The Chemistry and Biology of Rhazinilam and Analogues

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Abstract: Rhazinilam (**1**) is a natural substance with unique tubulin-binding properties. The present review describes the isolation and structure elucidation of (**1**), the available data regarding its biological properties, its semi- and total syntheses and its structure-activity relationships through the synthesis of analogues.

Keywords: Rhazinilam, tubulin, structure-activity relationships, semisynthesis, total synthesis, analogues

Dedicated to Dr. Claude Thal for his Major Contribution to this Field

INTRODUCTION

In the past decades, the mitotic spindle has been an attractive target for the development of anticancer agents. Microtubules are formed through the reversible assembly of a heterodimeric protein, tubulin. The tubulin-microtubule equilibrium generates a dynamic instability, which is crucial for the mitosis process [1]. Small molecules disturbing the assembly or disassembly of microtubules, in particular natural compounds, have shown a remarkable ability to impair cell proliferation and some of them have been successfully used as anticancer agents [2-4]. For instance the vinca alkaloids (vinblastine, vincristine and vinorelbine) as inhibitors of microtubule assembly and taxoids (paclitaxel and docetaxel) as inhibitors of microtubule disassembly have proven efficient anticancer agents.

The search for antimitotic compounds having a novel mode of action is still highly relevant, in order to solve issues of selectivity and drug resistance. Within this context, the discovery of the original biological properties of rhazinilam raised the interest of several chemistry groups. This review will focus on the isolation, structure elucidation and synthesis of rhazinilam, as well as on the literature data regarding structure-activity relationships (SAR) through the synthesis of analogues [5].

OCCURRENCE AND STRUCTURE

(–)-Rhazinilam (**1**) was first isolated from *Melodinus australis* (F. Mueller) Pierre [6]. It has later been found in other Apocynaceae such as *Rhazya stricta* Decaisne [7-8], *Aspidosperma quebracho-blanco* Schlecht. [9-10], *Leuconotis eugenifolia* A. DC. and *L. griffithii* Hook [11-12], *Kopsia singapurensis* Ridley [13], Fig. (**1**), and *Kopsia teoi* Allorge Remy [14]. More recently, (–)-rhazinilam was isolated from intergeneric somatic hybrid cell cultures of two Apocynaceae, *Rauvolfia serpentina* Benth. Ex Kurz and *Rhazia stricta* Decaisne [15].

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Fig. (1). *Kopsia singapurensis* flowers (left) and fruit (right); Photograph: T. Sévenet.

The structure of rhazinilam was elucidated concomitantly by X-ray analysis [9], Fig. (**2**), and degradation studies [8]. This molecule is characterised by the presence of four rings: the phenyl A-ring, the nine-membered lactam B-ring, the pyrrole C-ring and the piperidine D-ring. According to the X-ray data, the A-C dihedral angle of rhazinilam is ca. 90°, the amide bond possesses a *cis* conformation, and the median ring adopts a boat-chair conformation. This molecule bears two stereogenic elements: the quaternary carbon atom C-20 and the phenyl-pyrrole chirality axis. The absolute configuration (*R*, a*R*) was deduced by semisynthesis from (+)-1,2-didehydroaspidospermidine [16].

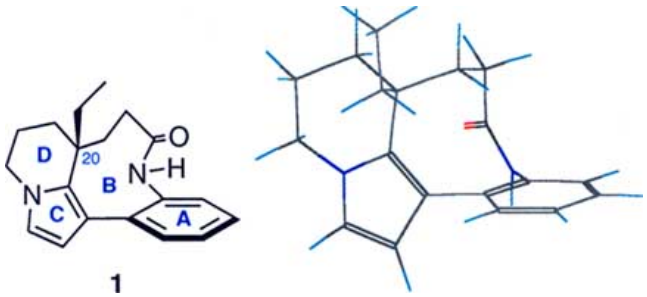

Fig. (2). Two and three-dimensional structure of rhazinilam (**1**) from X-ray data [9].

Rhazinilam was found to be actually an oxidative artifact, derived from the unstable natural 5,21-dihydrorhazinilam (**2**) [11-13], Fig. (**3**). Other natural compounds of the (–)-rhazinilam series have been isolated from various Apocynaceae such as 3-oxorhazinilam (**3**) (also called rhazinicine) [17-18], leuconolam (**4**) [11-12], 3-oxo-14,15-dehydrorhazinilam (**5**) [19] and rhazinal (**6**) [20].

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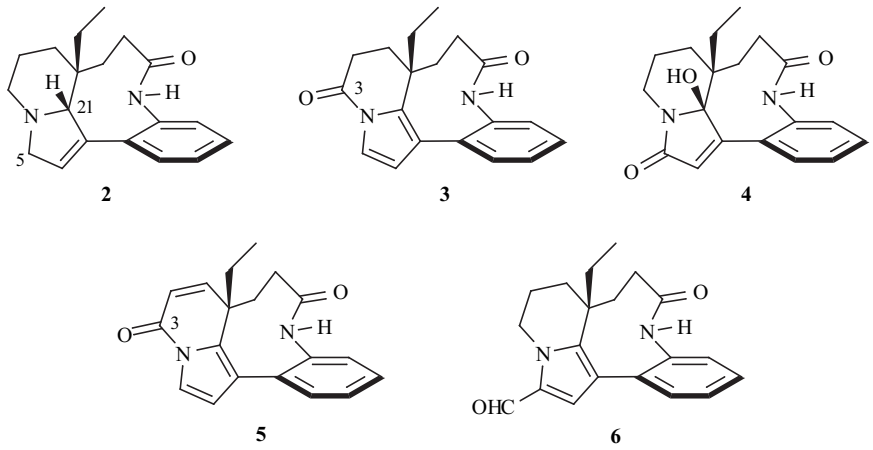


Fig. (3). Structure of naturally occurring rhazinilam congeners.

