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HEMISYNTHESIS OF RHAZINILAM ANALOGUES: STRUCTURE - ACTIVITY RELATIONSHIPS ON TUBULIN-MICROTUBULE SYSTEM

Bruno David, Thierry Sévenet,* Odile Thoison, Khalijah Awang, Mary Païs, Michel Wright‡ and Daniel Guénard

Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette Cedex, France

‡ *Laboratoire de Pharmacologie et Toxicologie Fondamentales CNRS, 205, route de Narbonne Toulouse, France*

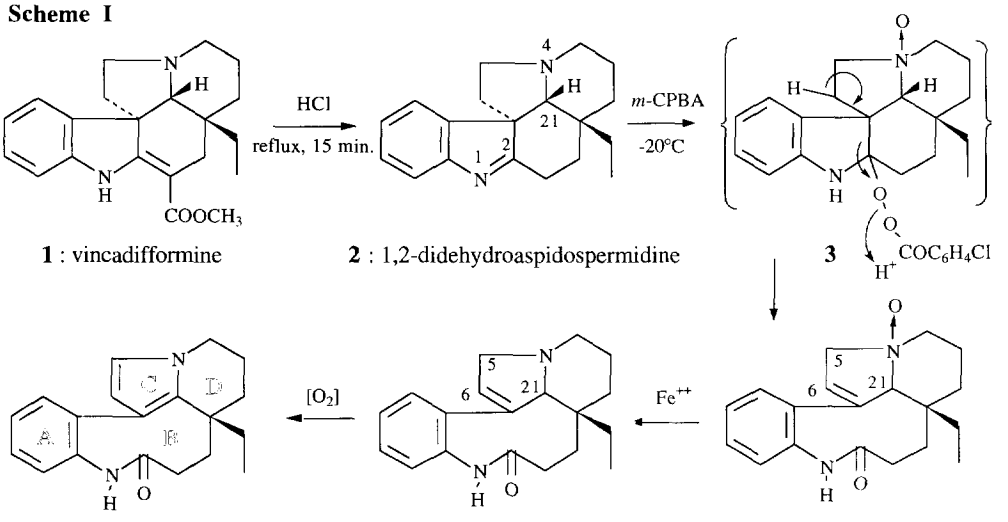
Abstract: Semi-synthesis of derivatives of rhazinilam, an antitubulin compound, delineated some molecular features necessary for biological activity. In the course of this study, the formation of rhazinilam from 1,2-didehydroaspidospermidine is reexamined and a new mechanism is proposed. © 1997 Elsevier Science Ltd.

Rhazinilam **6**, a substance first isolated from *Rhazya stricta*[1] (Apocynaceae), has been re-isolated from a Malaysian plant, *Kopsia singapurensis* Ridlay, in the framework of a screening of plant extracts acting on the tubulin-microtubule equilibrium.[2] Tubulin is an ubiquitous protein involved in the formation of the mitotic spindle during cell division. Colchicine, vinblastine and taxol are the main representatives of the three classes of inhibitors of the tubuline-microtubules equilibrium. It has been shown[2-4] that rhazinilam **6** can be regarded as a new leader among the mitotic spindle poisons because it possesses both the effects of the vinblastine and taxol classes of compounds: it induces a nonreversible assembly of tubulin, called spiralization, and it inhibits the cold-induced disassembly of the microtubules.

The interaction of rhazinilam **6** with tubulin occurs at concentrations similar to these observed for colchicine (IC_{50} 2.10^{-6} M). However, rhazinilam is less active on tubulin than vinblastine and taxol. Therefore, we have undertaken simple modifications of the molecule with the aim of obtaining a stronger activity in the microtubule disassembly assay. A preliminary structure - activity relationship study had indicated that the two aromatic rings and the lactam carbonyl group were essential features necessary for activity.[2] We report here the synthesis of functional derivatives of rhazinilam and the results of a more detailed structure - activity study.

It has been shown that rhazinilam **6** is an artefact of the plant extraction and that its natural precursor is 5,21-dihydrorhazinilam **5**.[5] Rhazinilam can also be synthesized starting from vincadiffomine **1**,[6,7] which is first transformed to 1,2-didehydroaspidospermidine **2**. Treatment of **2** by *m*-chloroperbenzoic acid followed by the action of aqueous $FeSO_4$ leads to rhazinilam. A mechanism for the latter reaction had been proposed.[6] However it was not fully satisfactory since the main intermediate was not isolated. Hence, we have reinvestigated the transformation of 1,2-didehydroaspidospermidine **2** into rhazinilam. Oxidation of compound **2** by *m*-chloroperbenzoic acid afforded two products, 1,2-didehydroaspidospermidine N-oxide and 5,21-didehydrorhazinilam N-oxide **4,** which was isolated from the aqueous layer after the usual workup procedure. Compound **4** upon treatment with $FeSO_4$ led to rhazinilam.

