Full Paper

Synthesis of Dimeric Quinazolin-2-one, 1,4-Benzodiazepin-2-one, and Isoalloxazine Compounds as Inhibitors of Amyloid Peptides Association

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The synthesis of dimeric compounds derived from quinazolin-2-one and 1,4-benzodiazepin-2-one possessing a piperazine or homopiperazine spacer was investigated. In addition, a piperazine spacered bis-isoalloxazine and a bis-riboflavin compound were prepared and their ability to interrupt the association of prion proteins and Alzheimer-specific Aβ peptides was investigated using a fast screening system based on flow cytometry. The bis-isoalloxazine **14** was identified as a new lead structure.

Keywords: Anti-Alzheimer / Antiprion / 1,4-Benzodiazepin-2-one / Isoalloxazine / Quinazolin-2-one

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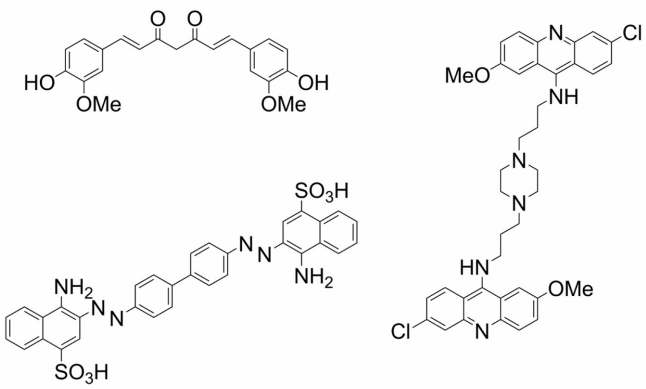
Introduction

The aggregation of β-sheet-enriched proteins in the central nervous system is a common process in a variety of neurodegenerative diseases, finally leading to a loss of neuronal tissue. Consequently, memory and cognitive skills decrease in the course of these diseases. Well known examples are the Alzheimer disease (AD), which affects more than 24 million people worldwide, and prion-based diseases like Creutzfeld – Jakob disease (CJD), Kuru, or the Gerstmann – Sträussler – Scheinker syndrome in humans, and BSE in cattle. In persons suffering from Alzheimer, Aβ-peptides consisting of 39-42 amino acids are deposited. They are released from the cellular APP protein by action of β- and γ-secretase. In contrast to AD, in prion-based diseases the generation of the β-sheet-enriched proteins is not provoked by enzymes. The cellular prion protein PrP^{c} is refolded into the pathogenic form PrP^{Sc} spontaneously or this process is catalyzed by already misfolded species. Additional factors for prion propagation have been discussed.



Scheme 1. Inhibitors of peptide - protein association.

During the last decade, different compounds have been investigated as potential therapeutics acting as inhibitors of these association processes. Prominent examples are curcumin **I** for Alzheimer-specific Aβ-peptides and congo red **II** [1] or the dimeric acridine compounds **III** [2] for prion proteins (Scheme 1). The binding of the acridine-derived compounds involves – among

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Abbreviation: flow cytometry assay (FACS)

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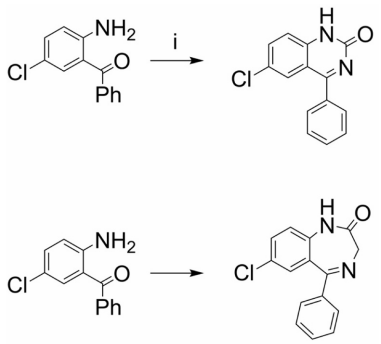


Reagent and conditions: (i) 1.) $ClSO_2NCO$, DCM, 5 h; 2.) H_2O, 5 h; (ii) 1.) chloroacetyl chloride TEA, DMAP, 1 h; 2.) KI, CH_3CN, 24 h; 3.) $(NH_4)_2CO_3$, CH_3CN, reflux, 10 h.

Scheme 2. Synthesis of quinazolin-2-one **1** and 1,4-benzodiazepin-2-one **2**.

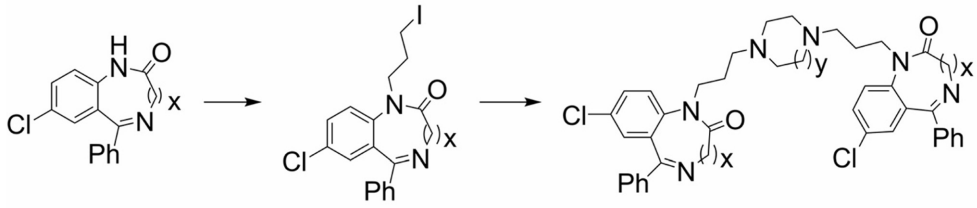
other things – the amino acid residues gln227, tyr225, and tyr226 of the PrP at the C-terminal helix [3], suggesting that π-π interactions are important for the inhibition of the protein association. As already shown, the potency of the bis-acridines [2] depends on the structure of the interacting heterocycle as well as on the spacer. However, best results were observed for the rigid 1,4-bis-(3-aminopropyl)piperazine spacer.

Results and discussion

Quite recently, we became interested in the synthesis of dimeric heterocyclic compounds possessing piperazine or homopiperazine spacer units. Hereby, we focused on quinazolin-2-one and the 7-membered ring analogue 1,4-benzodiazepin-2-one as interacting segments. In addition, the synthesis of a piperazine spacered isoalloxazine **14** and a related riboflavin-derived compound **17** was investigated. The screening for their anti-prion and anti-Alzheimer activity was performed using flow cytometry (FACS analysis).

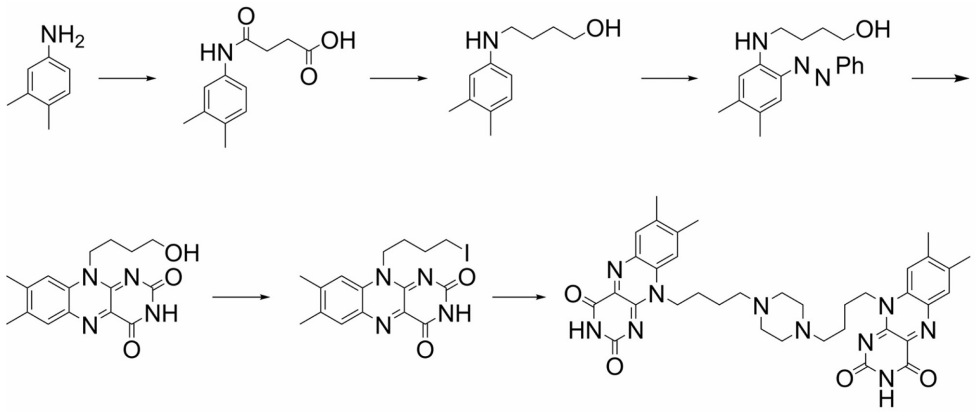
Quinazolin-2-one (Scheme 2, **1**) can be prepared in a one-pot synthesis starting from commercially available 2-amino-5-chlorobenzophenone by its reaction with chlorosulfonyl isocyanate [4]. Its 7-membered ring analogue, 1,4-benzodiazepin-2-one (Scheme 2, **2**), is also accessible from this starting material via a three-step synthesis [5]. Thus, 2-amino-5-chlorobenzophenone was allowed to react with chloroacetyl chloride followed by an exchange of the chloro-substituent by iodine and a ring-closure reaction using ammonium carbonate.

