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Synthesis of diphenylcarbazoles as cytotoxic DNA binding agents

Ulrich Jacquemard,[a] Sylvain Routier,*[a] Arnaud Tatibouët,[a] Jérôme Kluza,[b] William Laine,[b] Christine Bal,[b] Christian Bailly *[b] and Jean-Yves Mérour *[a]

[a] *Institut de Chimie Organique et Analytique, UMR 6005, Université d'Orléans, B.P. 6759, 45067 Orléans Cedex 2, France. E-mail: sylvain.routier@univ-orleans.fr; Fax: 33 2 38.41 72.81; Tel: 33 2384 94592*

[b] *INSERM U-524 et Laboratoire de Pharmacologie Antitumorale du Centre Oscar Lambret, IRCL, 59045 Lille, France*

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We report the synthesis of a series of novel diphenylcarbazoles designed to interact with DNA. The compounds bearing two or three dimethylaminoalkyloxy side chains were found to bind much more tightly to DNA, preferentially at AT-rich sites, than the corresponding hydroxy compounds. The DNA binding compounds exhibit potent cytotoxic activity toward P388 leukemia cells. The 3,6-diphenylcarbazole thus represent an interesting scaffold to develop antitumor agents interacting with nucleic acids.

Introduction

Small molecules capable of interacting selectively with A–T base pairs within the minor groove of DNA have been extensively developed over the past ten years.[1] These include (i) oligopyrrole/imidazole carboxamide, derived from the antibiotics netropsin and distamycin, which may serve as molecular tools to control gene expression in cells,[2,3] (ii) diamidine unfused aromatic cations, typified by the promising antiparasitic drug furamidine (DB75, Fig. 1), which is currently developed in the form of an amidoxime prodrug (DB289) for the treatment of African trypanosomiasis and opportunistic infections,[4] and (iii) benzimidazole-containing minor groove binders, derived from the fluorescent dye Hoechst 33258, which may be useful as anticancer agents.[1]

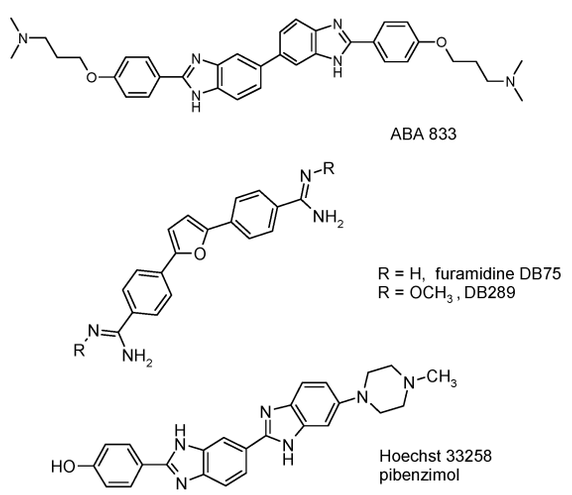

Fig. 1 Minor groove binders.

Hoechst 33258 has long been used as a DNA stain in cytometry experiments and in biology to study the condensation/decondensation of nucleic acids. In addition, this bis-benzimidazole derivative, also known as pibenzimol, has revealed potent cytotoxic activity and has undergone clinical trials as an anticancer drug.[5] The clinical responses were not sufficient to warrant a further development but nevertheless Hoechst 33258 remains an interesting scaffold from which potential anticancer drugs can be built. A promising development has been reported recently with the design by Mann *et al.*[6] of a new class of symmetric bisbenzimidazole-based minor groove binders showing *in vivo* antitumor activities.[6] The head-to-head bis-benzimidazole ABA833 (Fig. 1) has revealed strong cytotoxic activities against various cell lines *in vitro*, especially ovarian tumour cells sensitive or resistant to cisplatin and *in vivo* activity has been observed using the hollow fiber assay with ovarian carcinoma cells.[6]

More recently, a potent cytotoxic action against breast cancer cells has also been reported with this compound[7] which exploits water molecules to adapt its extended shape to that of the minor groove of DNA at AT-rich sequences.[8] This compound thus represents a novel promising model for the development of DNA-interacting anticancer agents. Bis-phenylbenzimidazole ABA833 and the diphenylfuran DB75 are built on the same model. They both contain a heterocycle "sandwiched" between two phenyl rings substituted with cationic side chains. This model can be exploited to design novel potential therapeutic agents. We decided to replace the benzimidazole system with a carbazole planar chromophore which may be better adapted to fit to the minor groove surface of DNA or to stack on DNA base pairs. The carbazole tricycle is frequently encountered in DNA binding agents, including intercalating topoisomerase II inhibitors (*e.g.* pyrrolocarbazoles,[9] pyridocarbazoles[10,11] and benzopyrimidocarbazoles[12]) topoisomerase I inhibitors (*e.g.* indolo carbazole),[13] and most importantly, in DNA minor groove binders such as the diamidinocarbazoles which have revealed interesting biological properties as antiparasitic drugs.[14]

Bisamidinocarbazole dications form tight DNA minor groove complexes and exhibit a pronounced selectivity for AT-rich sequences.[15–17] Based on these considerations, we therefore decided to synthesize a series of bis-phenylcarbazole derivatives substituted with different side chains, neutral or cationic, analogous to the dimethylaminoalkyl chains found in ABA833. Here we report the synthesis of a first series of bis substituted carbazoles obtained from 2,6-dibromocarbazole and we present preliminary data about the cytotoxic potential and DNA interacting capacity (Scheme 1).

Results

To complete the first step, it was reported that the commercially available 3,6-dibromocarbazole **1**, after *N*-protection, reacted in the presence of a base to perform a halogen–metal exchange. Addition of $B(OMe)_3$ or $Sn(CH_3)_3Cl$ also generates bis-boronic

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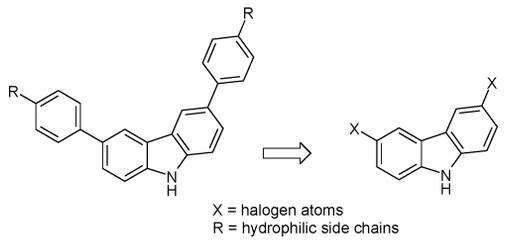


Scheme 1 Retrosynthetic scheme.

acid or bis-stannyl carbazoles[18] which were engaged in a Suzuki or a Stille cross coupling procedure, respectively. In addition, this synthesis reported a protection of the nitrogen carbazole by a tosyl group which was cleaved under strong basic conditions (*n*-BuLi, 9 eq.).

We first decided to introduce, on the nitrogen of **1**, a benzenesulfonyl group (Scheme 2), which could be easily removed under mild basic conditions and was also compatible with the presence of various reactive groups. Thus, compound **2** was obtained, using NaH as a base and benzenesulfonylchloride in dry THF at room temperature, in 86% yield. Halogen exchange, followed by the addition of $B(OMe)_3$ generated the bis boronic acid in 79% yield.[18] Unfortunately, despite numerous attempts (variation of temperature, reaction time, palladium catalyst entity, bases and solvents) the yield of the Suzuki reaction, between the bis-boronic acid and the 4-bromoanisole remained limited. Under our best conditions, using 4-bromoanisole (6 eq.) and an aqueous saturated solution of $NaHCO_3$ as a base, in a mixture of ethanol and toluene at reflux for 5 h, we obtained only 38% of the desired product **3**. A major drawback was the low solubility of compound **2**.