BIOLOGICAL PROPERTIES

The tubulin-binding properties of (–)-rhazinilam were discovered through screening of a number of Malaysian plant extracts [13]. Natural (–)-rhazinilam induces tubulin spiralisation [21] and therefore inhibits tubulin assembly in the same way as vinca alkaloids with an IC_{50} value of ca. 7 μM [22], Fig. (**4**). Besides it protects microtubules from cold disassembly such as with paclitaxel with an IC_{50} value of ca. 3 μM [22-23]. This mode of action was never observed with other microtubule poisons. In relation to these tubulin-binding properties, rhazinilam shows a relatively moderate cytotoxicity towards cancer cells, with IC_{50} values in the 0.5-5 μM range [22-23].

Rhazinilam was found inactive *in vivo* [21]. This could be inferred either to an insufficient binding affinity for tubulin, related to the moderate cytotoxicity towards cancer cells, or to a poor bioavailability. However, in light of the novel mode of interaction of this molecule with tubulin, structure-activity relationship (SAR) studies aimed at preparing analogues with better pharmacological properties were initiated. These analogues were prepared either by semisynthesis from indole alkaloids or by total synthesis.

ANALOGUES BY SEMISYNTHESIS AND SAR

Shortly after the structure elucidation of (–)-rhazinilam (**1**), Smith published its semisynthesis, which both proved

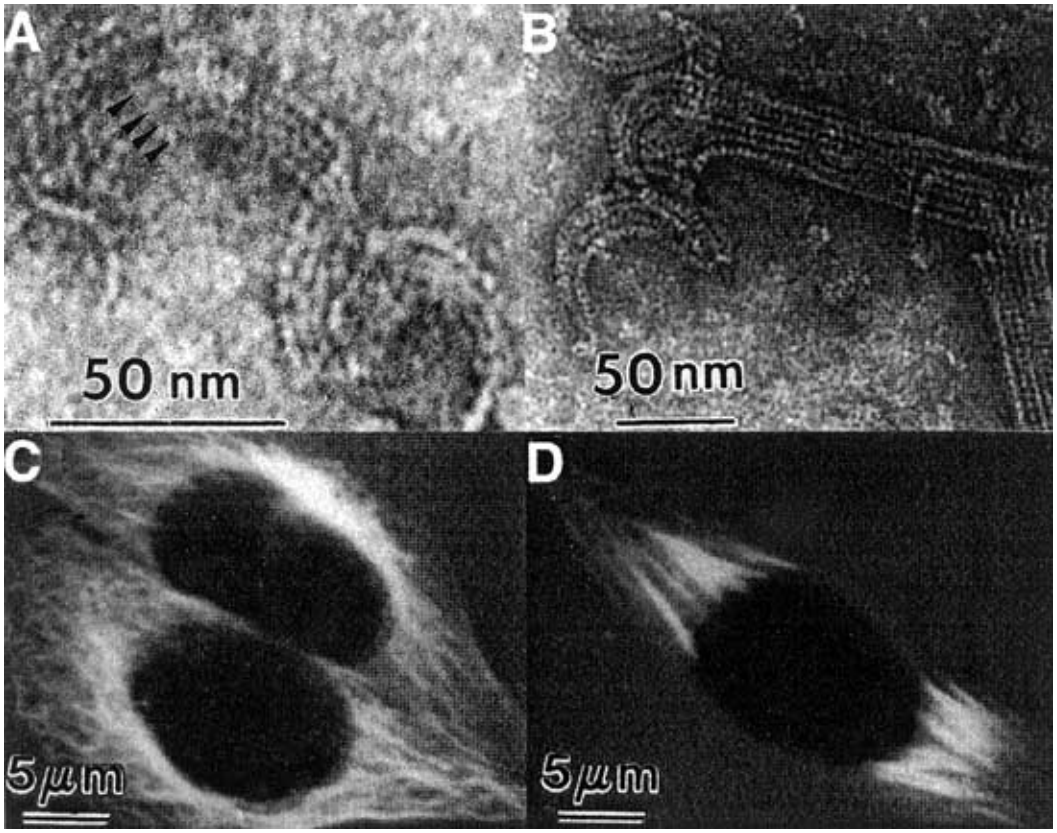
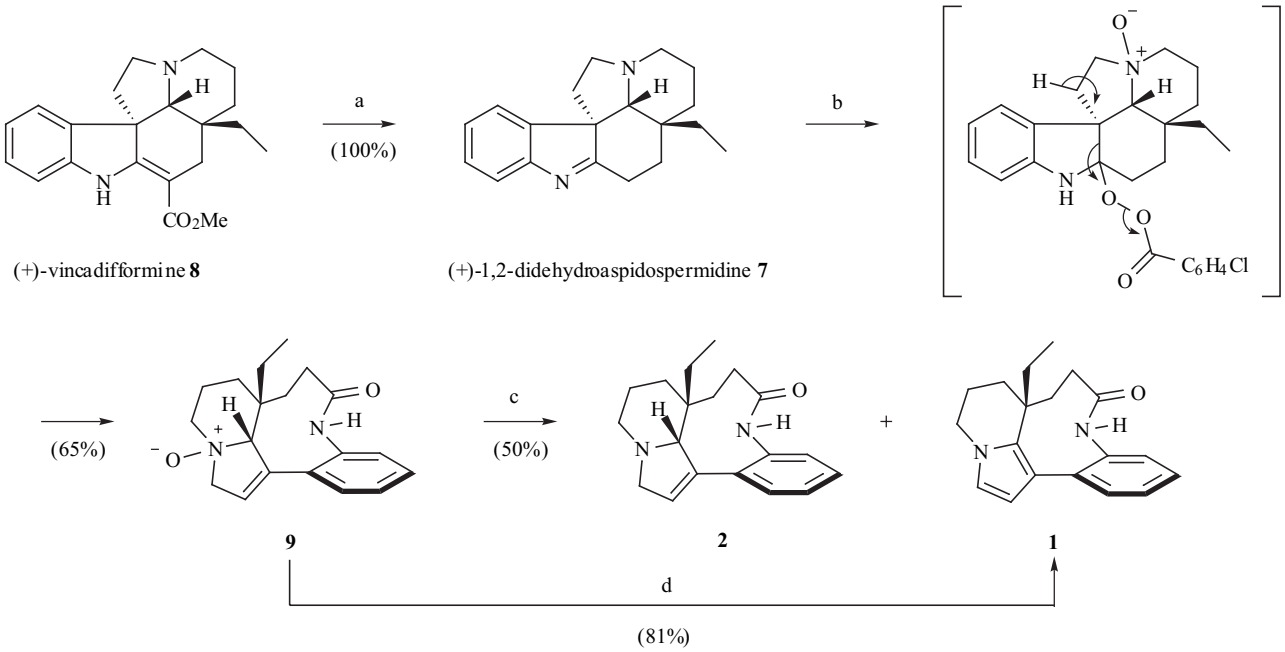