Both heterocyclic compounds can be transformed into the desired bis-derivatives (Scheme 3) by a similar



Reagent and conditions: (i) NaH, DMF, 15 min, 1,3-diiodopropane, 30 min; (ii) piperazine or homopiperazine, $NaHCO_3$, DMF, 40°C, 24 h.

Scheme 3. Synthesis route to target compounds **16**–**18**.



Reagent and conditions: (i) Succinic anhydride, TEA, DMAP, DCM, 5 h; (ii) $LiAlH_4$, THF, reflux, 24 h; (iii) 1.) aniline, $NaNO_2$, AcOH, aq. HCl, 5°C, 30 min; 2.) **10**, aq. NaOH, 10°C, 2 h; (iv) barbituric acid, 1,4-dioxane, AcOH, reflux, 5 h; (v) I_2, PPh_3, imidazole, DCM, 1 h; (vi) piperazine, $NaHCO_3$, DMF, 40°C, 24 h.

Scheme 4. Synthesis route to bis-isoalloxazine **14**.

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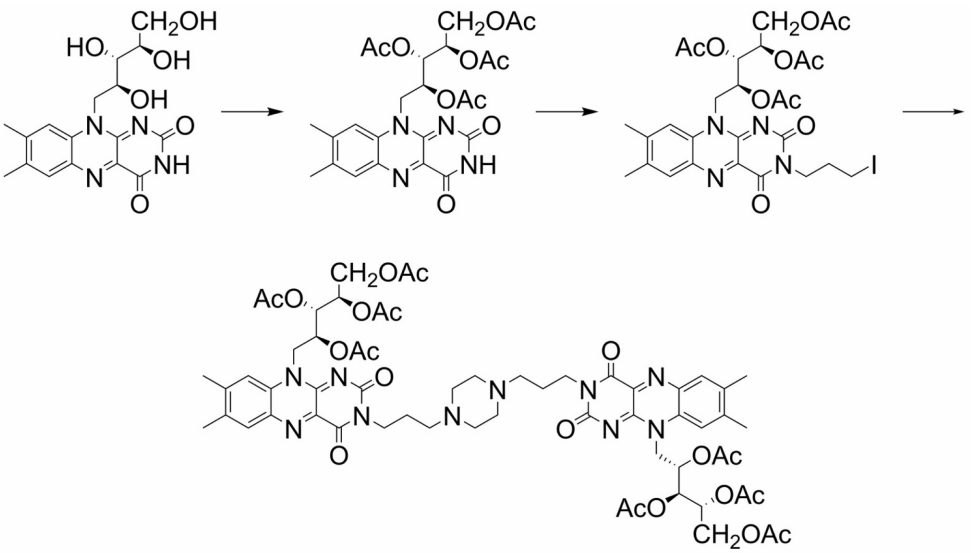

Reagent and conditions: (i) Ac_2O, AcOH, $HClO_4$, 40°C, 30 min; (ii) 1,3-diiodopropane, Cs_2CO_3, DMF, 1 h; (iii) piperazine, $NaHCO_3$, DMF, 40°C, 24 h.

Scheme 5. Synthesis of the bis-riboflavin **17**.

sequence. The alkylation of the lactame was performed using an excess of 1,3-diiodopropane and sodium hydride [6], thus affording compounds **3** and **4** in 70% and 62% yield, respectively. For the diazepam derivative possessing a 3-iodopropyl chain in equatorial position, two different NMR signals are observed for the ring methylene group; in addition, the protons of the 3-iodopropyl chain are magnetically unequal. These observations probably arise from restricted rotation. Finally, the alkylation of the piperazine or homopiperazine-moiety was achieved using $NaHCO_3$ as a base in DMF, affording compounds **5**–**8**. The use of other bases invariantly gave significant lower yields due to an increase in elimination reactions.

Different synthetic approaches have been reported for the synthesis of isoalloxazines. Following TISHLER'S approach [7], our own synthesis started from readily available 3,4-dimethylaniline. Acylation using succinic anhydride followed by reduction with $LiAlH_4$ [8] afforded the corresponding amino alcohol **10**; its treatment with phenyl diazonium salt furnished the azo compound **11**. By this sequence, the desired 4-(4,5-dimethyl-2-phenyldiazenium-phenylamino)-1-butanol (**11**) was obtained in 56% yield.

Finally, **11** was allowed to reacted with barbituric acid to yield the isoalloxazine derivative **12**. The hydroxy group was transformed into the corresponding iodinated compound **13** using triphenylphosphane, iodine, and imidazole. In the concluding step, the piperazine moiety was alkylated to afford the bis-isoalloxazine **14**.

The bis-riboflavin **17** was obtained in a three-step synthesis starting from riboflavin. To increase the solubility in organic solvents and to prevent side reaction from the D-ribityl side chain, the hydroxy groups were acetylated with acetic anhydride in the presence of catalytical amounts of $HClO_4$ [9]. Alkylation with 1,3-diiodopropane using Cs_2CO_3 [10] as a base afforded compound **16**; subsequent reaction with piperazine yielded the desired bis-riboflavin **17**. The synthesis of the analogous homopiperazine compounds, however, failed under these conditions.

Conclusion

Our compounds were investigated for their anti-Alzheimer and anti-prion activity using a flow cytometry assay (FACS). Using suitable fluorescence-labeled peptides and proteins, the association of peptides and proteins is measured as an appearance of particles with appropriate side and forward scattering and fluorescence intensities. The association can be quantified and the inhibitory effect of compounds is calculated with reference to standard association conditions. The ability of our compounds to interrupt the addition of fluorescence-marked monomeric Aβ or prion proteins to preformed fibrils was examined at concentrations of 0.1, 1.0, and 4.0 μM. As a standard, we used the dimeric 6-chlor-2-methoxyacridine derivative **III**. The quinazolin-2-one and 1,4-dibenzothiazepin-2-one compounds revealed only a weak inhibitory

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Table 1. Results of FACS analysis for Aβ and PrP.

Compound	% of control Aβ peptides (μM)			% of control PrPsc (μM)		
	0.1	1.0	4.0	0,1	1.0	4.0
I	48	2	6	86	46	28
5	65	61	77	85	79	82
6	66	24	5	73	56	72
7	85	81	80	77	73	79
8	76	78	75	71	71	70
14	45	3	2	71	54	36
17	63	68	18	73	66	32

effect onto the prion - protein association. For Alzheimer-specific Aβ peptides the homopiperazine-spacered quinazolin-2-one derivative **6** showed promising results, whereas the analogous piperazine compound **5** revealed only a low potency. This emphasizes the important role of the spacer. Unfortunately, the 1,4-benzodiazepin-2-one compounds exhibited only a negligible inhibitory effect on Aβ peptides, suggesting that the planarity of the heterocycle is essential. The bis-isoalloxazine compound **14**, however, showed comparable results to the lead structure **I** for PrP proteins as well as for Aβ peptides. Also the bis-riboflavine revealed a considerable inhibitory effect for PrP and at 4 μM also for the Alzheimer-specific Aβ peptides.

In summary, the dimeric quinazolin-2-one and 1,4-benzodiazepin-2-one compounds revealed only low activities as compared to the isoalloxazine and riboflavin-derived compounds. The latter compounds are comparable to the potent bis-acridine **I**, thus rendering them as interesting new lead structures.

The authors have declared no conflict of interest.