For these reasons, we continued to perform the coupling Suzuki procedure directly between the more soluble carbazole **1** and the 4-methoxyphenylboronic acid.[19] The described conditions involving $Pd(PPh_3)_4$ (40%), the boronic acid (2.4 eq.) and sodium hydrogen carbonate as the base, in a homogeneous mixture of DME/H_2O at 80 °C for 3 h afforded the desired compound **5** in 60% yield. Changing the solvent to dioxane and increasing the reaction time to 18 h gave a lower yield of 51%. Similar results were obtained by varying the nature of the base. Disappointed by these modest results, we tested biphasic conditions to perform this reaction. The use of a mixture of ethanol and toluene at reflux for 3 h resulted in a significant improvement of the synthesis with compound **5** now obtained in 97% yield using only 10% catalyst.[20] This considerable enhancement illustrates the versatility of cross coupling reactions. However, it is worth mentioning at this point that minor variations in the procedure can affect significantly the yield of the reaction. The protection of the free nitrogen atom was performed using NaH and benzenesulfonyl chloride in THF at room temperature to afford **3** in 91% yield. Demethylation was carried out on **3** and **5**, using boron tribromide, to obtain **4** and **6** in quantitative and 94% yields, respectively.[21]

The next step consisted of the introduction of various hydrophilic side chains (Fig. 2, Table 1). Three different side chains were chosen, in order to introduce hydrophilic functions spaced by 2 or 3 carbon atoms from the aromatic moieties (entries 1–6). First non-selective alkylation was realized on compound **6** with 2-chloroethyldimethylamine hydrochloride **7**, using an excess of caesium carbonate to afford trisubstituted compound **8**, in 49% yield, and disubstituted compound **13** in 34% yield. Decreasing the amounts of base and halogen derivative, or reaction time did not produce selective *O*-alkylation. This can be explained by the poor reactivity of halide **7** in nucleophilic substitutions. Decreasing the reaction time to a few hours, compound **6**, in the presence of more reactive halogen compounds such as 3-chloropropyldimethylamine hydrochloride **9** and 3-bromopropoxymethylbenzene **10**, leads to selective *O*-alkylation, although with limited yields. Compounds **11** and **12** were also obtained in 67% and 36% yields, respectively. Several other conditions were tested using the *N*-protected carbazole compound **4**.

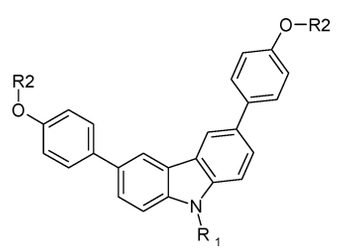
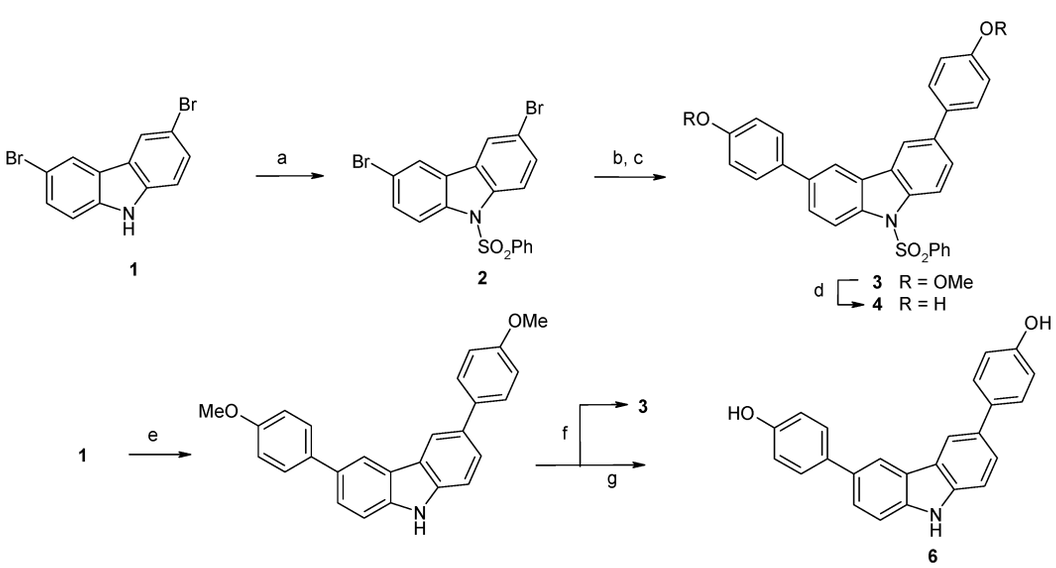


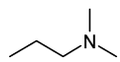
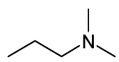
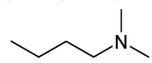
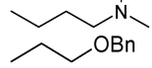
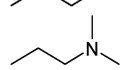
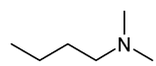
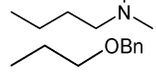
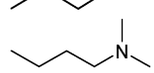
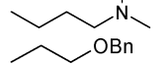
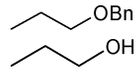
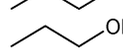
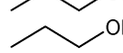
Fig. 2 Introduction of hydrophilic side chains (see Table 1).

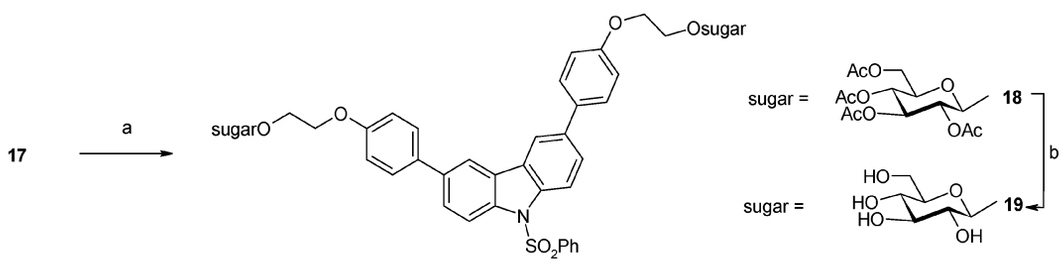
First, the reaction of compound **4** with compound **7** using K_2CO_3 as a base in DMF afforded, after the cleavage of the protecting group by Bu_4NF[22] (for an easier purification),



Scheme 2 **a**) NaH, THF, $PhSO_2Cl$, 0 °C to r.t., 12 h, 86%; **b**) *n*-BuLi (2.6 eq.), THF, −70 °C, 30 min, $B(OMe)_3$ (3 eq.), 30 min, then r.t., 12 h, 79%; **c**) $Pd(PPh_3)_4$ (10 mol%.), 4-bromoanisole (6.2 eq.), aq. sat. $NaHCO_3$, ethanol, toluene, reflux, 5 h, 38%; **d**) BBr_3 (2.3 eq.), CH_2Cl_2, 0 °C to r.t., 2 h, 97%; **e**) $Pd(PPh_3)_4$, 4-methoxyphenylboronic acid, aq. sat. $NaHCO_3$, toluene, reflux, 5 h, 97%; **f**) NaH, THF, $PhSO_2Cl$, 0 °C to r.t., 2 h, 91%; **g**) BBr_3 (2.3 eq.), CH_2Cl_2, 0 °C to r.t., 3 h, 94%.

