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Article

Electrophilic Substitution of Dimethyl 1-Methylcarbazole-2,3-dicarboxylate: Synthesis of New *b*-Fused Carbazoles as Potential Antitumor Agents

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O ácido dimetiléster 1-metilcarbazol-2,3-dicarboxílico, um intermediário chave na síntese de carbazóis *b*-fusionados, foi transformado com êxito nos seus 6-bromo e 6-nitro derivados via uma substituição eletrofílica numa solução de ácido acético, usando como reagentes *N*-bromosuccinimida ou nitrato de uréia, respectivamente. Com a *N*-clorosuccinimida foi também obtido o derivado 6,8-dicloro congênere, em quantidade apreciável. Os carbazodiésteres 6-substituídos foram usados como matéria-prima para a preparação de vários novos derivados piridazino- ou pirrolocarbazóis fusionados com cadeias laterais básicas, levando à estrutura básica de compostos antitumorais previamente desenvolvidos.

1-Methylcarbazole-2,3-dicarboxylic acid dimethyl ester, a key intermediate in the synthesis of *b*-fused carbazoles, was successfully transformed into its 6-bromo and 6-nitro derivative via electrophilic substitution in acetic acid solution, using *N*-bromosuccinimide or urea nitrate, respectively, as reagents. With *N*-chlorosuccinimide, a 6,8-dichloro congener was also obtained in substantial amounts. The 6-substituted carbazolediesters were used as building blocks for the preparation of several new pyridazine- or pyrrole-fused carbazoles with basic side chains, featuring the core structure of previously developed antitumor compounds.

Keywords: carbazoles, electrophilic substitution, bromination, nitration, antitumor activity

Introduction

Polycyclic heteroaromatic compounds featuring a *b*-fused carbazole skeleton have been known for some time as anticancer agents. As prototypical lead structures can be regarded the pyrido[4,3-*b*]carbazole alkaloids, ellipticine and olivacine[1-5] (Figure 1), which are targeting the DNA-topoisemerase-II complex. Their scaffolds (sharing a methyl group at position 5) have been used as the core motif for a larger number of structural variations, leading to compounds with significantly enhanced activity, such as the drugs (or drug candidates, respectively) elliptinium,[6] datelliptium,[7] retelliptine,[8] pazelliptine,[9] or S16020-2.[10]

Among several other research groups,[11] we had previously reported such structural modifications,[12] focusing mainly on the bioisosteric replacement of the pyridine unit (ring D) of the pyrido[4,3-*b*]carbazole system with a pyridazine ring.[13,14] Moreover, we had also employed a pyrimidine[15] and a pyrrole subunit[16] as ring D of such tetracyclic compounds, and various side chains have been introduced into this region and evaluated for their effect. In the context of our department's ongoing research program in the field of antitumor agents,[12,17-20] we became interested in the systematic enlargement of our compound library by also modifying the substitution pattern of ring A. This region of the scaffold is known to be relevant in terms of binding affinity, and some examples among the drug molecules mentioned above demonstrate that, e.g., an oxygen substituent at ring A is advantageous.[5] A comparable effect had been observed previously by ourselves in the pyridazino[4,5-*b*]carbazole series,[13] but the limited synthetic accessibility of the corresponding building blocks had prevented us from more extensive investigations. In particular, a 6-methoxy-

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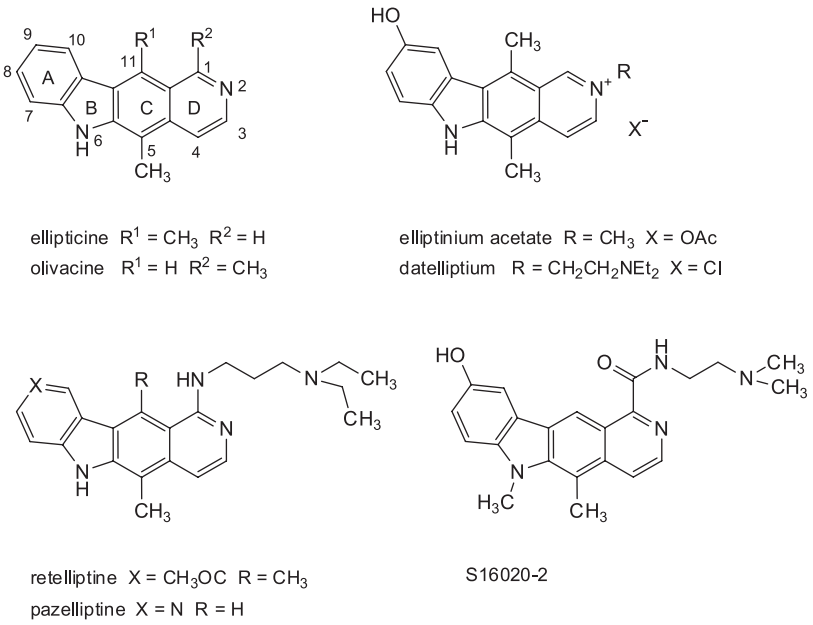


Figure 1. Structures of ellipticine, olivacine and anticancer agents derived thereof.

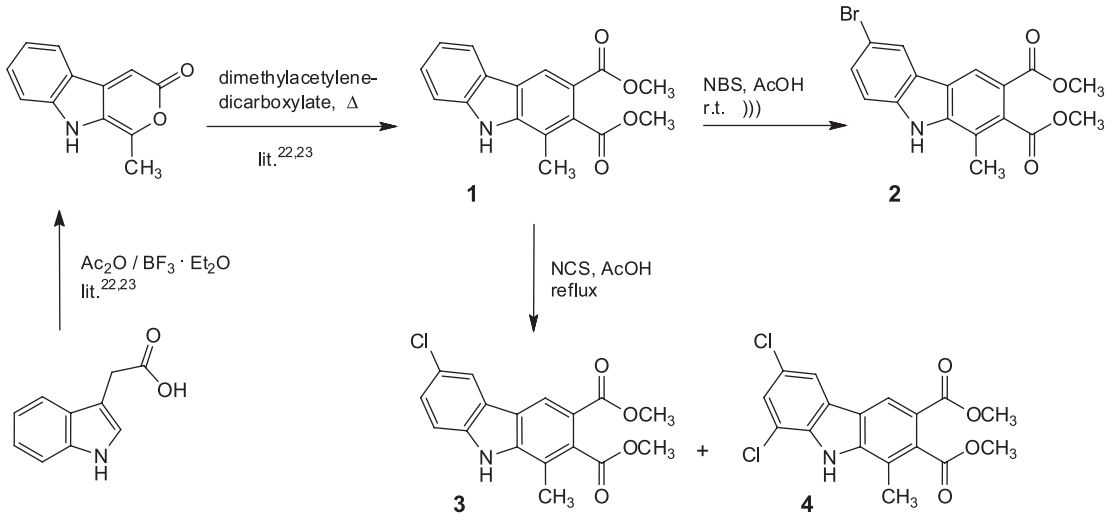
1-methylcarbazole-2,3-dicarboxylic ester which served as the key intermediate had to be prepared in a multistep synthesis from 5-methoxyindole[13] and such an approach was found to be unattractive for broader variation of the target structures. Here, we wish to report on the facile introduction of heteroatom substituents into position 6 of the 1-methylcarbazole-2,3-dicarboxylate intermediate by electrophilic substitution and on the exploitation of these new synthons for the preparation of several hitherto inaccessible *b*-fused carbazoles as potential antitumor agents.