Experimental

General

Melting points are uncorrected (*Leica* hot stage microscope; Leica, Wetzlar, Germany), NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000, or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si; Varian, Palo Alto, CA, USA), optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000 (Perkin Elmer, USA), MS spectra were taken on a Intectra GmbH (Harpstedt, Germany) AMD 402 (electron impact, 70 eV) or on a Finnigan MAT TSQ 7000 (electrospray, voltage 4.5 kV, sheath gas nitrogen; Thermo Electron Corporation, Bremen, Germany) instrument. TLC was performed on silica gel (Merck 5554; Merck, Darmstadt, Germany, detection by UV absorption). The solvents were dried according to usual procedures.

6-Chloro-4-phenylquinazolin-2(1H)-one ***1***

To a solution of 2-amino-5-chlorobenzophenone (10.0 g, 43.0 mmol) in CH_2Cl_2 (50 mL) chlorosulfonyl isocyanate (7.3 g, 52.0 mmol) was added dropwise at 0°C and stirred for an additional 5 h at 25°C. To the resulting solution H_2O (10 mL) was added and stirring was continued for 5 h. The precipitate was filtered off, washed with H_2O (10 mL) and dried *in vacuo*. Compound **1** (9.4 g, 85%) was obtained as a yellow solid. M.p.: 316–317°C (lit.: 321–323°C [11], 318°C [12], 316–318°C [13], 312°C [14]); IR (KBr) ν: 2821*m*, 1780*w*, 1650*s*, 1615*s*, 1591*s*, 1539*m*, 1476*s*, 1458*s*, 1403*m*, 1363*m*, 1338*m*, 1308*m*, 1257*m*, 1177*m*, 1156*w*, 1088*w*, 1073*w*, 1000*m* cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.10 (br s, 1H, NH), 7.76 (dd, $^3J_{H,H}$ = 9.1 Hz, $^4J_{H,H}$ = 2.5 Hz, 1H, CH(7)), 7.68–7.65 (m, 2H, CH(2′)), 7.62–7.58 (m, 3H, $H_{arom.}$), 7.52 (d, $^4JJ_{H,H}$ = 2.5 Hz, 1H, CH(5)), 7.37 (d, $^3J_{H,H}$ = 9.1 Hz, 1H, CH(8)); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 174.3 (s, C=N), 155.0 (s, C=O), 142.7 (s, C(8a)), 136.5 (s, C(1')), 135.3 (d, CH(7)), 131.0 (d, CH(4′)), 129.4 (d, CH(2′)), 129.0 (d, CH(3′)), 127.4 (d, CH(5)), 126.3 (s, C(6)), 118.3 (d, CH(8)), 115.5 (s, C(5a)); UV-vis (methanol) λ_{max} (log ε): 249 nm (4.48); MS (ESI, MeOH) *m/z*: 257.1 [M(^{35}Cl) + H]$^+$ (60%), 259.1 [M(^{37}Cl) + H]$^+$ (19%), 279.2 [M(^{35}Cl) + Na]$^+$ (30%), 281.2 [M(^{37}Cl) + Na]$^+$ (10%), 535.1 [M(M(^{35}Cl) + Na)]$^+$ (100%), 537.1 [M(M(^{37}Cl) + Na)]$^+$ (32%).

7-Chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one ***2***

To a solution of 2-amino-5-chlorobenzophenone (11.35 g, 49.00 mmol), TEA (10 mL) and DMAP (30 mg) in CH_2Cl_2 (100 mL) chloroacetyl chloride (5.9 g, 52.00 mmol) was added dropwise at 0°C and stirring at 25°C was continued for 1 h. The precipitate was filtered off and washed with CH_2Cl_2 (100 mL). The filtrate was washed with aq. Na_2CO_3 solution (150 mL) and H_2O (100 mL), dried over Na_2SO_4, and evaporated to dryness. After recrystallization from EtOH the intermediary chloro-acetyl compound (13.7 g, 91%) was obtained as light yellow solid. This intermediate and KI (18.6 g, 0.1 mol) were dissolved in CH_3CN (200 mL) and stirred at room temperature over night. The solvent was removed *in vacuo* and the residue treated with CH_2Cl_2 (100 mL) and H_2O (100 mL). The phases were separated, the organic layer was washed with H_2O (100 mL), dried over Na_2SO_4, and concentrated *in vacuo*. A solution of this iodoacetyl compound and ammonium carbonate (28.8 g, 0.3 mol) in CH_3CN (200 mL) was heated under reflux for 10 h. The solvent was then removed *in vacuo* and the residue treated with CH_2Cl_2 (200 mL) and H_2O (100 mL). The phases were separated, the organic layer was washed with H_2O (100 mL), dried over Na_2SO_4, and concentrated under diminished pressure. After purification by column chromatography, (silica gel, CH_2Cl_2 / methanol, 95 : 5) compound **2** (7.0 g, 52%) was obtained as light yellow solid. M.p.: 217–218°C (lit.: 215–221°C [15], 216-217°C [16]); R_F: 0.50 (CH_2Cl_2 / MeOH, 95 : 5); IR (KBr) ν: 3178*m*, 3042*m*, 2956*w*, 2361*w*, 1683*s*, 1606*s*, 1576*m*, 1509*m*, 1480*s*, 1446*m*, 1385*s*, 1360*s*, 1321*s*, 1285*m*, 1258*m*, 1234*s*, 1194*m*, 1180*w*, 1129*w*, 1098*w*, 1013*m* cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.43 (br s, 1H, NH), 7.52–7.37 (m, 6H, $H_{arom.}$), 7.28 (d, $^4J_{H,H}$ = 2.4 Hz, 1H, CH(6)), 7.13 (d, $^3J_{H,H}$ = 8.9 Hz, 1H, CH(9)), 4.31 (br s, 2H, CH_2(3)); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 171.7 (s, C=N), 169.8 (s, C=O), 138.7 (s, C(6a)), 137.3 (s, C(9a)), 131.9 (d, CH(8)), 130.7 (d, CH(4′)), 130.6 (d, CH(6)), 129.6 (d, CH(2′)), 128.8 (s, C(1′)), 128.5 (s, C(7)), 128.3 (d, CH(3′)), 122.6 (d, CH(9)), 56.6 (t, CH_2(3)); UV-vis (methanol) λ_{max} (log ε): 246 nm (4.52); MS (ESI, MeOH) *m/z*: 271.1 [M(^{35}Cl) + H]$^+$ (100%), 273.1 [M(^{37}Cl) + H]$^+$ (33%).

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*6-Chloro-1-(3-iodopropyl)-4-phenylquinazolin-2(1H)-one **3***

To a solution of **1** (6.0 g, 23.4 mmol) in DMF (50 mL), NaH (1.0 g, 60% dispersion in mineral oil, 25.0 mmol) was added in several portions. After the evolution of hydrogen had ceased, 1,3-diiodopropane (10.8 g, 35.0 mmol) was added and and stirring at r.t. was continued for 30 min. Finally, the solvent was evaporated *in vacuo* and the residue purified by column chromatography (silica gel, ethyl acetate). Compound **3** (7.0 g, 70%) was obtained as a light yellow solid. M.p.: 177-178°C; R_F: 0.75 (ethyl acetate); IR (KBr) ν 2924*m*, 1723*w*, 1646*s*, 1602*s*, 1538*s*, 1479*m*, 1457*m*, 1364*m*, 1280*m*, 1176*m*, 1103*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.80 (d, $^4J_{H,H}$ = 2.5 Hz, 1H, CH(5)), 7.72 – 7.64 (m, 3H, $H_{arom.}$), 7.57 – 7.48 (m, 4H, $H_{arom.}$), 4.37 (t, $^3J_{H,H}$ = 7.0 Hz, 2H, CH_2(1″)), 3.33 (t, $^3J_{H,H}$ = 6.6 Hz, 2H, CH_2(3″)), 2.40 – 2.30 (m, 2H, CH_2(2″)); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 174.0 (s, C=N), 155.0 (s, C=O), 141.8 (s, C(8a)), 135.8 (s, C(1′)), 135.4 (d, CH(7)), 131.0 (d, CH(4′)), 129.4 (d, CH(2′)), 129.2 (d, CH(3′)), 128.5 (d, CH(5)), 127.8 (s, C(6)), 116.9 (s, C(5a)), 115.4 (d, CH(8)), 45.1 (t, CH_2(1″)), 30.6 (t, CH_2(2″)), 2.0 (t, CH_2(3″)); UV-vis (methanol) λ_{max} (log ε): 252 nm (4.59); MS (ESI, MeOH) *m*/*z*: 424.9 [M(^{35}Cl) + H]$^+$ (100%), 426.9 [M(^{37}Cl) + H]$^+$ (33%), 447.0 [M(^{35}Cl) + Na]$^+$ (50%), 449.0 [M(^{37}Cl) + Na]$^+$ (16%).

