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SYNTHESIS OF ANALOGUES OF MAKALUVAMINE A

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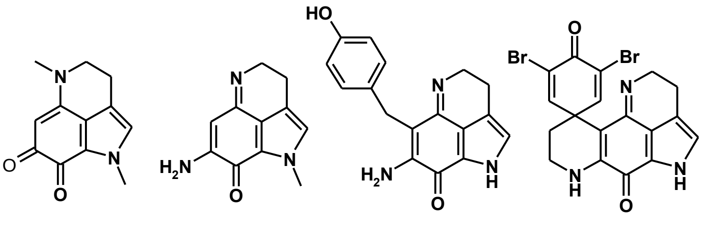
Abstract – A new and efficient synthesis of tricyclic pyridoquinonoxalinone **1** an analog of Makaluvamine A and its intermediates **2** and **3** has been developed starting from 5,8-dimethoxy-4,4-dimethyl[1,2,3,4]tetrahydroquinoline **4**. The synthesis was accomplished in 11 steps and 4% overall yield from commercially available 2,5-dimethoxyaniline.

Marine sponges are the most prolific producers of biologically active natural marine products.[1] An important class of bioactive sponge metabolites is that the pyrroloiminoquinone alkaloids confined to the sponge family *Latrunculiidae*.[2,3] This class of alkaloids includes damirones, discorhabdins, prianosines, batzellines, isobatzellines, wakayin, makaluvamines, tsitsikammanines, epinardins and veiutamine (Figure 1).[4-20]



damirone A makaluvamine A veiutamine discorhabdin C

Figure 1. Marine natural products from sponge family *Latrunculiidae*

Makaluvamines, based on a pyrrolo[4,3,2-*de*]quinoline skeleton, are an important class of marine metabolites isolated predominantly from sponges of the *Zyzzya genus.*[21] They were reported to show significant *in vitro* and *in vivo* cytotoxic activity towards several tumour cell lines like human colon tumour cell line HCT-116.[22] This class of metabolites was also shown to inhibit the function of topoisomerase II, to intercalate into DNA and to effect reductive cleavage of DNA.[22,23] As part of our continuing search for potential antineoplastic agents, we have investigated the synthesis of Makaluvamine mimics in which the pyrrole core has been replaced by a piperidine ring (Figure 2).

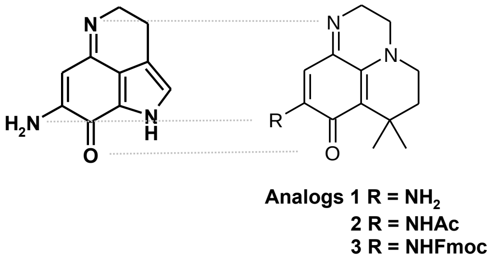
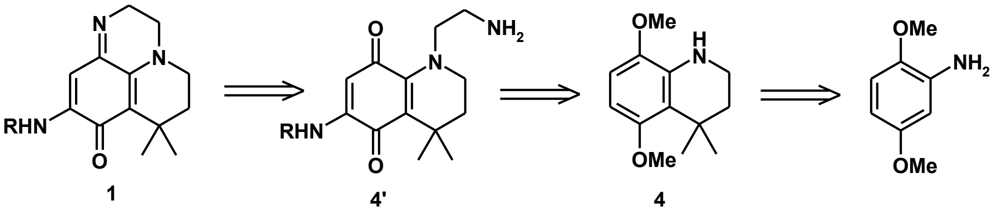