Results and Discussion

Chemistry

Electrophilic aromatic substitution was regarded as the method of choice for the synthesis of 6-halo and 6-nitro derivatives of the 1-methylcarbazole-2,3-dicarboxylic ester (**1**), because one of the two benzene rings in this substrate is strongly deactivated by the electron-withdrawing ester groups, whereas the other benzene unit should be sufficiently reactive towards electrophilic reagents. The directing effect of the (unprotected) NH function should ensure preferential attack of electrophiles at positions 6 and 8, with the latter site being somewhat less favored due to steric reasons.[21] This assumption could be verified when we subjected the ester **1** (which is an easily accessible synthon)[22,23] to bromination with a slight excess of *N*-bromosuccinimide (NBS) in acetic acid solution at room temperature under sonication (Scheme 1), as described recently for pyrrolo[3,2-*b*]quinolone derivatives.[24] After 1 hour, the starting material was completely consumed and a single product was formed. This compound was isolated in 68% yield and it was found to be the 6-bromo derivative **2**, based on its ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, infrared (IR), mass spectrometry (MS) and microanalytical data. In particular, the pronounced nuclear Overhauser enhancement (NOE) which can be observed between 4-H (singlet at 8.40 ppm) and 5-H (singlet at 8.12 ppm) and another NOE between the NH proton (broad singlet at 8.58 ppm) and 8-H (doublet at 7.30 ppm, *J* 8.7 Hz) are indicative for the substitution pattern in **2**.

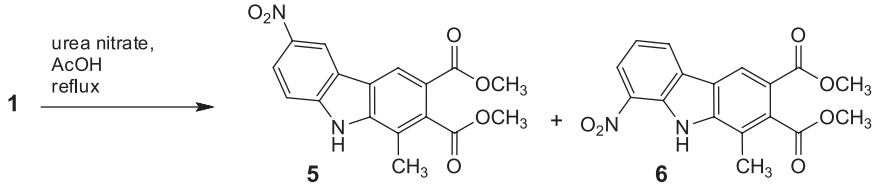
In an attempt to also prepare the analogous 6-chloro compound, the ester **1** was treated with a slight excess of *N*-chlorosuccinimide (NCS) under identical conditions as described above. However, at room temperature there was almost no conversion, obviously because of the lower reactivity of NCS compared to NBS. Thus, the reaction temperature was increased to 117 °C (reflux temperature of acetic acid), which effected complete consumption of the substrate within 2 hours, albeit at the expense of regioselectivity (Scheme 1). In contrast to the smooth and selective transformation of **1** into the 6-bromo compound **2**, two products were formed in the chlorination reaction. After chromatographic separation, the main product (isolated in 59% yield) was identified as the desired 6-chloro compound **3**, whereas the minor product (22% yield) was found to be the 6,8-dichloro derivative **4**. Performing the chlorination with a smaller amount of reagent gave a mixture of **3**, **4** and unreacted **1**, whereas the yield of the dichloro ester **4** could be raised to 72% by employing two equivalents of NCS.



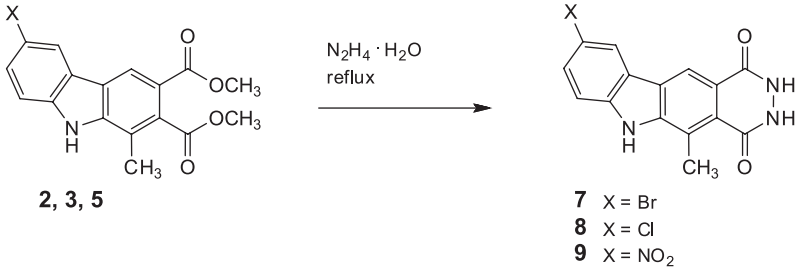
Scheme 1. Synthesis of bromo and chloro derivatives of the ester **1**.

As we were also interested in 1-methylcarbazole-2,3-dicarboxylic esters with a nitro substituent at position 6, the ester **1** was subjected to nitration both under classical conditions (concentrated nitric acid) as well as with the solid reagent, urea nitrate,[25] in acetic acid solution (Scheme 2). Similar results were obtained with both methods, but the slightly better yields and easier reagent handling made the variant with urea nitrate the method of choice, although a larger excess of reagent (3 equivalents) had to be employed in order to achieve complete conversion, as opposed to just one equivalent which had been described for use with non-deactivated carbazoles as substrates.[25] The resulting mixture was separated by column chromatography to afford the 6-nitro compound **5** as the main product (70% yield) and the 8-nitro isomer **6** as the minor component (27% yield). No disubstitution was observed in this case, which is obviously due to the strongly deactivating effect of the first nitro group that is introduced.

The three 6-substituted 1-methylcarbazole-2,3-dicarboxylic esters **2**, **3** and **5** are well suited for the construction of pyridazine-fused carbazole derivatives (featuring a 3-aza-ellipticine/olivacine skeleton), as demonstrated by their smooth cyclization into the tetracycles **7**, **8** and **9** upon heating in neat hydrazine hydrate (Scheme 3), in analogy to previously reported cyclocondensation reactions of similar building blocks.[16,26] These pyridazinediones can exist in different tautomeric forms, of which the dihydroxy form can be safely excluded at least for the solid state: the IR spectra of all compounds show a strong C=O stretching band at 1630-1650 cm^{-1}. In a solution of dimethyl sulfoxide-d_6 (DMSO-d_6), tautomeric exchange processes can be assumed, as indicated by marked signal broadening of the C=O carbon resonances



Scheme 2. Nitration of the ester **1** with urea nitrate.



Scheme 3. Cyclization of the diesters **2**, **3** and **5** into pyridazino[4,5-*b*]carbazoles.