*7-Chloro-1-(3-iodopropyl)-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one **4***

Compound **4** (3.6 g, 62%) was obtained from **2** (3.60 g, 13.30 mmol) following the procedure described for compound **3** as an orange-colored oil. R_F: 0.92 (ethyl acetate); IR (KBr) ν: 2931*w*, 1678*s*, 1607*m*, 1559*m*, 1480*m*, 1446*m*, 1405*m*, 1357*m*, 1322*m*, 1267*m*, 1226*m*, 1191*m*, 1191*m*, 1139*m*, 1100*w*, 1075*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.34 (m, 7H, $H_{arom.}$), 7.27 (d, $^4J_{H,H}$ = 2.4 Hz, 1H, CH(6)), 4.78 (d, $2J_{H,H}$ = 10.4 Hz, 1H, CH_a(3)), 4.38 (ddd, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 7.5, 5.8 Hz, 1H, CH_a(1″)), 3.75 – 3.68 (m, 2H, CH_b(3) + CH_b(1″)), 3.03 (ddd, $^2J_{H,H}$ = 10.1 Hz, $^3J_{H,H}$ = 12.5, 6.1 Hz, 1H, CH_a(3″)), 2.84 (ddd, $^2J_{H,H}$ = 10.1 Hz, $^3J_{H,H}$ = 8.3, 5.9 Hz, 1H, CH_b(3″)), 2.20 – 2.09 (m, 1H, CH_a(2″)), 1.94 – 1.83 (m, 1 H, CH_b(2″)); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 166.9 (s, C=N), 166.6 (s, C=O), 139.0 (s, C(6a)), 135.5 (s, C(9a)), 129.3 (d, CH(8)), 128.8 (s, C(1′)), 128.5 (d, CH(4′)), 127.6 (s, C(7)), 127.5 (d, CH(6)), 127.0 (d, CH(2′)), 126.2 (d, CH(3′)), 121.3 (d, CH(9)), 54.6 (t, CH_2(3)), 45.3 (t, CH_2(1″)), 29.0 (t, CH_2(2″)), 0.0 (t, CH_2(3″)); UV-vis (methanol) λ_{max} (log ε): 244 nm (4.48); MS (ESI, MeOH) *m*/*z*: 311.1 [(M(^{35}Cl) + H) − HI]$^+$ (100%), 313.1 [(M(^{37}Cl) + H) − HI]$^+$ (34%), 461.1 [M(^{35}Cl) + H]$^+$ (100%), 463.1 [M(^{35}Cl) + H]$^+$ (32%).

1,1′-(Piperazine-1,4-diyldipropane-3,1-diyl)bis(6-chloro-4-phenyl-quinazolin-2(1H)-one) 5

A mixture of **3** (0.50 g, 1.18 mmol), piperazine (42.00 mg, 0.49 mmol), and $NaHCO_3$ (0.1 g, 1.2 mmol) in DMF (10 mL) was heated to 40°C for 24 h. The reaction mixture was concentrated *in vacuo* and the residue purified by column chromatography (silica gel, CH_2Cl_2 / MeOH, 95 : 5). Compound **5** (0.13 g, 40%) was obtained as an off-white solid. M.p.: >250°C (decomp.); R_F: 0.28 (CH_2Cl_2 / MeOH, 95 : 5); IR (KBr) ν: 3039*m*, 2950*m*, 1658*s*, 1605*s*, 1539*s*, 1480*m*, 1460*m*, 1367*m*, 1280*m*, 1200*w*, 1158*w*, 1103*w*, 1014*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.79 (d, $^4J_{H,H}$ = 2.5 Hz, 2H, CH(5)), 7.70 – 7.66 (m, 6H, $H_{arom.}$), 7.58 – 7.50 (m, 8H, $H_{arom.}$), 4.35 (t, $^3J_{H,H}$ = 7.0 Hz, 4H, CH_2(1″)), 2.53 (br s, 12H, CH_2(3″) + piperazine), 2.07 – 1.97 (m, 4H, CH_2(2″)); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 173.6 (s, C=N), 155.0 (s, C=O), 142.3 (s, C(8a)), 135.9 (s, C(1′)), 135.1 (d, CH(7)), 130.8 (d, CH(4′)), 129.5 (d, CH(2′)), 128.9 (d, CH(5)), 128.5 (d, CH(3′)), 127.5 (s, C(6)), 116.9 (s, C(5a)), 116.0 (d, CH(8)), 55.2 (t, CH_2(3″)), 53.3 (t, piperazine), 42.5 (t, CH_2(1″)), 24.6 (t, CH_2(2″)); UV-vis (methanol) λ_{max} (log ε): 252 nm (4.40); MS (ESI, MeOH) *m*/*z*: 679.2 [M(2 x ^{35}Cl) + H]$^+$ (100%), 681.2 [M(^{35}Cl, ^{37}Cl) + H]$^+$ (64%), 683.2 [M(2 × ^{37}Cl) + H]$^+$ (13%), 701.3 [M(2 × ^{35}Cl) + Na]$^+$ (20%), 703.3 [M(^{35}Cl, ^{37}Cl) + Na]$^+$ (13%).

*1,1′-(1,4-Diazepane-1,4-diyldipropane-3,1-diyl)bis(6-chloro-4-phenylquinazolin-2(1H)-one) **6***

Compound **6** (0.09 g, 27%) was obtained from **3** (0.50 g, 1.18 mmol) and homopiperazine (49.00 mg, 0.49 mmol) following the procedure described for compound **5** as an amorphous slightly yellowish solid. R_F: 0.74 (CH_2Cl_2 / MeOH, 8 : 2); IR (KBr) ν: 2925*m*, 1658*s*, 1606*s*, 1536*s*, 1477*m*, 1362*m*, 1280*m*, 1103*w* cm^{-1}; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 7.90 (dd, $^3J_{H,H}$ = 9.1 Hz, $^4J_{H,H}$ = 2.5 Hz, 2H, CH(7)), 7.80 (d, $^3J_{H,H}$ = 9.1 Hz, 2H, CH(8)), 7.66 – 7.56 (m, 12H, $H_{arom.}$), 4.27 (t, $^3J_{H,H}$ = 7.0 Hz, 4H, CH_2(1″)), 3.08 – 2.90 (m, 12H, CH_2(3″) + homopiperazine), 2.00 – 1.86 (m, 6H, CH_2(2″) + homopiperazine); ^{13}C-NMR (100 MHz, DMSO-d_6) δ172.7 (s, C=N), 153.9 (s, C=O), 141.7 (s, C(3)), 135.5 (s, C(1′)), 135.0 (d, CH(7)), 130.4 (d, CH(4′)), 128.8 (d, CH(2′)), 128.4 (d, CH(3′)), 127.6 (d, CH(5)), 126.0 (s, C(6)), 117.0 (d, CH(8)), 116.1 (s, C(5a)), 53.9 (t, CH_2(3″)), 52.9 (t, homopiperazine), 51.4 (t, homopiperazine), 41.3 (t, CH_2(1″)), 24.0 (t, homopiperazine), 23.6 (t, CH_2(2″)); UV-vis (methanol) λ_{max} (log ε): 252 nm (4.81); MS (ESI, MeOH): *m*/*z*: 693.2 [M(2 × ^{35}Cl) + H]$^+$ (100%), 695.2 [M(^{35}Cl, ^{37}Cl) + H]$^+$ (67%), 697.2 [M(2 × ^{37}Cl) + H]$^+$ (100%).

