Synthesis and biological evaluation of hydrophilic embelin derivatives

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In continuance of our search for newer anti-cancer agents we were interested on embelin, a XIAP (X-linked inhibitor of apoptosis protein) inhibitor. This natural benzoquinone bear a lipophilic chain and we report here the synthesis of hydrophilic analogues of embelin. To allow a large flexibility in the nature of the hydrophilic group, three amines with different length of carbon chain bearing a protected benzoquinone were prepared. The cytotoxic effects of these derivatives were evaluated on KB cell line.

1. Introduction

Embelin (1), a benzoquinone extracted from the Embelia ribes plant (Myrsinaceae), displays many biological activities known since the antiquity. For instance, embelin showed effects on NF-κB signalling (nuclear factor kappa-light-chain-enhancer of activated B cells) and on immunity and inflammation. Its capacity to inhibit XIAP (X-linked inhibitor of apoptosis protein) and thus induce apoptosis has been also reported. It has been proven that embelin enhances therapeutic efficacy of ionizing radiation in prostate cancer. These rich biological activities make embelin an interesting scaffold for medicinal chemists.

Embelin bears a lipophilic chain attached to a hydrophilic benzoquinone moiety. A survey of literature precedents shows that other benzoquinone based natural products, such as ilimaquinone (2) and metachromine A (3) (Fig. 1) share this common feature (i.e., a lipophilic chain and a hydrophilic benzoquinone moiety) and display interesting anti-cancer activities. Many embelin analogues have been synthesized in the search of an improved anti-tumour activity.

Our group has been interested for a long time in the synthesis and the biological evaluation of anti-cancer agents including quinone based compounds. Considering that modifications reported in the literature so far, on the embelin scaffold, did not affect the polarity of the linear chain, we were interested in exploring this opportunity. Such drastic changes in the compound polarity, when applied to the naphthoquinone scaffold led to enhanced cytotoxic and pro-apoptotic activity. Introduction of a polar group may also improve water solubility and biodisponibility. We therefore envisioned the preparation of embelin derivatives bearing a long hydrophilic chain in order to evaluate the potential of such modification in the anti-cancer activity. We designed our targets following the structure depicted in Fig. 2. Indeed, we envisioned the introduction of an amine function on a short carbon chain for a further ligation with an amino-acid group. Such modification would provide useful information on the influence of the polarity of the side chain for anti-cancer activity.
**2. Results and discussion**

The synthesis of the embelin derivatives involved three common precursors, 11, 13 and 17, depending on the length of the carbon chain, that were prepared from commercially available 2,4,5-trimethoxybenzaldehyde 1 (Scheme 1). A Baeyer–Villiger oxidation of 4 followed by a hydrolysis with potassium hydroxide under alcoholic conditions furnished the 2,4,5-trimethoxyphenol 5 in 83% yield. Treatment of the phenol with allyl bromide provided 6, which can be submitted to a Claisen rearrangement upon heating in a microwave oven at 190 °C in neat conditions to lead to the allylphenol 7 in 92% yield. To access the two-carbon chain carbamate 9, an oxidative cleavage of the olefin should provide an easy way to functionalize aldehyde, thus the phenol function of 7 needed a suitable protection. The phenol was masked with a TBS group by a reductive carbamoylation with TBSCl in presence of imidazole. Olefin 8 was then submitted to a Lémieux–Johnson oxidative cleavage and 9 was obtained in 89% yield. The aldehyde 9 was converted into the carbamate 10 by a reductive carbamation with tert-buty carbamate in 89% yield. The TBS protecting group was easily removed by treatment with TBAF to furnish the two-carbon chain carbamate 11 in 90% yield.

In order to provide analogues bearing side chain of one- and three-carbon (Scheme 2), 2-hydroxy-3,5,6-trimethoxybenzaldehyde 12 was prepared by a Duff reaction on phenol 5. Subsequently, the aldehyde function was converted into the corresponding one-carbon chain carbamate 13 by a reductive carbamation with tert-butyl carbamate in 89% yield, to provide the one-carbon chain carbamate precursor of embelin derivatives. Alternatively, the phenol function of compound 12 was protected as a silyl ether and the resulting aldehyde 14 was transformed by a Wittig reaction to the phenylacrylonitrile 15. Hydrogenation of compound 15 could give a direct access to the corresponding three-carbon chain analogues of embelin.

As shown in Scheme 3, the precursors prepared above were used to prepare a variety of compounds. Carbamates 11 and 13 were Boc deprotected and the crude resulting amines were directly coupled with N-Boc protected amino-acids to generate a series of α-benzyl and α-phenethylamidocarbamates 16a–f and 17a–c, respectively. α-(Phenyl)propyl amidocarbamates 18a–c were prepared by hydrogenation of the phenylacrylonitrile 15 followed by coupling of the resulting amine with N-Boc protected amino-acids and TBAF deprotection of the phenol.

