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ACCESS TO NEW CYTOTOXIC BISINDOLE ALKALOIDS BY A MODIFIED BORCH REDUCTIVE AMINATION PROCESS

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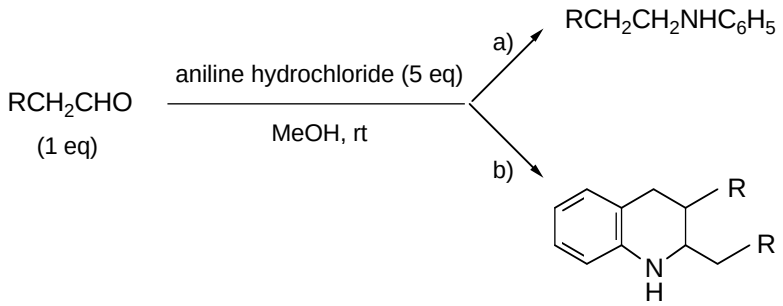
Abstract- A slight modification of the Borch reductive amination method (delayed addition of $NaBH_3CN$) was applied to an indole aldehyde compound, analog of the natural alkaloid, goniomitine. This reaction led to a series of new cytotoxic bis-indole alkaloids with a 1,2,3,4-tetrahydroquinoline bridge.

In a previous publication, we reported on a slight modification of the Borch reductive amination method which consists in delayed addition of $NaBH_3CN$.[1,2] In the case of reductive amination between an enolizable aldehyde and aniline, this modification allows a one-pot synthesis of 2,3-disubstituted 1,2,3,4-tetrahydroquinolines.

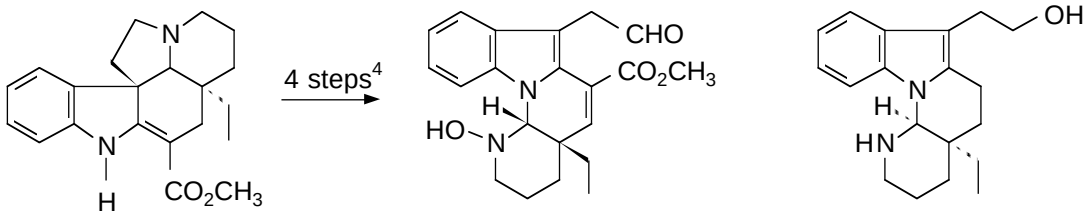


a) $NaBH_3CN$ added immediately

b) $NaBH_3CN$ added after 20 min

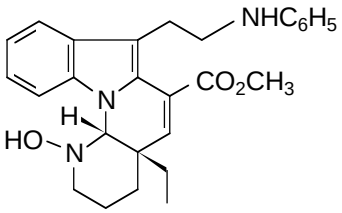
This cyclization implies a Schiff base intermediate and results at the beginning from the addition of the enamine form to the tautomeric Schiff base, as iminium salt. The application of this modified reductive amination to the indole alkaloid (**2**), a semi-synthetic analog of natural goniomitine (**3**) [3] derived from (-)-

vincadifformine (**1**),[4] provides a series of new cytotoxic bisindole alkaloids which are described in this paper.

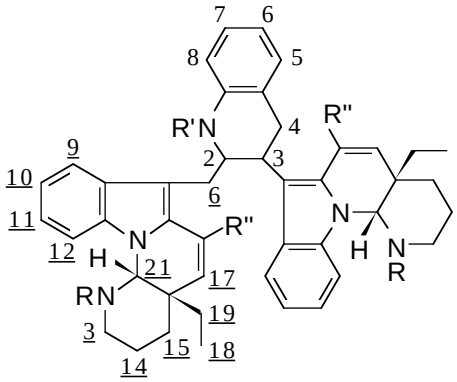


1 **2** **3**

Reaction of the aldehyde (**2**) with aniline hydrochloride (5 eq, MeOH, 16 h, rt) afforded under the Borch original conditions (immediate addition of $NaBH_3CN$) two main compounds (**4**) (23%) and (**5**) (28%) while a delayed addition (20 min) of $NaBH_3CN$ led only to **5** (45%). HR-EIMS of these two products proved the expected reductive amination structure for **4** and the dimeric structure for **5**. 2D homo- and heteronuclear NMR experiments were in full agreement with the 2,3 disubstituted 1,2,3,4-tetrahydroquinoline hypothesis and demonstrated that **5** was a pure compound and not a mixture of diastereoisomers at C2 and C3.[5]