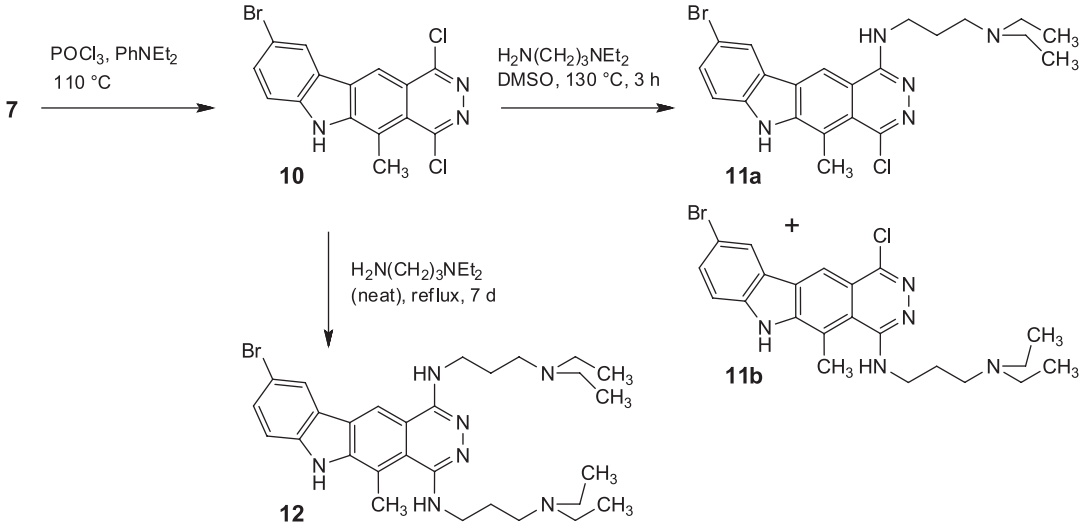
in the ^{13}C NMR spectra, in particular for the halogen compounds.

The bromo derivative **7**, being most conveniently accessible from the precursor **1** as described above, was chosen for further structural modification with the aim of increasing the molecule's solubility as well as its DNA affinity. For this purpose, the introduction of basic side chains is known to be a successful strategy.[8] Thus, the pyridazinedione **7** was first transformed into a 1,4-dichloro derivative (**10**) by heating in phosphorus oxychloride in the presence of *N*,*N*-diethylaniline, which afforded **10** in almost quantitative yield. This reactive intermediate was then treated with excess *N*,*N*-diethylpropane-1,3-diamine in DMSO solution at 130 °C. After 3 hours, the dichloro compound was completely consumed and the formation of two products was detected by thin layer chromatography (TLC). The mixture could be separated by column chromatography, giving the two isomeric monosubstitution products in yields of 36% (**11a**) and 17% (**11b**), respectively (Scheme 4). The position of the newly introduced alkylamino residues clearly follows from NOE experiments which show the spatial proximity of the alkylamino NH and the adjacent 11-H (for compound **11a**) or the 5-methyl group (for compound **11b**). Interestingly, no disubstituted product was found in the reaction mixture despite the excess of nucleophile and high reaction temperature. This is obviously caused by the strongly deactivating effect of the first alkylamino group that is introduced. Replacement of both chloro functions with *N*,*N*-diethylaminopropylamino side chains could be finally accomplished under even more drastic conditions, by refluxing **10** in the neat amine for 7 days under argon atmosphere. After work-up, the 1,4-disubstituted compound **12** was obtained in 62% yield. Like the two monosubstitution products **11a** and **11b**, compound **12** was selected for evaluation of its *in vitro* cytotoxicity.

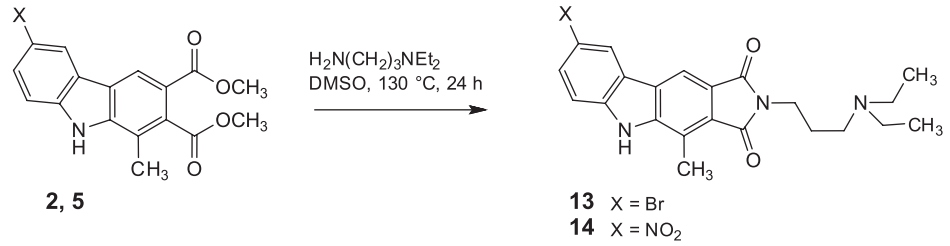
As a further structural modification, we envisaged ring closure of the *ortho*-diester function with an appropriate primary amine to afford carbazole-fused cyclic imides, again with a basic side chain attached to ring D of the tetracyclic skeleton thus formed. Previous investigations[16] had shown that the corresponding ring-A-unsubstituted pyrrolo[3,4-*b*] carbazole-1,3(2*H*,5*H*)-diones (bearing one or two methyl groups at ring C) can significantly inhibit the growth of various tumor cells *in vitro*. Accordingly, our new diesters **2** and **5** were subjected to this type of cyclocondensation with *N*,*N*-diethylpropane-1,3-diamine under the conditions we had previously used (prolonged heating with 10 equivalents of amine in DMSO solution at 130 °C). By this method, the 8-bromo compound **13** and the 8-nitro congener **14** could be obtained in acceptable yields (58 and 57%, respectively, Scheme 5). Also in this case, the spectral data of the new compounds are in excellent agreement with those of their ring-A-unsubstituted counterparts.[16]

Biological evaluation

Compounds **11a**, **11b**, **12**, **13** and **14** were tested *in vitro* for their cytotoxic activity against the human colon carcinoma cell line, SW480, at a fixed concentration of 10 μM, using the well-established XTT assay.[27] This is a colorimetric assay which measures the activity of the mitochondrial dehydrogenase of living cells. The water soluble, yellow-colored 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide (XTT) is taken up by living cells and it is reduced to an intensely colored formazan dye by a mitochondrial dehydrogenase. As the amount of product is proportional to the number of



Scheme 4. Introduction of basic side chains into the pyridazino[4,5-*b*]carbazole skeleton.



Scheme 5. Synthesis of carbazole-fused cyclic imides (**13**, **14**) with a basic side chain.

Table 1. *In vitro* evaluation of **11b** and **13** for cytotoxic activity against human tumor cells: percentage inhibition of tumor cell growth

Compound (concentration)	Cell line				
	SW480	A549	HEP3b	U373	HTB65
11b (10 μM)	83.1 ± 2.5	n.i.	18.6 ± 5.4	n.i.	56.5 ± 5.9
11b (5 μM)	10.7 ± 3.1	n.i.	2.4 ± 6.3	n.i.	5.8 ± 2.4
13 (10 μM)	15.0 ± 3.1	2.9 ± 2.0	60.3 ± 0.5	48.7 ± 2.9	81.6 ± 0.3
13 (5 μM)	8.0 ± 2.4	3.3 ± 2.6	29.4 ± 3.4	14.6 ± 8.6	19.7 ± 7.0

n.i.: no inhibition.

viable cells in the culture, this assay is suitable to assess the number of viable cells. The tested compounds showed moderate inhibitory activity of 15% (for **13**), 83% (for **11b**), 83% (for **11a**), 63% (for **12**), and 84% (for **14**). In addition, compounds **11b** and **13** were tested against a small panel of different cell lines consisting of A549 (lung carcinoma), Hep3b (hepatocarcinoma), U373 (glioblastoma) and HTB65 (melanoma) at two fixed concentrations of 10 and 5 μM. The results are summarized in Table 1: only at the higher concentration, significant inhibition of tumor cell growth was observed. Interestingly, the two compounds show (at least partially) marked differences in cell-type specificity, with **13** being most active against HTB65 and **11b** against the SW480 line. One must conclude, however, that in comparison with our previous lead structures,[13,16] the introduction of electron-withdrawing substituents at ring A does not improve cytotoxic activity.