Table 1 Alkylation procedure

Entry	Reactant	Conditions	Product (Yield)	R_1	R_2
1	**6**	**7** (6 eq.), Cs_2CO_3 (10 eq.), DMF, 100 °C, 12 h	**8** (49%)		
2	**6**	**9** (5 eq.), Cs_2CO_3 (10 eq.), DMF, 100 °C, 1 h	**11** (67%)	H	
3	**6**	**10** (5 eq.), Cs_2CO_3 (2.5 eq.), DMF, 100 °C, 1 h	**12** (36%)	H	
4	**4**	a) **7** (6 eq.), Cs_2CO_3 (11 eq.), DMF, 100 °C, 12 h b) Bu_4NF (1.2 eq.), THF, reflux, 2 h	**13** (70%)	H	
5	**4**	**9** (5 eq.), Cs_2CO_3 (10 eq.), DMF, 100 °C, 1 h	**14** (81%)	SO_2Ph	
6	**4**	**10** (5 eq.), Cs_2CO_3 (2.2 eq.), THF/DMF, reflux, 1 h	**15** (75%)	SO_2Ph	
7	**14**	Bu_4NF (1.2 eq.), THF, reflux, 2 h	**11** (80%)	H	
8	**15**	Bu_4NF (5.0 eq.), THF, reflux, 2 h	**12** (quant.)	H	
9	**12**	H_2, Pd(C) 10%, acetic acid/dioxane, r.t. 12 h	**16** (quant.)	H	
10	**15**	H_2, Pd(C) 10%, acetic acid/dioxane, r.t. 12 h	**17** (quant.)	SO_2Ph	
11	**17**	Bu_4NF (1.2 eq.), THF, reflux, 12 h	**16** (32%)	H	



Scheme 3 **a**) Penta-*O*-acetyl-β-D-glucopyranose, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å molecular sieves, r.t, 4 d, 56%; **b**) MeOH, MeONa, r.t, 2 h, quant.

compound **13** in only 35% yield. Replacing the base by Cs_2CO_3, followed by the deprotection increased the yield to 70% for the two steps. Under these conditions, treatment of **4** with halides **9** or **10** led to compounds **14** and **15** in 81 and 75% yields, respectively.

Cleavage of the benzenesulfonyl groups were always performed using Bu_4NF in refluxing THF,[22] to obtain compounds **11** and **12** from **14** and **15** in quantitative yields (entries 7 and 8). Catalytic hydrogenolysis of **12** and **15** afforded compounds **16** and **17** in quantitative yields (entries 9 and 10). Deprotection of benzenesulfonyl group of **17** with Bu_4NF led to **16** in only 32% yield (entry 11). Degradation of the starting material suggested also an incompatibility between the hydroxyalkyl group and the reagent.

Introduction of the glycosyl moiety was first attempted using compound **16** (Scheme 3), but no reaction was observed. Considering the possible implication of the free nitrogen atom of the carbazole ring in this result, protected carbazole **17** was used instead. Condensation of **17** and penta-*O*-acetyl-β-D-glucopyranose was undertaken with $BF_3{\cdot}Et_2O$ as the Lewis acid, and afforded in 56% yield glycosylated **18** which contains the expected β-D-glucoside moiety.[23] This was the only anomer isolated.

Deprotection of the acetates were performed under classical Zemplen conditions,[24] catalytic presence of MeONa in MeOH, leading to **19** in quantitative yield. Unfortunately, whatever the experimental conditions used, the deprotection of the benzenesulfonyl group of **19** always failed and we could never obtain the desired unsubstituted carbazole.

DNA interaction

The interaction of the newly synthesized compounds with DNA was probed by absorption spectroscopy to measure the capacity of the molecules to stabilize the double helix structure of the synthetic polynucleotide poly(dAT)$_2$. This alternating polymer melts at a much lower temperature than calf thymus DNA (41 °C *vs* 62 °C) and thus offers a more sensitive assay to compare small molecules, especially those targeted to AT-rich sequences, as it is the case here. Indeed, DNase I footprinting experiments have indicated that some of these compounds (**8** and **13** in particular) bind preferentially to AT-rich sequences (data not shown). For each molecule, we measured the difference in melting temperature for the drug–DNA complexes and the unbound DNA ($\Delta T\mathrm{m} = T\mathrm{m}^{\mathrm{complex}} - T\mathrm{m}^{\mathrm{DNA}}$). All compounds were tested at a fixed drug/DNA-nucleotide ratio of 0.1 with a heating rate of 1 °C min^{-1}. A typical example of melting curves obtained with poly(dAT)$_2$ and four compounds **6**, **8**, **13** and **11** is presented in Fig. 3 and Table 2.

It was clear that compound **6** had no effect on the thermal stability of the DNA whereas the other compounds strongly

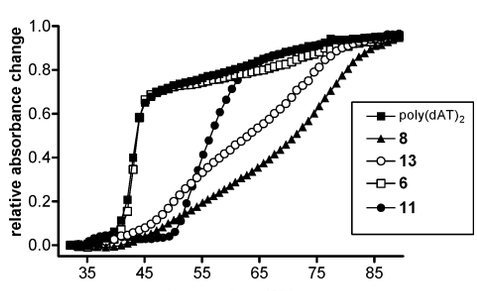


Fig. 3 Melting temperature variation $\Delta T\mathrm{m}$ ($T\mathrm{m}^{\mathrm{drug\text{–}DNA\ complex}} - T\mathrm{m}^{\mathrm{DNA\ alone}}$, in °C) of poly(dAT)$_2$ after incubation with the diphenylcarbazoles. The Tm measurements were performed at a constant drug/DNA-phosphate ratio of 10 (2 μM drug, 20 μM DNA-nucleotide). Tm measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA), in 1 cm quartz cuvettes at 260 nm with a heating rate of 1 °C min^{-1}. The Tm values were obtained from first-derivative plots.

Table 2 Binding to DNA and cytotoxicity

Compound	ΔTm/°C[a]	IC_{50}/μM[b]
6	0	7.23 ± 0.07
8	8.3–33.4	4.15 ± 0.05
11	13.2	0.77 ± 0.04
13	6.0–29.5	1.53 ± 0.27
16	2.2	1.83 ± 0.27
17	0	9.7 ± 0.5
19	0	>50

[a] Tm measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) using 20 μM drug and 20 μM poly(dAT)$_2$ (nucleotide concentration) with a heating rate of 1 °C min^{-1}. [b] Drug concentration (μM) that inhibits P388 leukemia cell growth by 50% after incubation in liquid medium for 72 h (n = 2–4).

stabilize the duplex structure of the polymer so as to increase considerably its melting temperature. Monophasic melting curves were obtained with the dimethylaminopropyloxy derivative **11** but biphasic curves were observed with the two ethyloxy analogues **8** and **13**, possibly due to a heterogeneous binding mode. A detailed analysis of the binding mode and sequence selectivity will be reported separately.[25] For each molecule, the melting temperature was determined from the first derivative analysis and the ΔTm data are collated in Table 2. In the case of biphasic melting curve, the Tm of the two transitions are indicated. The DNA binding capacity varies significantly from one drug to another with, as expected, the cationic dimethylaminoalkyloxy compounds being much more potent than the uncharged analogues. The hydroxy compounds **6** and **16** show very little interaction with DNA and similarly, the two carbazoles **19** and **17** (bearing a *N*-substituted benzenesulfonyl group) also failed to stabilize the duplex structure of DNA. The incorporation of a dimethylaminoalkyloxy chain thus appears as a profitable scheme to target DNA with these diphenylcarbazoles.