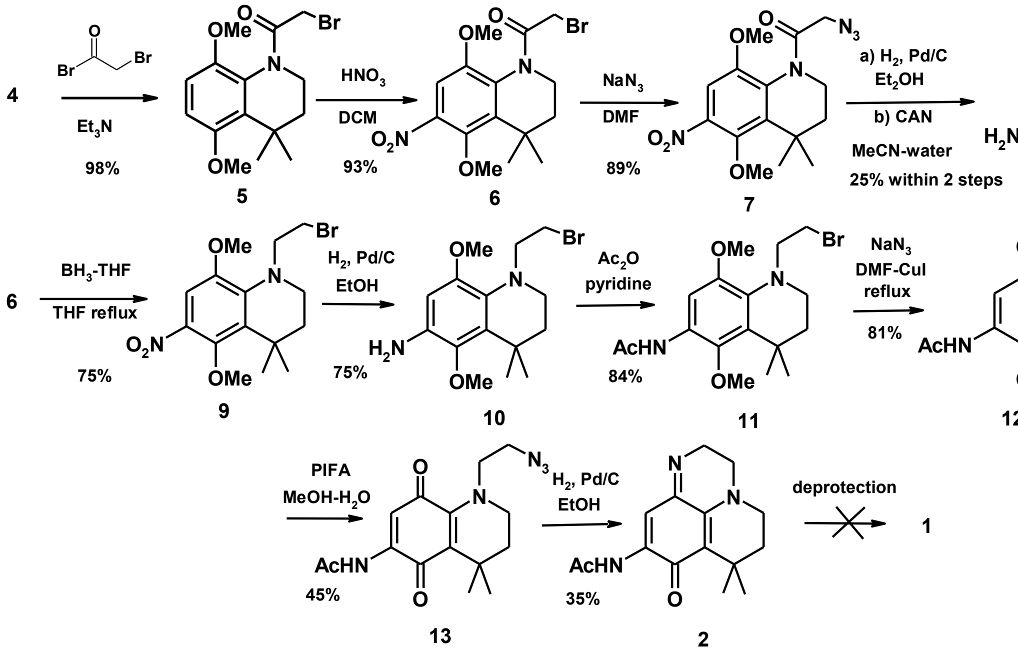
Figure 2. Analogues of Makaluvamine A

As illustrated in Scheme 1, our approach starts from 2,5-dimethoxyaniline which is readily transformed into tetrahydroquinoline **4** in two steps.[24] *N*-Alkylation of **4**, followed by oxidation of **4** with [bis(trifluoroacetoxy)iodo]benzene (PIFA) or cerium ammonium nitrate (CAN) should afford the desired quinone **4’** that should uneventfully give compound **1**.



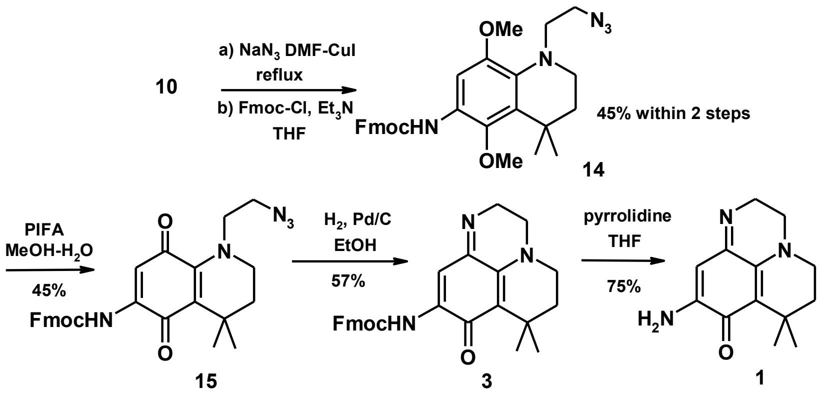
Scheme 1. Retrosynthetic analysis

In a first attempt, we tried to synthesize analog **1** starting from 5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinoline **4**.[24] *N*-Acylation of **4** with bromoacetyl bromide in the presence of triethylamine provided bromoacetamide **5** (98%). Nitration of **5** with extra pure nitric acid in dichloromethane afforded the nitro derivative **6** (93%). Bromine displacement with sodium azide in *N*,*N*-dimethylformamide led to the desired azido derivative **7** (89%). The azido and nitro group of **7** were then easily reduced under hydrogenation with Pd/C in ethanol. However, oxidation of the resulting glycinamide with CAN did not furnish the corresponding imino-quinone and only the aromatic product **8** was isolated in a poor yield (25%, 2 steps).[25] This unexpected result was believed to be due the amide function and so we decided to reduce this functional group earlier in the synthesis using BH_3-THF complex, thus obtaining **9** in 75% yield. Reduction of the nitro group of **9** followed by acetylation furnished compound **11** in good yield. Subsequently, treatment of **11** with sodium azide in *N*,*N*-dimethylformamide with a catalytic amount of copper iodide, provided azide **12** in 81% yield. Oxidation of **12** with PIFA led to desired quinone **13** in 45% yield.[26,27] This quinone **13** was then reduced by catalytic hydrogenation on Pd/C to afford the desired imine **2**. Unfortunately, all attempts to remove the acetyl protective group of **2** were unsuccessful under acidic or basic conditions.



Scheme 2. Synthesis of acetyl iminoquinone **2**

In view of these results, we decided to change the protecting group of the aromatic amine from intermediate **10** using the fluorenylmethyloxy carbonyl (Fmoc) group. Follow the same sequences previously outlined in Scheme 2, *i.e.* oxidation, reduction-cyclisation and deprotection, the desired compound **1** was obtained in satisfactory yield (Scheme 3).



Scheme 3. Synthesis of analogue **1**

Tricyclics (**1**-**3**) were screened for their ability to inhibit topoisomerase II enzymatic activity. Topoisomerase II functions by generating a break in double-stranded DNA, followed by resealing of the break. Topoisomerase II inhibitors interfere with the breakage-rejoining reaction thereby trapping the enzyme in a cleavage complex. In order to determine the ability of our compounds to inhibit topoisomerase II enzymatic activity, we used a topoisomerase-II drug screening kit that uses a supercoiled plasmid DNA substrate (pRYG) which contains one topoisomerase II cleavage/recognition site. Doxorubicin was used as a control in these assays. Unfortunately, all the molecules tested failed to showed (100μM) significant inhibition of topoisomerase II (>100μM).

To summarise, we have developed an efficient synthetic route to analogues **1-3**. The synthesis of the tetrahydropyrido[1,2,3-*de*]quinoxalin-8(*5H*)-one **1** was achieved in 11 steps from the commercially available 2,5-dimethoxyaniline with 4% overall yield. The synthetic methodology developed in this work will be extended to access other interesting makaluvamine analogues.