Fig. (4). A-B: Anomalous tubulin assembly observed after incubation of tubulin at 0°C (**A**) or microtubules at 37°C (**B**) with rhazinilam. **C-D:** PtK2 cells at 37°C untreated (**C**, normal microtubule network) or pre-incubated with rhazinilam (**D**, large microtubule bundles) [21].



Scheme 1. Semisynthesis of (–)-rhazinilam [16, 24]. (a) Concd. aq. HCl; (b) *m*-CPBA, CH_2Cl_2, –20°C; (c) $FeSO_4$, H_2O [9:1 mixture of (**1**):(**2**)]; (d) Ac_2O, Et_3N, CH_2Cl_2, 0°C. *m*-CPBA = *m*-chloroperbenzoic acid.

its absolute configuration and shed light on its plausible biogenetic origin [16]. Indeed the naturally occurring indole alkaloid (+)-1,2-didehydroaspidospermidine (**7**), Scheme (**1**), was sequentially treated with *m*-CPBA and ferrous sulfate to give (**1**) in ca. 30% overall yield. The mechanism of this stepwise conversion was first proposed by Smith and later refined by our own group [23-24]. Alkaloid (**7**) could be obtained quantitatively from the more readily available (+)-vincadifformine (**8**) by concd. hydrochloric acid-induced decarboxylation, Scheme (**1**). Treatment of (**7**) with *m*-CPBA at –20°C in methylene chloride afforded 5,21-dihydrorhazinilam *N*-oxide (**9**) in 65% isolated yield, *via* a plausible bis-oxidised intermediate. In the presence of Fe^{II}, step (c), compound (**9**) was reduced in 30 min to a 9:1 mixture of rhazinilam (**1**) and 5,21-dihydrorhazinilam (**2**), the latter itself giving rhazinilam upon exposure to air for several days. This slow conversion (**2**)→(**1**) suggested that the formation of rhazinilam from compound (**9**) occurred *via* a Polonovski-type reaction. Indeed, submission of (**9**) to the usual Polonovski conditions (Ac_2O, Et_3N), step (d), afforded rhazinilam in 81% yield. The reaction sequence (**7**)→(**1**) could also be performed in 50% yield in a one-pot fashion.

In plant, the unstable dihydrorhazinilam (**2**) is most certainly the direct biogenetic precursor of rhazinilam, which is an isolation artifact. Indeed, (**2**) was isolated together with (**1**) from the Apocynaceae *Leuconotis eugenifolia*, *L. griffithii* [11-12] and *Kopsia singapurensis* [13]. The question whether (+)-1,2-didehydroaspidospermidine (**7**) is the actual biogenetic precursor of (**2**) is still open.

The preceding semisynthetic scheme has been utilised by our group in order to conduct SAR studies [23-24], Table (**1**). The enantiomer of natural (–)-rhazinilam, (+)-rhazinilam, was obtained in the same manner as above starting from (–)-1,2-didehydroaspidospermidine, itself being obtained from (–)-tabersonine [13]. (+)-(**1**) was found inactive on tubulin (entry 1). Substituents were introduced either by derivatisation of (–)-rhazinilam itself [23] or by derivatisation of (+)-vincadifformine (**8**) followed by semisynthesis according to Scheme (**1**) [24]. The generic structure (**10**) encompasses the different modifications which were operated. Most analogues were markedly less active than (–)-(**1**) both on the inhibition of microtubule disassembly and on KB cells. However, C-5 substituted analogues such as ester (**10a**), the N-1 methyl-substituted analogue (**10b**) and the $\Delta^{14,15}$ unsaturated analogue (**10c**) retained significant microtubule disassembly inhibitory properties (entries 2-4).

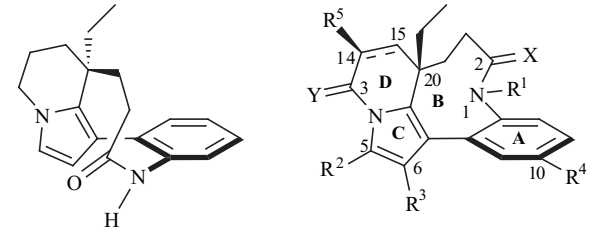
Lévy and co-workers have described the semisynthesis of D-ring *seco*-rhazinilam analogues from (–)-tabersonine using an Emde degradation and *m*-CPBA oxidation [25]. The *seco*-analogue (–)-(**11**) was only twice less active than rhazinilam on the inhibition of microtubules disassembly (entry 5), showing that the rigidity induced by the presence of D-ring is not strictly required for antitubulin activity [26].