*1,1′-(Piperazine-1,4-diyldipropane-3,1-diyl)bis(7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) **7***

Compound **7** (0.1 g, 31%) was obtained from **4** (0.50 g, 1.14 mmol) and piperazine (39.20 mg, 0.46 mmol) following the procedure described for compound **5** as a slightly yellowish solid. M.p.: 128 – 129°C; R_F: 0.56 (CH_2Cl_2 / MeOH, 9 : 1); IR (KBr) ν: 2937*m*, 2813*m*, 2361*w*, 1735*w*, 1677*s*, 1446*m*, 1608*m*, 1562*w*, 1480*m*, 1446*m*, 1406*m*, 1360*w*, 1323*m*, 1270*m*, 1188*w*, 1139*m*, 1323*m*, 1270*m*, 1188*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.60 – 7.34 (m, 14H, $H_{arom.}$), 7.25 (d, $^4J_{H,H}$ = 2.4 Hz, 2H, CH(6)), 4.77 (d, $^2J_{H,H}$ = 10.4 Hz, 2H, CH_a(3)), 4.30 (ddd, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 7.5, 2H, 5.8 Hz, CH_a(1″)), 3.72 (d, $^2J_{H,H}$ = 10.4 Hz, 2H, CH_b(3)), 3.64 – 3.58 (m, 2H, CH_b(1″)), 2.50 – 2.20 (m, 12H, CH_2(3″) + piperazine), 1.83 – 1.72 (m, 2H, CH_a(2″)), 1.70 – 1.60 (m, 2H, CH_b(2″)); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.2 (s, C=N), 168.7 (s, C=O), 140.8 (s, C(6a)), 137.8 (s, C(9a)), 131.6 (d, CH(8)), 131.3 (s, C(1′)), 130.9 (d, CH(4′)), 130.0 (s, C(7)), 129.8 (d, CH(6)), 129.3 (d, CH(2′)), 128.5 (d, CH(3′)), 123.6 (d, CH(9)), 57.1 (t, CH_2(3)), 54.5 (t, CH_2(3″)), 51.5 (t, piperazine), 44.5 (t, CH_2(1″)), 24.6 (t, CH_2(2″)); UV-vis (methanol) λ_{max} (log ε): 244 nm (4.73); MS (ESI, MeOH) *m*/*z*: 707.2 [M(2 x ^{35}Cl) + H]$^+$ (100%), 709.2 [M(^{35}Cl, ^{37}Cl) + H]$^+$ (64%), 711.2 [M(2 × ^{37}Cl) + H]$^+$ (14%).

*1,1′-(1,4-Diazepane-1,4-diyldipropane-3,1-diyl)bis(7-chloro-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one) **8***

Compound **8** (0.25 g, 76%) was obtained from **4** (0.50 g, 1.14 mmol) and homopiperazine (45.60 mg, 0.46 mmol) following the procedure as described for compound **5** as an amorphous slightly yellowish solid. R_F: 0.70 (CH_2Cl_2 / MeOH, 9 : 1); IR (KBr) ν: 2925*m*, 1672*s*, 1607*m*, 1480*m*, 1446*m*, 1406*m*, 1322*m*, 1183*m*, 1074*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.63 – 7.35 (m, 14 H, $H_{arom.}$), 7.26 (d, $^4J_{H,H}$ = 2.4 Hz, 2H, CH(6)), 4.72 (d, $^2J_{H,H}$ = 10.4 Hz,

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2H, $CH_a(3)$), 4.33 (ddd, $^2J_{H,H}$ = 13.8 Hz, $^3J_{H,H}$ = 7.5, 5.8 Hz, 2H, $CH_a(1'')$), 3.73–3.60 (m, 4H, $CH_b(3)$ + $CH_b(1'')$), 2.76–2.40 (m, 12H, $CH_2(3'')$ + homopiperazine), 1.97–1.68 (m, 6H, $CH_2(2'')$ + homopiperazine); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 169.4 (s, C=N), 168.7 (s, C=O), 140.5 (s, C(6a)), 137.6 (s, C(9a)), 131.8 (d, CH(8)), 131.1 (s, C(1′)), 130.9 (d, CH(4′)), 130.2 (s, C(7)), 129.8 (d, CH(6)), 129.3 (d, CH(2′)), 128.6 (d, CH(3′)), 123.8 (d, CH(9)), 57.1 (t, $CH_2(3)$), 54.5 (t, $CH_2(3'')$), 52.6 (t, homopiperazine), 51.9 (t, homopiperazine), 44.0 (t, $CH_2(1'')$), 24.3 (t, $CH_2(2'')$), 23.6 (t, homopiperazine); UV-vis (methanol) λ_{max} (log ε): 208 nm (5.19); MS (ESI, MeOH): *m*/*z*: 721.2 [M(2 × ^{35}Cl) + H]$^+$ (100%), 723.2 [M(^{35}Cl, ^{37}Cl) + H]$^+$ (63%), 725.2 [M(2 × ^{37}Cl) + H]$^+$ (13%).

4-[(3,4-Dimethylphenyl)amino]-4-oxo-butanoic acid ***9***

To a solution of 3,4-dimethylaniline (5.0 g, 41.3 mmol), TEA (10 mL) and DMAP (0.05 g, 0.41 mmol) in CH_2Cl_2 (100 mL), succinic anhydride (5.4 g, 54.0 mmol) was added in several portions; then, the mixture was stirred for 5 h at room temperature. The reaction was quenched by the addition of an aq. saturated solution of Na_2CO_3 (100 mL). The phases were separated and the aqueous layer was washed with CH_2Cl_2 (100 mL). The product was precipitated by the addition of hydrochloric acid and collected by filtration. The residue was washed with H_2O (2 × 30 mL) and dried *in vacuo*. Compound **9** was obtained (8.6 g, 95%) as a colourless solid. M.p.: 142–143°C (lit.: 143 [17]); IR (KBr) ν: 3309*s*, 3026*m*, 2931*m*, 1720*s*, 1659*s*, 1616*m*, 1534*s*, 1506*m*, 1448*m*, 1401*s*, 1371*w*, 1354*m*, 1304*w*, 1264*m*, 1236*s*, 1214*w*, 1194*s*, 1156*w*, 1118*w*, 1070*w*, 1020*w* cm^{-1}; ^{1}H-NMR (400 MHz, acetone-d_6) δ 9.00 (br s, 1H, NH), 7.40 (d, $^4J_{H,H}$ = 2.0 Hz, 1H, CH(2)), 7.35 (dd, $^3J_{H,H}$ = 8.1, $^4J_{H,H}$ = 2.0 Hz, 1H, CH(4)), 7.00 (d, $^3J_{H,H}$ = 7.9 Hz, 1H, CH(5)), 2.67–2.61 (m, 4H, $CH_2(2' + 3')$), 2.19 (s, 3H, CH_3), 2.16 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 173.8 (s, C=O(1′)), 170.3 (s, C=O(4′)), 138.0 (s, C(1)), 137.2 (s, C(3)), 131.8 (s, CH(6)), 130.3 (d, CH(2)), 121.2 (d, CH(4)), 117.5 (d, CH(5)), 30.3 (t, $CH_2(2' + 3')$), 19.9 (q, CH_3), 19.0 (q, CH_3); UV-vis (methanol) λ_{max} (log ε): 263 nm (4.02); MS (ESI, MeOH) *m*/*z*: 220.8 [M − H]$^-$ (100%), 441.4 [M(M − H)]$^-$ (98%).