EXPERIMENTAL

All reactions requiring anhydrous conditions were conducted over flame dried apparatus under an argon atmosphere. THF was freshly distilled from benzophenone-sodium. All reagents and starting materials were purchased from commercial sources and used as received. All lithiation reactions were carried out using Sigma-Aldrich LDA 2.0M in tetrahydrofuran/heptane/ethylbenzene. Analytical TLC were carried out on silica gel F_{254} plates. Visualization was achieved by UV light (254 nm). Flash column chromatography was carried using Sigma-Aldrich Versaflash silica gel (particle size 20-45 μm). Melting points (Mp) were measured on a Büchi B-540 capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ using a Bruker Avance 300 FT spectrometer at ambient temperature (operating frequencies: ^{1}H, 300,13 MHz; ^{13}C, 75,47 MHz). The chemical shifts (δ, ppm) for all compounds are listed in parts per million downfield from tetramethylsilane using the NMR solvent as an internal reference. The reference values used for deuterated chloroform ($CDCl_3$) were 7.26 and 77.00 ppm for ^{1}H and ^{13}C NMR spectra, respectively. Multiplicities are given as: s (singlet), brs (broad singlet), d (doublet), t (triplet), dd (doublet of doublets), td (triplet of doublets) or m (multiplet). Low-resolution mass (MS) were recorded on a Shimadzu GCMS-QP 2010 Gas Chromatograph Mass Spectrometer and reported in units of mass to charge (*m/z*). The mode of ionization used was electron-impact (EI, 70 eV) or chemical ionization (CI, methane reagent gas).

2-Bromo-1-(5,8-dimethoxy-4,4-dimethyl-3,4-dihydroquinolein-1(*2H*)-yl)ethanone (5)

Under an inert atmosphere, to a solution of **4** (1 g, 4.5 mmol) in dry THF at 0 °C was added dropwise bromoacetyl bromide (0.59 mL, 6.78 mmol). The mixture was left to stir for 2 h at room temperature. The mixture was poured onto ice and the product was extracted with EtOAc (3 x 30 mL). The organic layers were combined and successively washed with a saturated aqueous solution of sodium hydrogencarbonate and a saturated aqueous solution of sodium chloride. They were then dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **5** as brown solid (1.51 g, 98%). Mp 91-94 °C. ^{1}H NMR (300MHz, $CDCl_3$): δ = 1.37 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.68 (m, 1H, CH_2), 1.93 (m, 1H, CH_2), 2.97 (m, 1H, CH_2), 3.77 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.84 (d, 1H, J = 10.5 Hz, $C\mathbf{H_a}H_b$), 3.97 (d, 1H, J = 10.5 Hz, $CH_a\mathbf{H_b}$), 4.62 (m, 1H, CH_2), 6.75 (s, 1H, H_{Ar}), 6.76 (s, 1H, H_{Ar}) ppm. ^{13}C RMN (75 MHz, $CDCl_3$): δ = 28.0 (CH_2), 28.5 (2xCH_3), 34.7 (C), 42.1 (2xCH_2), 55.6 (OCH_3), 56.4 (OCH_3), 109.5 (CH_{Ar}), 109.9 (CH_{Ar}), 128.9 (C), 129.5 (C), 145.9 (C), 153.2 (C), 168.4 (C=O) ppm. MS (EI): *m/z* = 231 ([M-CH_3-CH_3-Br]$^+$, 100%), 206 ([M-$COCH_2$Br-CH_3]$^+$, 84%), 310 ([M-OCH_3, Br^{79}]$^+$, 83%), 312 ([M-OCH_3, Br^{81}]$^+$, 74%), 341 ([M, Br^{79}]$^+$, 39%), 343 ([M, Br^{81}]$^+$, 34%). Anal. Calcd for $C_{15}H_{20}BrNO_3$ (342.24) : C 52.64, H 5.89, N 4.09; Found : C 52.44, H 5.90, N 4.07.

2-Bromo-1-(5,8-dimethoxy-4,4-dimethyl-6-nitro-3,4-dihydroquinolein-1(*2H*)-yl)ethanone (6)

A solution of 2-bromo-1-(5,8-dimethoxy-4,4-dimethyl-3,4-dihydroquinolein-1(*2H*)-yl)ethanone **5** (1.8 g, 5.3 mmol) in dry CH_2Cl_2 (20 mL) was cooled to 0 °C under argon atmosphere. Extra pure nitric acid (99.99%) was added carefully and the mixture was allowed to stir at 0 °C for 2 h before addition of ice. The product was extracted with EtOAc (2 x 20 mL) and the combined organic layers were successively washed with an aqueous solution of sodium hydrogencarbonate (20 mL) and sodium chloride (20 mL), and dried over anhydrous $MgSO_4$. The solvents were then removed under reduced pressure. Recrystallization with CH_2Cl_2 provided the desired compound **6** as brown solid (1.89 g, 93%). Mp 126-130 °C. ^{1}H NMR (300 MHz, DMSO): δ = 1.47 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.75 (m, 1H, $C\mathbf{H_a}H_b$), 1.95 (m, 1H, $CH_a\mathbf{H_b}$), 3.16 (m, 1H, $C\mathbf{H_c}H_d$), 3.84 (s, 3H, OCH_3), 3.85 (m, 2H, CH_2), 3.87 (m, 3H, OCH_3), 3.90 (m, 1H, $CH_c\mathbf{H_d}$), 7.32 (s, 1H, H_{Ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.7 (CH_2), 27.9 (CH_3), 29.5 (CH_3), 35.8 (C), 41.2 (CH_2), 42.1 (CH_2), 56.6 (OCH_3), 62.4 (OCH_3), 106.6 (CH_{Ar}), 133.2 (C), 136.5 (C), 140.9 (C), 147.4 (C), 148.2 (C), 167.9 (C=O) ppm. MS (CI): *m/z* = 386 ([M, Br^{79}]$^+$, 100%), 388 ([M, Br^{81}]$^+$, 96%), 387 ([M+H, Br^{79}]$^+$, 46%), 356 ([M-OCH_3+H, Br^{79}]$^+$, 19%). Anal. Calcd for $C_{15}H_{19}BrN_2O_5$ (387.23) : C 46.53, H 4.95, N 7.23; Found : C 46.67, H 4.97, N 7.22.