Scheme I



1 : vincadifformine **2** : 1,2-didehydroaspidospermidine **3** COC_6H_4Cl

6 : rhazinilam **5** : 5,21-dihydrorhazinilam **4** : 5,21-dihydrorhazinilam N-oxide

Thus, we propose a mechanism (Scheme 1) involving the perester **3**, which however was not isolated. The perester function induces a fragmentation reaction proceeding through the abstraction of one H-6. This yielded compound **3** that was reduced to **5** by Fe^{++} and further transformed to rhazinilam **6**.

Rhazinilam derivatives

The rhazinilam molecule was modified at the six positions 1, 2, 3, 5, 6, and 10 (Table 1). This led to compounds **7a - t** and **8** listed in Table 1. We also added to the list the natural derivatives 5,21-dihydrorhazinilam **5** and leuconolam **9**. The latter, isolated from two plants belonging to the Apocynaceae family, *Leuconotis griffithii* and *L. eugeniifolia,*[8] may be regarded as an oxidation product of rhazinilam. Compounds **5**, **7o,** and **9** were studied in our preliminary work.[2] We have included them in the current paper in order to obtain a more complete overview of the structure - activity relationships.

The pyrrole site: Substitution by a formyl group at position 5 and 6 according to the Vilsmeier - Haack reaction provided 5-formylrhazinilam **7a** and 5,6-diformylrhazinilam **7b**. Treatment of **6** with trifluoroacetic anhydrid led to **7c,** and compound **7d** was obtained by a Friedel - Craft reaction.[9] Compounds **7e - h** were prepared from **7d** by addition of water or the corresponding alcohols. Compounds **7e** and **7f** derivatives are soluble in water, which is useful for the biological assays.

The phenyl ring: (-)-Bromorhazinilam **7i** and (-)-acetamidorhazinilam **7j** were prepared from (+)-vincadifformine by bromination (NBS/CF_3COOH) and by nitrosation (HNO_3,CF_3COOH/CH_3COOH).[10] After further transformation into the 1,2-didehydroaspidospermidine analogues by HCl, the same procedure as for rhazinilam (*m*-chloroperbenzoic acid followed by Fe^{++}) was performed.

The lactam site: Compounds **7k - n** were obtained from **6** according to the usual methods. Rhazinilamin **7o** was prepared by $LiAlH_4$ reduction of rhazinilam.[2] The compounds **7p** and **7q** were synthesized from **7o** in order to regenerate the amide function with a carbonyl position close to that of rhazinilam. Compound **7r** was made by action of Lawesson reagent[11] on rhazinilam. The opened rhazinilam **8** was easily obtained under alkaline conditions.

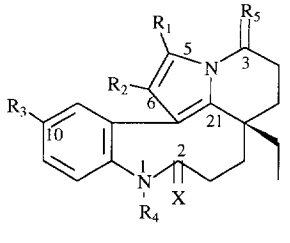
The piperidinic ring: Microbiological oxidation assays with oxidative bacteria have been carried out using different strains[12]: one of them, *Beau veria bassiana* led to an oxidation of ring C on C-3 thus allowing

the isolation of the 3-hydroxy and the 3-keto compounds **7s** and **7t.**

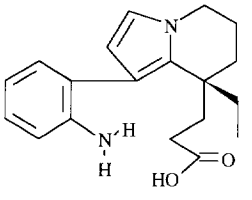
Activity evaluation

The easiest method to evaluate the activity of these compounds on tubulin is to use the same procedure as for paclitaxel derivatives :[13] the drug, dissolved in DMSO or ethanol at different concentrations, is added to a solution of microtubules (about 20 µM in tubulin) at 37 °C in a temperature controlled cell; then the absorbance decrease is monitored in a UV spectrophotometer at 350 nm for 2 min at 0 °C; this temperature allows the disassembly of microtubules. The maximum rate of disassembly is recorded and compared to a sample without drug. The IC_{50} of the compound is calculated from the effect of several concentrations and compared to IC_{50} of rhazinilam obtained within the same day with the same tubulin preparation.