4



	R	R'	R"
5	OH	H	CO_2CH_3
6	H	H	CO_2CH_3
7	$COCH_3$	H	CO_2CH_3
8	$COCH_3$	$COCH_3$	CO_2CH_3
9	$OCOCH_3$	H	CO_2CH_3
10	OH	CH_3	CO_2CH_3
11	OH	H	CH_2OH

Numbering system of indole part according to ref. 8

In view of biological studies and structure activity relationships establishment, several derivatives were prepared from **5**. Reduction of the hydroxylamino functions was performed by aqueous $TiCl_3$ in MeOH (6 eq, 20 h, rt) according to Murahashi's method [6] and led in 38% yield to the triamine (**6**). Acetylation of **6** (pyridine, Ac_2O, 48 h, rt) furnished in 48 and 22% yields respectively **7**, diacetylated on both indole moieties, and the triacetylated compound (**8**). Acetylation of the hydroxylamino groups of **5** (pyridine, Ac_2O, 3 h, rt) afforded in 68% yield the di-*O*-acetyl (**9**). A selective functionalization of **5** on the only tetrahydroquinoline secondary amino group was accomplished by methylation ($CH_2O/NaBH_3CN$, AcOH,

2 h, rt) and provided in 64% yield the *N*-methyl derivative (**10**). Finally the $LiAlH_4$ reduction of **5** (THF, 3 h, reflux) led to the tetrahydroxy compound (**11**) in 32% yield.[7]

Stereochemistry of **5** at C2 and C3 - Previously,[2] we had already observed that the relative stereochemistry at C2 and C3 of the 1,2,3,4-tetrahydroquinoline was highly dependent on the starting aldehyde, a bulky aldehyde yielding only the 2,3-*trans* disubstituted isomer because of steric hindrance of the substituents. The very likely *trans* stereochemistry was inferred from ^1H NMR experiments with the $TiCl_3$ reduction compound (**6**) (conclusions could not be drawn from **5** itself owing to the overlapping of H3, one H6 and two other protons into a multiplet). ^1H NMR spectrum of **6** displayed clearly both signals of H2 (td, J = 11 and 2.5 Hz) and H3 (ddd, J = 11.8, 11 and 5 Hz) at respectively δ 4.60 and 3.37 ppm. The observed J_{H2-H3} = 11 Hz by irradiation experiments unambiguously proved the 2,3-*trans* relative configuration. Unfortunately, the amorphous state of compounds (**5-11**) did not allow determination of the absolute configuration at C2 and C3 by X-Ray crystallography.

Biological results - The original bisindole structure of **5** and its derivatives led us to evaluate their cytotoxicity (IC_{50} and cell cycle effect) on L1210 leukemia cells in culture compared to monomers (**2**) and (**4**) (Table 1).

Table 1

Compound	IC_{50} (μM) [a]	Effect on cell cycle (% accumulation in G1) [b]
2	38	n s [c]
4	7.1	61% at 50 μM
5	2.7	80% at 25 μM
6	3.1	no specificity
7	> 100	n s
8	24.8	55% at 100 μM
9	> 100	n s
10	5.3	79% at 25 μM
11	3.4	no specificity

[a] Inhibition of L1210 cells proliferation measured by the microculture tetrazolium assay
[b] 46% of untreated cells are in the G1 phase
[c] no studied