4-[(3,4-Dimethylphenyl)amino]butan-1-ol ***10***

To a suspension of $LiAlH_4$ (2.7 g, 72.0 mmol) and $AlCl_3$ (0.26 g, 2.00 mmol) in THF (50 mL), a solution of **9** (10.7 g, 48.3 mmol) in THF (50 mL) was added dropwise and heated under reflux for 48 h. After cooling to room temperature, MeOH was added dropwise until the evolution of gas had ceased, H_2O (20 mL) was added and the precipitate was filtered off. The residue was washed thoroughly with $CHCl_3$ (3 × 100 mL). Then, the combined extracts were washed with brine (100 mL), dried over Na_2SO_4, and evaporated to dryness. After column chromatography (silica gel, hexane / ethyl acetate, 3 : 7) compound **10** (6.7 g, 72%) was obtained as colourless solid. M.p.: 75–76°C; R_F: 0.75 (hexane / ethyl acetate, 3 : 7); IR (KBr) ν: 3283*s*, 3016*w*, 2950*s*, 2933*s*, 2864*s*, 1613*s*, 1583*w*, 1502*s*, 1479*s*, 1452*m*, 1418*w*, 1383*w*, 1311*m*, 1255*m*, 1227*w*, 1171*m*, 1125*m*, 1105*m*, 1082*s*, 1042*w*, 1023*w* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.92 (d, $^3J_{H,H}$ = 8.0 Hz, 1H, CH(5)), 6.45 (d, $^4J_{H,H}$ = 2.3 Hz, 1H, CH(2)), 6.39 (dd, $^3J_{H,H}$ = 8.0, $^4J_{H,H}$ = 2.3 Hz, 1H, CH(6)), 3.73 (t, $^3J_{H,H}$ = 6.0 Hz, 2H, $CH_2(1')$), 2.49 (t, $^3J_{H,H}$ = 6.5 Hz, 2H, $CH_2(4')$), 2.18 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 1.70–1.62 (m, 4H, $CH_2(2'+3')$); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 146.4 (s, C(3)), 137.2 (s, C(1)), 130.2 (d, CH(5)), 125.6 (s, C(6)), 115.0 (d, CH(2)), 110.6 (d, CH(4)), 62.6 (t, $CH_2(1')$), 44.3 (t, $CH_2(4')$), 30.4 (t, $CH_2(2')$), 26.2 (t, $CH_2(3')$), 20.0 (q, CH_3), 18.6 (q, CH_3); UV-vis (methanol) λ_{max} (log ε): 263 nm (4.04); MS (ESI, MeOH) *m*/*z*: 194.2 [M + H]$^+$ (100%), 437.1 [(M(M + Na)]$^+$ (100%).

4-({4,5-Dimethyl-2-[(E)-phenyldiazenyl]phenyl}amino)-butan-1-ol ***11***

To a solution of aniline (9.3 g, 0.1 mol) in glacial acetic acid (150 mL), H_2O (21 mL) and conc. hydrochloric acid (21 mL) a solution of sodium nitrite (6.21 g, 0.09 mol) in H_2O (10 mL) was added at 0–5°C and stirring was continued for 15 min. Then, **10** (8.7 g, 45.0 mmol) was added, followed by NaOH (6.00 g, 0.15 mol) in H_2O (30 mL) at 0-5°C. After stirring for 2 h at 10°C, the reaction mixture was concentrated *in vacuo* and treated with diethyl ether (250 mL) and saturated Na_2CO_3 solution (250 mL). The phases were separated, the organic layer was washed with H_2O (100 mL) and brine (100 mL). The solution was dried over Na_2SO_4 and concentrated *in vacuo*. The crude material was purified by column chromatography (silica gel, hexane / ethyl acetate, 3 : 7) and **11** (6.4 g, 48%) was obtained as a red oil. IR (KBr) ν: 2928*s*, 1627*m*, 1566*s*, 1508*s*, 1458*s*, 1376*s*, 1278*s*, 1173*s*, 1054*m*, 1001*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.74–7.71 (m, 2H, CH(8')), 7.59 (s, 1H, CH(5)), 7.44–7.42 (m, 2H, ^4J_HCH(9')), 7.36–7.32 (m, 1H, CH(10′)), 6.57 (s, 1H, CH(2)), 3.72 (t, $^3J_{H,H}$ = 6.2 Hz, 2H, $CH_2(1')$), 3.30 (t, $^3J_{H,H}$ = 6.6 Hz, 2H, $CH_2(4')$), 2.27 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 1.85–1.67 (m, 4H, $CH_2(2' + 3')$; ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 153.0 (s, C(7′)), 142.5 (s, C(3)), 141.6 (s, C(1)), 134.8 (s, C(6)), 131.4 (d, CH(5)), 128.9 (d, CH(9′+10′)), 123.9 (s, C(4)), 121.6 (d, CH(8′)), 112.6 (d, CH(2)), 62.4 (t, $CH_2(4')$), 42.5 (t, $CH_2(1')$), 30.3 (t, $CH_2(3')$), 25.7 (t, $CH_2(2')$), 20.5 (q, CH_3), 18.5 (q, CH_3); UV-vis (methanol) λ_{max} (log ε): 343 nm (4.24); MS (ESI, MeOH) *m*/*z*: 298.1 [M + H]$^+$ (100%), 320.3 [M + Na]$^+$ (70%), 617.0 [M(M + Na)]$^+$ (70%).