Conclusions

We have demonstrated that the diester **1**, a key intermediate for the preparation of *b*-fused carbazoles, can be efficiently and selectively brominated at position 6 with NBS in acetic acid under sonication at room temperature. In contrast, chlorination of **1** with NCS lacks this selectivity and gives also disubstitution at positions 6 and 8. Nitration with urea nitrate in acetic acid affords the 6-nitro compound in satisfactory yields along with minor amounts of the 8-nitro isomer. Expectedly, the functionalized diesters **2** and **5** could be smoothly cyclized into the tetracyclic imides **13** and **14**, and compound **2** was transformed into the mono- or dialkylamino-substitited fused pyridazines **11a,b** and **12** via dichloropyridazine **10**. The target compounds showed only weak to moderate activity in an *in vitro* antitumor assay. On the other hand, our synthetic route offers some potential for further structural modifications, especially in view of the versatility of the bromo and the nitro functionality.

Experimental

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1605 FT-IR instrument. ^{1}H NMR (300 MHz) and ^{13}C NMR spectra (75 MHz) were obtained on a Varian Unity*Plus* 300 spectrometer, using the residual signals of undeuterated solvent for calibration; chemical shift values (δ) are reported in ppm, coupling constants (J) in Hz. Mass spectra (electron ionization, EI, 70 eV) were measured on a Shimadzu QP5050A DI50 instrument, high-resolution mass spectra were recorded on a Finnigan MAT 8230 at the Faculty of Chemistry, University of Vienna. Elemental analyses were performed at the Microanalytical Laboratory, Faculty of Chemistry, University of Vienna. For TLC, Merck aluminum sheets pre-coated with Kieselgel 60 F_{254} were used; column chromatography was done on silica (Merck Kieselgel 60, 0.063-0.200 mm). Dimethyl 1-methyl-9*H*-carbazole-2,3-dicarboxylate (**1**) was prepared according to literature procedures.[22,23]

Dimethyl 6-bromo-1-methyl-9*H*-carbazole-2,3-dicarboxylate (**2**)

To a suspension of 2.97 g (10 mmol) of the ester **1** in glacial acetic acid (100 mL) were added 1.87 g (10.5 mmol) of *N*-bromosuccinimide and the mixture was sonicated for

60 min at room temperature in an ultrasound bath. The solvent was distilled off under reduced pressure and the residue was taken up in CH_2Cl_2 (200 mL). This solution was washed consecutively with 5% aqueous Na_2SO_3 (50 mL) and water (2 × 50 mL), then it was dried (Na_2SO_4) and evaporated. The residue was recrystallized from methanol to afford 2.55 g (68%) of **2** as almost colorless needles, m.p. 220-221 °C; IR (KBr) ν_{max}/cm^{-1} 3450, 3322, 2950, 1706, 1576, 1480, 1438, 1288, 1246, 1152, 1058, 786; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H, 1-CH_3, shows positive NOE on irradiation at 8.58 ppm), 3.92 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.30 (d, 1H, *J* 8.7 Hz, 8-H, shows positive NOE on irradiation at 8.58 ppm), 7.51 (dd, 1H, *J* 8.7, 1.8 Hz, 7-H), 8.12 (s, 1H, 5-H, shows positive NOE on irradiation at 8.40 ppm), 8.40 (s, 1H, 4-H), 8.58 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 52.3, 52.7, 112.7, 113.5, 117.6, 119.3, 121.3, 121.4, 123.5, 125.0, 129.6, 133.0, 138.8, 141.3, 166.6, 170.4; MS *m/z* (%) 377 (M^+, 53), 375 (M^+, 51), 346 (54), 345 (40), 344 (59), 343 (54), 331 (45), 330 (47), 329 (49), 328 (28), 301 (21), 300 (20), 287 (56), 285 (75), 259 (85), 258 (33), 257 (75), 213 (23), 179 (42), 178 (94), 177 (66), 172 (36), 166 (22), 164 (30), 162 (22), 152 (37), 151 (52), 150 (77), 149 (20), 139 (26), 89 (32), 83 (25), 77 (36), 76 (31), 75 (37), 75 (71), 74 (31), 73 (47), 71 (38), 69 (78), 67 (34), 63 (61), 62 (32), 59 (41), 57 (100), 55 (81), 51 (38); anal. calcd. for $C_{17}H_{14}BrNO_4$: C, 54.27%; H, 3.75%; N, 3.72%; found: C, 53.97%; H, 3.34%; N, 3.61%.

Dimethyl 6-chloro-1-methyl-9*H*-carbazole-2,3-dicarboxylate (**3**)

To a solution of 0.297 g (1 mmol) of the ester **1** in glacial acetic acid (20 mL) were added 0.147 g (1.1 mmol) of *N*-chlorosuccinimide. The mixture was sonicated for 10 min at room temperature in an ultrasound bath, and then it was refluxed with stirring for 2 h. The solvent was distilled off under reduced pressure and the residue was taken up in CH_2Cl_2 (40 mL). This solution was washed consecutively with 5% aqueous Na_2SO_3 (20 mL) and water (2 × 20 mL), then it was dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography, eluting with light petroleum/ethyl acetate (2:1, v/v). Evaporation of the first fraction gave 0.080 g (22%) of the dichloro compound **4** (for characterization details, see below), evaporation of the second fraction yielded 0.196 g (59%) of **3** as colorless crystals, m.p. 183-185 °C (methanol); IR (KBr) ν_{max}/cm^{-1} 3320, 2950, 1732, 1694, 1606, 1440, 1340, 1292, 1264, 1150, 1060, 780, 598; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H, 1-CH_3, shows positive NOE on irradiation at 8.62 ppm), 3.92 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.34 (d, 1H, *J* 8.4 Hz, 8-H, shows positive NOE on irradiation at 8.62 ppm), 7.38 (dd, 1H, *J* 8.7, 1.8 Hz, 7-H), 7.96 (d, 1H, *J* 1.5 Hz, 5-H, shows positive NOE on irradiation at 8.40 ppm), 8.40 (s, 1H, 4-H), 8.62 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.7, 52.3, 52.7, 112.3, 117.6, 119.2, 120.5, 121.3, 121.5, 124.4, 126.2, 127.0, 132.9, 138.5, 141.5, 166.7, 170.5; MS *m/z* (%) 333 (M^+, 26), 331 (M^+, 60), 302 (23), 301 (38), 300 (68), 299 (68), 286 (21), 285 (69), 284 (64), 243 (29), 242 (27), 241 (90), 215 (31), 214 (46), 213 (100), 212 (30), 179 (22), 178 (46), 177 (35), 152 (26), 151 (39), 150 (91), 83 (22), 77 827), 76 (28), 75 (67), 74 (23), 73 (39), 71 (41), 69 (67), 67 (25), 63 (24), 59 (27), 57 (83), 56 (20), 55 (59), 51 (38); anal. calcd. for $C_{17}H_{14}ClNO_4 \cdot 0.4H_2O$: C, 60.25%; H, 4.40%; N, 4.14%; found: C, 60.21%; H, 4.01%; N, 4.03%.