Generation of the para-quinone cycle was performed by oxidation of phenols 16a–f, 17a–c and 18a–c with (diacetoxyiodo)benzene (Scheme 4). Hydrolysis of the methoxy of the benzoquinones moieties and Boc deprotection of amines were performed by hydrochloric acid treatment to furnish a series of embelin derivatives with three different carbon chain lengths and various terminal amino-acids. Compounds 16a–f, 17a–c, 18a–c, 19a–f, 20a–c, 21a–c, 22a–f, 23a–c and 24a–c (Table 1) were evaluated for their cytotoxic effect on KB cell line, with no activity, while embelin display an IC50 of 5.58 μM under the same conditions. Since none of the new synthesized analogues displayed any cytotoxic activity, we suspect that changing the polarity of embelin side chain might result in a poor cellular uptake. Amphiphilic disubstituted compounds,

![Scheme 1. Reagents and conditions:](image)

![Scheme 2.](image)

![Scheme 3.](image)

![Fig. 2. Benzoquinone based natural products bearing a lipophilic chain.](image)
bearing both hydrophobic and hydrophilic moiety could be the key to biological properties.

3. Conclusion

In summary, we have described the synthesis of a library of hydrophilic embelin analogues. An efficient diverted synthesis approach has been developed from the common precursor 5. Preliminary biological studies showed that our targets did not display any cytotoxic activity on tumoral cells. Although these results were disappointing, it provided useful information for the establishment of a comprehensive structure–activity relationship of embelin analogues. In order to improve water solubility and biodisponibility of embelin, but still maintaining a hydrophobe side chain required for the pro-apoptotic activity, we are now working on the preparation of amphiphilic agents. These results will be disclosed in due course.

4. Experimental section

4.1. General remarks

All commercial solvents and reagents were used as received from the Aldrich Chemical Company, Fischer Scientific Ltd, Alfa Aesar Company or TCI Europe company. All glassware was flame dried and allowed to cool under a stream of dry argon. Silica gel (40–63 μm) used in flash column chromatography was obtained from Merck and was pre-coated with oxalic acid. Analytical thin-layer chromatography (TLC) was performed on silica gel plates (TLC silica gel 60 F254 purchased from Merck), visualized with a Spectroline UV254 lamp, and stained with a basic solution of KMnO4. Solvent systems associated with Rf values and flash column chromatography are reported as percent by volume values. 1H and 13C NMR, recorded at 300 MHz and 75 MHz, respectively, were performed on a Bruker Advance 300 spectrometer. Proton chemical shifts were internally referenced to the residual proton resonance in CDCl3 (δ 7.26 ppm), CD3OD (δ 3.31 ppm). Carbon chemical shifts were internally referenced to the deuterated solvent signals in CDCl3 (δ 77.2 ppm), CD3OD (δ 49.0 ppm). FT-IR spectra were recorded on a Bruker IFS 55 spectrometer with samples loaded as neat films on ZnSe plates or as a powder mixed with KBr.

4.2. Experimental procedures

4.2.1. 2,4,5-Trimethoxyphenol (5). mCPBA (9.4 g, 70 wt %, 38.25 mmol, 1.5 equiv) was added dropwise to stirred solution of 2,4,5-trimethoxybenzaldehyde 1 (5 g, 25.5 mmol, 1 equiv) in 50 mL of DCM at 0 °C. After addition was complete, the ice bath was removed and the mixture allowed warming to room temperature and dissolved. The mixture was filtered through a Celite pad, and then washed with DCM. The combined filtrate was concentrated and purified by flash chromatography on silica gel 60 (230–400 μm).

Table 1

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Scheme 4. Reagents and conditions: (a) PhI(OAc)2, CH3CN/H2O, 54–77%; (b) HCl, MeOH, 73–89%.

Table 1 Structures of embelin derivatives and intermediates

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Table 1 Structures of embelin derivatives and intermediates
temperature for 2 h. The reaction mixture was filtered, then the organic solution was washed withaq NaHCO₃ (2–20 mL), H₂O (20 mL) and brine (20 mL). The solution was then concentrated giving a dark yellow oil that was dissolved in EtOH (25 mL) and stirred with 10% KOH (25 mL) at 50 °C for 2 h. The mixture was acidified to pH 1 with 1 M HCl and extracted with DCM (4×40 mL). The combined organic layers were washed with H₂O (50 mL), brine (50 mL) and then dried (MgSO₄). The solution was concentrated and the residual oil was purified by silica gel column chromatography (20% EtOAc in hexanes) providing the phenol 5 in 83% yield (3.9 g, 21.2 mmol). The spectroscopic data for these compounds are identical to those reported in the literature.⁴