Cytotoxicity

A tetrazolium-based assay was applied to determine the drug concentration required to inhibit the growth of murine P388 leukemia cells by 50% after incubation in the culture medium for 72 h. The IC_{50} values are collated in Table 2. Interestingly, the dimethylamino compounds **8**, **11** and **13** which bind strongly to DNA proved to be significantly more cytotoxic than the uncharged hydroxy derivative **6** and the phenyl sulfonyl substituted compounds **17** and **19**. The benzenesulfonyl protecting group, which unfortunately could not be removed, is clearly detrimental to both DNA interaction and cytotoxicity. This may be an indication that affinity for DNA contributes to the cytotoxicity and perhaps also that the free NH of the carbazole ring is necessary for DNA interactions. Although, there is not a simple correlation between the extent of DNA binding and the cytotoxic action of the compounds, it seems clear that DNA binding is necessary to inhibit cell proliferation. The uncharged molecule **16** is equally toxic to the cationic derivative **13** despite its poor capacity to stabilize duplex DNA against heat denaturation. However, the most active compound in the series is the propyloxy derivative **11** which is a potent DNA binder. A single parameter, such as the ΔTm, is often insufficient to account for a complex biological process. Uptake, distribution, metabolism and other biological parameters can vary among the different compounds, especially when comparing compounds with different size and hyphobic/hydrophilic characters, as is the case here. Nevertheless, this preliminary screening indicates that some of the diphenylcarbazole compounds exhibit an interesting cytotoxic profile, with IC_{50} values in the low μM range.

Table 3 Drug-induced apoptosis (sub-G_1 cells, %)[a]

Concentration/μM	1		5	
Time/h	24	48	24	48
6	3	2.3	7.7	14.6
8	3.5	23.7	12.6	25.2
11	20	34.3	42.9	75.8
13	17.8	43.6	30.4	57.1
16	6.8	26.6	16.6	37.1
17	2.4	2.6	5.2	10.2
19	2.5	2.3	2.8	4.7

[a] Cells (%) in the sub-G_1 phase, as determined by flow cytometry.

Cell cycle effects and apoptosis

Treatment of the P388 cells with the different compounds at 1 or 5 μM for 24 h or 48 h led, in most cases, to profound changes of the cell cycle profiles. The flow cytometry analysis of propidium iodide-labeled cells indicates that the most cytotoxic drugs **8**, **11** and **13** induce a decrease of the number of cells in the S phase and a concomitant marked accumulation of cells in the sub-G1 phase (Fig. 4).

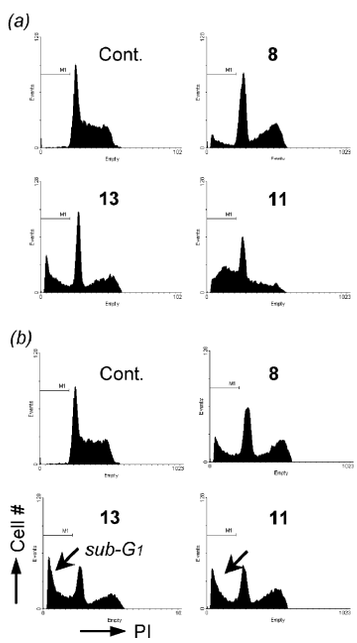


Fig. 4 Cell cycle distribution determined by flow cytometry in P388 cells treated for (a) 24 h or (b) 48 h with the indicated compound at (a) 5 μM or (b) 1 μM. Cells were stained with propidium iodide (PI) and analysed with a FACScan flow cytometer. In each case, the percentage of cells in the sub-G_1 phase was calculated (Table 3).

The cell cycle perturbations are dose- and time-dependent (Table 3). The sub-G_1 population, with a characteristic hypo-diploid DNA content, reflects apoptotic cells with fragmented DNA. Up to 75% of the cells undergo apoptosis with **11** after 48 h of treatment at 1 μM. Interestingly, there is a correlation between the extent of drug-induced apoptosis and the level of cytotoxicity. All these biological data augur well for a more extended *in vitro* and *in vivo* evaluation of compounds **11** and **13** which is currently in progress.

Experimental

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DPX 250 instrument using $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in ppm (δ scale) and all coupling constants (J) values are in hertz (Hz). The splitting patterns are designated as follows: s (singlet), d (doublet), t (triplet), q (quartet),

m (multiplet), and dd (doublet doublet). Melting points are uncorrected. IR absorption spectra were obtained on a Perkin Elmer PARAGON 1000 PC and values were reported in cm^{-1}. MS spectra (ion spray) were performed on a Perkin Elmer Sciex PI 300. Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F254). Spots were visualized by UV light at 254 nm and 356 nm. Column chromatography was performed using silica gel 60 (0.063–0.200 mm, Merck).

9-Benzenesulfonyl-3,6-dibromo-9*H*-carbazole (2)

A solution of 3,6-dibromocarbazole **1** (1 g, 3.07 mmol) in dry THF (25 mL) was cooled to 0 °C, under argon, then NaH 60% dispersion in oil (184 mg, 4.60 mmol) was added portionwise and the solution was stirred for 1 h. Benzenesulfonylchloride (0.590 mL, 4.60 mmol) was added, followed after 12 h at room temperature, by a saturated solution of NH_4Cl (30 mL). The mixture was extracted with CH_2Cl_2 (3 × 75 mL) and the combined organic layers were successively washed with a saturated solution of K_2CO_3 (30 mL), water (30 mL) then dried over $MgSO_4$. After filtration, the solvents were removed under reduced pressure to afford compound **2** as a white solid (1.230 g, 86%). *R*f (petroleum ether/ethyl acetate 96/4): 0.43; mp > 250 °C; IR (KBr) ν (cm^{-1}) 1465, 1425, 1372, 1208, 1182, 1125, 815, 729, 573; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 7.52 (t, 2H, J = 7.5 Hz), 7.67 (t, 1H, J = 7.5 Hz), 7.76 (dd, 2H, J = 2.0, 8.7 Hz), 7.86 (d, 2H, J = 7.5 Hz), 8.19 (d, 2H, J = 8.7 Hz), 8.52 (d, 2H, J = 2 Hz);^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 116.5 (2 × CH), 117.2 (2 × Cq), 124.2 (2 × CH), 126.3 (2 × CH), 126.7 (2 × Cq), 130.0 (2 × CH), 131.2 (2 × CH), 135.2 (CH), 136.1 (Cq), 136.7 (2 × Cq); MS (IS) : 464 (M + 1)$^+$; Anal. calcd for $C_{18}H_{11}Br_2NO_2S$: C, 46.48; H, 2.38; N, 3.01. Found: C, 46.85; H, 2.51; N, 2.83%.

9-Benzenesulfonyl-3,6-bis(4-methoxyphenyl)-9*H*-carbazole (3)

A solution of compound **5** (670 mg, 1.77 mmol) in dry THF (15 mL) was cooled to 0 °C under argon then NaH (60% dispersion in oil, 85 mg, 2.12 mmol) was added portionwise and the solution was stirred for 30 min. Benzenesulfonylchloride (468 mg, 2.65 mmol) was added dropwise, followed after 1 h at room temperature, by a saturated solution of NH_4Cl (30 mL). The mixture was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were successively washed with a saturated solution of K_2CO_3 (20 mL), water (20 mL) then dried over $MgSO_4$. After filtration, the solvents were removed under reduced pressure. Purification by flash chromatography (ether petroleum/ethyl acetate 9/1 to 1/1) afforded compound **3** as a pale yellow solid (0.84 g, 91%). *R*f (ether petroleum/ethyl acetate 8/2): 0.36; mp 160 °C; IR (KBr) ν (cm^{-1}) 1608, 1518, 1481, 1449, 1369, 1247, 1209, 1173, 819; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 3.86 (s, 6H), 7.00 (dd, 4H, J = 2.2, 6.5 Hz), 7.34 (t, 2H, J = 7.8 Hz), 7.45 (dd, 1H, J = 1.2, 7.3 Hz), 7.60 (dd, 4H, J = 2.2, 6.6 Hz), 7.68 (dd, 2H, J = 1.7, 8.8 Hz), 7.86 (d, 2H, J = 7.4 Hz), 8.08 (d, 2H, J = 1.7 Hz), 8.35 (d, 2H, J = 8.8 Hz); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 55.5 (2 × CH_3), 114.5 (4 × CH), 115.5 (2 × CH), 118.1 (2 × CH), 126.7 (4 × CH), 127.2 (2 × Cq), 128.4 (4 × CH), 129.2 (2 × CH), 133.5 (2 × Cq), 134.0 (CH), 137.3 (2 × Cq), 137.9 (2 × Cq), 138.0 (Cq), 159.3 (2 × Cq); MS (IS) : 520 (M + 1)$^+$; Anal. calcd for $C_{32}H_{25}NO_4S$: C, 73.97; H, 4.85; N, 2.70. Found: C, 74.38; H, 5.00; N, 2.84%.