Dimethyl 6,8-dichloro-1-methyl-9*H*-carbazole-2,3-dicarboxylate (**4**)

To a solution of 0.297 g (1 mmol) of the ester **1** in glacial acetic acid (10 mL) were added 0.268 g (2 mmol) of *N*-chlorosuccinimide. The mixture was sonicated for 10 min at room temperature in an ultrasound bath, and then it was refluxed with stirring for 40 h. The solvent was distilled off under reduced pressure and the residue was taken up in CH_2Cl_2 (40 mL). This solution was washed consecutively with 5% aqueous Na_2SO_3 (20 mL) and water (2 × 20 mL), then it was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography, eluting with CH_2Cl_2/ethyl acetate (19:1, v/v), followed by recrystallization from methanol to afford 0.270 g (72%) of **4** as colorless crystals, m.p. 170-171 °C; IR (KBr) ν_{max}/cm^{-1} 3294, 2948, 1716, 1568, 1486, 1438, 1326, 1288, 1260, 1152, 1060, 786; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.52 (s, 3H, 1-CH_3), 3.95 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 7.47 (d, 1H, *J* 1.8 Hz, 7-H), 7.92 (d, 1H, *J* 1.8 Hz, 5-H), 8.37 (s, 1H, NH), 8.49 (s, 1H, 4-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 52.4, 52.8, 117.0, 118.3, 119.2, 120.2, 121.7, 121.9, 125.3, 126.1, 126.4, 133.7, 135.9, 141.1, 166.3, 169.9; MS *m/z* (%) 369 (M^+, 22), 367 (M^+, 45), 365 (M^+, 44), 336 (31), 335 (39), 334 (72), 333 (53), 321 (28), 320 (33), 319 (31), 318 (51), 277 (59), 275 (100), 250 (20), 249 (62), 248 (53), 247 (100), 246 (34), 213 (27), 211 (31), 184 (21), 178 (21), 177 (51), 176 (35), 175 (39), 168 (54), 164 (23), 150 (29), 124 (25), 123 (21), 98 (20), 88 (25), 83 (22), 75 (38), 74 (38), 71 (26), 69 (539, 67 (24), 63 (22), 59 (369, 57 (56), 55 (50); anal. calcd. for $C_{17}H_{13}Cl_2NO_4 \cdot 0.5H_2O$: C, 54.42%; H, 3.76%; N, 3.73%; found: C, 54.27%; H, 3.25%; N, 3.69%.

Dimethyl 1-methyl-6-nitro-9*H*-carbazole-2,3-dicarboxylate (**5**) and dimethyl 1-methyl-8-nitro-9*H*-carbazole-2,3-dicarboxylate (**6**)

To a solution of 0.297 g (1 mmol) of the ester **1** in glacial acetic acid (5 mL) were added 0.462 g (3 mmol)

of urea nitrate and the mixture was refluxed with stirring for 20 h. It was then cooled, diluted with water (30 mL) and extracted with CH_2Cl_2 (3 × 15 mL). The extract was washed with water (2 × 10 mL), then it was dried (Na_2SO_4) and evaporated. The residue was subjected to column chromatography, eluting with CH_2Cl_2/ethyl acetate (19:1, v/v). Evaporation of the first fraction gave 0.093 g (27%) of the 8-nitro compound (**6**) as yellow crystals, m.p. 213-214 °C (methanol); IR (KBr) ν_{max}/cm^{-1} 3434, 3412, 2948, 1726, 1712, 1610, 1526, 1484, 1432, 1340, 1254, 1060, 740; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.57 (s, 3H, 1-CH_3, shows positive NOE on irradiation at 10.00 ppm), 3.95 (s, 3H, OCH_3), 4.04 (s, 3H, OCH_3), 7.39 (t, 1H, *J* 8.1 Hz, 6-H), 8.35 (d, 1H, *J* 8.4 Hz, 5-H, shows positive NOE on irradiation at 8.55 ppm), 8.38 (d, 1H, *J* 8.7 Hz, 7-H), 8.55 (s, 1H, 4-H), 10.00 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 52.5, 52.8, 112.3, 117.6, 118.8, 120.1, 121.3, 122.9, 124.4, 126.2, 128.0, 132.9, 138.5, 141.2, 166.2, 169.6; MS *m/z* (%) 342 (M$^+$, 34), 311 (57), 310 (75), 306 (20), 295 (32), 265 (30), 252 (100), 250 (47), 224 (68), 206 (20), 179 (25), 178 (70), 177 (37), 151 (29), 150 (31), 75 (31); anal. calcd. for $C_{17}H_{14}N_2O_6$: C, 59.65%; H, 4.12%; N, 8.18%; found: C, 59.43%; H, 4.06%; N, 7.98%. Evaporation of the second fraction gave 0.240 g (70%) of the 6-nitro compound (**5**) as yellow crystals, m.p. 168-169 °C (methanol); IR (KBr) ν_{max}/cm^{-1} 3632, 3352, 2954, 1718, 1612, 1580, 1516, 1484, 1436, 1332, 1304, 1270, 1238, 1132, 1060, 1014; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.46 (s, 3H, 1-CH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 7.63 (d, 1H, *J* 9.0 Hz, 8-H), 8.30 (dd, 1H, *J* 9.0, 2.4 Hz, 7-H), 8.81 (s, 1H, 4-H, shows positive NOE on irradiation at 9.24 ppm), 9.24 (s, 1H, 5-H), 12.4 (br s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 13.8, 52.2, 52.3, 111.8, 118.2, 118.9, 119.5, 121.7, 121.8, 122.1, 122.1, 133.0, 142.5, 144.2, 166.0, 168.8; MS *m/z* (%) 342 (M$^+$, 42), 311 (68), 310 (98), 295 (32), 266 (29), 265 (51), 253 (21), 252 (100), 250 (26), 249 (23), 224 (54), 206 (22), 179 (34), 178 (70), 177 (33), 164 (22), 152 (20), 151 (30), 150 (38), 140 (26), 139 (26), 76 (21), 75 (34), 69 (21), 63 (25), 59 (23), 57 (30), 55 (21); anal. calcd. for $C_{17}H_{14}N_2O_6$·0.5H_2O: C, 58.12%; H, 4.30%; N, 7.97%; found: C, 58.10%; H, 4.27%; N, 7.75%.