4.2.1. 2-(tert-Butyldimethylsilyloxy)-3,5,6-trimethoxyphenylacetdehyde (9). 3.15 mL (0.157 mmol, 0.025 equiv) of a 0.05 M solution of OsO₄ in BuOH were added to a stirred solution of alkenel in 0.157 mmol, 1 equiv) in Et₂O (125 mL). The resulting dark solution was stirred for 10 min prior to the addition of H₂O (125 mL) and finely powdered NaIO₄ (2.69 g, 12.6 mmol, 2 equiv). The reaction mixture was then vigorously stirred at room temperature for 20 h. 3.15 mL (0.157 mmol, 0.025 equiv) of a 0.05 M solution of OsO₄ in BuOH were added to the reaction and the mixture was further stirred for 20 h. The organic fraction was separated, then washed with 1 M Na₂SO₄ (50 mL), water (50 mL), brine (50 mL) and then dried (Na₂SO₄). The solution was concentrated and the residual oil was purified on silica gel chromatography (10–20% EtOAc in hexanes) to provide 1.78 g (5.23 mmol) of aldehyde 9 in 89% yield. Rf = 0.33 (20% EtOAc in hexanes); ¹H NMR (300 MHz, CDCl₃) δ 6.95 (1H, t, J = 1.8 Hz), 6.50 (1H, s), 3.85 (3H, s), 3.78 (3H, s), 3.72 (3H, s), 3.71 (2H, d, J = 1.8 Hz), 0.96 (9H, s), 0.16 (6H, s); ¹³C NMR (75 MHz, CDCl₃) δ 200.1, 146.5, 146.1, 141.7, 137.0, 119.0, 98.8, 60.6, 56.9, 55.3; 39.9, 26.1 (3C); IR (ZnSe) ν 3290, 1625, 1525, 1200, 855 cm⁻¹; HRMS (ESI-TOF) calcd for [M+Na⁺]: 363.1604; found: 363.1599.

4.2.6. tert-Butyl-2-(tert-butyldimethylsilyloxy)-3,5,6-trimethoxyphenylacetic acid (10). TBDMSH (1.18 mL, 73 mmol, 2 equiv) was added to a stirred solution of aldehyde 9 (1.24 g, 3.65 mmol, 1 equiv), tert-butyl carbamate (855 mg, 7.3 mmol, 2 equiv) and TFA (562 µL, 7.3 mmol, 2 equiv) in CH₂CN (15 mL). The solution was stirred at room temperature for 18 h, then diluted with Et₂O (30 mL) washed with aqueous NaHCO₃ (20 mL), brine (20 mL) and dried (MgSO₄). The solution was concentrated and the residual oil was purified on silica gel chromatography (20% EtOAc in hexanes) to provide 1.47 g (3.32 mmol of carbamate 10 in 89% yield. Rf = 0.25 (20% EtOAc in hexanes); ¹H NMR (300 MHz, CDCl₃) δ 6.43 (1H, s), 3.85 (3H, s), 3.80 (3H, s), 3.78 (3H, s), 3.33 (2H, q, J = 5.4 Hz), 2.88 (2H, t, J = 6.0 Hz), 1.42 (9H, s), 0.19 (6H, s); ¹³C NMR (75 MHz, CDCl₃) δ 156.0, 146.6, 145.9, 141.4, 136.8, 117.1, 96.9, 78.6, 60.9, 56.5, 55.2, 41.2, 28.4 (3C), 26.2 (3C), 24.2, 18.8, –3.8 (2C); IR (ZnSe) ν 3001, 2852, 1682, 1604, 1499, 1392, 1245, 1056, 1032, 970, 851 cm⁻¹; HRMS (ESI-TOF) calcd for [M+Na⁺]: 464.2444; found: 464.2401.

4.2.7. tert-Butyl-2-hydroxy-3,5,6-trimethoxyphenethylcarbamate (11). TBAD (3.34 mL of a 1 M solution in THF, 3.34 mmol, 2 equiv) was added to a stirred solution of silylated phenol 1 (717 mg, 1.67 mmol, 1 equiv) and Et₂N (700 µL, 5.01 mmol, 3 equiv) in THF (10 mL). The solution was stirred at room temperature for 1 h then concentrated and directly loaded on silica gel chromatography (50% EtOAc in hexanes) to provide 493 mg (1.5 mmol) of phenol 11 in 90% yield. Rf = 0.15 (30% EtOAc in hexanes); ¹H NMR (300 MHz, CDCl₃) δ 6.48 (1H, s), 3.82 (3H, s), 3.80 (3H, s), 3.43–3.27 (2H, m), 2.90 (2H, t, J = 7.0 Hz), 1.43 (9H, s); ¹³C NMR (75 MHz, CDCl₃) δ 156.2, 145.6, 142.3, 142.0, 138.1, 119.9, 97.1, 78.8, 61.1, 56.9, 56.4, 40.7, 28.4 (3C), 24.0; IR (ZnSe) ν 3405 (br), 2975, 2840, 1694, 1505, 1392, 1366, 1250, 1088, 974, 862 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 328.1760; found: 328.1783.

