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Synthesis of a Novel Series of Cytotoxic Bisindole Alkaloids

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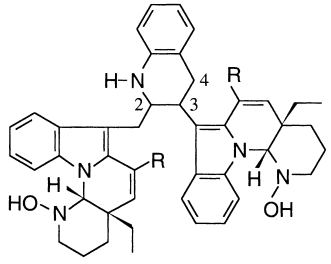
Abstract—Original cytotoxic bisindole alkaloids with a 1,2,3,4-tetrahydroquinoline bridge were synthesized by reductive amination with various anilines. The most cytotoxic compounds display a high and dose-dependent cell cycle effect with accumulation in the G1 phase. Influence of substitution of the starting aniline on the reaction and on cytotoxicity of produced dimers was pointed out.

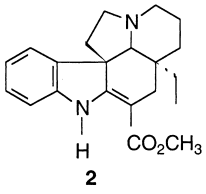
Compound **1** is an original semi-synthetic bisindole alkaloid displaying on L1210 leukemia cells in culture a moderate cytotoxicity (IC_{50} 2.7 μM) but a high and dose-dependent accumulation in the G1 phase of the cell cycle (80% accumulation at 25 μM).[1] This bisindole compound was synthesized in five steps from the easily available natural alkaloid, (−)-vincadifformine **2**. The first four steps constitute a biomimetic conversion of **2** into **3**,[2] a compound with the same skeleton as the natural alkaloid goniomitine **4**.[3] The last step is a dimerization by reductive amination of **3** with aniline·HCl and $NaBH_3CN$. We have previously pointed out that yield of dimerization was closely dependent on experimental conditions: Borch classical conditions[4] (immediate addition of $NaBH_3CN$) led to a mixture of the expected monomer **5** (23%) and **1** (28%) while a delayed addition (20 min) of $NaBH_3CN$[5] provided only the dimer **1** (45%). Preliminary pharmacomodulation studies at the amine, hydroxyamine and ester functions proved **1** to remain the most promising structure.[1] This paper reports on new analogues of **1** which were synthesized with the goal of increasing the cytotoxicity and establishing structure–activity relationships in this original series.

In order to determine the influence of the chirality, synthesis of **6** [amorphous, $\alpha_D = +156$ (*c* 0.3, $CHCl_3$)], the enantiomer of **1**, was perfomed from (+)-vincadifformine[6] under exactly the same conditions as described for **1** Biological activity of **6** appeared slightly



1 R = CO_2CH_3

(2,3-*trans* relative configuration)



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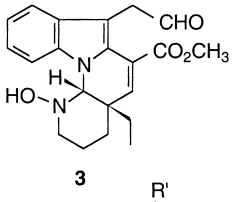

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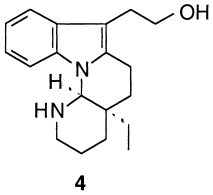

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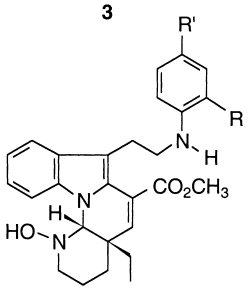

5 R = R' = H

7 R = R' = Cl

2 2 R = H; R' = F

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less interesting than **1** (IC_{50} 4.5 μM, 71% accumulation in the G1 phase at 50 μM), so following studies were undertaken in the optical series of **1**. Taking **1** as pattern, we synthesized analogues substituted on the aromatic ring of the tetrahydroquinoline (THQ) by reductive amination of **3** with different aromatic amines.[7] These amines were either symmetrical anilines (*p*-, *mm*′- or *mpm*′-substituted) or anilines with only one *ortho* free position to avoid the formation of isomers at the cyclization step into tetrahydroquinoline. Substituents were chosen owing to their possible influence on the reductive amination reaction and on cytotoxicity of the produced dimers. The selected anilines were:

- the *p*-monosubstituted (classified in descending order of the σ_p substituent) 4-(trifluoromethyl)-aniline, ethyl 4-aminobenzoate, 4-chloroaniline, 4-fluoroaniline and *p*-anisidine; the *mm*′-disubstituted 3,5-dichloroaniline; the *mpm*′-trisubstituted 3,4,5-trimethoxyaniline; the *o*-substituted 2-chloroaniline and 2-fluoroaniline; the *op*-disubstituted 2,4-dichloroaniline.

