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Synthesis and Radioprotective Properties of Pulvinic Acid Derivatives

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Dedicated to Dr. Charles Mioskowski, deceased June 2007, who initiated this work.

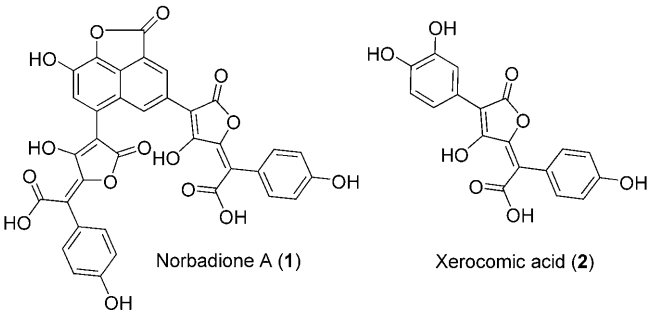
A high-throughput screening method has highlighted the marked antioxidant activity of some pulvinic acid derivatives (PADs) towards oxidation of thymidine, under γ and UV irradiation, and Fenton-like conditions. Here, we report the synthesis of a series of new hydrophilic PADs and the evaluation of their radioprotective efficacy in cell culture. Using a cell-based fluorescent assay, we show that some of these compounds have a pronounced ability to prevent cell death caused by radiation and to allow the subsequent resumption of proliferation. Thus, PADs may be considered as a novel class of radioprotective agents.

Introduction

The medical and industrial use of ionizing radiation has expanded considerably during the last few decades, with applications ranging from the treatment and diagnosis of cancer to power supply in nuclear plants. Consequently, the risk of accidental exposure to ionizing radiation has appreciably increased. Ionizing radiation consists of electromagnetic waves (X-rays and γ-rays), subatomic particles (α and β-particles, protons, neutrons) and atomic ions. When irradiated, water contained in the cells generates reactive oxygen species (ROS) and other free radicals. In turn, ROS react with DNA and additional cellular material and components, including proteins and membrane lipids. Living organisms have developed a number of systems to cope with oxidative injuries (i.e., glutathione, thioredoxin, superoxide dismutase). However, in the case of acute or protracted irradiation, these mechanisms can be rapidly overwhelmed. Whole-body irradiation results in acute radiation syndrome (ARS).[1] Ionizing radiation can immediately affect all organs (i.e., heart, brain, liver, lungs, kidneys, eyes) to varying extents. The lympho-hematopoietic tissues and the gastrointestinal tract are the first tissues to be affected, due to the acute sensitivity of lymphocytes and progenitor cells to radiation. Death, cancers, or irreversible damage to the organism can result from irradiation. The threat of accidental exposure to ionizing radiation therefore represents a public health concern, which fully justifies the need to develop novel radioprotectants.[2] A radioprotectant is defined as a compound that, when given before irradiation, may protect normal tissues from the damaging effects of radiation by promoting cell recovery and survival.

There are currently several pharmacological approaches to reduce the harmful consequences of irradiation.[3,4,5] Among them, antioxidants such as polyphenols and thiols have been broadly studied.[6] These compounds act as radical scavengers by trapping free radicals before they attack DNA and other cellular materials. One of the most efficient radioprotectants discovered to date is amifostine.[7] This compound is currently used as a cytoprotective agent in cancer patients undergoing radiotherapy, but so far, its severe side-effects have hampered its wider clinical applications. Thus, much remains to be done to identify radioprotective agents without such drawbacks.

A high-throughput screening method that determines the ability of a given chemical agent to protect thymidine from γ



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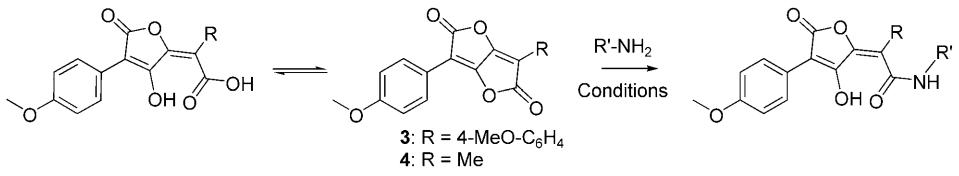
irradiation has revealed that norbadione A (**1**), a polyphenol present in mushrooms (*Xerocomic badius and Pisolithus tinctorius*), exhibits strong antioxidant activity under γ and UV exposure.[8a,b] Subsequently, tests in cell culture and mice revealed that norbadione A was also endowed with radioprotective properties, but was too toxic to be used as a radioprotector.[9] Norbadione A is constituted of two similar subunits derived from xerocomic acid (**2**), a member of the family of the pulvinic acid, which are well known as potent antioxidant compounds.[8a,b,10] In order to develop new radioprotective agents, we focused on the synthesis of hydrophilic pulvinic acid derivatives (PADs). We describe here their synthesis and their radioprotective activity on cultured lymphoid cells, evaluated using a fluorometric assay. Results indicate that some of these compounds can efficiently protect cells against radiation.

Results and Discussion

Chemistry

Pulvinic acids are the opened form of a bis-lactone (Scheme 1). When reacted with an amine, one lactone is opened to furnish the corresponding amide. We recently reported that unsymmetrical mono-aromatic bis-lactones can selectively be ring-opened by an amine in the presence of tetra-*n*-butylammonium fluoride (TBAF).[11] This ability to be easily opened by amines will be used to insert hydrophilic groups on both mono-aromatic and di-aromatic bis-lactones **3** and **4**.



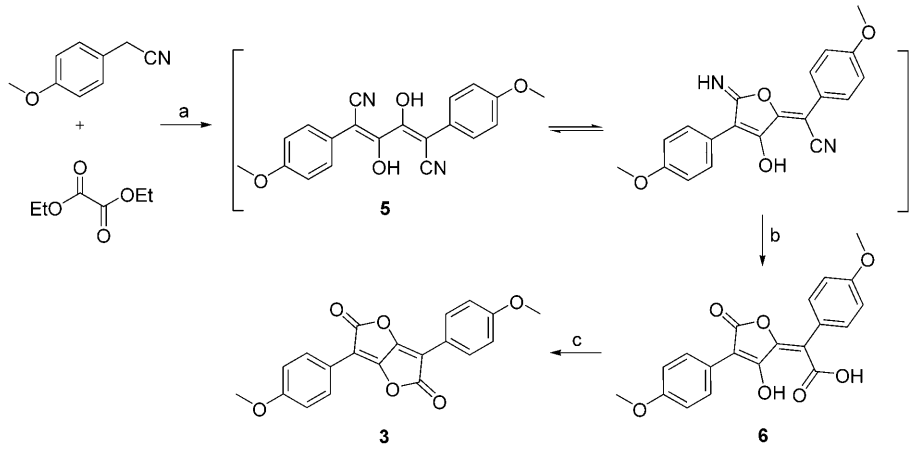
Scheme 1. Synthesis and reactivity toward amines of bis-lactones **3** and **4**.

Bis-lactone **3** was synthesized according to Volhard's method (Scheme 2).[12] 4-Methoxyphenylacetonitrile was condensed on diethyl oxalate in refluxing ethanol in the presence of sodium ethoxide to give **5**, which exists in the cyclized form. Acid hydrolysis gave access to **6**, which was then dehydrated in refluxing acetic anhydride (Ac_2O) to give bis-lactone **3** in 10% yield over three steps.

Bis-lactone **4** was synthesized from a tetronic acid according to Mioskowski's procedure.[13] Methyl 4-methoxyphenylacetate and ethyl glycolate were refluxed in tetrahydrofuran (THF) in the presence of sodium *tert*-butoxide to furnish the tetronic acid **7** in 72% yield (Scheme 3). After protection by dimethyl sulfate to give **8** in quantitative yield, deprotonation with *n*-butyl lithium and condensation on methyl pyruvate gave access to the tertiary alcohol **9**, which was then dehydrated to give **10** as a mixture of *Z* and *E* isomers (*E*/*Z* = 70:30) with a total yield of 88% over three steps.[14] The mixture of both isomers was then reacted with magnesium bromide in *N*,*N*-dimethylformamide (DMF) to demethylate the enol and the ester in one pot to give acid **11** as a single isomer in 78% yield. The acid was then cyclized in refluxing Ac_2O to give lactone **4** with a total yield of 46% from the starting material, methyl 4-methoxyphenylacetate. This procedure has been developed for scale-up synthesis and using on a multiple gram scale.