4.2.8. 2-Hydroxy-3,5,6-trimethoxybenzaldehyde (12). A solution of phenol 5 (3.6 g, 19.5 mmol, 1 equiv) and HMTA (3.0 g, 21.5 mmol, 1.1 equiv) in TFA (40 mL) under Ar was refluxed for 20 h. The solution was cooled to room temperature and concentrated in vacuo. Toluene (20 mL) was added to the residue and the solution was further concentrated to remove traces of TFA. 20 mL of THF and 20 mL of 2 M HCl were added to the residual oil and the mixture...
was refluxed for 2 h. The solution was cooled to room temperature and extracted with DCM (4×50 mL), combined organic layers were washed with brine (50 mL) and dried (MgSO₄). The solution was concentrated and the residual oil was purified on silica gel chromatography (40% EtOAc in hexanes) to provide 1.94 g (9.16 mmol) of benzaldehyde 12 in 92% yield. *R*<sub>f</sub> = 0.45 (40% EtOAc in hexanes); <sup>1</sup>H NMR (300 MHz, CDCl₃) δ 11.38 (1H, s), 10.31 (1H, s), 6.89 (1H, s), 3.95 (3H, s), 3.90 (3H, s), 3.88 (3H, s); <sup>13</sup>C NMR (75 MHz, CDCl₃) δ 195.4, 146.4, 144.9, 143.8, 143.6, 114.5, 109.5, 62.4, 57.7, 570; mp = 82–83 °C (lit. 84 84 °C); IR (ZnSe) ν 3210, 1660, 1605, 1543, 1397, 1236, 1261, 1071, 951, 742, 713 cm⁻¹; HRMS (ESI-TOF) calc'd for [M+Na⁺]: 372.1607; found: 372.1611.

4.3. General procedure for deprotection of tert-buty carbamates 11 and 13 and peptide coupling, synthesis of 16a–f and 17a–c

TFA (3 mL) was added to a solution of carbamate 11, or 13 (1 mmol, 1 equiv) in DCM (3 mL) at 0 °C. The solution was stirred at 0 °C for 30 min then toluene (6 mL) was added and the solution was concentrated in vacuum. Toluene (6 mL) was added to the residue and the solution was further concentrated to remove the residual TFA providing crude amino salts, which were used without further purification in peptide coupling. The corresponding amino salt was dissolved in DMF (10 mL) then the N-protected amino-acid (1 mmol, 1 equiv), DIPEA (697 µL, 4 mmol, 4 equiv), HOBt (675 µL, 5 mmol, 5 equiv) were added. The mixture was cooled to 0 °C, then EDC·HCl (211 mg, 1.1 mmol, 1 equiv) was added. The mixture was stirred at room temperature overnight, then diluted with EtOAc (60 mL), washed with 1 M NaOH (20 mL), H₂O (20 mL), 1 M HCl (20 mL), H₂O (20 mL) and dried (Na₂SO₄). The reaction was concentrated in vacuum providing the product, which was used pure enough for the next step.

4.3.1. (S)-tert-Butyl-1-(2-hydroxy-3,5,6-trimethoxybenzylamino)-1-oxopropan-2-ylcarbamate (16a).
Yield 80%; *R*<sub>f</sub> = 0.41 (80% EtOAc in hexanes); <sup>1</sup>H NMR (300 MHz, CDCl₃, δ ppm) δ 6.53 (1H, s), 4.46 (2H, d, J = 6.2 Hz), 4.25–4.09 (1H, m), 3.86 (3H, s), 3.83 (3H, s), 3.82 (3H, s), 1.49 (9H, s), 1.33 (3H, d, J = 7.1 Hz); <sup>13</sup>C NMR (75 MHz, CDCl₃, δ ppm) δ 172.0, 156.3, 146.3, 145.2, 142.2, 139.9, 120.1, 100.5, 81.0, 62.1, 57.8, 57.5, 34.5, 29.1 (3C), 19.2; IR (ZnSe) ν 3401 (br), 2975, 2942, 1717, 1492, 1366, 1245, 1088, 974, 733 cm⁻¹; HRMS (ESI-TOF) calc'd for [M+Na⁺]: 407.1794; found: 407.187.

4.3.2. (S)-tert-Butyl-3-tert-butoxy-1-(2-hydroxy-3,5,6-trimethoxybenzylamino)-1-oxopropan-2-ylcarbamate (16b).
Yield 93%; *R*<sub>f</sub> = 0.64 (80% EtOAc in hexanes); <sup>1</sup>H NMR (300 MHz, CDCl₃, δ ppm) δ 6.52 (1H, s), 4.47–4.36 (2H, m), 4.25–4.10 (1H, br, s), 3.84 (3H, s), 3.78–3.64 (1H, br, s), 3.44–3.22 (1H, br, s), 1.42 (9H, s), 1.09 (9H, s); <sup>13</sup>C NMR (75 MHz, CDCl₃, δ ppm) δ 172.3, 155.4, 144.4, 141.5, 139.1, 119.5, 100.0, 80.0, 73.8, 61.7, 61.2, 60.3, 57.0, 56.7, 33.6, 28.2 (3C), 27.2 (3C); IR (ZnSe) ν 3389 (br), 2939, 1715, 1661, 1490, 1395, 1360, 1250, 1224, 1132, 1089, 1025, 914, 738 cm⁻¹; HRMS (ESI-TOF) calc'd for [M+Na⁺]: 479.2369; found: 479.2383.