Apart from reaction with 2,4-dichloroaniline which afforded the monomer **7**, all the isolated compounds were dimers. These dimers displayed either a 2,3-disubstituted THQ structure as **1** or a 2,3,4-trisubstituted THQ ring with an anilino or a methoxy group as additional substituent at C4 (Table 1).

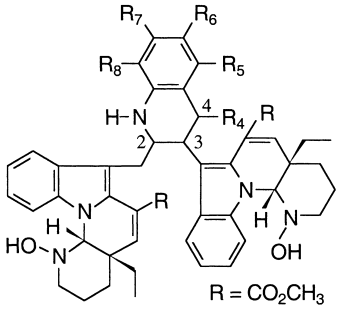
Dimers Isolated from a *p*-Monosubstituted Aniline

The structure of these dimers is closely dependent on the electrical effect of substituent of the aniline and can be related to its σ_p. An electron-withdrawing substituent provides only a 2,3,4-trisubstituted dimer with an anilino group at C4 (**8**, **9**). A gradual decreasing σ_p value produces first a mixture of 2,3,4-trisubstituted and 2,3-disubstituted dimers (**10**, **11**), then a 2,3-disubstituted dimer as the only isolated compound of the reaction (**12**, **1**). Lastly, a strong electron-donating group (as in *p*-anisidine) furnishes besides the 2,3-disubstituted dimer **13** the 2,3,4-trisubstituted compound **14** having an additional methoxy at C4 of the THQ ring.

Dimers Isolated from other Anilines

Additivity rule of σ_p and σ_m values applies to 3,4,5-trimethoxyaniline which affords the attempted 2,3-disubstituted dimer **15** but fails with 3,5-dichloroaniline which gives the 2,3-disubstituted dimer **16** besides the expected 2,3,4-trisubstituted dimer **17**. Finally, anilines with only one *ortho* free position never provide 2,3-disubstituted dimers since 2,4-dichloroaniline, 2-chloroaniline and 2-fluoroaniline produce, respectively, the monomer **7** (vide supra) and the 2,3,4-trisubstituted dimers with an anilino group at C4 **18** and **19** (Table 1).

Table 1. Structure and cytotoxicity of **1** and its analogues **8**–**20**



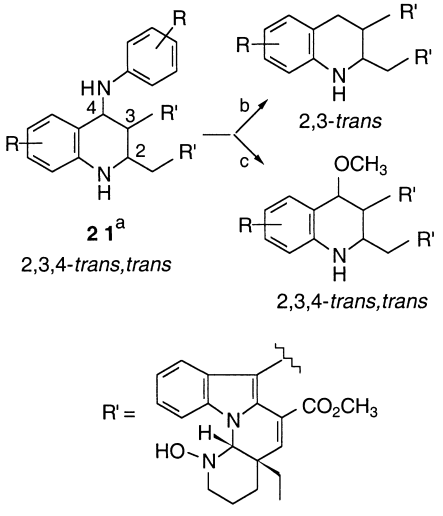
No (yield)[a]	(Subst. aniline)[b]		R_4	R_5	R_6	R_7	R_8	IC_{50}[c] (μM)	% Cells in the G1 phase[d]
	σ_p	σ_m							
8 (47)	+0.53		$NHC_6H_4pCF_3$	H	CF_3	H	H	>10	ns[e]
9 (74)	+0.44		$NHC_6H_4pCO_2Et$	H	CO_2Et	H	H	>10	ns
10 (28)	+0.24		NHC_6H_4pCl	H	Cl	H	H	>10	ns
11 (24)	+0.24		H	H	Cl	H	H	3.6	77% at 10 μM
12 (74)	+0.15		H	H	F	H	H	1.8	81% at 5 μM
1 (45)			H	H	H	H	H	2.7	80% at 25 μM
13 (16)	−0.28		H	H	OMe	H	H	2.2	69% at 20 μM
14 (23)	−0.28		OMe	H	OMe	H	H	1.7	81% at 20 μM
15 (37)	−0.28	+0.10	H	OMe	OMe	OMe	H	2.4	61% at 5 μM
16 (18)		+0.37	H	Cl	H	Cl	H	>10	ns
17 (35)		+0.37	$NHC_6H_3m,m'Cl$	Cl	H	Cl	H	>10	ns
18 (37)			NHC_6H_4oCl	H	H	H	Cl	>10	ns
19 (47)			NHC_6H_4oF	H	H	H	F	14.1	ns
20 (54)			OMe	H	OH	H	H	0.9	[f]

[a]Yield from **3**.
[b]From ref 8.
[c]Inhibition of L1210 cell proliferation measured by the microculture tetrazolium assay.
[d]In the control, 46% cells are in the G1 phase.
[e]Not studied.
[f]Not specific at 5 μM, toxic at 10 μM.