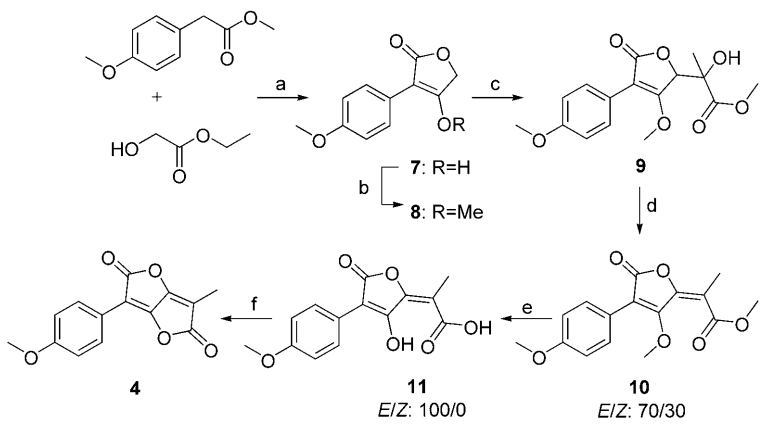
With intermediates **3** and **4** in hand, we focused then on the insertion of hydrophilic amines. This was achieved by the introduction of amines bearing different hydrophilic groups, such as amines, alcohols, ethers, carboxylic acid, or a glucose moiety (Figure 1).

N-(2-Aminoethyl)morpholine was successfully introduced on **3** and **4** to afford **12a** and **13a** in 95% and 88% yield, respectively. The insertion of a glucose moiety required the prior synthesis of amine **16**, which was made according to the procedure described by Osborne et al. (Scheme 4).[15] Glycosylation of β-D-(+)-glucose pentaacetate with 2-bromoetanol in the presence of boron trifluoride etherate ($BF_3{\cdot}Et_2O$) gave access to bromo derivative **14** in 51% yield. Substitution of the bromine by sodium azide in DMF gave access to azide **15** in quantitative yield, which was then reduced under a hydrogen atmosphere in the presence of Pd/C to yield **16** in 98% yield. Lactone opening of **3** and **4** gave access to the protected pulvinamides **12h** and **13f** in 98% and 57% yield, respectively. These were then deprotected using a catalytic amount of sodium methoxide in methanol to give **12b** and **13b**, in 94% and 58%, respectively.



Scheme 2. *Reagents and conditions*: a) EtONa, EtOH, reflux; b) H_2SO_4, H_2O; c) Ac_2O, reflux, 10% (three steps).

A diethyleneglycol chain was inserted giving compounds **12c** and **13c** in 98% and 24% yield, respectively. Compound **3** was then opened by glycine, *N*,*N*-dimethylethylenediamine, ethyle-



Scheme 3. *Reagents and conditions*: a) *t*BuOK, THF, reflux, o/n, 72%; b) Me_2SO_4, K_2CO_3, acetone, reflux, o/n, quant.; c) methyl pyruvate, LDA, THF, −78 °C, 1 h; d) TFAA, Et_3N, DMAP (cat), CH_2Cl_2, 0 °C 1 h; then 25 °C, o/n, 88% (two steps); e) $MgBr_2$, DMF, 120 °C, 5 h, 78%; f) Ac_2O, reflux, 1 h, 94%.

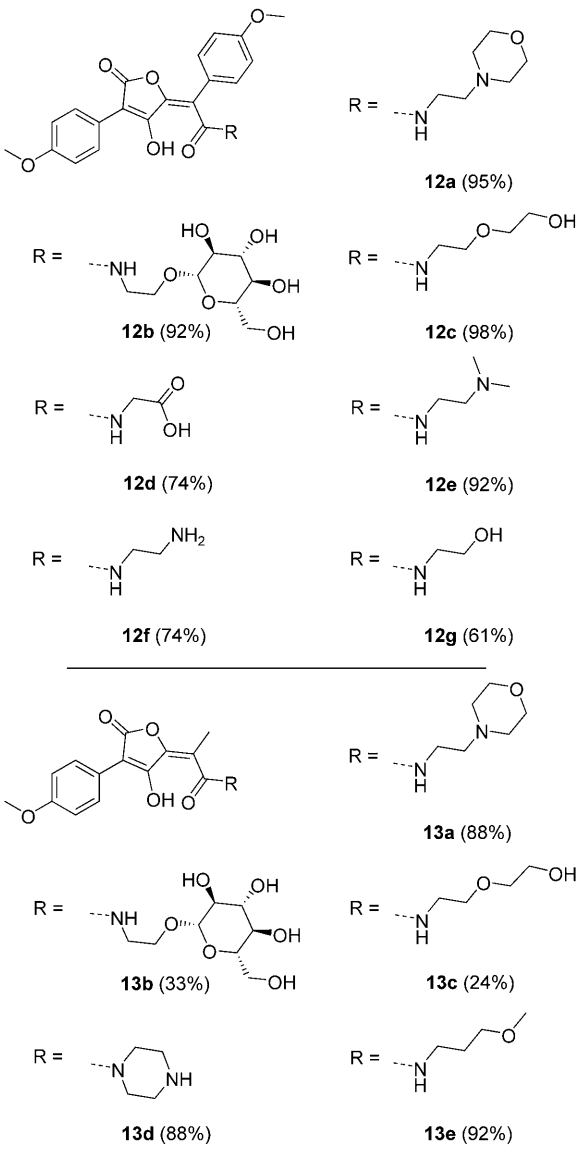


Figure 1. Structures of the different synthesized hydrophilic PADs with yield from corresponding bis-lactone **3** or **4**.

nediamine and 2-aminoethanol to afford **12d** (74%), **12e** (92%), **12f** (74%) and **12g** (61%). Finally **4** was reacted with *N*-Boc-piperazine and 3-methoxypropylamine to afford **13d** and **13e** in 88% and 92%, respectively. Derivative **13d** was obtained as the trifluoroacetate salt after quantitative deprotection using trifluoroacetic acid (TFA) (Scheme 5).

After synthesizing all of the derivatives, we turned our attention to their aqueous solubility in phosphate-buffered saline (PBS; Figure 2). The most soluble derivatives were those bearing a carboxylic acid functionality or a glucose moiety, with solubility values ranging from 4.02 mM (**12d**) to 15.10 mM (**11**). Derivatives bearing an amine or an alcohol functionality were less soluble, with solubility values ranging from 0.003 mM (**12a**) to 1.04 mM (**13c**).

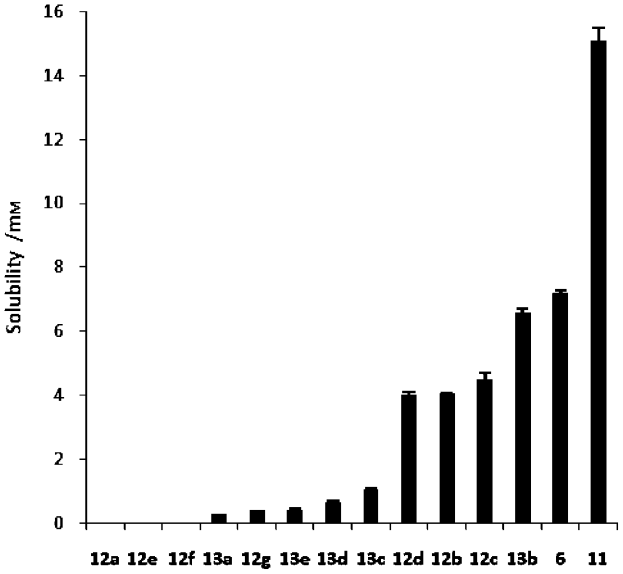
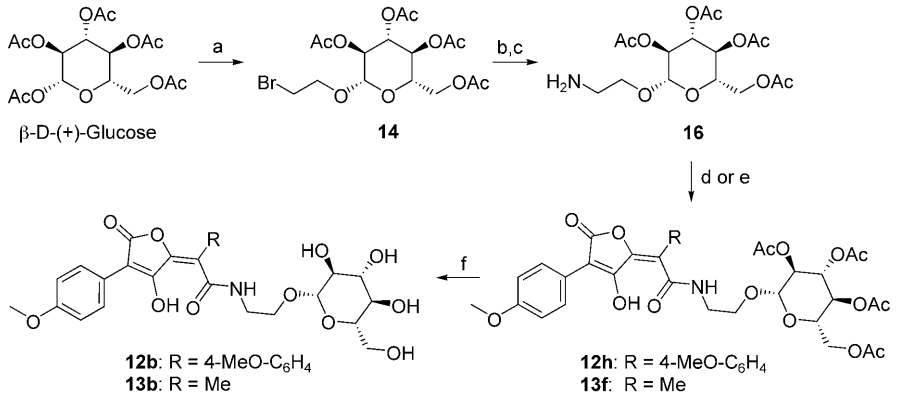