4.3.3. tert-Butyl-3-tert-butoxy-1-(2-hydroxy-3,5,6-trimethoxyphenylamino)-1-oxobutan-2-ylcarbamate (16d).
Yield 94%; *R*<sub>f</sub> = 0.70 (80% EtOAc in hexanes); <sup>1</sup>H NMR (300 MHz, CDCl₃, δ ppm) δ 6.51 (1H, s), 4.48 (1H, dd, J = 14.1, 6.3 Hz), 4.37 (1H, dd, J = 14.1, 6.3 Hz), 4.02–4.15 (2H, m), 3.85 (3H, s), 3.81 (3H, s), 3.80 (3H, s), 3.77–3.79 (1H, m), 1.40 (9H, s), 1.14 (9H, s), 0.95 (3H, d, J = 6.2 Hz); <sup>13</sup>C NMR (75 MHz, CDCl₃, δ ppm) δ 172.0, 156.4, 146.3, 145.4, 142.3, 139.9, 120.4, 100.9, 80.6, 75.9, 67.8, 62.1, 57.8, 57.7, 34.9, 29.2 (3C), 29.1 (3C), 15.0; IR (ZnSe) ν 3407 (br), 2907, 1715, 1496, 1367, 1257, 1079, 863, 737 cm⁻¹; HRMS (ESI-TOF) calc'd for [M+Na⁺]: 493.2526; found: 493.2536.

4.3.4. tert-Butyl-2-(5R,3R)-3-tert-butoxy-1-(2-hydroxy-3,5,6-trimethoxyphenylamino)-1-oxobutan-2-ylcarbamate (16f).
Yield 82%; *R*<sub>f</sub> = 0.57 (80% EtOAc in hexanes); <sup>1</sup>H NMR (300 MHz, CDCl₃, δ ppm) δ 7.39–7.22 (4H, m), 7.20–7.10 (1H, m), 6.52 (1H, s), 4.67–4.04 (6H, m), 3.83 (3H, s), 3.81 (3H, s), 3.75 (3H, s), 1.44 (9H, s), 1.15 (3H, d, J = 6.4, 21.7 Hz); <sup>13</sup>C NMR (75 MHz, CDCl₃, δ ppm) δ 171.7, 155.8, 145.4, 144.4, 141.4, 139.0, 137.8, 128.4, 784 cm⁻¹; HRMS (ESI-TOF) calc'd for [M+Na⁺]: 372.1607; found: 372.1611.
3.4. General procedure for peptide coupling from cyanostyrenes, synthesis of 18a–c

A stirred suspension of cyanostyrene 15 (1 mmol, 1 equiv) in absolute EtOH (5 mL) and CHCl₃ (250 μL) containing PtO₂ (0.1 g%) was placed under H₂ at atmospheric pressure and the mixture was stirred at room temperature for 20 h. The reaction mixture was then filtered through a Celite pad and washed with EtOH. Evaporation of solvent afforded the corresponding amine, which was directly submitted to peptide coupling. The amine was dissolved in DMF (10 mL) then the N-protected amino acid (1.1 mmol, 1.1 equiv), DIPEA (697 μL, 4 mmol, 4 equiv), HOBt (675 mmol, 5 mmol, 5 equiv) were added. The mixture was cooled to 0 °C, then EDC·HCl (211 mg, 1.1 mmol, 1.1 equiv) was added. The mixture was stirred at room temperature overnight, then diluted with Et₂O (50 mL) and washed with 1 M NaOH (20 mL), H₂O (20 mL), and dried (Na₂SO₄). The reaction was concentrated in vacuum providing a mixture of TBS protected and deprotected phenol. Thus, the mixture was then submitted to TBAF deprotection: TBAF (1 mL of a 1 M solution in THF, 1 mmol, 1 equiv) was added to a stirred solution of the mixture and Et₂N (278 μL, 2 mmol, 2 equiv) in THF (5 mL). The solution was stirred at room temperature for 1 h then concentrated and directly loaded on silica gel chromatography (50% EtOAc in hexanes).

4.4.1. (S)-tert-Butyl-1-(3-(2-hydroxy-3,5,6-trimethoxyphenyl)propylamino)-1-oxopropan-2-ylcarbamate (18a). Yield 74%; Rf=0.51 (50% EtOAc in hexanes); ¹H NMR (300 MHz, CDCl₃, δ ppm) δ 6.46 (1H, s), 4.22–4.10 (1H, m), 3.84 (3H, s), 3.81 (3H, s), 3.78 (3H, s), 3.80–3.75 (1H, m), 3.32–3.18 (1H, m), 1.76 (2H, t, J=6.6 Hz), 1.45 (9H, s), 1.16 (9H, s); ¹³C NMR (75 MHz, CDCl₃, δ ppm) δ 170.2, 155.3, 145.7, 142.2, 141.8, 137.6, 121.4, 96.4, 77.2, 61.1, 56.8, 56.4, 37.8, 29.7, 28.4 (3C), 22.6, 20.5, 19.0; IR (ZnSe) ν 3368 (br), 2944, 2250, 1706, 1670, 1590, 1490, 1401, 1370, 1079, 1025, 976, 735 cm⁻¹; HRMS (ESI-TOF) calc'd for [M⁺Na⁺]: 435.2107; found: 435.2107.