TOTAL SYNTHESES OF RHAZINILAM

To date, four total syntheses of rhazinilam, three racemic and one enantioselective, have been reported. The main purpose of these studies was to address the synthetic challenges posed by the intriguing structure of this compound, rather than the possible application to medicinal chemistry. In particular, the axially chiral phenyl-pyrrole A-C biaryl bond, the fused pyrrole-piperidine C-D rings, the stereogenic quaternary carbon (C-20) *ortho* to the phenyl-pyrrole axis and the nine-membered lactam B-ring seemed to be challenging structural features for organic synthesis.

Along with the semisynthesis of rhazinilam from (+)-1,2-didehydroaspidospermidine, Smith and co-workers

Table 1. Evaluation of Semisynthetic Analogues of Rhazinilam [23-24, 26]



(+)-**1**

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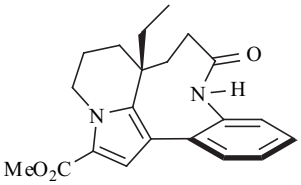
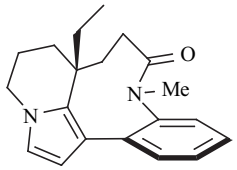


10a



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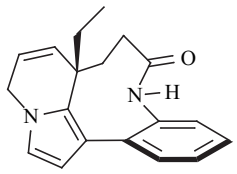

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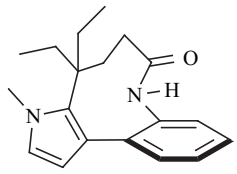

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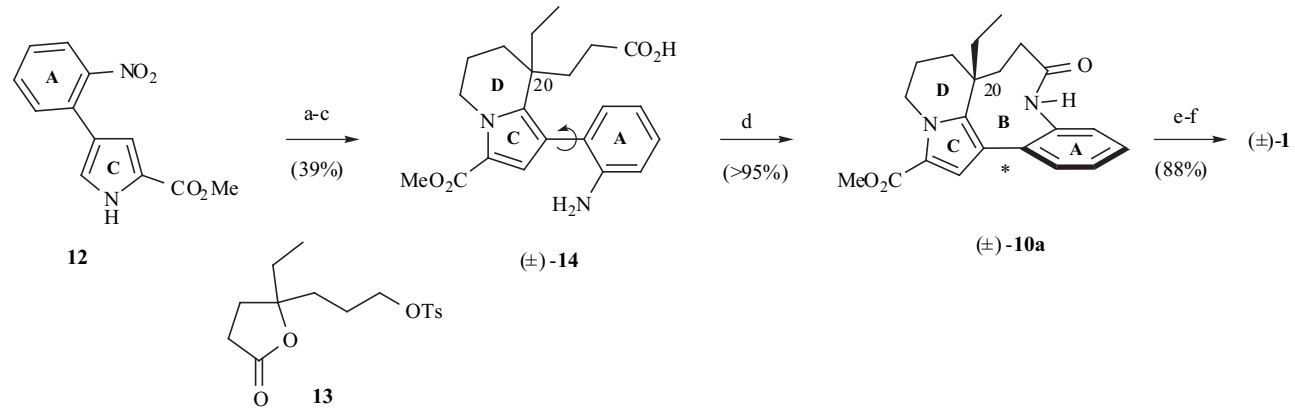
entry	cpd	IMD[a]	CT-KB[b]
1	(+)-**1**	inact	
2	(–)-**10a**	1/5	1/8
3	(–)-**10b**	1/4	1/120
4	(–)-**10c**	1/2	1/2
5	(–)-**11**	1/2	

[a] Inhibition of cold-induced microtubule disassembly; [b] cytotoxicity towards KB cell lines. Results are expressed as fractions of IC_{50}(rhazinilam)/IC_{50}(cpd).

reported in 1973 the first total synthesis of the racemic compound [16], Scheme (**2**). Phenyl-pyrrole (**12**), obtained by a Knorr-type condensation, was *N*-alkylated with racemic lactone tosylate (**13**) to give, after $AlCl_3$-induced cyclisation and hydrogenation of the nitro group, intermediate (**14**) having the A-C-D ring system of rhazinilam. The installation of the lactam B-ring was performed by cyclisation in the presence of DCC to give (**10a**) in quantitative yield. The final steps of the synthesis proceeded uneventfully through hydrolysis of the methyl ester and decarboxylation. As compound (**10a**) adopts only the (a*R*, 20*R*) relative configuration, the quantitative conversion of amino-acid (**14**) to (**10a**) indicates that free rotation around the phenyl-pyrrole bond occurs in (**14**) at room temperature. The (a*S*, 20*R*) conformer of (**14**) is unable to cyclise and atropisomerises to the (a*R*, 20*R*) conformer, which undergoes irreversible ring closure to give (**10a**). Therefore, the absolute configuration of the biaryl axis can be completely controlled by the absolute configuration at C-20, a property that was further exploited in other total syntheses.

In 2000 and 2002, Sames and co-workers described a total synthesis first of (±)-rhazinilam [27], then of (–)-rhazinilam [28], using an original strategy to build the quaternary C-20 stereogenic centre, Scheme (**3**). Both syntheses arrive at the achiral intermediate (**17**) having the A-C-D ring system of rhazinilam. This compound was built from allyl-iminium (**15**), which in the presence of silver carbonate, underwent a 1,5-electrocyclisation according to Grigg [29] to give phenyl-pyrrole (**16**) in 70% yield. Protection of the 2-pyrrole position followed by reduction of the nitro group afforded compound (**17**) in high yield. The asymmetric version of the synthesis is described in the second part of Scheme (**3**). The desymmetrisation of the two enantiotopic ethyl groups of (**17**) at C-20 to form the ethyl-vinyl-bearing chiral compound (**22**) was effected by metal-induced C(sp^3)-H activation (dehydrogenation) according to the following sequence: after Schiff base formation with chiral oxazoline (**18**), the platinum complex (**19**) was formed by complexation with the dimethylplatinum reagent $[Me_2Pt(\mu\text{-}SMe_2)]_2$. Addition of one equiv of triflic acid caused liberation of methane and formation of cationic complex (**20**), the thermolysis (70°C) of which generated the platinum alkene-hydride complex (**21**) in a diastereomeric ratio of 4.4:1. Lower temperatures gave higher diastereoselectivities but lower conversions. Decomplexation of the platinum with potassium cyanide followed by removal of the chiral auxiliary from the major diastereoisomer provided the enantiopure phenyl-pyrrole (**22**)