Figure 2. Water solubility of compounds **6**, **11**, **12a-g** and **13a-e**.

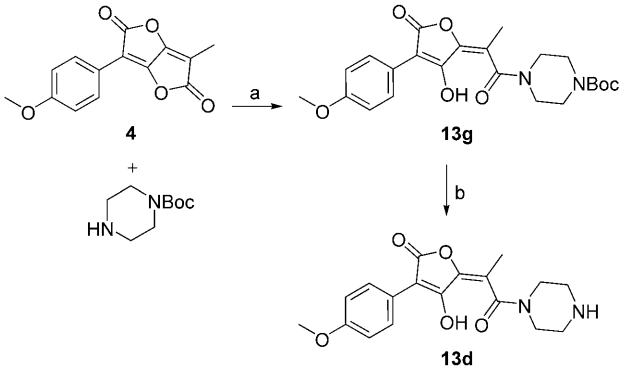
Biology

The biological evaluation of radioprotectants relies predominantly on in vivo assays, which require the use of significant numbers of animals, in most cases mice. In contrast, the assessment of the antioxidant potency of compounds can be determined using a variety of in vitro techniques, such as the telomere repeat amplification protocol (TRAP),[16] oxygen radical absorbance capacity (ORAC),[17] total oxyradical scavenging capacity (TOSC),[18] ferric reducing ability of plasma (FRAP),[19] and 2,2-diphenyl-l-picrylhydrazyl (DPPH) trapping assays. However, the antioxidant potency and radioprotective activity of a molecule do not always correlate. For this reason, we focused our study on an in vitro assay based on X-ray irradiation of radiosensitive cells, and followed the effects of irradiation on their viability and ability to resume division in the presence and absence of varying concentrations of our PADs.

As the cellular assay requires sterile solutions, we were forced to use the most soluble pulvinic acid derivatives. There-



Scheme 4. *Reagents and conditions*: a) 2-bromoethanol, BF_3:Et_2O, CH_2Cl_2, 0 °C→RT, o/n, 51%; b) NaN_3, DMF, 60 °C, 4 h, quant.; c) H_2, Pd/C 10%, THF, o/n, quant.; d) R=Me: TBAF, THF, see Ref. [11]; e) R=4-$MeOC_6H_4$: CH_2Cl_2, RT, o/n; f) MeONa (cat), MeOH, RT, o/n, R=Me: 58%, R=4-$MeOC_6H_4$: 94%.



Scheme 5. *Reagents and conditions*: a) TBAF, THF, −78 °C→RT, 1 h (see Ref. [11]); b) TFA, CH_2Cl_2, 1 h, 88%

fore, we chose to assess compounds **12b–d**, **13b**, **6** and **11** for their toxicity on cultured cells. For this purpose, we selected the TK6 cell line,[20] a human lymphoid, p53 wild-type, actively dividing cell line. TK6 cells are frequently used in radiobiological investigations due to their high radiosensitivity. Indeed, the surviving fraction after irradiation by X-rays at 2 Gy (SF2) is 6.2%, and only 8.29×10^{-6}% after irradiation at 8 Gy (SF8), as determined by clonogenic survival. They also have a pronounced tendency to undergo apoptosis in response to radiation and thus are well suited for the analysis of molecular mechanisms underlying the induction of this type of cell death.[21] Since the inhibition of apoptosis does not always reflect the ability of antioxidants to protect against radiation,[22] we measured the number of viable cells using the fluorescent reagent, alamar blue.[23] In radioprotection assays, compounds are usually tested at relatively high concentrations, up to 1 mM;[24] however, we decided to tested PADs at 50 μM and 100 μM. First, the toxicity of PADs at those concentrations was evaluated by incubating cells in the presence of the compound for 24 h and comparing the number of living cells to those of the PBS-treated controls (Figure 3). Using this procedure, no loss of viability was observed. Under the same conditions, amifostine appeared to be toxic toward TK6 cells, and thus was not incorporated in our tests.

TK6 cells were irradiated by X-rays at 8 Gy in the presence or absence (control) of the respective PADs. Two days (48 h) after irradiation, the culture medium was removed by centrifugation and the cell pellets were resuspended in the same volume of fresh medium to prevent any possible interference of the PADs on cell proliferation. Cells were then seeded in 96-well flat-bottomed microplates and maintained for variable lengths of time at 37 °C in a 5% CO_2, humidified atmosphere. For each experiment group, five wells

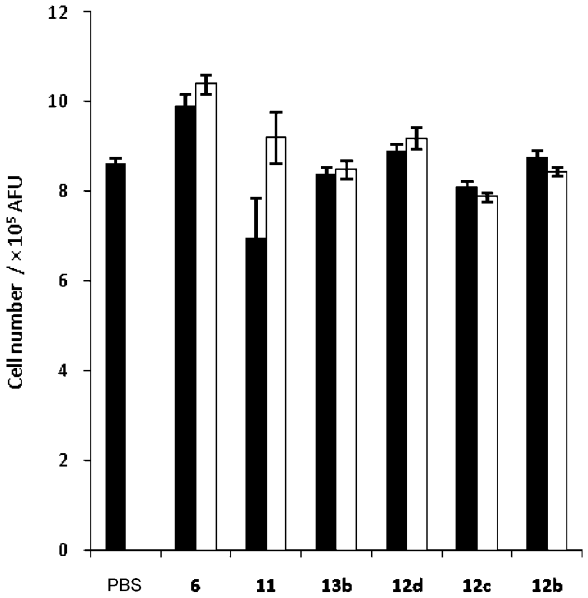


Figure 3. Viable cell numbers were recorded 24 h after incubation in the presence of six pulvinic acid derivatives at 50 (■) and 100 μM (□), or PBS (control). No significant cytotoxicity was detected. Data are the mean of three independent determinations ± standard error (SE). Cell number is expressed in arbitrary fluorescence unit (AFU).

were prepared. Measurements were performed on day 2, 4, 7 and 9 post-irradiation. Results of experiments carried out with compounds **6** and **12c** are represented in Figure 4.

We observed that the cell numbers progressively decreased during the first week after irradiation, irrespective of the treatment they had received (Figure 4). This is due to the massive loss of viability of TK6 cells following an irradiation at this dose. However, on day 7, in the presence of **12c**, the rate of cell loss was reduced. By day 9, treated and nontreated cells had resumed proliferation. In the control cells, the proliferation levels were low, but in cells treated with **6** and **12c** prior to irradiation, the rate of proliferation recovered faster. Five times more living cells were observed in cells treated with the pulvinamide **12c**.