9-Benzenesulfonyl-3,6-bis(4-hydroxyphenyl)-9*H*-carbazole (4)

To a solution of compound **3** (840 mg, 1.62 mmol) in CH_2Cl_2 (20 mL) at 0 °C, BBr_3 (6.50 mL, 1 M in CH_2Cl_2, 6.50 mmol) was added dropwise. After 2 h at room temperature, the reaction mixture was poured into ice (100 g), extracted with EtOAc (2 × 50 mL) then dried over $MgSO_4$, and filtered. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/ethyl acetate 1/1) to afford compound **4** as a white solid (565 mg, 97%). *R*f (petroleum ether/ethyl acetate 1/1): 0.53; mp 140 °C; IR (KBr) ν (cm^{-1}) 3418, 1610, 1518, 1481, 1447, 1364, 1169, 818; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 6.90 (d, 4H, J = 8.6 Hz), 7.49 (d, 2H, J = 8.1 Hz), 7.52 (s, 2H), 7.58–7.66 (m, 5H), 7.81 (dd, 2H, J = 1.7, 8.8 Hz), 7.87 (dd, 2H, J = 1.5, 8.8 Hz), 8.26 (d, 2H, J = 8.6 Hz), 9.60 (s, 2H); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 114.9 (2 × CH), 115.8 (4 × CH), 118.2 (2 × CH), 125.9 (2 × CH), 126.2 (2 × CH), 126.8 (2 × Cq), 128.0 (4 × CH), 129.8 (2 × CH), 130.3 (2 × Cq), 134.7 (CH), 136.6 (2 × Cq), 136.7 (2 × Cq), 139.2 (Cq), 157.2 (2 × Cq); MS (IS): 492 (M + 1)$^+$; Anal. calcd for $C_{30}H_{21}NO_4S$: C, 73.30; H, 4.31; N, 2.85. Found: C, 73.02; H, 4.48; N, 2.99%.

3,6-Bis(4-methoxyphenyl)-9*H*-carbazole (5)

A solution of 3,6-dibromocarbazole **1** (500 mg, 1.53 mmol) and 4-methoxyphenylboronic acid (600 mg, 3.92 mmol) in a mixture of toluene (31.6 mL), ethanol (19.2 mL) and aqueous saturated $NaHCO_3$ solution (12.6 mL) was degassed by argon bubbling for 20 min. $Pd(PPh_3)_4$ (10 mol%, 0.153 mmol) was added and the mixture was immediately transfered to a pre-heated oil bath and refluxed for 5 h. After hydrolysis (50 mL), the mixture was extracted with ethyl acetate (2 × 50 mL), washed with brine (50 mL), dried over $MgSO_4$, then filtered. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/ethyl acetate 8/2 to ethyl acetate) to afford compound **5** as a white solid (565 mg, 97%). *R*f (petroleum ether/ethyl acetate 8/2): 0.19; mp 198 °C; IR (KBr) ν (cm^{-1}) 3428, 1607, 2980, 1514, 1482, 1455 1274, 1245, 1036, 820; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 3.81 (s, 6H), 7.05 (d, 4H, J = 8.6 Hz), 7.52 (d, 2H, J = 8.6 Hz), 7.68 (m, 6H), 8.48 (s, 2H), 11.27 (s, NH);^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 55.1 (2 × CH_3), 111.3 (2 × CH), 114.3 (4 × CH), 118.0 (2 × CH), 123.3 (2 × Cq), 124.4 (2 × CH), 127.6 (4 × CH), 130.8 (2 × Cq), 133.8 (2 × Cq), 139.4 (2 × Cq), 158.2 (2 × Cq); MS (IS) : 380 (M + 1)$^+$; Anal. calcd for $C_{26}H_{21}NO_2$: C, 82.3; H, 5.58; N, 3.69. Found: C, 82.57; H, 5.71; N, 3.54%.

3,6-Bis(4-hydroxyphenyl)-9*H*-carbazole (6)

Same procedure as described for compound **5**. To a solution of compound **4** (519 mg, 1.37 mmol) in CH_2Cl_2 at 0 °C, BBr_3 (2.76 mL, 1 M in CH_2Cl_2, 2.76 mmol) was added dropwise. After 3 h at room temperature, the reaction mixture was poured into ice (100 g), extracted with ethyl acetate (2 × 50 mL), dried with $MgSO_4$, then filtered. The solvents were removed under reduced pressure and the crude material was purified by flash chromatography (petroleum ether/ethyl acetate 1/1) to afford compound **6** as a white solid (500 mg, 94%). *R*f (ether petroleum/ethyl acetate 1/1): 0.32; mp > 240 °C; IR (KBr) ν (cm^{-1}) 3514, 3422, 1610, 1517, 1484, 1272, 1205, 819; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 6.90 (d, 4H, J = 8.6 Hz), 7.49–7.64 (m, 8H), 8.44 (s, 2H), 9.43 (s, 2H), 11.22 (s, NH); ^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 111.2 (2 × CH), 115.7 (4 × CH), 117.7 (2 × CH), 123.3 (2 × Cq), 124.2 (2 × CH), 127.6 (4 × CH), 131.2 (2 × Cq), 132.2 (2 × Cq), 139.2 (2 × Cq), 156.3 (2 × Cq); MS (IS) : 352 (M + 1)$^+$; Anal. calcd for $C_{24}H_{17}NO_2$: C, 82.03; H, 4.88; N, 3.99. Found: C, 73.02; H, 4.72; N, 4.14%.

3,6,9-Tris[4-(2-dimethylaminoethoxy)phenyl]carbazole (8)

Cs_2CO_3 (280 mg, 0.86 mmol) was added to a solution of compound **6** (100 mg, 0.28 mmol) in DMF (10 mL) under argon at room temperature. At the same time, a solution of 2-chloroethyldimethylamine hydrochloride **7** (248 mg, 1.72 mmol) in DMF (10 mL) was stirred in the presence of Cs_2CO_3 (746 mg, 2.29 mmol). After 30 min, this solution was added to the solution containing **6**. The mixture was warmed up to 100 °C for 12 h. Hydrolysis was performed with water (10 mL), and the mixture was concentrated under reduced pressure. The crude residue was further purified by flash chromatography

(THF/MeOH 1/1) to afford compound **8** as a white hygroscopic solid (80 mg, 49%) and compound **13** as a brown hygroscopic solid (49 mg, 34%). Compound **8**, *R*f (THF/MeOH 1/1): 0.36; IR (KBr) ν (cm^{-1}) 1654, 1483, 1233, 1109, 805; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 2.46 (s, 6H), 2.62 (s, 12H), 3.18–3.23 (m, 4H), 4.10–4.60 (m, 8H), 7.09 (d, 4H, J = 8.3 Hz), 7.72–7.77 (m, 8H), 8.54 (s, 2H); ^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 43.9 (4 × CH_3), 44.5 (2 × CH_3), 56.3 (3 × CH_2), 63.8 (3 × CH_2), 109.9 (2 × CH), 115.1 (4 × CH), 118.2 (2 × CH), 123.1 (2 × CH), 124.6 (2 × Cq), 127.7 (4 × CH), 131.1 (2 × Cq), 134.0 (2 × Cq), 139.6 (2 × Cq), 157.0 (2 × Cq) MS (IS): 565 (M + 1)$^+$; Anal. calcd for $C_{36}H_{44}N_4O_2$: C, 76.56; H, 7.85; N, 9.92. Found: C, 76.29; H, 7.98; N, 10.04%.