General procedure for the preparation of 9-substituted 5-methyl-2,3-dihydro-1*H*-pyridazino[4,5-*b*]carbazole-1,4(6*H*)-diones (**7-9**)

A solution of the corresponding precursor, 6-substituted dimethyl 1-methyl-9*H*-carbazole-2,3-dicarboxylate (**2**, **3**, or **5**) (1 mmol) in 100% hydrazine hydrate (6 mL) was heated to reflux for 2 h. Excess reagent was distilled off under reduced pressure, the residue was taken up in ethanol (10 mL) and again evaporated under reduced pressure. The last step was repeated three times in order to remove residual hydrazine hydrate. The solid residue was recrystallized from ethanol to afford the pure product.

9-Bromo-5-methyl-2,3-dihydro-1*H*-pyridazino[4,5-*b*]carbazole-1,4(6*H*)-dione (**7**)

Yield: 0.333 g (97%) as yellow crystals; m.p. > 350 °C; IR (KBr) ν_{max}/cm^{-1} 3422, 3162, 3010, 1646, 1466, 1394, 1282, 1240, 1122, 1050, 802; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.09 (s, 3H, CH_3), 7.54 (d, 1H, *J* 8.7 Hz, 7-H), 7.64 (dd, 1H, *J* 8.7, 1.8 Hz, 8-H), 8.61 (d, 1H, *J* 1.8 Hz, 10-H, shows positive NOE on irradiation at 8.78 ppm), 8.78 (s, 1H, 11-H), 11.21 (s, 2H, NH), 11.88 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.2, 111.8, 113.5, 115.8, 119.6, 121.2, 122.9, 124.2, 124.4, 124.5, 130.3, 140.5, 142.3, 154.0, 158.6; MS *m/z* (%) 345 (M$^+$, 10), 343 (M$^+$, 10), 71 (30), 69 (45), 67 (20), 59 (100), 57 (71), 55 (50); anal. calcd. for $C_{15}H_{10}BrN_3O_2$·0.5H_2O: C, 51.01%; H, 3.14%; N, 11.90%; found: C, 51.19%; H, 3.06%; N, 11.64%.

9-Chloro-5-methyl-2,3-dihydro-1*H*-pyridazino[4,5-*b*]carbazole-1,4(6*H*)-dione (**8**)

Yield: 0.259 g (86%) as pale yellow crystals, m.p. > 350 °C; IR (KBr) ν_{max}/cm^{-1} 3450, 3152, 2950, 1646, 1470, 1282, 1122, 1060, 866, 818, 732, 700; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.09 (s, 3H, CH_3), 7.52 (dd, 1H, *J* 8.7, 1.8 Hz, 8-H), 7.60 (d, 1H, *J* 8.7 Hz, 7-H), 8.46 (d, not resolved, 1H, 10-H, shows positive NOE on irradiation at 8.78 ppm), 8.78 (s, 1H, 11-H), 11.19 (s, 2H, NH), 11.86 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.1, 113.0, 115.8, 119.5, 121.1, 121.3, 122.8, 123.5, 124.0, 124.5, 127.6, 140.1, 142.4, 154.0, 158.4; MS *m/z* (%) 301 (M$^+$, 11), 299 (M$^+$, 36), 83 (20), 71 (42), 69 (46), 67 (28), 57 (100), 56 (24), 55 (78); anal. calcd. for $C_{15}H_{10}ClN_3O_2$·0.85H_2O: C, 57.19%; H, 3.74%; N, 13.34%; found: C, 57.25%; H, 3.51%; N, 12.95%.

5-Methyl-9-nitro-2,3-dihydro-1*H*-pyridazino[4,5-*b*]carbazole-1,4(6*H*)-dione (**9**)

Yield: 0.230 g (74%) as yellow crystals, m.p. > 350 °C; IR (KBr) ν_{max}/cm^{-1} 3414, 2892, 1626, 1518, 1472, 1324, 1242, 1128, 822; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.04 (s, 3H, CH_3), 7.60 (d, 1H, *J* 9.0 Hz, 7-H), 8.29 (d, *J* 8.7 Hz, 1H, 8-H), 8.80 (s, 1H, 11-H), 9.20 (s, 1H, 10-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.1, 111.5, 116.2, 118.7, 120.5, 121.8, 121.9, 122.9, 123.5, 125.2, 140.4, 142.9, 145.0, 153.8, 158.4; MS *m/z* (%) 311 ([M+1]$^+$, 22), 310 (M$^+$, 98), 207 (48), 179 (22), 178 (48), 177 (24), 151 (20), 150 (32), 83 (22), 77 (22), 76 (25), 75 (34), 74 (29), 73 (24),

71 (27), 70 (21), 69 (62), 63 (34), 57 (67), 56 (40), 55 (70), 53 (20), 51 (27); anal. calcd. for $C_{15}H_{10}N_4O_4 \cdot 0.6H_2O$: C, 56.11%; H, 3.52%; N, 17.45%; found: C, 55.96%; H, 3.35%; N, 17.68%.

9-Bromo-1,4-dichloro-5-methyl-6*H*-pyridazino[4,5-*b*]carbazole (**10**)

A mixture of 1.72 g (5 mmol) of compound **7**, *N*,*N*-diethylaniline (6.5 mL) and phosphorus oxychloride (75 mL) was refluxed for 2 h under exclusion of moisture. During the reaction, a clear solution was obtained which later became turbid and a yellowish precipitate was formed. The excess reagent was distilled off under reduced pressure and the solid residue was neutralized with dilute ammonia under ice cooling. The material was collected by filtration and washed several times with cold water. After air-drying, the filter cake was washed several times with diethyl ether and dried *in vacuo* to afford 1.85 g (97%) of **10** as yellow crystals, m.p. > 350 °C (decomp.); ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.12 (s, 3H, CH_3), 7.55 (d, 1H, *J* 8.7 Hz, 7-H), 7.73 (dd, 1H, *J* 8.7, 1.8 Hz, 8-H), 8.78 (d, 1H, *J* 1.8 Hz, 10-H), 9.09 (s, 1H, 11-H), 12.19 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.9, 112.4, 113.5, 116.0, 117.0, 120.6, 122.7, 123.3, 125.3, 127.4, 131.9, 141.4, 143.8, 152.9, 155.2; MS *m/z* (%) 385 (M^+, 6), 383 (M^+, 36), 381 (M^+, 100), 379 (M^+, 51), 363 (23), 361 (21), 311 (20), 309 (23), 230 (20), 202 (18), 176 (18), 101 (23), 87 (24), 75 (26), 69 (28), 57 (41); anal. calcd. for $C_{15}H_8BrCl_2N_3 \cdot 0.3H_2O$: C, 46.62%; H, 2.24%; N, 10.87%; found: C, 46.71%; H, 2.61%; N, 10.80%.