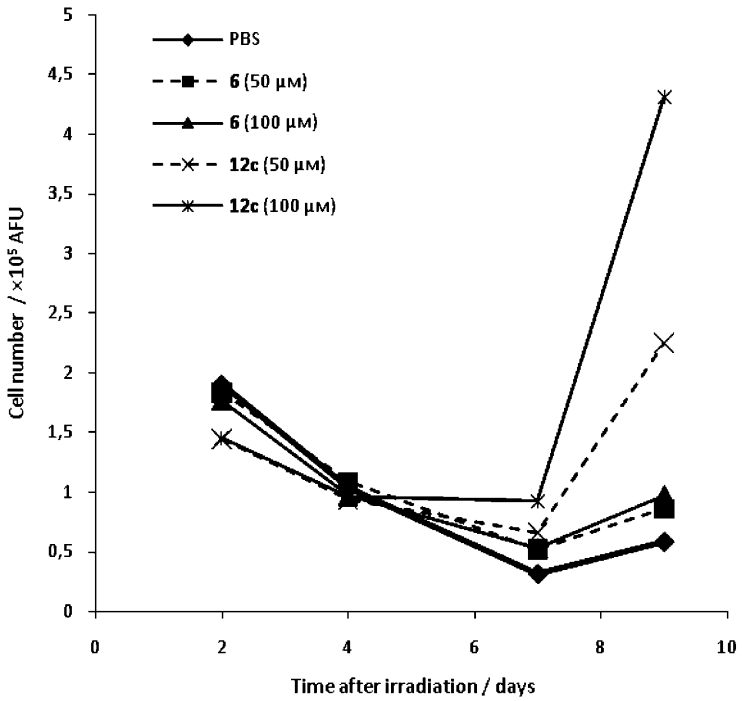


Figure 4. Viable cell number of TK6 treated with **6**, **12c** or untreated and irradiated at 8 Gy, as a function of the time post-irradiation.

The other derivatives were tested using the same protocol. Figure 5 shows the normalized data recorded at day 9 (nontreated cells value set at 100). Surprisingly, compound **6** was found to be less active than expected, with only 1.5 and 1.7 times more cells surviving at 50 and 100 μM, respectively, as compared to nontreated cells. The other derivatives also provided good levels of protection, with 3.5–5.1 times more living cells observed at 50 μM than untreated cells, and 3.1–6.2 times more living cells at 100 μM. Interestingly, compound **11** was less active at 100 μM than at 50 μM, suggesting the presence of a pro-oxidant activity at higher concentrations. Indeed, some polyphenols, such as resveratrol, are known to be both pro-oxidant and antioxidants,[25] and this possibility cannot be ruled out.

Next, we selected **12c** for a concentration effect study. This derivative was assessed for its radioprotective activity at concentrations ranging from 12.5 μM to 400 μM, and cell number measurements were carried out on day 9 after irradiation by X-rays at 8 Gy (Figure 6).[26] We observed a steady concentration-dependent effect. At the highest tested concentration (400 μM), no loss of viability was recorded. However, the rate of cell growth recovered more slowly at lower concentrations than at higher ones. Indeed, from 100 μM to 400 μM, the number of viable cells only increased by a factor of 1.5.

Conclusions

In conclusion, we have synthesized several hydrophilic PADs and assessed their capacity to protect lymphoid cells from radiation. We described the scalable synthesis of a common precursor, the bis-lactone **4**, and the easy insertion of several hydrophilic groups on **3** and **4**. These compounds were found to be devoid of cytotoxicity and afforded moderate (**11**, **12b**, **12d**) to pronounced (**12c**, **13b**) protection to TK6 cells against radiation. No loss of viability was observed with **12c**, even at 400 μM, the highest concentration used. Among the six derivatives, **13b** displayed the best protection, with greater than sixfold more living cells at 100 μM than untreated cells. It is also interesting to note that **6** was weakly active, that **11** was less active at 100 μM than at 50 μM, and that **12d** gave the same protection at 50 and 100 μM. Together, these observations lead us to suggest that the presence of a carboxylic acid moiety could decrease the activity by developing a pro-oxidant activity. On the other hand, it remains to be established whether aqueous insolubility of the agent and the radioprotective effect are related. Forthcoming work will consist of verifying the ability of PADs to protect animals from the harmful consequences of a whole-body irradiation. We also intend to provide a better understanding of the mechanisms underlying the radioprotective activity of PADs, in an attempt to improve their efficiency as radioprotectants.

Experimental Section

Chemistry

General method: Commercial reagents were used without additional purification. Anhydrous tetrahydrofuran (THF) was obtained by distillation over sodium and benzophenone. Analytical thin-layer chromatography (TLC) was performed using plates cut from glass sheets (silica gel 60F-254; Merck). Visualization was achieved

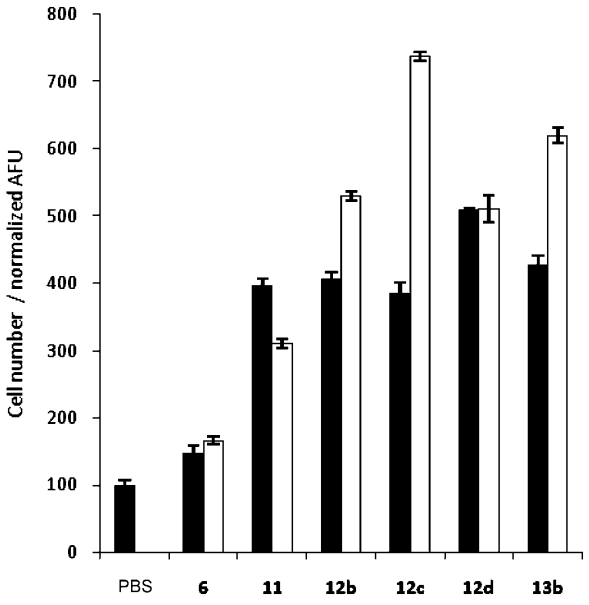


Figure 5. Effect of pulvinic acid derivatives upon TK6-cell survival following exposure to 8 Gy radiation. Compound concentrations were 50 (■) or 100 μM (□); control cells were incubated in PBS. Alamar blue test was performed nine days after irradiation. Results are from a single representative experiment. Values are the mean of five determinations ± standard error (SE). Cell number is expressed in normalized arbitrary fluorescence unit (AFU).

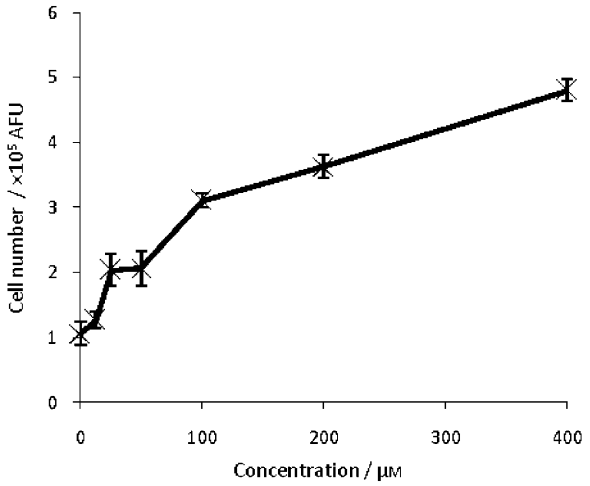


Figure 6. The dose-dependent radioprotective efficacy of derivative **12c** on TK6-cell survival measured nine days after exposure to 8 Gy radiation.

under a 254 or 365 nm ultraviolet (UV) light and by immersion in an EtOH solution of $Ce(SO_4)_2$, followed by treatment with a heat gun. Purification of compounds was performed by column chromatography using silica gel 60 (Merck). Infrared (IR) spectra were recorded on a Nicolet 380 FT-IR spectrometer (Thermo Electron Corporation) with samples prepared as a CH_2Cl_2 solution or neat solid on a diamond plate. ^{1}H and ^{13}C NMR spectra were recorded at 23 °C on a Bruker 400 MHz, Bruker 300 MHz or Brucker 200 MHz spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) and calibrated using the residual nondeuterated solvent peak. Mutiplicity is given as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br s = broad singlet; coupling constants (*J*) are given in Hz. High-resolution mass spectra (HRMS) were obtained using a Agilent Q-TOF 6520 and mass spectra (MS) using a Agilent MSD 1200 SL (ESI/APCI) with a Agilent HPLC 1200 SL. Melting points were measured on a Stuart Scientific SMP3 apparatus (Bibby) and are uncorrected.