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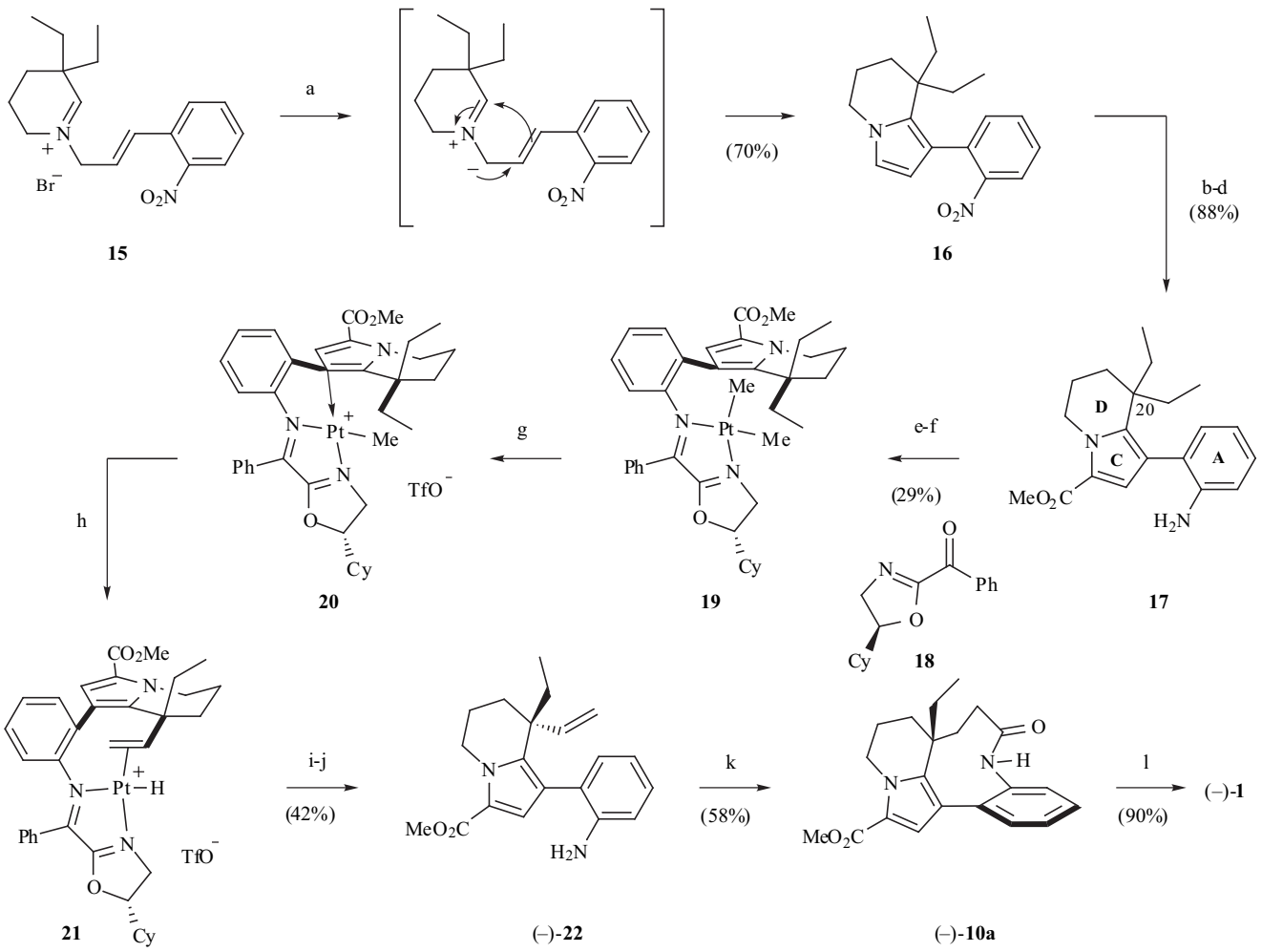
13

(±)-**14**

(±)-**10a**

Scheme 2. Smith's total synthesis of (±)-rhazinilam [16]. (a) NaH, **13**; (b) $AlCl_3$, CH_3NO_2; (c) H_2, PtO_2, AcOEt; (d) DCC, THF; (e) NaOH, MeOH-H_2O; (f) 240°C/vacuum. DCC= 1,3-dicyclohexylcarbodiimide.



Scheme 3. Sames' total synthesis of (–)-rhazinilam [28]. (a) Ag_2CO_3, toluene, reflux; (b) Cl_3CCOCl, CH_2Cl_2; (c) NaOMe, MeOH; (d) H_2, PtO_2, AcOEt; (e) **18**, *p*-TsOH, toluene, reflux; (f) $[Me_2Pt(\mu\text{-}SMe_2)]_2$, toluene; (g) TfOH, CH_2Cl_2, –40°C; (h) CF_3CH_2OH, 70°C; (i) aq. KCN, CH_2Cl_2; (j) NaOAc, NH_2OH•HCl, MeOH; (k) Pd/C, dppb, HCO_2H, DME, CO (10 atm), 150°C; (l) aq. NaOH, MeOH then aq. HCl, 50°C.

in satisfying overall yield. The latter was directly converted to the 9-membered lactam (**10a**) in 58% yield *via* a palladium-catalysed carbonylation. Removal of the methyl ester protection as previously described furnished (–)-rhazinilam in enantiomerically pure form. This elegant work constitutes the only asymmetric total synthesis of the natural product to date.