3,6-Bis[4-(3-dimethylaminopropoxy)phenyl]-9*H*-carbazole (11)

To a solution of **14** (70 mg, 0.106 mmol) in dry THF (10 mL), Bu_4NF (0.53 mL, 1 M in THF, 0.53 mmol) was added. The solution was refluxed with stirring for 2 h. The reaction mixture was concentrated under reduced pressure. Water was added (2 × 10 mL) and the suspension was filtered off. The crude solid was purified by flash chromatography (dichloromethane/MeOH/triethyl amine 1/1/0.01) to afford compound **11** as a white solid (44 mg, 80%). *R*f (dichloromethane/MeOH/triethyl amine 1 /1/0.01): 0.22; mp 175 °C; IR (KBr) ν (cm^{-1}) 3436, 1608, 1515, 1483, 1458, 1271, 1232, 812, 799; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 2.00 (q, 4H, J = 6.5 Hz), 2.29 (s, 12H), 2.50 (t, 4H, J = 7.0 Hz), 6.98 (d, 4H, J = 7.0 Hz), 7.40 (d, 2H, J = 8.3 Hz), 7.59 (d, 6H, J = 8.8 Hz), 8.26 (s, 2H), 8.65 (s, 1H); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 27.7 (2 × CH_2), 45.6 (4 × CH_3), 56.6 (2 × CH_2), 66.4 (2 × CH_2), 111.0 (2 × CH), 114.9 (4 × CH), 118.4 (2 × CH), 124.1 (2 × Cq), 125.3 (2 × CH), 128.3 (4 × CH), 132.7 (2 × Cq), 134.7 (2 × Cq), 139.3 (2 × Cq), 158.1 (2 × Cq); MS (IS): 522 (M + 1)$^+$; Anal. calcd for $C_{34}H_{39}N_3O_2$: C, 78.28; H, 7.54; N, 8.05. Found: C, 77.90; H, 7.63; N, 8.12%.

3,6-Bis[4-(2-benzyloxyethoxy)phenyl]-9*H*-carbazole (12)

Same procedure as described for compound **11**. Bu_4NF (3.29 mL, 1 M in THF, 3.29 mmol) was added to a solution of compound **15** (500 mg, 0.65 mmol) in dry THF (20 mL). The sample was refluxed with stirring for 2 h prior to concentrating the reaction mixture under reduced pressure. Water was added (2 × 10 mL) and the suspension was filtered off. The crude solid was purified by flash chromatography (petroleum ether/ethyl acetate 7/3) to afford compound **12** (407 mg, quant.). *R*f (petroleum ether/ethyl acetate 8/2): 0.23; mp 124 °C; IR (KBr) ν (cm^{-1}) 3426, 1628, 1516, 1483, 1452, 1271, 1234, 1123, 739, 696; ^{1}H-NMR ($CDCl_3$): δ 3.87 (t, 4H, J = 4.4 Hz), 4.22 (t, 4H, J = 4.6 Hz), 4.66 (s, 4H), 7.03 (d, 4H, J = 8.8 Hz), 7.32–7.47 (m, 12H), 7.62 (d, 6H, J = 8.8 Hz), 8.07 (s, 1H), 8.26 (s, 2H); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 67.7 (2 × CH_2), 68.7 (2 × CH_2), 73.5 (2 × CH_2), 111.0 (2 × CH), 115.1 (4 × CH), 118.5 (2 × CH), 124.1 (2 × Cq), 125.4 (2 × CH), 127.9 (2 × CH), 127.9 (4 × CH), 128.3 (4 × CH), 128.6 (4 × CH), 132.7 (2 × Cq), 135.0 (2 × Cq), 138.2 (2 × Cq), 139.2 (2 × Cq), 157.9 (2 × Cq); MS (IS): 620 (M + 1)$^+$; Anal. calcd for $C_{42}H_{37}NO_4$: C, 81.40; H, 6.02; N, 2.26. Found: C,81.77; H,6.12; N,2.40%.

3,6-Bis[4-(2-dimethylaminoethoxy)phenyl]-9*H*-carbazole (13)

Same procedure as described for **8**. To a solution of compound **4** (100 mg, 0.20 mmol) in DMF (10 mL) under argon was added at room temperature Cs_2CO_3 (725 mg, 3.21 mmol). At the same time, a solution of 2-chloroethyldimethylamine hydrochloride **7** (175 mg, 1.21 mmol) in DMF (10 mL) was stirred in the presence of Cs_2CO_3 (746 mg, 2.29 mmol). After 30 min, this solution was added to the solution containing **4**. The mixture was warmed up to 100 °C for 12 h. After cooling, the reaction mixture was concentrated under reduced pressure, then THF (10 mL) and Bu_4NF (0.24 mL, 1 M in THF, 0.24 mmol) was added. The solution was stirred to reflux for 2 h. The reaction mixture was concentrated under reduced pressure. Water was added (2 × 10 mL) and the suspension was filtered off. The crude solid was purified by flash chromatography (ethyl acetate/MeOH 1/1) to afford compound **13** as a brown hygroscopic solid (70 mg, 70%). *R*f (ethyl acetate/MeOH 1/1): 0.54; IR (KBr) ν (cm^{-1}) 3408, 1618, 1517, 1483, 1271, 1037, 805; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 2.15 (s, 12H), 3.31–3.32 (m, 4H), 3.99–4.03 (m, 4H), 6.96 (d, 4H, J = 7.8 Hz), 7.52–7.64 (m, 9H), 8.44 (s, 2H); ^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 45.6 (4 × CH_3), 57.7 (2 × CH_2), 65.9 (2 × CH_2), 109.7 (2 × CH), 114.9 (4 × CH), 118.1 (2 × CH), 123.0 (2 × Cq), 124.4 (2 × CH), 127.6 (4 × CH), 130.9 (2 × Cq), 133.5 (2 × Cq), 139.6 (2 × Cq), 157.5 (2 × Cq); MS (IS): 494 (M + 1)$^+$; Anal. calcd for $C_{32}H_{35}N_3O_2$: C, 77.86; H, 7.15; N, 8.51. Found: C, 77.53; H, 7.29; N, 8.65%.