3,6-Bis(4-methoxyphenyl)fuoro[3,2-*b*]furan-2,5-dione (3): Sodium (3.6 g, 156 mmol, 2 equiv) were dissolved in cooled absolute EtOH (50 mL). After complete consumption of sodium, diethyl oxalate (10.8 mL, 80 mmol, 1 equiv) and 4-methoxyphenylacetonitrile (22.6 mL, 156 mmol, 2 equiv) were added. The mixture was then stirred at 70 °C for 90 min, and then cooled to RT. Water (8 mL) was then added, followed by acidification to pH 5 using acetic acid (AcOH). The precipitate was then removed by filtration to give an orange solid (9.55 g). This solid was then dissolved in AcOH (20 mL) and water (10 mL), and concd H_2SO_4 (8.5 mL) was added dropwise. After a red solid is formed, the mixture was heated at reflux for 30 min, then cooled in an ice bath. The solid was collected by filtration and resuspended in Ac_2O (15 mL). The suspension was heated at reflux for 30 min then cooled to RT. The bis-lactone **3** was collected by filtration and washed with heptane (yield: 10%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 3.89$ (s, 6H), 7.01 (d, $J = 8.6$ Hz, 4H), 8.01 ppm (d, $J = 8.6$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 54.8, 96.4, 100.3, 115.3, 117.6, 130.0, 134.4, 159.5 ppm; IR (neat): $\tilde{\nu} = 595$, 869, 1019, 1157, 1257, 1354, 1506, 1600, 1657, 1782, 1909 cm^{-1}.

4-Hydroxy-3-(4-methoxyphenyl)furan-2(5*H*)-one (7): Ethyl glycolate (28 mmol) *t*BuOK (56 mmol) was added to a solution of methyl 4-methoxyphenylacetate (28 mmol) in THF (180 mL). The mixture was refluxed overnight. After cooling to RT, the reaction was treated with concd HCl to pH 1 and the mixture was extracted with EtOAc. The combined organic phases were dried ($MgSO_4$), filtered and concentrated. Compound **7** crystallized out of solution during evaporation and was collected and washed with Et_2O. The filtrate was concentrated, and the operation repeated until no further crystals were obtained (total yield: 78%); ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 3.75$ (s, 3H), 4.74 (s, 3H), 6.94 (d, $J = 9.0$ Hz, 2H), 7.84 (d, $J = 9.0$ Hz, 2H), 12.58 ppm (s, 1H); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 55.0$, 66.0, 97.3, 113.5, 122.9, 127.6, 157.6, 173.1, 173.3 ppm; MS (ESI): *m/z*: 207.1 $[M+H]^+$.

4-Methoxy-3-(4-methoxyphenyl)furan-2(5*H*)-one (8): K_2CO_3 (20 mmol) and dimethyl sulfate (20 mmol) were added to a suspension of **7** (20 mmol) in acetone (85 mL). The mixture was refluxed for 4 h. After cooling to RT, the mixture is filtered through celite, and the filtrate was concentrated to give **8** as a white solid (yield: 99%); ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 3.76$ (s, 3H), 3.86 (s, 3H), 4.72 (s, 2H), 6.86 (d, $J = 8.8$ Hz, 2H), 7.77 ppm (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 55.3$, 58.0, 64.6, 102.1, 113.7, 121.9, 128.9, 158.9, 172.3, 173.1 ppm; MS (ESI): *m/z*: 221.1 $[M+H]^+$.

Methyl 2-(3-methoxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)propanoate (10): A solution of diisopropylamine (30 mmol) in anhydrous THF (125 mL) was cooled to −20 °C, then *n*BuLi (30 mmol) was added dropwise. The mixture was stirred for 30 min at that temperature and then cooled further to −78 °C. A solution of **8** (20 mmol) in anhydrous THF (65 mL) was added dropwise, and the mixture was stirred for 30 min at that temperature. Methyl pyruvate (60 mmol) was then added dropwise. The mixture was stirred for 30 min at −78 °C and then warmed to RT. Saturated aq NH_4Cl was added, the aqueous layer was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$), filtered and concentrated. The resulting oil was diluted in CH_2Cl_2 (200 mL) and cooled to 0 °C. Et_3N (120 mmol) and DMAP (0.2 mmol) were then added, followed by the addition of trifluoroacetic anhydride (TFAA; 60 mmol) dropwise. The mixture was stirred overnight at RT, and then hydrolyzed by a 1 M aq HCl. The aqueous phase was extracted with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$), filtered and concentrated. The crude was then purified by column chromatography (cyclohexane/EtOAc, 90:10→50:50) to give **10** (yellow solid) as a mixture of *E* and *Z* isomers (*E*/*Z* = 70:30; yield: 88%); ^{1}H NMR (300 MHz, $CDCl_3$): *E* isomer: $\delta = 2.15$ (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.92–6.95 (m, 2H), 7.41–7.43 ppm (m, 2H); *Z* isomer: $\delta = 2.27$ (s, 3H), 3.80 (s, 3H), 3.83 (s, 3H), 3.86 (s, 3H), 6.93–6.96 (m, 2H), 7.40–7.43 ppm (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): *E* isomer: $\delta = 15.2$, 52.8, 55.6, 61.1, 109.0, 113.4, 114.2, 120.8, 131.4, 143.3, 160.3, 161.3, 168.2, 168.7 ppm; MS (ESI): *m/z*: 305.1 $[M+H]^+$.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-propanoic acid (11): $MgBr_2$ (1 mmol) was added to a solution of **10** (1 mmol) in DMF (10 mL). The mixture was stirred at 120 °C until no more starting material remained. The solvent was removed in vacuo, and the residue was diluted with water. The aqueous phase was washed with CH_2Cl_2, and acidified with 1 M aq HCl. The aqueous phase was then extracted with EtOAc. Combined organic phases were washed with brine, dried (Na_2SO_4), filtered and concentrated to give **11** as an orange solid (yield: 78%); ^{1}H NMR (200 MHz, CD_3OD): $\delta = 2.11$ (s, 3H), 3.81 (s, 3H), 6.92 (d, $J = 9.0$ Hz, 2H), 8.02 ppm (d, $J = 9.0$ Hz, 2H); ^{13}C NMR (50 MHz, CD_3OD): $\delta =$ 14.5, 55.6, 103.9, 114.1, 114.6, 123.4, 129.9, 154.6, 160.6, 160.9, 168.3, 174.7 ppm; MS (ESI): *m/z* (%): 231.0 (100%) $[M-CO_2]^-$.

3-(4-Methoxyphenyl)-6-methylfuro[3,2-*b*]furan-2,5-dione (4): Compound **13** (3.6 mmol) was suspended in Ac_2O (17 mL) and

heated to reflux for 1 h. After cooling to RT, the crystallized compound was collected by filtration and washed with pentane to give **4** as a yellow crystalline solid (yield: 94%); ^{1}H NMR (300 MHz, $CDCl_3$). 2.06 (s, 3H); 3.86 (s, 3H); 6.98 (d, J=9.1 Hz, 2H); 7.94 (d, J=9.1 Hz, 2H)2.06 (s, 3H); 3.86 (s, 3H); 6.98 (d, J=9.1 Hz, 2H); 7.94 ppm (d, J=9.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=7.64, 55.5, 98.8, 101.1, 114.8, 119.0, 129.9, 155.6, 159.3, 161.0, 166.1, 168.8 ppm; MS (ESI+APCI): *m/z* (%): 258.0 (100%) $[M]^+$.

General procedure for the synthesis of compounds 12a and 12c–h: The bis-lactone **3** (200 mg, 0.6 mmol) was suspended in CH_2Cl_2 (10 mL), and the amine was added (1 equiv). The mixture was stirred at RT for 15 min before concentration in vacuo and purification by column chromatography (CH_2Cl_2/MeOH, 100:0→90:10) to furnish **12a**, **12c–h** (yield: 61–98%).