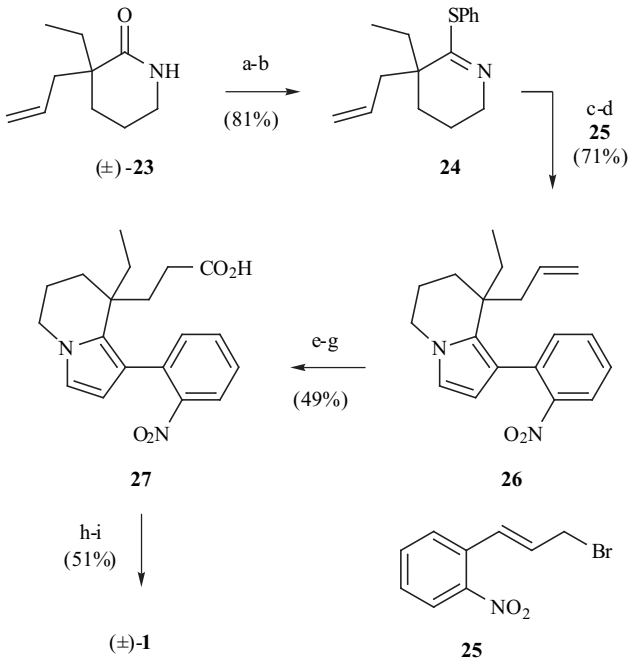
In 2001, Magnus and co-workers reported a straightforward synthesis of racemic rhazinilam, Scheme (**4**) [30]. The pyrrole ring was constructed from a piperidone, in an analogous manner to the Sames synthesis. The racemic piperidone (**23**), containing the C-20 quaternary carbon of rhazinilam, was obtained by sequential alkylations of piperidone. After formation of the thiophenyl iminoether (**24**), *N*-alkylation with allyl bromide (**25**) furnished the corresponding iminium intermediate, which underwent 1,5-electrocyclisation/thiophenol elimination in basic medium to form phenyl-pyrrole (**26**) in good yield. Compound (**26**) was transformed into rhazinilam by sequential hydroboration and oxidation, furnishing carboxylic acid (**27**), then hydrogenation and cyclisation under Mukayama conditions [31]. Racemic rhazinilam (**1**) was thus obtained with an overall yield of 8% for nine steps. Compared to the two previous total syntheses, this approach was significantly shorter for the following reasons: 1). the conversion of piperidone (**23**) to phenyl-pyrrole (**26**) was effected without changing the oxidation state of the piperidine ring; 2). the pyrrole ring protection/deprotection steps were avoided.

The above total syntheses, in spite of their elegance and efficiency, have not been utilised to obtain analogues of rhazinilam for biological screening. The following paragraph will focus on the synthesis of analogues by total syntheses employing different routes.

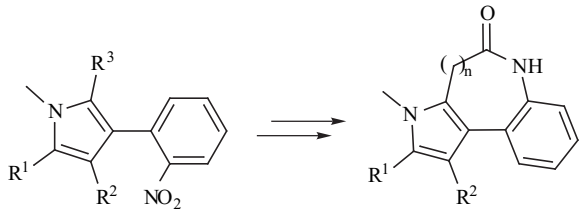
ANALOGUES BY TOTAL SYNTHESIS AND SAR

The Thal group was the first to conduct SAR studies by total synthesis of phenyl-pyrrole analogues [26, 32-33]. In particular the role of the lactam B-ring of rhazinilam was evaluated and to this purpose a number of achiral phenyl-pyrroles lacking this ring or having different lactam ring sizes (6 to 9-membered) were synthesised, Scheme (**5**). Substituted phenyl-pyrroles such as (**28**) were prepared using the Barton-Zard [34] or the Gupton [35] methods.

Functionalisation of the R^3 chain and cyclisation afforded various tricyclic lactams (**29**). All intermediates such as (**28**) lacking the lactam B-ring were inactive on tubulin. This is a constant feature in the rhazinilam series, which was confirmed in subsequent studies. Tricyclic analogues (**29**), including those having a 9-membered lactam ring (such as rhazinilam), showed only a very weak inhibition of microtubule disassembly (1/200 activity compared to rhazinilam). These important results provided the first evidence that both the lactam B-ring and the substitution at the C-20 atom were essential for the interaction with tubulin. The presence of bulky substituents at C-20 induces the lactam ring of rhazinilam to adopt the rigid boat-chair conformation, which is probably the active conformation.



Scheme 4. Magnus' total synthesis of (±)-rhazinilam [30]. (a) PCl_5, toluene, reflux; (b) PhSH, Et_3N, THF; (c) **25**, toluene, heat; (d) DBU, THF, 0°C; (e) BH_3•THF, THF, 0°C, then aq. NaOH, aq. H_2O_2, 0°C; (f) SO_3•py, Et_3N, DMSO-THF; (g) aq. $AgNO_3$, aq. KOH, EtOH; (h) Raney Ni, H_2 (20 psi), MeOH; (i) 2-chloro-1-methylpyridinium iodide, Et_3N, toluene-THF. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, py = pyridine.

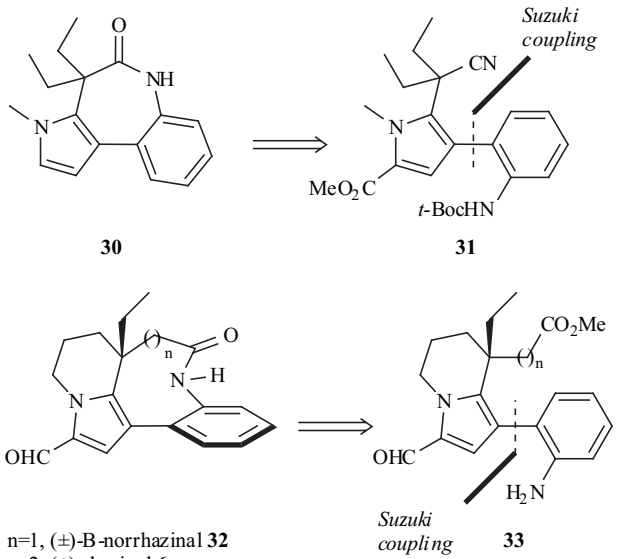


R^1=H, *n*-Bu; R^2=H, Me; R^3=H, CO_2Et

n = 0-3; R^1=H, *n*-Bu; R^2 = H, Me

Scheme 5. Synthesis of phenyl-pyrrole analogues [26, 32-33].