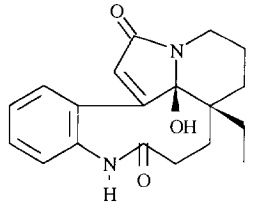
Table 1: Activity of rhazinilam analogues against the cold-induced disassembly of microtubules



7a-t



8



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compound	R_1	R_2	R_3	R_4	R_5	X	Act 1	Act 2
(-)-Rhazinilam **6**	H	H	H	H	H,H	O	1	0.5
(+)-Rhazinilam	H	H	H	H	H,H	O	inactive	
2							inactive	
5							inactive	
7a	CHO	H	H	H	H,H	O	6	
7b	CHO	CHO	H	H	H,H	O	inactive	
7c	$COCF_3$	H	H	H	H,H	O	4	15
7d	$COCCl_3$	H	H	H	H,H	O	8	25
7e	COOH	H	H	H	H,H	O	2,5	75
7f	COO^-Na^+	H	H	H	H,H	O	3,5	75
7g	$COOCH_3$	H	H	H	H,H	O	5	4
7h	G_1	H	H	H	H,H	O	7	20
7i	H	H	Br	H	H,H	O	100	inactive
7j	H	H	$NHCOCH_3$	H	H,H	O	inactive	inactive
7k	H	H	H	CH_3	H,H	O	4	60
7l	H	H	H	tBoc	H,H	O	80	inactive
7m	H	H	H	G_2	H,H	O	80	inactive
7n	H	H	H	CH_2CO_2H	H,H	O	80	inactive
7o	H	H	H	H	H,H	H,H	20	1
7p	H	H	H	tBoc	H,H	H,H	80	10
7q	H	H	H	$COCH_3$	H,H	H,H	80	inactive
7r	H	H	H	H	H,H	S	50	10
7s	H	H	H	H	OH,H	O	15	3
7t	H	H	H	H	O	O	10	3
8							inactive	
9							inactive	

$G_1 = CO_2CH_2C_6H_2(OCH_3)_3$; $G_2 = CH_2CO_2CH_2C_6H_5$; Act 1 = $IC_{50}/IC_{50\ Rhazinilam}$ ($IC_{50\ Rhazinilam} = 2.10^{-6}M$); Act 2 = IC_{50} on KB cells (µM).

Results and discussion

These results have shown that none of these analogues have a higher activity than rhazinilam. Changes on the pyrrole moiety have led to interesting compounds with an activity level close to that of **6**, while the

tubulin binding has been lowered with the bulk of the substituent at this position. 5,21-Dihydrorhazinilam **5** and leuconolam **9**, which exhibit a nonaromatic ring are completely devoided of activity on tubulin. Substitution on the phenyl ring (**7i - j**) has led to a dramatic loss of activity possibly due to either the electronic effect or the volume of the substituent. These results underline the importance of the aromatic rings on the binding to tubulin especially for their electronic properties. Substitution of the piperidinic ring C on C-3 leading to compounds (**7s - t**) decreased the activity, probably because the electronic distribution of the pyrrole ring, and consequently its aromaticity, is modified for the oxo derivative.

Molecular modeling studies[14] show that the conformation of the 9-membered ring does not change if the carbonyl is replaced by a thiocarbonyl or if the nitrogen bears various substituents. This is further supported for the thiocarbonyl compound **7r** by X-ray crystallography.[14] The loss of antitubulin activity of compound **7r,** as well as the decrease of activity observed from the N-methyl derivative **7k** to the analogues with bulky substituents **7l - n**, suggests that the carbonyl group participates in a hydrogen bond with the receptor. The bulky substituents in **7l - n** hinder the carbonyl from interacting with an acidic hydrogen of the protein, while the thiocarbonyl can form only weak hydrogen bonds. Rhazinilamine **7o** and its derivatives **7p,q** as well as the opened compound **8** are also devoided of antitubulin activity thus confirming that the presence and the position of the lactam in relation to the phenyl group are essential features. Absolute configuration is of crucial importance since only the (-)-enantiomer **2** is active on tubulin.[3]

In summary, 23 derivatives of rhazinilam have been analyzed in order to study the structural elements necessary for activity. Some of them, although slightly less active than rhazinilam **6**, have kept a good capacity to interact with tubulin (**7c,e,l**). Consequently, aromaticity of the two rings A and C is necessary, but substitution on the pyrrole ring has less consequence than on the phenyl ring on the binding to tubulin. The lactam site is an important feature because it is probably directly involved in a bond with tubulin.

Rhazinilam represents probably the smallest entity recognized by tubulin and leading to this kind of activity: two aromatic groups linked by a rigid bridge bearing a function able to exchange a hydrogen bond with the receptor site. Further attempts are currently in progress in order to investigate other ways for modifying the hydrophobicity or polarity of this molecule.

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