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(4-methoxyphenyl)-*N*-(2-morpholinoethyl)acetamide (12a): Compound **12a** was obtained by opening **3** with *N*-(2-*a*minoethyl)morpholine according to the general procedure. Yield: 95%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=3.32 (m, 6H), 3.60 (m, 6H), 3.73 (s, 3H), 3.78 (s, 3H), 4.01 (br s, 4H), 6.85 (d, J=9.2 Hz, 2H), 6.98 (d, J=9.2 Hz, 2H), 7.57 (d, J=9.2 Hz, 2H), 8.07 ppm (d, J=9.2 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=32.7, 51.6, 54.9, 55.2, 56.7, 63.2, 91.5, 112.1, 113.0, 113.8, 125.6, 125.9, 127.1, 129.8, 144.1, 155.7, 158.6, 168.4, 170.8, 174.3 ppm; IR (neat): $\tilde{\nu}$=431, 592, 630, 659, 829, 925, 950, 1035, 1103, 1148, 1179, 1249, 1293, 1509, 1552, 1603, 1659, 1698, 2358, 2832, 2946, 3279 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{29}N_2O_7$: 481.1976, found: 481.1969.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-*N*-(2-(2-hydroxyethoxy)ethyl)-2-(4-methoxyphenyl)acetamide (12c): Compound **12c** was obtained by opening **3** with 2-(2-aminoethoxy)ethanol according to the general procedure. Yield: 98%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=3.39–3.51 5 (m, 8H), 3.78 (s, 3H), 3.83 (s, 3H), 7.01 (d, J=9.2 Hz, 2H), 7.05 (d, J=9.2 Hz, 2H), 7.32 (d, J=9.2 Hz, 2H), 7.96 (d, J=9.2 Hz, 2H), 8.04 ppm (t, J=5.6 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=40.2, 55.1, 55.2, 60.2, 67.8, 72.0, 113.9, 114.1, 117.9, 122.1, 123.6, 128.2, 131.6, 150.4, 158.4, 159.6, 166.8, 168.4 ppm; IR (neat): $\tilde{\nu}$=457, 483, 524, 580, 697, 832, 915, 957, 1025, 1062, 1116, 1176, 1242, 1290, 1506, 1548, 1594, 1659, 1747, 2838, 2933, 3354 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{26}NO_8$: 456.1659, found: 456.1653.

2-(2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(4-methoxyphenyl)acetamido)acetic acid (12d): Compound **12d** was obtained by opening **3** with glycine according to the general procedure, and subsequent purification by column chromatography (CH_2Cl_2/MeOH/AcOH, 90:9:1). Yield: 74%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=3.77 (s, 3H), 3. 82 (s, 3H), 3.90 (d, J=6.0 Hz, 2H), 7.00 (d, J=9.2 Hz, 2H), 7.06 (d, J=9.2 Hz, 2H), 7.36 (d, J=9.2 Hz, 2H), 7.98 ppm (d, J=9.2 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=42.4, 55.1, 55.2, 113.8, 114.2, 116.9, 122.5, 123.8, 128.0, 131.2, 150.3, 158.3, 159.6, 167.1, 168.7, 169.8 ppm; IR (neat): $\tilde{\nu}$=541, 583, 651, 836, 953, 993, 1033, 1108, 1168, 1178, 1250, 1291, 1303, 1341,1417, 1454, 1484, 1505, 1547, 1589, 1658, 1715, 1750, 2839, 2934, 3290, 3397 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{20}NO_8$: 426.1178, found: 426.1183.

***N*-(2-(Dimethylamino)ethyl)-2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(4-methoxyphenyl)acetamide (12e)**: Compound **12e** was obtained by opening **3** with *N*,*N*-dimethylethylenediamine according to the general procedure. Yield: 92%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=2.97 (s, 3H), 2.98 (s, 3H), 3.29 (t, J=4.8 Hz, 2H), 3.58 (m, 2H), 3.72 (s, 3H), 3.78 (s, 3H), 6.85 (d, J=9.2 Hz, 2H), 7.57 (d, J=9.2 Hz, 2H), 8.19 (d, J=9.2 Hz, 2H), 8.70 (d, J=9.2 Hz, 2H), 10.87 ppm (br s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=33.9, 42.8, 54.8, 55.1, 57.1, 91.1, 112.1, 113.0, 113.8, 125.2, 126.1, 127.4, 129.8, 144.1, 155.5, 158.5, 168.3, 170.8, 174.7 ppm; IR (neat): $\tilde{\nu}$=428, 518, 592, 666, 768, 809, 833, 901.3, 978, 1031, 1102, 1146, 1181, 1245, 1271, 1289, 1508, 1562, 1603, 1632, 1719, 2710, 2836, 3040, 3217 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{24}H_{27}N_2O_6$: 439.1866, found: 439.1864.

***N*-(2-Aminoethyl)-2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(4-methoxyphenyl)acetamide (12f)**: Compound **12f** was obtained by opening **3** with ethylenediamine according to the general procedure. The compound was purified by trituration in CH_2Cl_2 and Et_2O. Yield: 74%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=3.01 (m, 2H), 3.48 (m, 2H), 3.72 (s, 3H), 3.78 (s, 3H), 6.81 (d, J=9.2 Hz, 2H), 6.98 (d, J=9.2 Hz, 2H), 7.58 (d, J=9.2 Hz, 2H), 8.10 (br s, 2H), 8.19 (d, J=9.2 Hz, 2H), 8.62 ppm (t, J=6.0 Hz, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=36.2, 54.8, 55.1, 90.1, 112.0, 112.9, 113.7, 125.2, 126.3, 127.8, 129.8, 144.3, 155.4, 158.4, 168.4, 171.1, 175.1 ppm; IR (neat): $\tilde{\nu}$=434, 522, 667, 682, 832, 929, 988, 1030, 1092, 1152, 1179, 1259, 1298, 1411, 1509, 1547, 1604, 1639, 1712, 2836, 2934, 3065, 3227, 3371, 3501 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{23}N_2O_6$: 411.1557, found: 411.1551.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-*N*-(2-hydroxyethyl)-2-(4-methoxyphenyl)acetamide (12g): Compound **12g** was obtained by opening **3** with 2-aminoethanol according to the general procedure. Yield: 61%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ=3.31 (q, J=6.0 Hz, 2H), 3.46 (t, J=6.0 Hz, 2H), 3.78 (s, 3H), 3.83 (s, 3H), 7.01 (d, J=9.2 Hz, 2H), 7.05 (d, J=9.2 Hz, 2H), 7.32 (d, J=9.2 Hz, 2H), 7.95–7.98 ppm (m, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ=43.1, 55.1, 55.2, 58.7, 113.8, 114.1, 117.8, 122.2, 123.6, 128.1, 131.6, 150.5, 158.4, 159.6, 166.9, 168.4 ppm; IR (neat): $\tilde{\nu}$=420, 443, 457, 496, 539, 581, 700, 750, 797, 831, 840, 959, 993, 1027, 1055, 1160, 1178, 1218, 1237, 1289, 1301, 1320, 1421, 1462, 1486, 1509, 1552, 1575, 1594, 1656, 1731, 2949, 3258, 3488 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{22}N_2O_7$: 412.1394, found: 412.1391.

(2*S*,3*S*,4*R*,5*S*,6*S*)-2-(Acetoxymethyl)-6-(2-(2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(4-methoxyphenyl)acetamido)ethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (12h): Compound **12h** was obtained by opening **3** with **16** (1.5 equiv) according to the general procedure (reaction run overnight). Yield: 99%; ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.99 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 3.48–3.78 (m, 5H), 3.83 (s, 3H), 3.88 (s, 3H), 4.03–4.14 (m, 2H), 4.46 (d, J=7.6 Hz, 1H), 4.84 (dd, J=7.6, 9.6 Hz, 1H), 4.99 (t, J=9.6 Hz, 1H), 5.16 (t, J=9.6 Hz, 1H), 6.46 (t, J=5.6 Hz, 1H), 6.94 (d, J=8.8 Hz, 2H), 7.03 (d, J=8.8 Hz, 2H), 7.24 (d, J=8.8 Hz, 2H), 8.11 (d, J=8.8 Hz, 2H), 15.70 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.6, 20.7, 20.8, 40.6, 55.4, 55.5, 62.1, 67.2, 68.4, 71.3, 72.1, 72.8, 100.5, 103.5, 113.9, 114.9, 116.8, 122.5, 123.8, 129.1, 131.6, 152.9, 159.2, 160.5, 160.6, 167.3, 169.2, 169.3, 169.5, 170.2, 170.5 ppm; MS (ESI): *m/z* (%): 650.21 (100%) $[M+H]^+$.