9-Benzenesulfonyl-3,6-bis[4-(3-dimethylaminopropoxy)phenyl]-9*H*-carbazole (14)

Same procedure as described for **8**. Cs_2CO_3 (498 mg, 1.53 mmol) was added to a solution of compound **4** (150 mg, 0.30 mmol) in DMF (10 mL) under argon at room temperature. In parallel, a solution of 3-chloropropyldimethylamine hydrochloride **9** (241 mg, 1.53 mmol) in DMF (10 mL) was stirred in the presence of Cs_2CO_3 (746 mg, 2.29 mmol). After 30 min, this solution was added to the solution containing **4**. The mixture was warmed up to 100 °C for 1 h. After cooling, water was added and the mixture was extracted successively with ethyl acetate (2 × 25 mL) and CH_2Cl_2 (2 × 25 mL). The combined organic layers were dried with $MgSO_4$, filtered off and concentrated under reduced pressure. Compound **14** was obtained as a brown oil (164 mg, 81%). *R*f (ethyl acetate/MeOH/triethylamine 1/1/0.01): 0.22; IR (KBr) ν (cm^{-1}) 1608, 1518, 1481, 1449, 1265, 1174, 821, 738; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 2.02 (q, 4H, J = 6.3 Hz), 2.32 (s, 12H), 2.55 (t, 4H, J = 6.4 Hz), 4.07 (t, 4H, J = 6.4 Hz), 6.90 (d, 4H, J = 8.6 Hz), 7.34 (t, 2H, J = 7.7 Hz), 7.46–7.70 (m, 7H), 7.85 (d, 2H, J = 7.1 Hz), 8.07 (s, 2H), 8.34 (d, 2H, J = 8.7 Hz); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 27.4 (2 × CH_2), 45.4 (4 × CH_3), 56.5 (2 × CH_2), 66.3 (2 × CH_2), 115.0 (4 × CH), 115.5 (2 × CH), 118.0 (2 × CH), 126.6 (4 × CH), 127.2 (2 × Cq), 128.4 (4 × CH), 129.2 (2 × CH), 133.4 (2 × Cq), 134.0 (1 × CH), 137.2 (2 × Cq), 137.8 (2 × Cq), 137.9 (1 × Cq), 158.7 (2 × Cq); MS (IS): 662 (M + 1)$^+$; Anal. calcd for $C_{40}H_{43}N_3O_4S$: C, 72.59; H, 6.55; N, 6.35. Found: C, 72.98; H, 6.47; N, 6.44%.

9-Benzenesulfonyl-3,6-bis[4-(2-benzyloxyethoxy)phenyl]-9*H*-carbazole (15)

A solution of **4** (530 mg, 1.08 mmol) and 3-bromopropoxymethylbenzene (1.16 g, 5.40 mmol) in a mixture of THF (20 mL) and DMF (10 mL) was stirred under argon at room temperature. Cs_2CO_3 (498 mg, 1.53 mmol) was added and the mixture was warmed up to reflux. After 1 h, the solvents were removed under reduced pressure and the crude solid was purified by flash chromatography (petroleum ether/ethyl acetate 7/3 to 4/6) to afford compound **15** as a white solid (615 mg, 75%). *R*f (ethyl acetate/petroleum ether 3/7): 0.55; mp 116 °C; IR (KBr) ν (cm^{-1}) 1654, 1608, 1482, 1448, 1371, 1235, 1171, 1129, 824, 735, 591; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 3.85 (t, 4H, J = 5.1 Hz), 4.19 (t, 4H, J = 4.4 Hz), 4.65 (s, 4H), 7.01 (d, 4H, J = 8.8 Hz), 7.32–7.37 (m, 13H), 7.58 (d, 4H, J = 8.5 Hz), 7.67 (d, 2H, J = 6.8 Hz), 7.85 (d, 2H, J = 8.5 Hz), 8.07 (s, 2H), 8.34 (d, 2H, J = 6.8 Hz); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 67.6 (2 × CH_2), 68.6 (2 × CH_2), 73.5 (2 × CH_2), 115.2 (4 × CH), 115.5 (2 × CH), 118.0 (2 × CH), 126.6 (4 × CH), 127.2 (2 × Cq), 127.9 (2 × CH), 127.9 (4 × CH), 128.3 (4 × CH), 128.6 (4 × CH), 129.2 (2 × CH), 133.6 (2 × Cq), 133.9 (CH), 137.2 (2 × Cq), 137.8 (2 × Cq), 137.9 (Cq), 138.1 (2 × Cq), 158.5 (2 × Cq); MS (IS): 760 (M + 1)$^+$; Anal. calcd for $C_{48}H_{41}NO_6S$: C, 75.87; H, 5.44; N, 1.84. Found: C,75.50; H,5.62; N,1.66%.

3,6-Bis[4-(2-hydroxyethoxy)phenyl]-9*H*-carbazole (16)

A solution of compound **12** (164 mg, 0.26 mmol) and acetic acid (36 μL) in dioxane (10 mL) was stirred at room temperature. Pd(C) 10% (60 mg) was added and the mixture was stirred under hydrogen (atmospheric pressure) for 12 h. After filtration through Celite, the catalyst was washed with MeOH (30 mL) and the solvents were removed under reduced pressure. The crude solid was purified by flash chromatography (dichloromethane/MeOH 9/1) to afford compound **16** as a white solid (114 mg, quantitative). *R*f (dichloromethane/MeOH 9/1): 0.40; mp 225 °C; IR (KBr) ν (cm^{-1}) 3452, 1607, 1515, 1484, 1455, 1271, 1240, 825; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 3.75 (t, 4H, J = 4.8 Hz), 4.04 (t, 4H, J = 5.0 Hz), 4.87 (s, 2H), 7.05 (d, 4H, J = 8.7 Hz), 7.52 (d, 2H, J = 8.5 Hz), 7.64–7.72 (m, 6H), 8.48 (s, 2H), 11.27 (s, NH); ^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 60.3 (2 × CH_2), 70.1 (2 × CH_2), 112.2 (2 × CH), 115.7 (4 × CH), 118.5 (2 × CH), 124.0 (2 × CH), 125.3 (2 × Cq), 128.4 (4 × CH), 131.7 (2 × Cq), 134.4 (2 × Cq), 139.9 (2 × Cq), 158.3 (2 × Cq); MS (IS): 440 $(M + 1)^+$; Anal. calcd for $C_{28}H_{25}NO_4$: C, 76.52; H, 5.73; N, 3.19. Found: C,76.23; H,5.90; N,3.04%.

9-Benzenesulfonyl-3,6-bis[4-(2-hydroxyethoxy)phenyl]-9*H*-carbazole (17)

Same procedure as described for **16**. A solution of compound **15** (108 mg, 0.14 mmol) and acetic acid (30 μL) in dioxane (10 mL) was stirred at room temperature. Pd(C) 10% (60 mg) was added and the mixture was stirred under hydrogen (balloon pressure) for 12 h. After filtration through Celite, the catalyst was washed with MeOH (30 mL) and the solvents were removed under reduced pressure. The crude solid was purified by flash chromatography (petroleum ether/ethyl acetate 1/9) to give compound **17** as a white solid (82 mg, quantitative). *R*f (petroleum ether/ethyl acetate 1/9): 0.32; mp 197 °C; IR (KBr) ν (cm^{-1}) 3414, 1607, 1519, 1482, 1449, 1369, 1209, 1172, 979, 799; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 3.72 (t, 4H, J = 4.6 Hz), 4.01 (t, 4H, J = 4.9 Hz), 4.91 (s, 2H), 7.03 (d, 4H, J = 8.5 Hz), 7.46 (t, 2H, J = 8.0 Hz), 7.53–7.72 (m, 5H), 7.84 (dd, 4H, J = 7.6, 12.5 Hz), 8.25 (d, 2H, J = 8.5 Hz), 8.47 (s, 2H); ^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 59.6 (2 × CH_2), 69.6 (2 × CH_2), 114.9 (4 × CH), 118.4 (2 × CH), 125.4 (2 × CH), 126.1 (2 × CH), 126.2 (2 × CH), 126.7 (2 × Cq), 127.9 (4 × CH), 129.8 (2 × CH), 131.8 (2 × Cq), 134.8 (CH), 136.3 (2 × Cq), 136.6 (Cq), 136.9 (2 × Cq), 158.4 (2 × Cq); MS (IS): 580 $(M + 1)^+$; Anal. calcd for $C_{34}H_{29}NO_6S$: C, 70.45; H, 5.04; N, 2.42. Found: C, 70.81; H, 4.88; N, 2.23%.