4.4.2. (S)-tert-Butyl-3-tert-butoxy-1-(3-(2-hydroxy-3,5,6-trimethoxyphenyl)propylamino)-1-oxopropan-2-ylcarbamate (18b). Yield 64%; Rf=0.19 (50% EtOAc in hexanes); ¹H NMR (300 MHz, CDCl₃, δ ppm) δ 6.43 (1H, s), 4.22–4.10 (1H, m), 3.84 (3H, s), 3.81 (3H, s), 3.78 (3H, s), 3.80–3.75 (1H, m), 3.48–3.36 (1H, m), 3.32–3.18 (1H, m), 1.76 (2H, t, J=6.6 Hz), 1.76 (2H, t, J=6.6 Hz), 1.45 (9H, s), 1.16 (9H, s); ¹³C NMR (75 MHz, CDCl₃, δ ppm) δ 170.4, 155.4, 145.6, 142.2, 141.9, 137.7, 121.5, 96.5, 79.8, 73.5, 61.9, 61.0, 56.8, 56.3, 54.7, 37.9, 28.6, 28.3 (3C), 27.4 (3C), 20.6; IR (ZnSe) ν 3469 (br), 2976, 2954, 2260, 1725, 1668, 1495, 1392, 1367, 1351, 1248, 1077, 970, 859 cm⁻¹; HRMS (ESI-TOF) calc'd for [M⁺Na⁺]: 507.5728; found: 507.5731.

4.4.3. (S)-tert-Butyl-25(3R)-3-tert-butoxy-1-(2-hydroxy-3,5,6-trimethoxyphenyl)propylamino)-1-oxobutan-2-ylcarbamate (18c). Yield 77%; Rf=0.25 (50% EtOAc in hexanes); ¹H NMR (300 MHz, CDCl₃, δ ppm) δ 6.45 (1H, s), 4.25–4.12 (1H, m), 4.11–4.02 (1Hm), 3.87 (3H, s), 3.83 (3H, s), 3.80 (3H, s), 3.38–3.14 (2H, m), 2.47 (2H, t, J=7.1 Hz), 1.79 (2H, q, J=6.7 Hz), 1.48 (9H, s), 1.27 (3H, s), 1.24 (9H, s); ¹³C NMR (75 MHz, CDCl₃, δ ppm) δ 169.8, 155.6, 145.6, 142.0, 141.9, 137.7, 121.8, 96.5, 79.5, 77.2, 74.7, 69.9, 61.0, 56.9, 56.3, 38.4, 28.4 (6C), 20.9, 18.2, 14.1; IR (ZnSe) ν 3398 (br), 2951, 2938, 1723, 1659, 1494, 1401, 1366, 1250, 1090, 1026,
5.74 (1H, s), 4.66
5.69 (1H, s), 4.44–4.34 (1H, m), 4.31 (1H, d, J = 6.7 Hz), 4.27 (1H, d, J = 6.7 Hz), 4.05 (3H, s), 3.79 (3H, s), 3.36–3.04 (2H, m), 1.43 (9H, s); 13C NMR (75 MHz, CDCl3, δ ppm) δ 183.1, 181.9, 171.1, 158.4, 155.5, 153.9, 153.1, 127.4, 123.1, 121.2, 119.7, 118.9, 111.9, 110.7, 105.9, 80.0, 61.9, 56.4, 55.5, 32.2, 28.6 (3C, 28.3; IR (ZnSe) v 3354 (br), 2979, 2929, 2867, 1716, 1668, 1602, 1520, 1453, 1366, 1217, 1166, 1045, 913, 850 cm−1; HRMS (ESI-TOF) calcd for [M+Na]$: 506.1903; found: 506.1924.

5.6. (S)-tert-Butyl-3-(4-(tert-butoxyphenyl)-1-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-1-oxopropan-2-ylcarbamate (19f)

Yield 63%; Rf = 0.56 (80% EtOAc in hexanes); 1H NMR (300 MHz, CDCl3, δ ppm) δ 7.03 (2H, d, J = 8.4 Hz), 6.83 (2H, d, J = 8.4 Hz), 5.76 (1H, s), 4.38–4.08 (3H, m), 4.16 (3H, s), 3.82 (3H, s), 3.05–3.26 (2H, m), 1.40 (9H, s), 1.30 (9H, s); 13C NMR (75 MHz, CDCl3, δ ppm) δ 183.1, 182.3, 170.6, 158.5, 156.1, 155.1, 154.2, 131.3, 129.6 (2C), 124.3 (2C), 123.7, 106.0, 80.1, 78.3, 62.0, 56.5, 56.0, 38.2, 32.2, 28.8 (3C), 28.2 (3C); IR (ZnSe) v 3400 (br), 2939, 1716, 1660, 1624, 1455, 1365, 1217, 1149, 1045, 917 cm−1; HRMS (ESI-TOF) calcd for [M+Na]$: 539.2369; found: 539.2369.