General procedure for the synthesis of compounds 13a, 13c and 13e–g: Bis-lactone **4** (200 mg, 0.8 mmol) was suspended in dry THF (10 mL) and cooled to −35 °C. TBAF in THF (1 M) was then added dropwise (1.6 mL, 2 equiv) and the solution was stirred for 15 min at −35 °C, and then cooled to −78 °C. A solution of amine (2.5 equiv) in dry THF (5 mL) was added dropwise, and the reaction was stirred for 15 min before warming to RT. EtOAc was added, and the solution was washed with a 1 M aq HCl and brine, dried ($MgSO_4$), filtered and concentrated. The crude was purified by column chromatography (CH_2Cl_2/MeOH, 100:0→90:10) to give **13a**, **13c** and **13e–g** (yield: 24–88%).

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-*N*-(2-morpholinoethyl)propanamide (13a): Compound **13a** was obtained by opening **4** with *N*-(2-*a*minoethyl)morpholine according to the general procedure. Yield: 88%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.07 (s, 3H), 2.64 (t, J = 4.2 Hz, 4H), 2.72 (t, J = 5.7 Hz, 2H), 3.47 (q, 5.7 Hz, 2H), 3.76 (t, J = 4.2 Hz, 4H), 3.80 (s, 3H), 6.88 (d, J = 9.0 Hz, 2H), 7.18 (t, J = 5.4 Hz, 1H), 8.04 ppm (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.7, 36.4, 53.2, 55.4, 55.9, 66.6, 66.9, 111.2, 113.9, 112.9, 128.8, 158.9, 161.6, 167.8, 168.3, 169.0 ppm; IR (neat): $\tilde{\nu}$ = 534, 587, 661, 841, 910, 1074, 1102, 1237, 1294, 1441, 1511, 1548, 1658, 2340, 2361, 3268 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{20}H_{25}N_2O_6$: 389.1707, found: 389.1692.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-*N*-(2-(2-hydroxyethoxy)ethyl)propanamide (13c): Compound **13c** was obtained by opening **4** with 2-(2-aminoethyl)ethanol according to the general procedure. Yield: 24%; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 2.12 (s, 3H), 3.44–3.60 (m, 9H), 3.77 (s, 3H), 7.00 (d, J = 9.2 Hz, 2H), 7.97 ppm (d, J = 9.2 Hz, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): 13.5, 40.4, 55.1, 60.2, 67.8, 72.1, 113.5, 113.8, 122.2, 128.0, 151.0, 158.3, 161.0, 166.6, 168.6 ppm; IR (neat): $\tilde{\nu}$ = 418, 457, 513, 554, 577, 633, 653, 698, 719, 745, 806, 833, 888, 910, 936, 1022, 1055, 1087, 1110, 1158, 1246, 1270, 1416, 1454, 1510, 1557, 1605, 1747, 2879, 2934, 3079, 3307, 3458 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{18}H_{22}NO_7$: 364.1389, found: 364.1391.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-*N*-(3-methoxypropyl)propanamide (13e): Compound **13e** was obtained by opening **4** with 3-methoxypropylamine according to the general procedure. Yield: 92%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.84 (m, 2H), 2.08 (s, 3H), 3.36 (s, 3H), 3.49 (q, J = 6.8 Hz, 2H), 3.56 (t, J = 6.8 Hz, 2H), 3.80 (s, 3H), 6.92 (d, J = 9.0 Hz, 2H), 7.73 (br s, 1H), 8.10 ppm (d, J = 9.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.3, 27.7, 40.8, 55.3, 59.1, 73.0, 102.2, 111.9, 113.7, 122.7, 128.7, 152.4, 158.8, 161.2, 167.6, 168.4 ppm; IR (neat): $\tilde{\nu}$ = 418, 464, 498, 514, 558, 585, 670, 831, 923, 1027, 1088, 1121, 1154, 1180, 1248, 1289, 1381, 1449, 1511, 1552, 1605, 1746, 2819, 2841, 2879, 2930, 3343 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{19}H_{25}N_2O_5$: 361.1758, found: 361.1764.

(2*S*,3*S*,4*R*,5*S*,6*S*)-2-(Acetoxymethyl)-6-(2-(2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)propanamido)ethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (13f): Compound **13f** was obtained by opening **4** with **16** (1.1 equiv) according to the general procedure. Yield: 57%; ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.98 (s, 3H); 2.00 (s, 3H), 2.01 (s, 3H), 2.04 (s, 3H), 2.14 (s, 3H), 3.5–3.8 (m, 9H), 4.0–4.2 (m, 3H), 4.51 (d, J = 7.8 Hz, 1H), 4.9–5.3 (m, 3H), 6.91 (d, J = 9.0 Hz, 2H), 8.06 ppm (d, J = 9.0 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.4, 20.6, 20.7, 40.5, 55.3, 61.8, 67.5, 68.2, 71.3, 72.2, 72.5, 100.8, 102.8, 111.5, 113.8, 122.5, 128.8, 152.8, 159.0, 160.5, 169.1, 169.5, 170.1, 170.6 ppm; HRMS (ESI): *m/z* $[M+Li]^+$ calcd for $C_{30}H_{35}LiNO_{15}$: 656.2162, found: 656.2153.

***tert*-Butyl 4-(2-(3-hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)propanoyl)piperazine-1-carboxylate (13g).** Compound **13g** is obtained by opening **4** with *N*-Boc-piperazine according to the general procedure. Yield: 88%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.47 (s, 9H), 2.13 (s, 3H), 3.51 (br s, 4H), 3.59 (br s, 4H), 3.81 (s, 3H), 6.93 (d, J = 8.9 Hz, 2H), 8.01 ppm (d, J = 8.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.2, 28.4, 43.9, 45.8, 55.3, 80.9, 104.6, 112.7, 113.9, 121.8, 129.1, 149.6, 154.4, 158.7, 159.3, 166.8, 171.4 ppm; IR (neat): $\tilde{\nu}$ = 538, 836, 1155, 1245, 1360, 1420, 1593, 1687, 1764, 2361, 2974 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_7$: 445.1969, found: 445.1966.

(2*S*,3*S*,4*R*,5*S*,6*S*)-2-(Acetoxymethyl)-6-(2-bromoethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (14): A solution of β-D-glucose pentaacetate (500 mg, 1.3 mmol) and 2-bromoethanol (91 μL, 1.3 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C, and $BF_3{\cdot}Et_2O$ (552 μL, 4.3 mmol) was added dropwise. The mixture was stirred for 30 min at 0 °C and then allowed to stand at RT overnight. The mixture was quenched with saturated aq $NaHCO_3$. After separation, the aqueous phase was extracted with CH_2Cl_2, and the combined organic phases were washed with water, dried ($MgSO_4$), filtered and concentrated. The crude was purified by column chromatography (cyclohexane/EtOAc, 80:20→70:30) to give **14** as a white solid (yield: 51%); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.00 (s, 3H), 2.02 (s, 3H), 2.06 (s, 3H), 2.08 (s, 3H), 3.44–3.48 (m, 2H), 3.69–3.73 (m, 1H), 3.79–3.85 (m, 1H), 4.12–4.16 (m, 2H), 4.23–4.27 (dd, J = 4.8, 12.3 Hz, 1H), 4.57 (d, J = 7.9 Hz, 1H), 4.98–5.10 (m, 2H), 5.21 ppm (t, J = 9.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.7, 21.0, 30.3, 61.7, 62.1, 68.6, 70.0, 71.3, 72.1, 72.8, 89.3, 101.2, 169.1, 169.2, 169.9, 170.4 ppm; MS (ESI): *m/z* (%): 455.05 $[M+H]^+$; 472.08 (50%) $[M+NH_4]^+$.