Other B-norrhazinilam analogues having the crucial quaternary carbon α to the pyrrole ring have been recently synthesised, Fig. (**5**). Achiral tricyclic 7-membered lactam (**30**) was obtained by basic hydrolysis of the bicyclic precursor (**31**) followed by cyclisation in acid medium [36]. In turn, the phenyl-pyrrole bond of compound (**31**) was constructed by Suzuki-Miyaura coupling of 2-*t*-Boc-aminophenylboronic acid and a functionalised 3-bromopyrrole. Such cross-coupling was also utilised by Ghosez and co-workers to build the phenyl-pyrrole scaffold of rhazinilam [37-38]. Unlike rhazinilam, compound (**30**) was inactive on the cold-induced disassembly of microtubules. However, it showed inhibition of tubulin polymerisation with an IC_{50} value of 27 μM (IC_{50} = 7 μM for rhazinilam) and a significant toxicity towards KB cells (IC_{50} = 7 μM, ca. 1/14 compared to rhazinilam). This suggested a different mode of action of (**30**) towards tubulin compared to (**1**). Similarly, Banwell and co-workers have reported the total synthesis of racemic B-norrhazinal (**32**) and rhazinal (**6**), Fig. (**5**) [39-40]. The synthesis also implied a construction of the phenyl-pyrrole bond *via* Suzuki coupling [intermediate (**33**)] followed by macrocyclisation. Compounds (**6**), (**32**) and (**1**) showed close tubulin-binding properties and comparable cytotoxicities towards cancer cells. As (**32**) and (**1**) have markedly different B-ring conformations [dihedral angle between the two aromatic rings: 56° for (**32**) and ca. 90° for (**1**)], this result suggests that there is a certain degree of freedom of the molecule within its active site.



n=1, (±)-B-norrhazinal **32**
n=2, (±)-rhazinal **6**

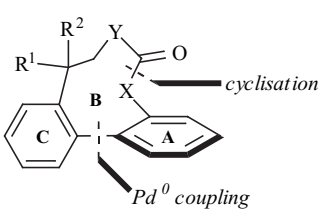
Fig. (5). Rhazinal and norrhazinilam analogues [36, 39-40].

Concomitantly, studies aimed at replacing the pyrrole C-ring of rhazinilam by other aromatic rings have been undertaken, Table (**2**). In the first place, a number of racemic biphenyl analogues having the general structure (**34**) have been synthesised using a strategy similar to that leading to (**30**), with construction of the biphenyl bond by a Stille or Suzuki-Miyaura coupling and final cyclisation to form the bridging ring [41-42]. Analogue (**34a**) having the structure closest to rhazinilam, but with no D-ring was less active than (**1**) (entry 1). Decreasing the size and the number of alkyl substituents R^1 and R^2 (entries 2-3) induced a dramatic decrease of the antitubulin activity in accordance with observations from Thal and co-workers. While the replacement of the amide group by a lactone (entry 4) or a urea (entry 5) proved unfruitful, the installation of a

carbamate group was particularly interesting. Indeed racemic compound (**34f**) was equipotent to rhazinilam on the inhibition of both microtubule assembly and disassembly, and was only twice less cytotoxic towards KB and MCF7 cells (entry 6). Analogue (**34f**) has, like rhazinilam, stable axial chirality and two atropisomeric enantiomers could be separated by chiral HPLC. It was found that, as with rhazinilam, the (–)-enantiomer is responsible for the antimitotic activity of the racemic mixture (entries 7-8). Based on circular dichroism experiments, it was deduced that the active atropisomer (–)-(**34f**) has the same (a*R*) axial configuration than (–)-rhazinilam. Finally, it was shown that the presence of a cyclohexane ring fused with C-ring as in rhazinilam [compound (**34g**)] is not particularly beneficial (entry 9).

A further optimisation of structure (**34f**) was next attempted and to this purpose an improved synthetic sequence was proposed, based on a one-pot borylation-Suzuki coupling protocol developed in our laboratories [22, 43]. Racemic biaryl analogues (**35**) bearing substituents on the A-ring were thus synthesised in a straightforward manner from substituted anilines. Most analogues were less active than unsubstituted (**34f**) on tubulin, pointing out the negative influence of steric effects for this ring (Table (**2**),

Table 2. Biaryl Rhazinilam Analogues [22, 41-42, 44]



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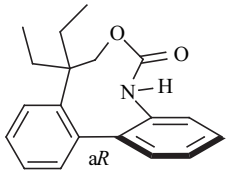
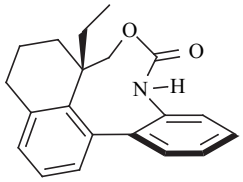
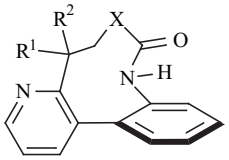