5.7. (S)-tert-Butyl-1-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-1-oxopropan-2-ylcarbamate (20a)

Yield 64%; Rf = 0.37 (80% EtOAc in hexanes); 1H NMR (300 MHz, CDCl3, δ ppm) δ 5.73 (1H, s), 4.15–4.05 (1H, m), 4.11 (3H, s), 3.79 (3H, s), 3.29–3.44 (2H, m), 2.65 (2H, t, J = 6.6 Hz), 1.41 (9H, s), 1.28 (3H, d, J = 7.0 Hz); 13C NMR (75 MHz, CDCl3, δ ppm) δ 183.1, 182.4, 172.8, 158.7, 156.7, 155.3, 126.2, 105.6, 79.9, 61.6, 56.4, 50.1, 38.3, 28.3 (3C); mp = 82–83 °C; IR (ZnSe) v 3418 (br), 3271, 2939, 1715, 1652, 1600, 1520, 1455, 1367, 1234, 1217, 1167, 1045, 915, 845, 733 cm−1; HRMS (ESI-TOF) calcd for [M+Na]$: 405.1638; found: 405.1623.

5.8. (S)-tert-Butyl-3-tert-butoxy-1-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-1-oxopropan-2-ylcarbamate (20b)

Yield 70%; Rf = 0.45 (80% EtOAc in hexanes); 1H NMR (300 MHz, CDCl3, δ ppm) δ 5.72 (1H, s), 4.11 (3H, s), 4.10–4.02 (1H, s), 3.78 (3H, s), 3.75–3.66 (1H, m), 3.45–3.10 (1H, m), 2.65 (2H, t, J = 6.5 Hz), 1.42 (9H, s), 1.14 (9H, s); 13C NMR (75 MHz, CDCl3, δ ppm) δ 183.1, 182.2, 170.7, 158.7, 156.6, 126.0, 105.6, 79.8, 73.8, 62.0, 61.7, 61.6, 56.4, 54.2, 38.3, 28.3 (3C), 27.3 (3C); mp = 55–56 °C; IR (ZnSe) v 3341 (br), 2976, 2875, 2251, 1652, 1600, 1505, 1392, 1366, 1217, 1046, 915, 845, 733 cm−1; HRMS (ESI-TOF) calcd for [M+Na]+: 477.2213; found: 477.2230.

5.9. tert-Butyl-(25S,3R)-3-tert-butoxy-1-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-1-oxopropan-2-ylcarbamate (20c)

Yield 74%; Rf = 0.61 (80% EtOAc in hexanes); 1H NMR (300 MHz, CDCl3, δ ppm) δ 5.72 (1H, s), 4.11 (3H, s), 4.03–4.10 (1H, m), 3.98 (1Br, br, s), 3.79 (3H, s), 3.50–3.25 (2H, m), 2.66 (2H, t, J = 7.2 Hz), 1.42 (9H, s), 1.20 (9H, s), 1.00 (3H, d, J = 6.2 Hz); 13C NMR (75 MHz, CDCl3, δ ppm) δ 183.1, 182.1, 170.0, 158.7, 156.7, 155.2, 126.0, 105.5, 79.5, 74.9, 66.8, 61.6, 58.6, 56.4, 38.1, 28.3 (3C), 28.2 (3C), 23.0, 17.8; mp = 94–95 °C; IR (ZnSe) v 3391 (br), 2977, 2251, 1715, 1667, 1600, 1487, 1367, 1217, 1168, 1067, 964, 845, 733 cm−1; HRMS (ESI-TOF) calcd for [M+Na]+: 491.2369; found: 491.2374.

5.10. (S)-tert-Butyl-1-(2,5-dimethoxy-3,6-dioxocyclohexa-1,4-dienyl)propylamino)-1-oxopropan-2-ylcarbamate (21a)

Yield 60%; Rf = 0.40 (80% EtOAc in hexanes); 1H NMR (300 MHz, CDCl3, δ ppm) δ 5.75 (1H, s), 4.23–4.06 (1H, m), 4.12 (3H, s), 3.82 (3H, s), 3.34–3.12 (2H, m), 2.48 (2H, t, J = 7.2 Hz), 1.72–1.56 (2H, m), 1.45 (9H, s), 3.38 (3H, d, J = 7.1 Hz); 13C NMR (75 MHz, CDCl3, δ ppm) δ 183.2, 182.5, 172.5, 158.7, 155.5, 128.4, 105.7, 77.2, 61.5, 56.4, 38.4, 29.7, 28.3 (3C), 19.9, 18.6, 14.1; IR (ZnSe) v 3379 (br), 2975, 1717, 1659,
5.4.6. (S)-1-((2,5-Dihydroxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-3-(1H-indol-3-yl)-1-oxo-2-aminopropan-2-one (22f). Yield 82%; 1H NMR (300 MHz, CD3OD, δ ppm) δ 6.01 (1H, d, J=9.7 Hz), 3.78 (2H, t, J=6.7 Hz); 13C NMR (75 MHz, CD3OD, δ ppm) δ 182.5, 182.3, 196.5, 160.4, 156.0, 115.3, 110.4, 103.3, 54.4, 36.5, 31.2; IR (ZnSe): v 3221 (br), 1648, 1591, 1516, 1388, 1255, 1089 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 356.1241; found: 356.1237.