N'-(9-Bromo-4-chloro-5-methyl-6*H*-pyridazino[4,5-*b*]carbazol-1-yl)-*N*,*N*-diethylpropane-1,3-diamine (**11a**) and *N'*-(9-bromo-1-chloro-5-methyl-6*H*-pyridazino[4,5-*b*]carbazol-4-yl)-*N*,*N*-diethylpropane-1,3-diamine (**11b**)

A mixture of 0.381 g (1 mmol) of the dichloro compound (**10**) and *N*,*N*-diethylpropane-1,3-diamine (4.0 mL, 25.3 mmol) in DMSO (20 mL) was stirred and heated to 130 °C for 3 h. The volatile components were removed by Kugelrohr distillation (10^{-1} mbar, 80 °C) and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with dilute ammonia (3 × 10 mL). The solution was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was subjected to column chromatography, eluting with CH_2Cl_2/methanol/triethylamine in v/v ratios of 97:3:2 (elution of apolar side products), followed by 95:5:2 (elution of **11a**) and finally 90:10:2 (elution of **11b**). Evaporation of the corresponding fraction gave 0.170 g (36%) of **11a** as yellow crystals, m.p. 261-263 °C (CH_2Cl_2/diethyl ether); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.17 (t, 6H, *J* 6.9 Hz, $NCH_2C\underline{H}_3$), 2.10 (quint, 2H, *J* 7.5 Hz, $NHCH_2C\underline{H}_2CH_2NR_2$), 3.13 (s, 3H, 5-$CH_3$), 3.12-3.33 (m, 6H, $NHCH_2CH_2C\underline{H}_2NR_2$, $NC\underline{H}_2CH_3$), 3.66 (q, 2H, *J* 9.6 Hz, $NHC\underline{H}_2CH_2CH_2NR_2$), 7.59 (d, 1H, *J* 8.4 Hz, 7-H), 7.72 (dd, 1H, *J* 8.7, 1.8 Hz, 8-H), 7.80 (m, 1H, 1-N<u>H</u>R, shows positive NOE on irradiation at 9.27 ppm), 8.37 (d, 1H, *J* 1.5 Hz, 10-H, shows positive NOE on irradiation at 9.27 ppm), 9.27 (s, 1H, 11-H), 12.01 (s, 1H, carbazole-NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 8.4, 17.1, 23.0, 46.0, 48.9, 111.6, 113.6, 114.0, 114.5, 115.0, 121.8, 123.7, 123.8, 125.1, 130.8, 141.0, 142.7, 143.2, 154.6; MS *m/z* (%) 446 (2), 444 (2), 440 (2), 439 (2), 438 (2), 403 (13), 402 (18), 401 (20), 400 (14), 375 (25), 373 (19), 367 (12), 312 (21), 311 (13), 310 (23), 231 (10), 230 (12), 203 (11), 202 (10), 201 (11), 161 (9), 155 (9), 113 (21), 112 (53), 100 (22), 98 (23), 86 (100), 84 (33), 72 (30), 71 (16), 58 (79), 57 (27), 56 (64); HRMS *m/z* calcd. for $C_{22}H_{26}BrClN_5$ ($[M+H]^+$): 474.1060; found: 474.1063. Evaporation of the last fraction gave 0.081 g (17%) of **11b** as yellow crystals, m.p. 231-235 °C (ethanol/diethyl ether); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (t, 6H, *J* 6.9 Hz, $NCH_2C\underline{H}_3$), 1.85 (quint, 2H, *J* 6.6 Hz, $NHCH_2C\underline{H}_2CH_2NR_2$), 2.47-2.58 (m, 6H, $NHCH_2CH_2C\underline{H}_2NR_2$, $NC\underline{H}_2CH_3$), 3.10 (s, 3H, 5-CH_3, shows positive NOE on irradiation at 6.68 ppm), 3.54 (q, 2H, *J* 5.7 Hz, $NHC\underline{H}_2CH_2CH_2NR_2$), 6.72-6.64 (m, 1H, 4-N<u>H</u>R), 7.56 (d, 1H, *J* 8.7 Hz, 7-H), 7.69 (dd, 1H, *J* 8.7, 1.8 Hz, 8-H), 8.74 (d, not resolved, 1H, 10-H), 8.87 (s, 1H, 11-H), 11.93 (s, 1H, carbazole–NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.5, 16.4, 25.6, 46.5, 50.8, 111.7, 113.3, 115.2, 115.4, 118.3, 119.9, 123.8, 124.7, 125.3, 130.8, 141.0, 142.6, 145.8, 155.1; MS *m/z* (%) 475 (M^+, 1), 473 (M^+, 1), 440 (4), 438 (4), 403 (5), 401 (4), 389 (5), 387 (4), 376 (4), 374 (4), 369 (9), 367 (10), 363 (4), 361 (7), 359 (4), 312 (8), 310 (8), 231 (4), 230 (6), 203 (5), 202 (6), 113 (15), 112 (100), 100 (8), 98 (10), 86 (64), 84 (17), 72 (13), 70 (7), 58 (31), 56 (27); anal. calcd. for $C_{22}H_{25}BrClN_5 \cdot 0.2H_2O$: C, 55.23%; H, 5.35%; N, 14.64%; found: C, 55.37%; H, 5.13%; N, 14.25%.

9-Bromo-*N*,*N'*-bis[3-(diethylamino)propyl]-5-methyl-6*H*-pyridazino[4,5-*b*]carbazole-1,4-diamine (**12**)

A mixture of 0.533 g (1.4 mmol) of the dichloro compound (**10**) and *N*,*N*-diethylpropane-1,3-diamine (12 mL, 76 mmol) was stirred and heated to 160 °C for 168 h under an argon atmosphere. The volatile components were removed by Kugelrohr distillation (10^{-1} mbar, 80 °C) and the residue was subjected to column chromatography, eluting with ethyl acetate/ethanol/triethylamine (25:25:2, v/v) to afford 0.246 g (62%) of **12** as yellow crystals, m.p. > 350 °C (ethyl acetate/ethanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.92-1.09 (m, 12H, $NCH_2C\underline{H}_3$), 1.92 (quint, not resolved, 4H, $NHCH_2C\underline{H}_2CH_2NR_2$), 2.62-2.79 (m, 12H,