The most interesting compounds are **5** and **10** which induced a high and dose-dependent accumulation in the G1-phase of the L1210 cell cycle. It is noteworthy that hydroxylamino group on the indole moieties seems essential for the cytotoxicity with accumulation in the G1-phase but not the tetrahydroquinoline N-H function. These observations could be related to probable occurence of intramolecular hydrogen bonds between the carboxyl group and the hydroxylamino group of the two indole moieties. A computational conformational analysis of **5** with Systematic Search within Sybyl v 6.2 confirmed these hydrogen bond patterns in both 2*S*,3*S* and 2*R*,3*R* *trans* stereoisomers, which does not allow to specify the absolute

configuration at C2 and C3. Synthesis and biological study of analogs of **5** modified on the only tetrahydroquinoline ring are in progress and will be the subject of a further article.

ACKNOWLEDGEMENTS

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2. G. Lewin and C. Schaeffer, *Heterocycles*, 1998, **48**, 171.

3. L. Randriambola, J.-C. Quirion, C. Kan-Fan, and H.-P. Husson, *Tetrahedron Lett.*, 1987, **28**, 2123.

4. G. Lewin, C. Schaeffer, and P.-H. Lambert, *J. Org. Chem.*, 1995, **60**, 3282.

5. Selected data for compounds (**4**) and (**5**).

4: $[(\alpha)_D$ = -74° (c = 0.6, $CHCl_3)]$; UV (EtOH) λ_{max} nm (log ε) 220 (4.34), 234 (4.31), 248 (4.17), 322 (3.92); IR (CH_2Cl_2) ν cm^{-1} 3500-3200, 1715; ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: 0.76 (3 H-18), 3.82 (CO_2CH_3), 4.00 (N-H), 4.53 (H-21), 4.87 (N-OH), 6.58, 6.65 and 7.14 (5 H of the aniline ring), 6.62 (H-17), 7.10 (H-10), 7.24 (H-11), 7.53 (H-12), 7.62 (H-9); HR-EIMS: M^+ 445.2357, calcd for $C_{27}H_{31}N_3O_3$ 445.2365.

5: $[(\alpha)_D$ = -150° (c = 1.6, $CHCl_3)]$; UV (EtOH) λ_{max} nm (log e) 224 (4.62), 250 (4.30), 320 (4.33); IR (CH_2Cl_2) ν cm^{-1} 3500-3250, 1715 (br); ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: tetrahydroquinoline part 2.75 (1 H-4), 3.36 (H-3), 3.50 (1 H-4), 4.33 (td, *J* = 11 and 2.5 Hz, H-2), 6.40 (H-8), 6.55 (H-6), 6.91 (H-5), 6.94 (H-7); indole part (both indole moieties were not distinguished) 0.69 and 0.82 (6 H-18), 2.70 (2 H-3), 3.09 (dd, *J* = 15.6 and 11 Hz, 1 H-6), 3.32 (2 H-3), 3.35 (1 H-6), 3.55 and 3.89 (2 CO_2CH_3), 4.46 and 4.59 (2 H-21), 6.40 and 6.45 (2 H-17), 7.05 (2 H-10), 7.22 (2 H-11), 7.51 and 7.58 (2 H-12), 7.71 (2 H-9); ^{13}C NMR ($CDCl_3$, 125 MHz), δ ppm: tetrahydroquinoline part 38.5 (C-4), 41.6 (C-3), 61.2 (C-2), 117.7 (C-8), 120.3 (C-6), 130.8 (C-5), 132.8 (C-7); indole part (both indole moieties were not distinguished) 14.5 and 15.2 (2 C-18), 25.8 and 25.9 (2 C-14), 33.8 (C-6), 35.1 and 35.4 (2 C-19), 36.8 and 37.0 (2 C-15), 56.5 and 56.6 (2 $CO_2\underline{C}H_3$), 60.1 and 60.2 (2 C-3), 82.7 and 82.9 (2 C-21), 114.4 and 114.9 (2 C-9), 123.6 and 123.8 (2 C-12), 125.6 (2 C-10), 127.1 and 127.3 (2 C-11), 145.9 and 147.1 (2 C-17); HR-EIMS: M^+ 795.4032, calcd for $C_{48}H_{53}N_5O_6$ 795.3996.