5.4.6. (S)-1-((2,5-Dihydroxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-3-(4-hydroxyphenyl)-1-oxo-2-aminopropan-2-one (22g). Yield 74%; 1H NMR (300 MHz, CD3OD, δ ppm) δ 7.03 (2H, t, J=8.0 Hz), 6.71 (1H, d, J=8.6 Hz), 6.67 (1H, d, J=6.8 Hz), 5.90 (1H, d, J=13.5 Hz), 4.31-3.92 (2H, m), 3.00 (2H, t, J=7.5 Hz); 13C NMR (75 MHz, CD3OD, δ ppm) δ 182.5, 182.3, 196.5, 160.4, 156.0, 115.3, 110.4, 103.3, 54.4, 36.5, 31.2; IR (ZnSe): v 3221 (br), 1648, 1591, 1516, 1388, 1255, 1089 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 333.1081; found: 333.1092.

5.4.6. (S)-1-((2,5-Dihydroxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-3-(4-ethylphenyl)-1-oxo-2-aminopropan-2-one (22h). Yield 89%; 1H NMR (300 MHz, CD3OD, δ ppm) δ 7.03 (2H, t, J=8.0 Hz), 6.71 (1H, d, J=8.6 Hz), 6.67 (1H, d, J=6.8 Hz), 5.90 (1H, d, J=13.5 Hz), 4.31-3.92 (2H, m), 3.00 (2H, t, J=7.5 Hz); 13C NMR (75 MHz, CD3OD, δ ppm) δ 182.5, 182.3, 196.5, 160.4, 156.0, 115.3, 110.4, 103.3, 54.4, 36.5, 31.2; IR (ZnSe): v 3221 (br), 1648, 1591, 1516, 1388, 1255, 1089 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 356.0975; found: 355.0969.

5.4.6. (S)-1-((2,5-Dihydroxy-3,6-dioxocyclohexa-1,4-dienyl)methylamino)-3-(4-ethyl-1-oxo-2-aminopropan-2-one (22i). Yield 89%; 1H NMR (300 MHz, CD3OD, δ ppm) δ 7.03 (2H, t, J=8.0 Hz), 6.71 (1H, d, J=8.6 Hz), 6.67 (1H, d, J=6.8 Hz), 5.90 (1H, d, J=13.5 Hz), 4.31-3.92 (2H, m), 3.00 (2H, t, J=7.5 Hz); 13C NMR (75 MHz, CD3OD, δ ppm) δ 182.5, 182.3, 196.5, 160.4, 156.0, 115.3, 110.4, 103.3, 54.4, 36.5, 31.2; IR (ZnSe): v 3221 (br), 1648, 1591, 1516, 1388, 1255, 1089 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 356.0975; found: 355.0969.
H NMR (300 MHz, CD3OD, δ ppm) δ 5.93 (1H, d, J = 2.1 Hz), 4.36 (2H, m, J = 7.5 Hz), 3.27 (2H, t, J = 7.5 Hz), 2.68 (2H, t, J = 7.5 Hz), 2.50 (2H, m), 1.66 (2H, m); 13C NMR (75 MHz, CD3OD, δ ppm) δ 182.6, 182.5, 169.5, 160.3, 154.3, 117.5, 116.2, 103.2, 102.8, 49.0, 38.9, 27.1, 19.31, 19.27, 16.4; IR (ZnSe): v 3250 (br), 3085 (br), 1651, 1595, 1504, 1397, 1286, 1210, 1092 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 285.1081; found: 285.1099.

6.4.11. (S)-1-(3-(2,5-Dihydroxy-3,6-dioxocyclohexa-1,4-dienyl)propylamino)-3-hydroxy-1-oxopropan-2-aminium chloridé (24b). Yield 75%; 1H NMR (300 MHz, CD3OD, δ ppm) δ 5.94 (1H, d, J = 2.1 Hz), 5.82 (1H, d, J = 6.9 Hz), 3.20 (2H, m), 1.67 (2H, m). 13C NMR (75 MHz, CD3OD, δ ppm) δ 182.6, 182.5, 169.5, 160.3, 154.3, 117.5, 116.2, 102.8, 49.0, 38.9, 27.1, 19.31, 19.27, 16.4; IR (ZnSe): v 3250 (br), 3085 (br), 1651, 1595, 1504, 1397, 1286, 1210, 1092 cm⁻¹; HRMS (ESI-TOF) calcd for [M+H⁺]: 269.1132; found: 269.1199.

4.7. Biology, cytotoxic assay

KB cells were originated from the NCI and grown in D-MEM medium supplemented with 10% foetal calf serum, in the presence of penicillin, streptomycin and fungizone in 75 cm² flasks under 5% CO₂. Cells were seeded in 96-well tissue culture plates in 200 μL medium and treated 24 h later with 2 μL stock solution of compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72 h exposure, MTS reagent (Celltiter 96 AQueous One, Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [1−(OD490 treated/OD490 control)]×100 in triplicate experiments.

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