2-(4-{9-Benzenesulfonyl-6-[4-(2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyrannosyl)ethoxy)phenyl]-9*H*-carbazol-3-yl}phenoxy)-ethanol (18)

To a solution of β-pentaacetoglucose (810 mg, 2.08 mmol) in dichloromethane (20 mL) under argon was added, at 0 °C molecular sieves, $BF_3{\cdot}Et_2O$ (90 μL, 0.62 mmol), and a solution of compound **17** (300 mg, 0.52 mmol) in dichloromethane (10 mL). The mixture was then stirred at room temperature for four days. Then, molecular sieves were filtered off and washed with dichloromethane. The mixture was washed with water (3 × 10 mL), and the solvents were removed under reduced pressure. The crude residue was further purified by flash chromatography (ethyl acetate/petroleum ether 6/4) to afford compound **18** as a brown solid (360 mg, 56%). *R*f (petroleum ether/ethyl acetate 4/6): 0.15; mp 120 °C; IR (KBr) ν (cm^{-1} 2946, 1752, 1450, 1374, 1229, 1037; ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 1.94–2.08 (m, 24H), 3.71–3.77 (m, 2H), 3.96–4.03 (m, 2H), 4.11–4.32 (m, 10H), 4.70 (d, 2H, J = 7.7 Hz), 5.01–5.25 (m, 6H), 6.99 (d, 4H, J = 8.7 Hz), 7.34 (t, 2H, J = 7.7 Hz), 7.47 (t, 1H, J = 7.5 Hz), 7.59 (d, 4H, J = 8.7 Hz), 7.68 (dd, 2H, J = 8.7, 1.8 Hz), 7.86 (d, 2H, J = 7.1 Hz), 8.09 (d, 2H, J = 1.8 Hz), 8.35 (d, 2H, J = 8.7 Hz); ^{13}C-NMR ($CDCl_3$, 62.5 MHz): δ 20.7 (4 × CH_3), 20.7 (2 × CH_3), 20.8 (2 × CH_3), 62.0 (2 × CH_2), 67.4 (2 × CH_2), 68.4 (2 × CH), 68.5 (2 × CH_2), 71.3 (2 × CH), 72.0 (2 × CH), 72.9 (4 × CH), 101.3 (2 × CH), 115.1 (4 × CH), 115.5 (2 × CH), 118.1 (2 × CH), 126.6 (2 × CH), 127.1 (2 × Cq), 128.4 (4 × CH), 129.2 (2 × CH), 133.8 (Cq), 134.0 (CH), 137.1 (2 × Cq), 137.8 (2 × Cq), 137.9 (2 × Cq), 158.3 (2 × Cq), 169.5 (2 × Cq), 169.6 (2 × Cq), 170.4 (2 × Cq), 170.8 (2 × Cq); MS (IS): 1240 $(M + 1)^+$; Anal. calcd for $C_{62}H_{65}NO_{24}S$: C, 60.04; H, 5.28; N, 1.13. Found: C, 59.67; H, 5.37; N, 1.02%.

2-(4-{9-Benzenesulfonyl-6-[4-(2-β-D-glucopyrannosyl)ethoxy)-phenyl]-9*H*-carbazol-3-yl}phenoxy)ethanol (19)

To a solution of compound **18** (100 mg, 0.08 mmol) in methanol (10 mL) under argon, at 0 °C was added sodium (5 mg, 0.21 mmol). The mixture was stirred at room temperature for 4 h, then acidified with a 10% solution of hydrochloric acid (5 mL). A white precipitate was formed and filtered off to afford a white solid corresponding to compound **19** (70 mg, quant.). *R*f (dichloromethane/MeOH 7/3): 0.39; mp 132 °C; IR (KBr) ν(cm^{-1}) 3414, 2946, 1450, 1374, 1229, 1037; ^{1}H-NMR (DMSO-d_6, 250 MHz): δ 3.67–4.27 (m, 32H), 7.09 (d, 4H, J = 8.5 Hz), 7.52 (t, 2H, J = 7.3 Hz), 7.64 (t, 1H, J = 7.5 Hz), 7.76 (d, 4H, J = 8.5 Hz), 7.88 (t, 4H, J = 8.0 Hz), 8.27 (d, 2H, J = 8.7 Hz), 8.58 (s, 2H) ^{13}C-NMR (DMSO-d_6, 62.5 MHz): δ 61.1 (2 × CH_2), 67.1 (2 × CH_2), 67.3 (2 × CH), 70.1 (2 × CH_2), 73.4 (2 × CH), 76.8 (2 × CH), 77.0 (4 × CH), 103.2 (2 × CH), 114.9 (4 × CH), 115.5 (2 × CH), 118.5 (2 × CH), 126.2 (2 × CH), 126.7 (2 × Cq), 127.9 (4 × CH), 129.8 (2 × CH), 131.9 (Cq), 134.8 (CH), 136.2 (2 × Cq), 136.6 (2 × Cq), 136.8 (2 × Cq), 158.2 (2 × Cq), MS (IS): 931 $(M + 1)^+$; Anal. calcd for $C_{46}H_{29}NO_6S$: C, 61.12; H, 5.46; N, 1.55. Found: C, 61.49; H, 5.33; N, 1.32%.

DNase I footprinting

The complete experimental procedure has been recently detailed.[26]

Melting temperature studies

Melting curves were measured using an Uvikon 943 spectrophotometer coupled to a Neslab RTE111 cryostat. The *T*m measurements were performed in BPE buffer pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA). The temperature inside the cuvette (10 mm pathlength) was increased over the range 20–100 °C with a heating rate of 1 °C min^{-1}. The melting temperature *T*m was taken as the mid-point of the hyperchromic transition.

Cell cultures and survival assay

P388 murine leukemia cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2 in RPMI 1640 medium, supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 UI ml^{-1}) and streptomycin (100 μg ml^{-1}). The cytotoxicity of the studied molecules was assessed using a cell proliferation assay developped by Promega (CellTiter 96® Aqueous one solution cell proliferation assay). Briefly, 2×10^4 exponentially growing cells were seeded in 96-well microculture plates with various drug concentrations in a volume of 100 μl. After 72 h incubation at 37 °C, 20 μl of the tetrazolium dye solution were added to each well and the samples were incubated for a further 2 h at 37 °C. Plates were analyzed on a Labsystems Multiskan MS (type 352) reader at 492 nm.

Cell cycle analysis

For flow cytometric analysis of DNA content, 10^6 cells in exponential growth were treated with the test compound at the indicated concentration for 24 h or 48 h and then washed with 1 mL of PBS. After centrifugation, the cell pellet was

resuspended in 1 mL of cold ethanol for 1 h at 4 °C. The ethanol was removed, the pellet was washed with 1 mL PBS and then incubated for 30 min in a solution of propidium iodide (PI at 50 μg mL^{-1}) containing 100 μg mL^{-1} RNase. Samples were analyzed on a Becton Dickinson FACScan flow cytometer using the CellQuest software which was also used to determine the percentage of cells in the different phases of the cell cycle. PI was excited at 488 nm, and fluorescence analyzed at 620 nm (on channel Fl-2).

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