6. S.-I. Murahashi and Y. Kodera, *Tetrahedron Lett.*, 1985, **26**, 4633.

7. Selected data for compounds (**6-11**).

6: $[(\alpha)_D$ = -124° (c = 1.3, $CHCl_3)]$; UV (EtOH) λ_{max} nm 229, 253, 319; ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: tetrahydroquinoline part 3.37 (ddd, *J* = 11.8, 11 and 5 Hz, H-3), 3.55 (dd, *J* = 16 and 11.8 Hz, 1 H-4), 4.60 (td, J = 11 and 2.5 Hz, H-2); indole part: 3.63 and 3.89 (2 CO_2CH_3), 4.77 and 4.89 (2 H-21), 6.53 (2 H-17); HR-EIMS: M^+ 763.4130, calcd for $C_{48}H_{53}N_5O_4$ 763.4098.

7: UV (EtOH) λ_{max} nm 220, 230 (sh), 251, 320; IR (CH_2Cl_2) ν cm^{-1} 3380, 1720, 1645; ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: 2.24 and 2.33 (2 $NCOCH_3$), 3.73 and 3.78 (2 CO_2CH_3); HR-EIMS: M^+ 847.4309, calcd for $C_{52}H_{57}N_5O_6$ 847.4315.

8: UV (EtOH) λ_{max} nm 223, 255 (sh), 320; IR (CH_2Cl_2) ν cm^{-1} 1720, 1645; ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: 2.12, 2.21 and 2.28 (3 $NCOCH_3$), 3.67 and 3.74 (2 CO_2CH_3); HR-EIMS: M^+ 889.4414, calcd for $C_{54}H_{59}N_5O_7$ 889.4437.

9: $[(\alpha)_D$ = +36° (c = 0.7, $CHCl_3)]$; UV (EtOH) λ_{max} nm 220, 230 (sh), 254, 316; IR (CH_2Cl_2) ν cm^{-1} 3380, 1760, 1720; ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: 1.28 and 1.30 (2 $OCOCH_3$), 3.82 and 3.87 (2 CO_2CH_3), 4.62 and 4.77 (2 H-21), 6.43 and 6.55 (2 H-17); HR-EIMS: M^+ 879.4205, calcd for $C_{52}H_{57}N_5O_8$ 879.4170.

10: $[(\alpha)_D$ = -170° (c = 0.75, $CHCl_3)]$; UV (EtOH) λ_{max} nm 218, 230 (sh), 320; IR (CH_2Cl_2) ν cm^{-1} 3400, 1715 (br); ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: 2.44 ($N\text{-}CH_3$), 3.56 and 3.76 (2 CO_2CH_3), 4.43 and 4.60 (2 H-21), 6.24 and 6.30 (2 H-17); EIMS: M^+ 809.

11: $[(\alpha)_D$ = -22° (c = 0.9, $CHCl_3)]$; UV (EtOH) λ_{max} nm 222, 250, 312, 322; ^1H NMR ($CDCl_3$, 500 MHz), δ ppm: tetrahydroquinoline part 6.34 (H-8), 6.58 (H-6), 6.94 (H-5 and H-7); indole part (both indole moieties were not distinguished) 0.63 and 0.73 (6 H-18), 4.39, 4.43 and 4.73 (2 $C\underline{H}_2OH$), 5.11 and 5.61 (2 H-17), 6.91 and 7.12 (2 H-10), 7.12 and 7.23 (2 H-11), 7.44 (2 H-12), 7.58 and 7.70 (2 H-9); HR-EIMS: M^+ 739.4098, calcd for $C_{46}H_{53}N_5O_4$ 739.4105.

8. Numbering system proposed by J. Le Men and W.I.Taylor, *Experientia*, 1965, **21**, 508.