (CH3), 23.5 (CH2), 16.0 (CH3), 15.7 (CH2); IR (neat, cm⁻¹) 3349 (OH), 1761 (C=O); HRMS (ES⁻) m/z: [M−H]⁻ calc. for C22H28FNO3: 372.2175, found: 372.2172; mp: 192–194 °C; [α]D²⁰ = −65.8° (c 4, CHCl3).

4.2.16. Synthesis of 6 3-((Phenylamino)methyl)dihydrofuran-2(3H)-one

Following general procedure A, a mixture of tulipane (100 mg, 1.00 mmol), aniline (112 mg, 1.20 mmol) and squaric acid (11 mg, 0.10 mmol) was stirred at 50 °C in 1:1 water-methanol (20 mL) for 48 h. The title compound was obtained as a colourless solid in 95% yield (181 mg, 0.948 mmol); IR (neat) 3421 (dichloromethane/methanol, 95:5%); 1H NMR (400 MHz, CDCl₃): δ 7.22–7.14 (2H, m, ArH), 6.73 (1H, tt, J 7.3 & 10.0, ArH), 6.68–6.62 (2H, m, ArH), 4.40–4.32 (1H, td, J 8.8 & 2.6, CH2), 4.26 (1H, br s, NH), 4.24–4.15 (1H, td, J 9.8 & 6.7, CH2), 3.52–3.37 (2H, m, CH2), 2.92–2.82 (1H, m, CH), 2.45–2.31 (1H, m, CH2), 2.15–2.05 (1H, m, CH); 13C NMR (100 MHz, CDCl₃): δ 178.5 (C=O), 147.6 (C=N), 129.4 (CH), 118.1 (CH), 113.2 (CH), 66.8 (CH2), 43.9 (CH2), 39.2 (CH), 26.8 (CH2); IR (neat, cm⁻¹) 3345 (OH), 1765 (C=O); HRMS (ES⁺) m/z: [M+Na⁺] calc. for C19H23NO3Na+: 214.0844, found: 214.0837; mp: 60–62 °C.

4.2.16.1. Synthesis of 7 (3aR,5aR,6aR,9aS,9bS)-9-hydroxy-6-methoxy-6,9-dimethyl-3-methylenedecahydroazuleno[4,5-b]furan-2(3H)-one

To a solution of parlenolide (100 mg, 0.403 mmol) in 10 mL of methanol was added squaric acid (5 mg, 0.04 mmol) and the resulting mixture was stirred at room temperature for three days. After completion of the reaction, the solvent was removed in vacuo, the residual was extracted with ethyl acetate (3 × 25 mL), the combined organic layers were washed with brine and then dried over anhydrous magnesium sulphate, filtered, and solvent removed in vacuo. The product was isolated by flash chromatography over silica gel. The title compound was obtained as a colourless solid in 40% yield (45 mg, 0.16 mmol); IR (neat) 3421 (OH), 3060 (C=O), 2925 (CH3), 1740 (C=O), 1620 (C=C), 1460 (C=C), 1383 (C–C), 1260 (C–C), 1145 (C–C), 1040 (C–C), 860 (C–C).
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