2-Azido-1-(5,8-dimethoxy-4,4-dimethyl-6-nitro-3,4-dihydroquinolein-1(*2H*)-yl)ethanone (7)

Under an inert atmosphere, to a solution of 2-bromo-1-(5,8-dimethoxy-4,4-dimethyl-6-nitro-3,4-dihydroquinolein-1(*2H*)-yl)ethanone **6** (0.4 g, 1.03 mmol) in dry DMF (5 mL) was added sodium azide (0.15 g, 1.8 mmol). The mixture was warmed to reflux for 2 h. After addition of a saturated aqueous solution of sodium hydrogencarbonate (30 mL), the product was extracted with EtOAc (2 x 20 mL) and the combined organic layers were washed with a saturated aqueous solution of sodium chloride (20 mL), dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **7** as yellow solid (0.32 g, 89%). Mp 113-116 °C. ^{1}H NMR (300MHz, $CDCl_3$): δ = 1.42 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.80 (m, 1H, $C\mathbf{H_a}H_b$-N_3), 3.13 (m, 1H, $CH_a\mathbf{H_b}$-N_3), 3.53 (d, 1H, *J* = 15.3 Hz, $C\mathbf{H_a}H_b$), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.95 (d, 1H, *J* = 15.3 Hz, $CH_a\mathbf{H_b}$), 4.46 (m, 2H, CH_2), 7.28 (s, 1H, H_{Ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.9 (CH_3), 29.5 (CH_3), 35.8 (C), 41.5 (CH_2), 41.8 (CH_2), 51.1 (CH_2), 56.5 (OCH_3), 62.6 (OCH_3), 106.5 (CH_{Ar}), 132.4 (C), 136.6 (C), 141.1 (C), 147.6 (C), 147.9 (C), 168.7 (C=O) ppm. MS (EI): *m/z* = 290 ($[M\text{- }N_2\text{-}OCH_3]^+$, 100%), 321 ($[M\text{-}N_2]^+$, 4%). Anal. Calcd for $C_{15}H_{19}N_5O_5$ (349.35) : C 51.57, H 5.48, N 20.05; Found : C 51.72, H 5.45, N 20.03.

9-Amino-8-methoxy-7,7-dimethyl-6,7-dihydropyrido[1,2,3-*de*]quinoxalin-3(5*H*)-one (8)

A solution of 2-azido-1-(5,8-dimethoxy-4,4-dimethyl-6-nitro-3,4-dihydroquinolein-1(2*H*)-yl)ethanone **7** (0.2 g, 0.57 mmol) in EtOH (10 mL) was hydrogenated (15 psi) over Pd/C (0.04 g) for 18 h at room temperature. The catalyst was filtered on Celite and the filtrate concentrated *in vacuo* to afford the desired amino compound as brown oil (40 mg, 30%), which was used without further purification. A solution of CAN (0.286 g, 0.51 mmol) in water (3 mL) was added dropwise to a solution of 2-amino-1-(6-amino-5,8-dimethoxy-4,4-dimethyl-3,4-dihydroquinolin-1(2*H*)-yl)ethanone (40 mg, 0.17 mmol) in MeCN (9 mL). Once addition was finished, the reaction mixture was allowed to stir at room temperature until the reaction was completed. Water (10 mL) was then added and the product was extracted with CH_2Cl_2 (2 x 10 mL). The organic layers were combined and washed with a saturated aqueous solution of sodium chloride (2 x 10 mL). They were then dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **8** as yellow oil (11 mg, 25% over 2 steps). ^1H NMR (300 MHz, $CDCl_3$): δ = 1.51 (s, 6H, $2xCH_3$), 1.90 (m, 2H, CH_2), 3.89 (s, 3H, OCH_3), 4.10 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 7.12 (s, 1H, H_{Ar}), 8.19 (s, 1H, $H_{C=N}$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.9 (2 x CH_3), 32.4 (C), 37.4 (CH_2), 37.6 (CH_2), 59.9 (OCH_3), 113.8 (CH_{Ar}), 123.1 (C), 125.9 (C), 129.6 (C), 137.3 (C), 147.8 (N=CH), 150.8 (C), 154.2 (C=O) ppm. MS (CI): *m/z* = 260 ($[M+1]^+$, 100%). Anal. Calcd for $C_{14}H_{17}N_3O_2$ (259.31): C 64.85, H 6.61, N 16.20; Found : C 64.73, H 6.63, N 16.19.