$NHCH_2CH_2C\underline{H}_2NR_2$, $NC\underline{H}_2CH_3$), 3.09 (s, 3H, 5-CH_3), 3.40-3.50 (m, 4H, $NHC\underline{H}_2CH_2CH_2NR_2$), 4.04-4.12 (m, 2H, 1-N<u>H</u>R and 4-N<u>H</u>R), 7.56 (d, 1H, *J* 8.7 Hz, 7-H), 7.66 (dd, not resolved, 1H, 8-H), 8.30 (d, not resolved, 1H, 10-H), 9.10 (s, 1H, 11-H), 12.03 (s, 1H, carbazole–NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 10.3, 10.5, 16.8, 25.2, 25.5, 45.7, 46.0, 46.1, 46.3, 49.9, 50.3, 111.4, 113.5, 114.5, 116.3, 118.7, 123.4, 123.9, 130.2, 140.7, 142.3, 150.2, 150.5; MS *m/z* (%) 567 (M+, 0.2), 538 (1), 496 (29), 494 (30), 467 (4), 465 (3), 422 (3), 410 (7), 408 (10), 396 (8), 394 (9), 382 (9), 380 (6), 368 (9), 367 (9), 354 (4), 340 (3), 327 (2), 310 (5), 247 (4), 230 (6), 203 (6), 177 (3), 157 (3), 143 (2), 112 (19), 98 (10), 86 (100), 84 (12), 73 (14), 72 (27), 58 (70), 56 (16); HRMS *m/z* calcd. for $C_{29}H_{43}BrN_7$ ($[M+H]^+$): 568.2758; found: 568.2765.

8-Bromo-2-[3-(diethylamino)propyl]-4-methylpyrrolo[3,4-*b*]carbazole-1,3(2*H*,5*H*)-dione (**13**)

A solution of 0.376 g (1 mmol) of the diester **2** and 1.30 g (10 mmol) of *N*,*N*-diethylpropane-1,3-diamine in DMSO (5 mL) was stirred and heated to 130 °C for 24 h under an argon atmosphere. The volatile components were removed by Kugelrohr distillation (10^{-1} mbar, 80 °C) and the residue was purified by column chromatography, eluting with CH_2Cl_2/ethyl acetate/triethylamine (95:95:10, v/v), followed by recrystallization from ethanol to afford 0.258 g (58%) of **13** as yellow needles, m.p. 223-226 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, 6H, *J* 7.2 Hz, $NCH_2C\underline{H}_3$), 1.71 (quint, 2H, *J* 6.9 Hz, $NCH_2C\underline{H}_2CH_2NEt_2$), 2.41-2.50 (m, 6H, $NCH_2CH_2C\underline{H}_2NEt_2$, $NC\underline{H}_2CH_3$), 2.85 (s, 3H, 4-CH_3), 3.58 (t, 2H, *J* 7.5 Hz, $NC\underline{H}_2CH_2CH_2NEt_2$), 7.54 (d, 1H, *J* 8.7 Hz, 6-H), 7.61 (dd, 1H, *J* 8.7, 2.1 Hz, 7-H), 8.56 (s, 1H, 10-H), 8.58 (d, 1H, *J* 2.1 Hz, 9-H), 12.22 (s, 1H, carbazole–NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.4, 12.0, 25.5, 35.7, 46.1, 49.9, 112.3, 113.8, 114.7, 121.4, 122.5, 123.8, 124.0, 124.5, 125.3, 129.6, 139.5, 142.6, 168.0, 168.9; MS *m/z* (%) 443 (M^+, 1.2), 441 (M^+, 1.3), 428 (2), 426 (2), 414 (4), 412 (4), 371 (2), 369 (2), 343 (3), 341 (3), 262 (2), 259 (2), 257 (2), 214 (2), 203 (3), 178 (4), 177 (4), 151 (3), 150 (3), 112 (5), 87 (8), 86 (100), 84 (6), 72 (20), 58 (14), 56 (6); anal. calcd. for $C_{22}H_{24}BrN_3O_2 \cdot 0.1H_2O$: C, 59.49%; H, 5.49%; N, 9.46%; found: C, 59.48%; H, 5.45%; N, 9.06%.

8-Nitro-2-[3-(diethylamino)propyl]-4-methylpyrrolo[3,4-*b*]carbazole-1,3(2*H*,5*H*)-dione (**14**)

A solution of 0.547 g (1.6 mmol) of the diester **5** and 2.08 g (16 mmol) of *N*,*N*-diethylpropane-1,3-diamine in DMSO (8 mL) was stirred and heated to 130 °C for 24 h under an argon atmosphere. The volatile components were removed by Kugelrohr distillation (10^{-1} mbar, 80 °C) and the residue was purified by column chromatography, eluting with ethyl acetate/triethylamine (19:1, v/v), followed by recrystallization from ethyl acetate/diethyl ether to afford 0.372 g (57%) of **14** as yellow crystals, m.p. 248-251 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.90 (t, 6H, *J* 7.2 Hz, $NCH_2C\underline{H}_3$), 1.70 (quint, 2H, *J* 7.2 Hz, $NCH_2C\underline{H}_2CH_2NEt_2$), 2.39-2.46 (m, 6H, $NCH_2CH_2C\underline{H}_2NEt_2$, $NC\underline{H}_2CH_3$), 2.79 (s, 3H, 4-CH_3), 3.56 (t, 2H, *J* 6.9 Hz, $NC\underline{H}_2CH_2CH_2NEt_2$), 7.60 (d, 1H, *J* 9.0 Hz, 6-H), 8.25 (dd, 1H, *J* 9.0, 2.1 Hz, 7-H), 8.56 (s, 1H, 10-H), 9.22 (d, 1H, *J* 2.4 Hz, 9-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 11.5, 11.9, 25.6, 35.8, 46.1, 49.9, 112.0, 115.0, 118.6, 121.8, 122.1, 122.2, 123.5, 125.0, 126.0, 140.8, 143.7, 144.0, 167.7, 168.5; MS *m/z* (%) 408 (M^+, 1), 393 (1), 379 (2), 336 (1), 308 (3), 265 (1), 262 (2), 249 (3), 203 (14), 178 (3), 177 (3), 112 (4), 105 (11), 91 (4), 87 (6), 86 (100), 84 (3), 77 (4), 72 (15), 58 (21), 57 (7), 56 (10), 55 (5); HRMS *m/z* calcd. for $C_{22}H_{25}N_4O_4$ ($[M+H]^+$): 409.1876; found: 409.1874.

Biological evaluation

Antiproliferative activity of compounds was assessed using an XTT[27] assay (EZ4U®) on SW480 (colon carcinoma), A549 (lung carcinoma), Hep3B (liver carcinoma), U373 (glioblastoma) and HTB65 (melanoma) cell lines. Cells were seeded at a density of 10000 cells (SW480, A549, HEP3B) or 6000 cells (U373, HTB65) *per* well into 96-well plates and after treatment with active compounds at fixed concentrations of 10 μM and 5 μM for 24 h, the supernatant was replaced with 100 μL of freshly prepared EZ4U® solution (EZ4U®, Biomedica, Vienna, Austria). The cells were incubated at 37 °C for 3 h and the absorbance was measured at 450 and 620 nm in a Tecan Infinite 200 Pro microplate reader (Tecan, Männedorf, Switzerland).

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

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