The structure of dimers **8**–**19** was deduced from MS, UV and ^{1}H NMR data. The 2,3-relative configuration of disubstituted dimers was fixed *trans* on account of our previous results,[5] and by analogy with the reference compound **1**. The 2,3,4-*trans*,*trans* relative configuration of trisubstituted dimers was infered from ^{1}H NMR spectra of **10** and **14** and the results applied to the other trisubstituted dimers. The conclusions were based on: (a) the observation of the H4 signal at 5.01 (d, $J = 10$ Hz) and 5.03 (d after D_2O, $J = 10$ Hz) ppm for **14** and **10**; (b) a strong NOE between H4 and H2 (at 4.31 and 4.53 ppm for **14** and **10**); and (c) lack of observed NOE between H2 and H3.

Mechanism (Scheme 1)

These results agree with our previously reported general mechanism which implies the key intermediate **21**.[5] According to the substituent R, either the intermediate form is stabilized (R highly electron-withdrawing group) and recovered at the end of the reaction (**8**–**10**, **17**–**19**) or it undergoes substitution of the amino group at C4 by $NaBH_3CN$ and leads to the 2,3-disubstituted dimers (**1**, **11**–**13**, **15**, **16**). Lastly, a strong electron-donating group as in *p*-anisidine promotes elimination of the anilino group at C4 and provides the 2,3,4-trisubstituted dimer **14** by addition of the solvent from the less hindered face. Compound **14** can be isolated in a better yield (59%) when the reaction is carried out in absence of $NaBH_3CN$. In the same manner, condensation of **3** (1 equiv) and 4-aminophenol·HCl (5 equiv) in MeOH gives the phenolic 2,3,4,-trisubstituted dimer **20** in 54% yield.



Scheme 1.

Biological Results[9] (Table 1)

Evaluation of the cytotoxicity of dimers **8**–**20** was carried out on L1210 leukemia cells in culture and allowed observation of clear structure–activity relationships within this original series. In comparison with **1**, all the 2,3,4-trisubstituted dimers with an anilino group at C4 display a marked decrease of cytotoxicity (IC_{50} >10 μM) and even, for some of them, a stimulation of cell proliferation at 10 μM (118, 126, 152 and 156% for **9**, **10**, **8** and **17**, respectively). 2,3-Disubstituted dimers (dichloro compound **16** excepted) and 2,3,4-trisubstituted dimers with a methoxy group at C4 reveal a cytotoxicity of the same order as **1**. Furthermore, these last compounds (**20** excepted) exhibit a high and dose-dependent accumulation in the G1 phase of the cell cycle. Particularly, strong effect on cell cycle of the fluoro dimer **12** led us to compare it with the related monomer **22**.[10] The very weak cytotoxicity of **22** (15.4 μM) confirmed the role of the dimerization in the biological activity. The mechanism of this accumulation effect in the G1 phase by a possible interaction of these dimers with some cyclin-dependent kinases (CDKs) is actually under investigation.

Acknowledgements

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References and Notes

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6. Thanks to Dr. J. Hannart (Omnichem, Belgium) for gift of (+)-vincadifformine.
7. General procedure. To a mixture of **3** (0.15 mmol) and amine·HCl (0.75 mmol) in MeOH (3 mL) at room temperature, $NaBH_3CN$ was added after 20 min then the reaction was left at room temperature for 16 h. The reaction mixture was taken up in water, raised to pH 10 with 0.5 N NaOH and extracted with CH_2Cl_2. Standard work up of the organic layer provided a dried residue which was purified by flash and thin layer chromatography on silica gel. Characterization and purity of all dimers followed from HRESIMS, ^{1}H NMR and HPLC.
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10. Compound **22** was prepared according to the general procedure but with immediate addition of $NaBH_3CN$ (yield 54%).