(2*S*,3*S*,4*R*,5*S*,6*S*)-2-(Acetoxymethyl)-6-(2-azidoethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (15): NaN_3 (860 mg, 13 mmol) was added to a solution of **14** (1 g, 2.2 mmol) in DMF (15 mL), and the solution was heated to 60 °C for 5 h. After cooling to RT, the solvent was partially removed in vacuo before water was added. The aqueous phase was extracted with EtOAc, and the combined organic phases were washed with a 1 M aq HCl, brine, and then dried ($MgSO_4$), filtered and concentrated to give **15** as a white solid (yield: quant); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.98 (s, 3H), 1.99 (s, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 3.24–3.29 (ddd, J = 3.5, 4.8, 13.4 Hz, 1H), 3.44–3.50 (ddd, J = 3.4, 8.3, 13.3 Hz, 1H), 3.64–3.72 (m, 2H), 3.98–4.03 (ddd, J = 3.5, 4.9, 0.7 Hz, 1H), 4.12–4.15 (dd, J = 2.5, 12.3 Hz, 1H), 4.21–4.25 (dd, J = 4.6, 12.3 Hz, 1H), 4.58 (d, J = 7.9 Hz, 1H), 4.97–5.02 (dd, J = 8.0, 9.7 Hz, 1H), 5.07 (t, J = 9.8 Hz, 1H), 5.19 ppm (t, J = 9.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.3, 20.4, 50.3, 61.6, 68.1, 68.3, 70.9, 71.6, 72.5, 100.4, 169.0, 169.1, 169.8, 170.2 ppm; MS (ESI): *m/z* (%): 418.15 $[M+H]^+$; 435.17 (96%) $[M+NH_4]^+$.

(2*S*,3*S*,4*R*,5*S*,6*S*)-2-(Acetoxymethyl)-6-(2-aminoethoxy)tetrahydro-2*H*-pyran-3,4,5-triyl triacetate (16): 10% Pd/C (20 mg) was added to a solution of **15** (200 mg, 0.5 mmol) in THF (7 mL). The suspension was then stirred under H_2 overnight. The suspension was filtered through celite and concentrated to give **16** as a white solid (yield: quant); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.98 (s, 3H), 2.00 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 3.56–3.62 (m, 1H), 3.67–3.72 (m, 1H), 3.84–3.89 (m, 1H), 4.12–4.16 (dd, J = 2.5, 12.3 Hz, 1H), 4.20–4.25 (dd, J = 4.8, 12.3 Hz, 1H), 4.52 (d, J = 7.9 Hz, 1H), 4.95–4.99 (dd, J = 8.0, 9.7 Hz, 1H), 5.06 (t, J = 9.8 Hz, 1H), 5.19 ppm (t, J = 9.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5, 20.6, 20.7, 20.8, 41.6, 61.9, 68.4, 71.3, 71.8, 72.3, 72.7, 100.9, 169.3, 169.4, 170.6 ppm; MS (ESI): *m/z* (%): 392.16 (100%) $[M+H]^+$.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-2-(4-methoxyphenyl)-*N*-(2-((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)ethyl)acetamide (12b): Sodium methoxide (16 mg, 3.1 equiv) was added to a solution of **12h** (70 mg, 0.09 mmol) in MeOH (3 mL), and the mixture was stirred overnight at RT. After addition of DOWEX 50WX8–200 cation-exchange resin, the mixture was filtered over celite and concentrated to give **12b** as an orange solid (yield: 94%); ^{1}H NMR (400 MHz, CD_3OD): δ = 3.11 (dd, J = 7.8, 9.3 Hz, 1H), 3.19–3.26 (m, 3H), 3.33 (t, J = 8.8 Hz, 2H), 3.50–3.63 (m, 3H), 3.68–3.76 (m, 1H), 3.81 (s, 3H), 3.86 (s, 3H), 3.89–3.95 (m, 1H), 4.24 (d, J = 7.8 Hz, 1H), 6.95 (d, J = 9.0 Hz, 2H), 7.04 (d, J = 8.8 Hz, 2H), 7.31 (d, J = 8.8 Hz,

2H), 8.05 ppm (d, $J=9.0$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=$ 41.2, 55.6, 55.7, 62.7, 69.4, 71.6, 75.2, 77.9, 94.6, 104.5, 114.2, 114.7, 115.4, 127.8, 127.9, 131.8, 147.3, 158.0, 160.7, 171.1, 175.7, 177.7 ppm; IR (neat): $\tilde{\nu}=$458, 524, 580, 698, 833, 915, 957, 1024, 1072, 1161, 1178, 1244, 1294, 1338, 1507, 1548, 1595, 1748, 2838, 3363 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{28}H_{32}NO_{12}$: 574.919, found: 574.1920.

2-(3-Hydroxy-4-(4-methoxyphenyl)-5-oxofuran-2(5*H*)-ylidene)-*N*-(2-((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)ethyl)propanamide (13b): **13b** was synthesized according to the procedure given for **12b**. Purification by preparative HPLC afforded **13b** as an orange solid (yield: 58%); ^{1}H NMR: (400 MHz, CD_3OD): $\delta=$2.18 (s, 3H), 3.19–3.24 (dd, $J=$7.8, 9.0 Hz, 1H), 3.28–3.33 (m, 3H), 3.37 (t, $J=$9.0 Hz, 1H), 3.52–3.59 (ddd, $J=$4.5, 6.9, 13.9 Hz, 1H), 3.64–3.71 (m, 2H), 3.81 (s, 3H), 3.86–3.89 (dd, $J=$1.6, 11.7 Hz, 1H), 4.00–4.04 (m, 1H), 4.32 (d, $J=$7.8 Hz, 1H), 6.93 (d, $J=$9.0 Hz, 2H), 8.04 ppm (d, $J=$9.0 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta=$13.6, 41.8, 55.7, 62.7, 69.0, 71.6, 75.1, 78.1, 103.1, 104.5, 114.6, 114.5, 123.8, 129.7, 152.7, 160.4, 162.1, 169.0, 170.4 ppm; IR (neat): $\tilde{\nu}=$458, 512, 581, 633, 699, 746, 833, 919, 1025, 1160, 1180, 1247, 1298, 1417, 1511, 1552, 1602, 1744, 2930, 3334 cm^{-1}; HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{28}NO_{11}$: 482.1670, found: 482.1657.

Biology

Cell line and culture: The human B-lymphoblastoid cell line TK6 was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). It was routinely cultured in RPMI 1640-Glutamax (Invitrogen; Cergy Pontoise, France) supplemented with 10% heat-inactivated fetal calf serum (Invitrogen), 1 mM sodium pyruvate, 1 mM nonessential amino acids and 50 μg gentamycin mL^{-1} (Dominique Dutscher; Brumath, France) Cultures were maintained in a humid atmosphere of 5% CO_2 at 37 °C.

Irradiation and treatment schedule: Irradiations were carried out at RT by 6 MV X-rays produced by a medical linear accelerator. Cells were contained in 25 cm^2 or 12.5 cm^2 culture flasks filled with 10 mL or 5 mL cell suspension, respectively. Cell concentrations were adjusted to 2×10^5 cells mL^{-1}. Dose rate was 0.8 Gy min^{-1}, and doses were 2–16 Gy, but most commonly 8 Gy.

Cell viability assay: Cell growth was determined using the alamar blue assay (UptiBlue, Interchim; Montluçon, France), according to the manufacturer's instructions. Flasks were centrifuged 24 h after irradiation, and the medium was replaced by an identical volume of fresh medium. Aliquots of the suspension (200 μL) were transferred into 96-well flat-bottomed microplates (Falcon F-3072). Five replicates were prepared for each concentration. At various time intervals, 20 μL of UptiBlue solution were then added to the wells. After incubation at 37 °C for 4 h, microplates were read at 590 nm (excitation at 560 nm), using a Perkin–Elmer Victor X2 2030–0020 microplate reader.

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