1-(2-Bromoethyl)-5,8-dimethoxy-4,4-dimethyl-6-nitro-1,2,3,4-tetrahydroquinoleine (9)

Under an inert atmosphere, a solution of borane-tetrahydrofuran complex (11.6 mL, 1 M solution in THF, 11.6 mmol) was added dropwise to a solution of compound **6** (1.5 g, 3.8 mmol) in dry THF to 0 °C. The addition finished, the reaction mixture was then warmed to reflux for 2 h. After the mixture was poured onto ice and the product was extracted with EtOAc (3 x 30 mL). The organic layers were combined and successively washed with water (2 x 20 mL), a saturated aqueous solution of sodium hydrogen carbonate (2 x 20 mL) and a saturated aqueous solution of sodium chloride (20 mL). They were then dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **9** as yellow solid (1.08 g, 75%). Mp 96-99 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.45 (s, 6H, $2xCH_3$), 1.72 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 3.51 (m, 4H, 2 x CH_2), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 7.39 (s, 1H, H_{Ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 29.2 (2xCH_3), 29.7 (CH_2), 33.6 (C), 38.6 (CH_2), 46.5 (CH_2), 56.3 (CH_2), 57.1 (OCH_3), 61.7 (OCH_3), 106.6 (CH_{Ar}), 129.4 (C), 133.8 (C), 143.5 (C), 145.5 (C), 150.8 (C) ppm. MS (EI): *m/z* = 279 ($[M-CH_2Br]^+$, 100%), 280 ($[M-Br-CH_3]^+$, 16%), 374 ($[M, Br^{81}]^+$, 12%), 372 ($[M, Br^{79}]^+$, 12%). Anal. Calcd for $C_{14}H_{16}BrN_2O_4$ (373.25) : C 48.27, H 5.67, N 7.51; Found : C 48.51, H 5.70, N 7.50.

1-(2-Bromoethyl)-5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinolein-6-amine (10)

A solution of 1-(2-bromoethyl)-5,8-dimethoxy-4,4-dimethyl-6-nitro-1,2,3,4-tetrahydroquinoleine **9** (0.5 g, 1.34 mmol) in EtOH (10 mL) was hydrogenated (15 psi) over Pd/C (0.1 g) for 18 h at room temperature. The catalyst was filtered on Celite and the filtrate concentrated *in vacuo*. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **10** as black solid (0.34 g, 75%). Mp 120-124 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.41 (s, 6H, 2 x CH_3), 1.67 (m, 2H, CH_2), 3.07 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 3.64 (m, 2H, CH_2), 3.76 (s, 3H, OCH_3), 3.81 (brs, 3H, OCH_3), 6.25 (brs, 1H, H_{Ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 29.6 (2 x CH_3), 30.3 (CH_2), 33.1 (C), 36.6 (CH_2), 45.5 (CH_2), 55.6 (CH_2), 57.6 (OCH_3), 59.4 (OCH_3), 98.8 (CH_{Ar}), 125.5 (C), 131.5 (C), 140.5 (C), 148.9 (C), 154.0 (C) ppm. SM (EI): *m/z* = 249 ($[M-CH_2Br]^+$, 100%), 342 ($[M, Br^{79}]^+$, 81%), 344 ($[M, Br^{81}]^+$, 78%), 233 ($[M-(Br+CH_3+CH_3)]^+$, 43%), 234 ($[M-(Br+OCH_3)]^+$, 31%). Anal. Calcd for $C_{15}H_{23}BrN_2O_2$ (343.27) : C 52.49, H 6.75, N 8.16; Found : C 52.38, H 6.78, N 8.15.

***N*-(1-(2-Bromoethyl)-5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinolein-6-yl)acetamide (11)**

To a solution of 1-(2-bromoethyl)-5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinolein-6-amine **10** (0.3 g, 0.87 mmol) in dry pyridine (1 mL) was added acetic anhydride (4 mL). The mixture was stirred for 18 h at room temperature. A solution of 1N HCl (70 mL) was then added and the product was extracted with EtOAc (2 x 20 mL). The organic layers were washed several times with a solution of 1N HCl and finally with a saturated aqueous solution of sodium chloride (10 mL). The extract was dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **11** as brown solid (0.28 g, 84%). Mp 159-162 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.37 (s, 6H, 2 x CH_3), 1.64 (m, 2H, CH_2), 2.20 (s, 3H, $COCH_3$), 3.05 (m, 2H, CH_2), 3.22 (m, 2H, CH_2), 3.60 (m, 2H, CH_2), 3.71 (s, 3H, OCH_3), 3.82 (brs, 3H, OCH_3), 7.31 (s, 1H, H_{Ar}), 7.57 (s, 1H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.7 (NCO$\mathbf{CH_3}$), 29.6 (2 x CH_3), 33.1 (C), 45.5 (CH_2), 45.7 (CH_2), 55.8 (CH_2), 57.2 (CH_2), 57.4 (OCH_3), 61.3 (OCH_3), 104.0 (CH_{Ar}), 125.1 (C), 130.1 (C), 134.3 (C), 143.8 (C), 148.2 (C), 168.2 (C=O) ppm. MS (EI): *m/z* = 291 ($[M-CH_2Br]^+$, 100%), 290 ($[M-CH_3-Br]^+$, 64%), 384 ($[M, Br^{79}]^+$, 54%), 386 ($[M, Br^{81}]^+$, 56%). Anal. Calcd for $C_{17}H_{23}BrN_2O_3$ (385.30) : C 52.99, H 6.54, N 7.27; Found : C 52.98, H 6.51, N 7.29.