10-(4-Hydroxybutyl)-7,8-dimethylbenzo[g]pteridine-2,4(3H, 10H)-dione ***12***

A mixture of **11** (4.5 g, 15.0 mmol), barbituric acid (3.8 g, 30.0 mmol) in 1,4-dioxane (50 mL) and glacial acetic acid (8 mL) was heated under reflux for 5 h. After cooling to room temperature, the product was filtered off and washed with diethyl ether until the filtrate was colourless. The crude material was purified by column chromatography (silica gel, CH_2Cl_2 / MeOH, 8 : 2) and afforded compound **12** (2.6 g, 55%) as an orange coloured solid. M.p.: 305–306°C (lit.: 304–307 [18]); R_F: 0.80 (CH_2Cl_2 / MeOH, 8 : 2); IR (KBr) ν: 3511*m*, 3193*m*, 3060*w*, 2940*w*, 1710*s*, 1676*s*, 1582*s*, 1546*s*, 1508*s*, 1462*m*, 1400*w*, 1348*m*, 1320*w*, 1251*m*, 1206*w*, 1162*w*, 1104*w*, 1056*w*, 1027*w* cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.21 (br s, 1H, NH), 7.80 (s, 1H, CH(9)), 7.72 (s, 1H, CH(6)), 4.53 (t, $^3J_{H,H}$ = 7.5 Hz, 2H, $CH_2(1')$), 3.44 (t, $^3J_{H,H}$ = 6.2 Hz, 2H, $CH_2(4')$), 2.46 (s, 3H, CH_3), 2.34 (s, 3H, CH_3); 1.77–1.68 (m, 2H, $CH_2(2')$), 1.58–1.51 (m, 2H, $CH_2(3')$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 159.9 (s, C=O), 155.7 (s, C=O), 149.9 (s, C=N(1a)), 146.7 (s, C(8)), 136.9 (s, C(6a)), 135.8 (s, C(7)), 133.7 (s, C(9a)), 131.0 (d, CH(9)), 130.6 (s, C=N(4a)), 116.0 (d, CH(6)), 60.4 (t, $CH_2(4')$), 44.1 (t, $CH_2(1')$), 29.3 (t, $CH_2(2')$), 23.4 (t, $CH_2(3')$), 20.2 (q, CH_3), 18.8 (q, CH_3); UV-vis (methanol) λ_{max} (log ε): 240 nm (4.43); MS (ESI, MeOH) *m*/*z*: 315.2 [M + H]$^+$ (20%), 337.2 [M + Na]$^+$ (30%), 651.6 [M(M + Na)]$^+$ (100%).

10-(4-Iodobutyl)-7,8-dimethyl-benzo[g]pteridine-2,4(3H, 10H)-dione ***13***

To a mixture of **12** (2.0 g, 6.4 mmol), iodine (3.4 g, 13.2 mmol) und imidazole (0.9 g, 1.3 mmol) in CH_2Cl_2 (100 mL), PPh_3 (3.3 g, 12.7 mmol) was added at 0°C, stirring was continued for 1 h at

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room temperature. The solvent was evaporated and the residue subjected to column chromatography (silica gel, CH_2Cl_2 / MeOH, 95 : 5). Compound **13** (1.6 g, 60%) was obtained as an orange coloured solid. M.p.: >200°C (decomp.); R_F: 0.20 (CH_2Cl_2 / MeOH, 95 : 5); IR (KBr) ν: 3446*s*, 1717*m*, 1654*s*, 1577*s*, 1538*s*, 1503*m*, 1459*m*, 1350*m*, 1262*m* cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.00 (br s, 1H, NH), 7.99 (s, 1H, CH(9)), 7.49 (s, 1H, CH(6)), 4.70 (br, 2H, CH_2(1')), 3.29 (t, $^3J_{H,H}$ = 6.2 Hz, 2H, CH_2(4')), 2.56 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 2.05-1.97 (m, 4H, CH_2(2'+3')); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 160.0 (s, C=O), 156.4 (s, C=O), 149.8 (s, C=N(1a)), 148.6 (s, C(8)), 137.3 (s, C(6a)), 135.7 (s, C(7)), 134.8 (s, C(9a)), 131.8 (d, CH(9)), 130.7 (s, C=N(4a)), 115.4 (d, CH(6)), 43.9 (t, CH_2(1')), 29.9 (t, CH_2(2')), 27.6 (t, CH_2(3')), 21.4 (q, CH_3), 19.2 (q, CH_3), 5.5 (t, CH_2(4')); UV-vis (methanol) λ_{max} (log ε): 240 nm (4.59); MS (ESI, MeOH) *m*/*z*: 425.0 $[M + H]^+$ (10%), 447.0 $[M + Na]^+$ (20%), 871.0 $[M(M + Na)]^+$ (100%).

10,10'(Piperazine-1,4-diyldibutane-4,1-diyl)bis(7,8-dimethylbenzo[g]pteridine-2,4(3H,10H)-dione) **14**

Compound **14** (0.1 g, 31%) was obtained from **13** (0.50 g, 1.18 mmol) and piperazine (40.40 mg, 0.47 mmol) following the procedure described for compound **3** as an orange coloured solid. M.p.: >250°C (decomp.); R_F: 0.33 (CH_2Cl_2 / MeOH, 7 : 3); IR (KBr) ν: 3447*m*, 1654*m*, 1578*m*, 1541*s*, 1458*m*, 1400*m*, 1350*m*, 1260*m*, 1008*w* cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6 + D_2SO_4) δ 7.91 (s, 2H, CH(9)), 7.85 (s, 2H, CH(6)), 4.64 (br, 4H, CH_2(1')), 3.48 – 3.30 (m, 12H, CH_2(4′) + piperazine), 2.51 (s, 6H, CH_3), 2.39 (s, 6H, CH_3), 1.86 – 1.76 (m, 8H, CH_2(2′+3′)); UV-vis (methanol) λ_{max} (log ε): 240 nm (4.00); MS (ESI, MeOH) *m*/*z*: 679.3 $[M + H]^+$ (100%).

O^2,O^3,O^4,O^5-Tetraacetylriboflavin **15**

To a mixture of glacial acetic acid (200 mL) and acetic anhydride (200 mL), riboflavin (5.0 g, 13.3 mmol) was added followed by $HClO_4$ (1 mL). The reaction mixture was stirred for 30 min at 40 C, then cooled in an ice bath, diluted with water (400 mL), and extracted with $CHCl_3$ (3 × 25 mL). The combined organic extracts were washed with H_2O (4 × 25 mL) and brine (25 mL). The solution was dried over Na_2SO_4 and evaporated to dryness. After recrystallisation from EtOH, compound **15** (5.8 g, 80%) was obtained as an orange colored solid. M.p.: 240 – 241°C (decomp.) (lit.: 242 [19], 240 [20], 238 – 242 [21], 238-239 [22]); $[\alpha]_D$: 119.5° (*c* = 2.2, $CHCl_3$) (lit.: 80.0° (*c* = 0.25, 0.1N NaOH) [23]); R_F: 0.22 (CH_2Cl_2 / MeOH, 95: 5); (IR (KBr) ν: 3159*w*, 3040*w*, 2813*w*, 1749*s*, 1716*s*, 1663*s*, 1578*s*, 1538*s*, 1508*m*, 1439*w*, 1400*s*, 1374*m*, 1212*s*, 1157*w*, 1056*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.93 (br s, 1H, NH), 7.96 (s, 1H, CH(9)), 7.52 (s, 1H, CH(6)), 5.62 (br s, 1H, CH (2')), 5.42 (dd, $^3J_{H,H}$ = 7.0, $^3J_{H,H}$ = 6.2 Hz, 1H, CH (3')), 5.37 (ddd, $^3J_{H,H}$ = 6.2, $^3J_{H,H}$ = 5.8, $^3J_{H,H}$ = 2.9 Hz, 1H, CH_2(4′)), 4.85 (br, 2H, CH_2(1′)), 4.40 (dd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 2.9 Hz, 1H, CH_a(5′)), 4.20 (dd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 5.8 Hz, 1H, CH_b(5′)), 2.53 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.25 (s, 3H, Ac), 2.18 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.71 (s, 3H, Ac); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5 (s, C=O), 170.1 (s, C=O), 169.7 (s, C=O), 169.6 (s, C=O), 159.2 (s, C=O), 154.5 (s, C=O), 150.6 (s, C=N(1a)), 147.9 C(8)), 136.8 (s, C(6a)), 136.0 (s, C(7)), 134.5 (s, C(9a)), 132.8 (d, CH(9)), 131.1 (s, C=N(4a)), 115.5 (d, CH(6)), 70.5 (d, CH(3′)), 69.4 (d, CH(2′)), 69.0 (d, CH(4′)), 61.8 (t, CH_2(5′)), 45.0 (t, CH_2(1′)), 21.4 (q, CH_3), 21.0 (q, OAc), 20.7 (q, OAc), 20.6 (q, OAc), 20.3 (q, OAc), 19.4 (q, CH_3); UV-vis (methanol) λ_{max} (log ε): 240 nm (4.47); MS (ESI, MeOH) *m*/*z*: 545.1 $[M + H]^+$ (30%), 567.1 $[M + Na]^+$ (100%), 1110.2 $[M(M + Na)]^+$ (100%).