***N*-(1-(2-Azidoethyl)-5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinolein-6-yl)acetamide (12)**

Under an inert atmosphere, to a solution of **11** (0.2 g, 0.52 mmol) in dry DMF (5 mL) was added sodium azide (0.1 g, 1.56 mmol) and copper iodide (0.01 g). Once addition was complete, the mixture was heated to reflux for 3 h. After addition of a saturated aqueous solution of sodium hydrogen carbonate (20 mL), the product was extracted with EtOAc (2 x 20 mL) and the combined organic layers were washed with a saturated aqueous solution of sodium chloride (20 mL), dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **12** as pink solid (0.14 g, 81%). Mp 136-138 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (s, 6H, 2 x CH_3), 1.61 (m, 2H, CH_2), 2.20 (s, 3H, $COCH_3$), 3.04 (m, 4H, 2xCH_2), 3.53 (m, 2H, CH_2), 3.71 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 7.35 (brs, 1H, NH), 7.55 (s, 1H, H_{Ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.6 (NCO**CH_3**), 29.6 (2 x CH_3), 33.1 (C), 37.0 (CH_2), 45.3 (CH_2), 50.9 (CH_2), 53.7 (CH_2), 55.6 (OCH_3), 61.2 (OCH_3), 104.0 (CH_{Ar}), 125.0 (C), 130.3 (C), 134.1 (C), 143.9 (C), 148.3 (C), 168.2 (C=O) ppm. MS (EI): *m/z* = 319 ($[M-N_2]^+$, 100%), 219 ($[M-CH_2CH_2N_3-NHAc]^+$, 42%). Anal. Calcd for $C_{17}H_{25}N_5O_3$ (347.42) : C 58.77, H 7.25, N 20.16; Found : C 58.92, H 7.27, N 20.14.

_N_-(1-(2-Azidoethyl)-4,4-dimethyl-5,8-dioxo-1,2,3,4,5,8-hexahydroquinolein-6-yl)acetamide (13)

To a suspension of **12** (0.1 g, 0.28 mmol) in mixture of H_2O/MeOH (9/1, 1 mL) at room temperature was added PIFA (0.37 g, 0.86 mmol). The mixture was left to stir for 2 h at room temperature. Water (10 mL) was then added and the product was extracted with CH_2Cl_2 (2 x 10 mL). The organic layers were combined and washed with a saturated aqueous solution of sodium chloride (10 mL). They were then dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **13** as red oil (0.041 g, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.34 (s, 6H, 2 x CH_3), 1.69 (m, 2H, CH_2), 2.19 (s, 3H, CH_3), 3.40 (m, 2H, CH_2), 3.67 (m, 4H, 2 x CH_2), 7.14 (s, 1H, H_{Ar}), 8.60 (brs, 1H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 25.0 (CH_3), 28.1 (2 x CH_3), 31.6 (C), 38.8 (CH_2), 49.4 (CH_2), 51.5 (CH_2), 53.7 (CH_2), 110.6 (CH), 117.6 (C), 139.7 (C), 149.0 (C), 169.4 (**CO**CH_3), 176.4 (C=O), 185.9 (C=O) ppm. MS (CI): *m/z* = 318 ($[M+H]^+$, 100%), 290 ($[M-N_2]^+$, 46%), 261 ($[M-CH_2N_3]^+$, 30%). Anal. Calcd for $C_{15}H_{19}N_5O_3$ (317.35) : C 56.77, H 6.03, N 22.07; Found : C 56.61, H 6.05, N 22.06.

_N_-(7,7-Dimethyl-8-oxo-2,3,5,6,7,8-hexahydropyrido[1,2,3-_de_]quinoxalin-9-yl)acetamide (2)

A solution of *N*-(1-(2-azidoethyl)-4,4-dimethyl-5,8-dioxo-1,2,3,4,5,8-hexahydroquinolein-6-yl)acetamide **13** (0.04 g, 0.12 mmol) in EtOH (10 mL) was hydrogenated (15 psi) over Pd/C (0.04 g) for 3 h at room temperature. The catalyst was filtered on Celite and the filtrate concentrated *in vacuo*. The crude was purified by flash chromatography (eluent cyclohexane/EtOH) to give compound **2** as orange oil (0.012 g, 35%). ^{1}H NMR (300MHz, $CDCl_3$): δ = 1.34 (s, 6H, 2 x CH_3), 1.75 (m, 2H, CH_2), 2.15 (s, 3H, CH_3), 3.14 (d, 2H, *J* = 6.9 Hz, CH_2), 3.20 (m, 2H, CH_2), 3.99 (d, 2H, *J* = 6.9 Hz, CH_2), 7.53 (s, 1H, H_{Ar}), 8.52 (brs, 1H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 24.8 (CH_2), 28.3 (2 x CH_3), 31.6 (C), 38.5 (CH_3), 45.7 (CH_2), 46.9 (CH_2), 48.2 (CH_2), 113.8 (C), 114.1 (CH), 134.9 (C), 140.5 (C), 155.9 (C), 169.0

(**CO**CH_3), 177.4 (C=O) ppm. MS (EI): *m/z* = 216 ([M-NHAc+H]$^+$, 100%), 273 ([M]$^+$, 49%), 258 ([M-$COCH_3$]$^+$, 27%). Anal. Calcd for $C_{15}H_{19}N_3O_2$ (273.34) : C 65.91, H 7.01, N 15.37; Found : C 65.90, H 7.02, N 15.35.

(*9H*-Fluoren-9-yl)methyl-1-(2-azidoethyl)-5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinolein-6-ylcarbamate (14)

Under an inert atmosphere, to a solution of 1-(2-bromoethyl)-5,8-dimethoxy-4,4-dimethyl-1,2,3,4-tetrahydroquinolein-6-amine **10** (0.5 g, 0.88 mmol) in dry DMF (5 mL) was added sodium azide (0.17 g, 2.65 mmol) and copper iodide (0.01 g). Once addition was complete, the mixture was heated to reflux for 3 h. After addition of a saturated aqueous solution of sodium hydrogen carbonate (20 mL), the product was extracted with ethyl acetate (2 x 20 mL) and the combined organic layers were washed with a saturated aqueous solution of sodium chloride (20 mL), dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure to afford the desired azido compound, which was used without further purification. To a solution of the latter in dry THF (5 mL) was added dry pyridine (0.16 mL, 1.76 mmol) and Fmoc-Cl (0.27 g, 1.06 mmol). The mixture was then stirred for 1h at room temperature. Subsequently, water (15 mL) was added and the product was extracted with EtOAc (2 x 20 mL). The organic layers were combined and washed with a saturated aqueous solution of sodium chloride (20 mL), then dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **14** as brown solid (0.34 g, 45% within 2 steps). Mp 101-107 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.39 (s, 6H, 2 x CH_3), 1.64 (m, 2H, CH_2), 3.08 (m, 4H, 2 x CH_2), 3.57 (m, 2H, CH_2), 3.65 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.29 (t, 1H, *J* = 6.6 Hz, CH), 4.56 (d, 2H, *J* = 6.6 Hz, CH_2), 6.72 (brs, 1H, NH), 7.32 (m, 2H, 2 x H_{Ar}), 7.41 (m, 3H, 3 x H_{Ar}), 7.62 (m, 2H, m, 2 x H_{Ar}), 7.78 (m, 2H, 2 x H_{Ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 26.9 (2 x CH_3), 29.6 (CH), 33.1 (C), 45.4 (CH_2), 45.7 (CH_{Fmoc}), 47.2 (CH_2), 53.8 (CH_2), 55.8 (CH_2), 57.2 (OCH_3), 61.1 (OCH_3), 66.7 (CH_{2Fmoc}), 102.8 (CH_{ar}), 120.1 (2 x CH_{ArFmoc}), 125.0 (2 x CH_{ArFmoc}), 127.1 (2 x CH_{ArFmoc}), 127.8 (2 x CH_{ArFmoc}), 130.8 (C), 141.4 (2xC_{Fmoc}), 143.4 (C), 143.7 (2xC_{Fmoc}), 148.6 (C), 153.7 (C), 166.6 (C=O) ppm. MS (CI): *m/z* = 528 ([M+1]$^+$, 60%). Anal. Calcd for $C_{30}H_{33}N_5O_4$ (527.63) : C 68.29, H 6.30, N 13.27; Found : C 68.41, H 6.33, N 13.25.

(*9H*-Fluoren-9-yl)methyl-1-(2-azidoethyl)-4,4-dimethyl-5,8-dioxo-1,2,3,4,5,8-hexahydroquinolein-6-ylcarbamate (15)

To a suspension of **14** (0.3 g, 0.57 mmol) in mixture of H_2O/MeOH (9/1,5 mL) at room temperature was added PIFA (0.73 g, 1.7 mmol). The mixture was continued to stir for 2 h at room temperature. Then, water (10 mL) was added and the product was extracted with CH_2Cl_2 (2 x 20 mL). The organic layers were combined and washed with a saturated aqueous solution of sodium chloride (20 mL). They were then dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **15** as red oil (0.127 g, 45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.36 (s, 6H, 2 x CH_3), 1.71 (m, 2H, CH_2), 3.41 (m, 2H, CH_2), 3.69 (m, 4H, 2 x CH_2), 4.24 (t, 1H, *J* = 6.9 Hz, CH), 4.50 (d, 2H, *J* = 6.9 Hz, CH_2), 6.84 (s, 1H, H_{Ar}), 7.32 (m, 2H, 2 x H_{Ar}), 7.42 (m, 2H, 2 x H_{Ar}), 7.59 (m, 2H, 2 x H_{Ar}), 7.79 (m, 2H, 2 x H_{Ar}), 8.18 (brs, 1H, NH) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 26,9 (CH_3), 28,1 (CH_3), 31,7 (C), 38,8 (CH_2), 46,8 (CH_{Fmoc}), 49,4 (CH_2), 51,4 (CH_2), 53,8 (CH_2), 67,8 (CH_{2Fmoc}), 108,9 (CH_{Ar}), 117,7 (C), 120,1 (2 x CH_{ArFmoc}), 124,9 (2 x CH_{ArFmoc}), 127,2 (2 x CH_{ArFmoc}), 127,9 (2 x CH_{ArFmoc}), 140,7 (C), 141.3 (2 x C_{Fmoc}), 143,3 (2 x C_{Fmoc}), 149,0 (C), 152,2 (C), 175,8 (C=O), 175,8 (C=O) ppm. MS (CI): *m/z* = 498 ($[M+1]^+$, 33%). Anal. Calcd for $C_{28}H_{27}N_5O_4$ (497.56) : C 67.59, H 5.47, N 14.08; Found : C 67.55, H 5.45, N 14.06.