3-(3-Iodopropyl)-O^2,O^3,O^4,O^5-tetraacetylriboflavin **16**

To a solution of **15** (5.0 g, 9.0 mmol) and Cs_2CO_3 (4.5 g, 13.5 mmol) in DMF (50 mL), 1,3-diiodopropane (7.5 g, 25.0 mmol) was added, then stirred for 1 h at room temperature. The solvent was removed *in vacuo*, the residue dissolved in CH_2Cl_2 (200 mL) and washed with H_2O (2 × 100 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. After purification by column chromatography (silica gel, CH_2Cl_2 / MeOH, 95 : 5), compound **16** (4.5 g, 70%) was obtained as an orange coloured oil. $[\alpha]_D$: 88.2° (*c* = 4.1, $CHCl_3$); R_F: 0.60 (CH_2Cl_2 / MeOH, 9 : 1); IR (KBr) ν: 2930*w*, 1747*s*, 1709*s*, 1662*s*, 1587*s*, 1549*s*, 1437*m*, 1372*m*, 1219*s*, 1157*w*, 1092*m*, 1051*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H, CH(9)), 7.52 (s, 1H, CH(6)), 5.64 (br s, 1H, CH (2′)), 5.44 (dd, $^3J_{H,H}$ = 7.0, $^3J_{H,H}$ = 6.2 Hz, 1H, CH (3′)), 5.39 (ddd, $^3J_{H,H}$ = 6.2, $^3J_{H,H}$ = 5.8, $^3J_{H,H}$ = 2.9 Hz, 1H, CH_2(4′)), 4.90 (br, 2H, CH_2(1′)), 4.42 (dd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 2.9 Hz, 1H, CH_a(5′)), 4.23 (dd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 5.8 Hz, 1H, CH_b(5′)), 4.12 (t, $^3J_{H,H}$ = 7.0 Hz, 2H, CH_2(1″)), 3.20 (t, $^3J_{H,H}$ = 7.5 Hz, 2H, CH_2(3″)), 2.53 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 2.33 – 2.22 (m, 5H, Ac + CH_2(2″)), 2.20 (s, 3H, Ac), 2.05 (s, 3H, Ac), 1.72 (s, 3H, Ac); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3 (s, C=O), 170.0 (s, C=O), 169.6 (s, C=O), 169.4 (s, C=O), 160.1 (s, C=O), 155.5 (s, C=O), 149.0 (s, C=N(1a)), 147.7 C(8)), 136.7 (s, C(6a)), 135.4 (s, C(7)), 134.8 (s, C(9a)), 132.9 (d, CH(9)), 131.1 (s, C=N(4a)), 115.5 (d, CH(6)), 70.5 (d, CH(3′)), 69.5 (d, CH(2′)), 69.1 (d, CH(4′)), 61.9 (t, CH_2(5′)), 44.7 (t, CH_2(1′)), 42.7 (t, CH_2(1″)), 32.0 (t, CH_2(2″)), 21.5 (q, CH_3), 21.0 (q, OAc), 20.7 (q, OAc), 20.6 (q, OAc), 20.3 (q, OAc), 19.4 (q, CH_3), 1.6 (t, CH_2(3″)); UV-vis (methanol) λ_{max} (log ε): 240 nm (4.46); MS (ESI, MeOH) *m*/*z*: 713.0 $[M + H]^+$ (20%), 735.0 $[M + Na]^+$ (100%), 1446.2 $[M(M + Na)]^+$ (50%).

1,4-Bis-[3-(O^2,O^3,O^4,O^5-tetraacetylriboflavin-3-yl)propyl]piperazine **17**

Compound **17** (0.2 g, 20%) was obtained from **16** (1.5 g, 2.1 mmol) following the procedure described for compound **2**, followed by column chromatography (silica gel, CH_2Cl_2 / MeOH, 95 : 5) as an amorphous orange coloured oil. $[\alpha]_D$: 65.6° (*c* = 3.4, $CHCl_3$); R_F: 0.50 (CH_2Cl_2 / MeOH, 9 : 1);IR (KBr) ν: 2926*m*, 1749*s*, 1709*m*, 1656*s*, 1587*s*, 1549*s*, 1437m, 1372*m*, 1217*s*, 1157*w*, 1049*m* cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.99 (s, 2H, CH(9)), 7.52 (s, 2H, CH(6)), 5.64 (br s, 2H, CH (2k)), 5.43 (dd, $^3J_{H,H}$ = 7.0, $^3J_{H,H}$ = 6.2 Hz, 2H, CH(3′)), 5.38 (ddd, $^3J_{H,H}$ = 6.2, $^3J_{H,H}$ = 5.8, $^3J_{H,H}$ = 2.9 Hz, 2H, CH_2(4′)), 4.90 (br, 4H, CH_2(1′)), 4.41 (dd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 2.9 Hz, 2H, CH_a(5′)), 4.22 (dd, $^2J_{H,H}$ = 12.5, $^3J_{H,H}$ = 5.8 Hz, 2H, CH_b(5′)), 4.10 (t, $^3J_{H,H}$ = 7.0 Hz, 4H, CH_2(1″)), 3.60 (t, $^3J_{H,H}$ = 6.6 Hz, 4H, CH_2(3″)), 2.80 (br s, 8H, piperazine), 2.52 (s, 6H, CH_3), 2.41 (s, 6H, CH_3), 2.24 (s, 6H, Ac), 2.18 (s, 6H, Ac), 1.96 – 2.08 (m, 10H, Ac + CH_2(2″)), 1.71 (s, 6H, Ac); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.5 (s, C=O), 170.2 (s, C=O), 169.8 (s, C=O), 169.6 (s, C=O), 159.9 (s, C=O), 154.8 (s, C=O), 149.1 (s, C=N(1a)), 147.5 C(8)), 136.6 (s, C(6a)), 135.6 (s, C(7)), 134.7 (s, C(9a)), 133.0 (d, CH(3)), 131.2 (s, C=N(4a)), 115.4 (d, CH(6)), 70.5 (d, CH (3′)), 69.3 (d, CH (2′)), 69.1 (d, CH (4′)), 61.8 (t, CH_2(5′)), 55.0 (t, CH_2(3″)), 44.6 (t, CH_2(1′)), 39.7 (t, CH_2(1″)), 34.8 (t, piperazine), 29.6 (t, CH_2(2″)), 21.4 (q, CH_3), 21.0 (q, OAc), 20.7 (q, OAc), 20.6 (q, OAc), 20.3 (q, OAc), 19.4 (q, CH_3); UV-vis (methanol) λ_{max} (log ε): 240 nm (4.83); MS (ESI, MeOH) *m*/*z*: 1255.4 $[M + H]^+$ (100%), 1277.4 $[M + Na]^+$ (20%).

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