(*9H*-Fluoren-9-yl)methyl-7,7-dimethyl-8-oxo-2,3,5,6,7,8-hexahydropyrido[1,2,3-*de*]quinoxalin-9-yl-carbamate (3)

A solution of (*9H*-fluoren-9-yl)methyl 1-(2-azidoethyl)-4,4-dimethyl-5,8-dioxo-1,2,3,4,5,8-hexahydroquinolein-6-ylcarbamate **15** (0.05 g, 0.1 mmol) in EtOH (3 mL) was hydrogenated (15 psi) over Pd/C (0.1 g) for 5 h at room temperature. The catalyst was filtered on Celite and the filtrate concentrate in vacuo. The crude was purified by flash chromatography (eluent cyclohexane/EtOAc) to give compound **3** as orange oil (0.025 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.37 (s, 6H, 2 x CH_3), 1.77 (m, 2H, CH_2), 3.15 (m, 2H, CH_2), 3.21 (m, 2H, CH_2), 3.99 (m, 2H, CH_2), 4.24 (t, 1H, *J* = 6.9 Hz, C**H**CH_2), 4.45 (d, 2H, *J* = 6.9 Hz, CHC**H$_2$**), 7.22 (s, 1H, H_{Ar}), 7.32 (m, 2H, 2 x H_{Ar}), 7.40 (m, 2H, 2 x H_{Ar}), 7.61 (m, 2H, 2 x H_{Ar}), 7,76 (m, 2H, 2 x H_{Ar}), 8,09 (m, 1H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.4 (CH_3), 30.2 (CH_3), 31.6 (C), 38.5 (CH_2), 43.5 (CH_2), 45.8 (CH_{Fmoc}), 46.9 (CH_2), 48.0 (CH_2), 67.3 (CH_{2Fmoc}), 111.9 (CH_{Ar}), 114.0 (C), 120.0 (2 x CH_{ArFmoc}), 125.1 (2 x CH_{ArFmoc}), 127.1 (2 x CH_{ArFmoc}), 127.8 (2 x CH_{ArFmoc}), 135.5 (C), 140.5 (2 x C_{Fmoc}), 141.3 (2 x C_{Fmoc}), 143.6 (C), 152.7 (C), 155.7 (C=O), 177.0 (C=O) ppm. MS (EI): *m/z* = 178 ($[Indene]^+$, 100%), 231 ($[M-Fmoc]^+$, 28%), 453 ($[M]^+$, 5%). Anal. Calcd for $C_{28}H_{27}N_3O_3$ (453.55) : C 74.15, H 6.00, N 9.26; Found : C 74.18, H 6.04, N 9.29.

9-Amino-7,7-diméthyl-2,3,6,7-tetrahydropyrido[1,2,3-*de*]quinoxalin-8(*5H*)-one (1)

Under an inert atmosphere, to a solution of **3** (0.04 g, 0.08 mmol) in dry THF (2 mL) was added slowly the piperidine (0.04 mL, 0.35 mmol). After hydrolysis with a saturated aqueous solution of ammonium chloride during 1 h, the product was extracted with EtOAc (2 x 10 mL) and the combined organic layers were washed with a saturated aqueous solution of sodium chloride (10 mL), dried over anhydrous $MgSO_4$ and the solvents were removed under reduced pressure. The crude was purified by flash chromatography (eluent DCM/MeOH) to give compound **1** as red oil (0.027 g, 75%). ^{1}H NMR (300MHz, $CDCl_3$): δ = 1.37 (s, 6H, 2 x CH_3), 1.77 (m, 2H, CH_2), 3.20 (m, 4H, 2 x CH_2), 3.87 (m, 2H, CH_2), 5.03 (brs, 1H, NH_2), 5.83 (s, 1H, H_Q) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.3 (2 x CH_3), 31.6 (C), 38.6 (CH_2), 46.3 (CH_2), 46.4 (CH_2), 47.1 (CH_2), 99.3 (CH_q), 114.6 (C), 141.4 (C), 144.8 (C), 155.6 (C), 178.2 (C=O) ppm. MS

(EI): m/z = 231 ($[M]^+$, 5%). Anal. Calcd for $C_{13}H_{17}N_3O$ (231.30) : C 67.51, H 7.41, N 18.17; Found : C 67.28, H 7.39, N 18.15.

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