(–)-**34f**

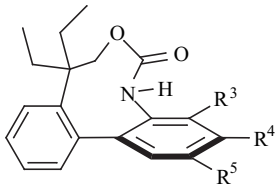


34g

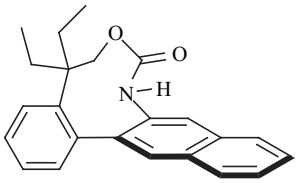


36

R^1,R^2=alkyl groups;
X=NH,O; Y= CH_2, NH, O



35



35f

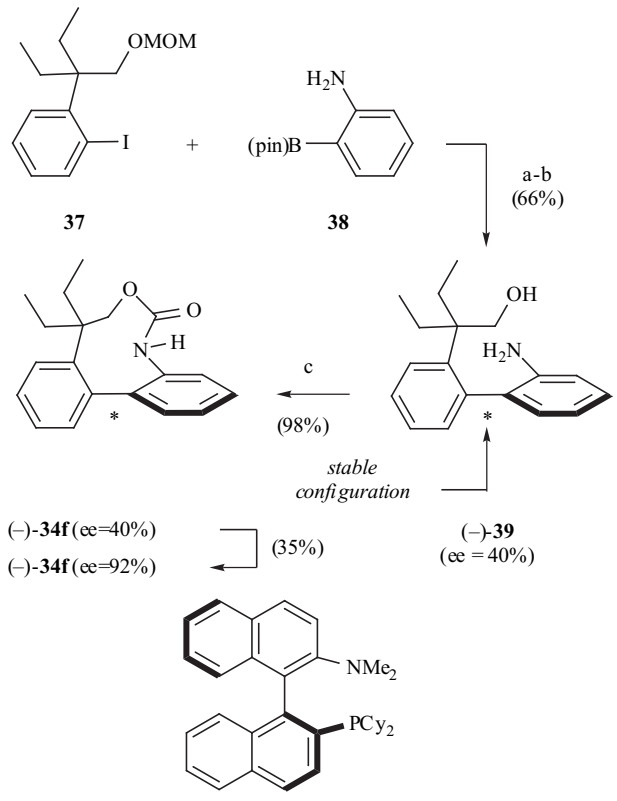
entry	cpd[a]	substitution	IMD[b]	IMA[c]	CT-KB[d]	CT-MCF7[e]
1	**34a**	X=NH, Y=CH_2, R^1=R^2=Et	1/8		1/11	
2	**34b**	X=NH, Y=CH_2, R^1=Et, R^2=H	1/21		inact	
3	**34c**	X=NH, Y=CH_2, R^1=R^2=Me	1/17		1/26	
4	**34d**	X=O, Y=CH_2, R^1=R^2=Et	inact		1/11	
5	**34e**	X=NH, Y=NH, R^1=R^2=Et	1/15		1/40	
6	**34f**	X=NH, Y=O, R^1=R^2=Et	1	1	1/2	1/1.5
7	**(–)-34f**		2		1	
8	**(+)-34f**		inact		1/5	
9	**34g**		1/3		1/10	
10	**35a**	R^3=R^4=H, R^5=Me	inact	1/24	1/5	1/4
11	**35b**	R^3=H, R^4=R^5=OMe	inact	1/28	<1/30	1/5
12	**35c**	R^3=R^4=H, R^5=NO_2	1/55	1/15	1/18	1/1.6
13	**35d**	R^3=R^5=H, R^4=NO_2	1/1.7	1/1.8	1/6	1/2
14	**35e**	R^3=R^4=R^5=F	1/5	1/11	1/7	1/2
15	**35f**				1/9	1.3
16	**36a**	X=CH_2, R^1=R^2=Et	1/10			
17	**36b**	X=O, R^1=R^2=Et	1/6		1/8	

[a] Racemic compounds, unless otherwise stated; [b] cold-induced inhibition of microtubule disassembly; [c] inhibition of microtubule assembly; [d] cytotoxicity towards KB cells; [e] cytotoxicity towards MCF7 cells. Results are expressed as fractions of IC_{50}(rhazinilam)/IC_{50}(cpd).

entries 10-14). However, the naphthyl-phenyl analogue (**35f**) was more cytotoxic than (**1**) and (**34f**) towards the MCF7 cell line (entry 15), but its effect on tubulin could not be determined. Interestingly, it appears in most studies that rhazinilam analogues, having low or no activity on tubulin, can retain significant cytotoxicity towards cancer cells [see for instance entries 4, 8, 10 in Table (**2**)]. This could indicate that other intracellular targets, as yet unidentified, are involved.

Finally, phenyl-pyridine analogues (**36**) of rhazinilam have been synthesised by Rocca, Quéguiner and co-workers, Table (**2**) [44]. The synthesis started with the functionalisation of the pyridine ring by picolinic metalation, then again a Suzuki coupling to build the phenyl-pyridine bond and a final B-ring cyclisation. However, these analogues were less active than their biphenyl surrogates (entries 16-17), which might be imputable to the basicity of the pyridine nitrogen.

At this point, methodologic studies were initiated in order to synthesise biphenyl-carbamate analogue (–)-(**34f**), the most active rhazinilam congener so far, in an enantiomerically pure form. Indeed the separation of (–) and (+) enantiomers by HPLC on chiral column was relatively laborious and, therefore, unadapted to the provision of significant quantities of (–)-(**34f**) for further biological testing. Besides, the control of the axial chirality of (**34f**) seemed to be a chemical challenge in the light of the available literature methods. Following these considerations, an asymmetric synthesis of (–)-(**34f**) was recently reported by our group, Scheme (**6**) [45]. The synthetic strategy is close to the previously reported racemic synthesis [22] and utilises recent literature data on the asymmetric Suzuki biaryl coupling [46-47]. After chiral ligand screening and optimisation of the reaction conditions, it was shown that the coupling of phenyl iodide (**37**) and pinacolboronic ester (**38**) in the presence of Pd^0, Buchwald ligand (**40**) [47] and barium hydroxide afforded, after MOM-group deprotection, biphenyl (**39**) in 66% yield and with 40% ee. Under these particular conditions, the coupling was sufficiently fast so that no epimerisation at the biaryl bond was observed. After triphosgene - induced cyclisation, the optic purity of target (–)-(**34f**) was improved by crystallisation (92% ee), affording quantities of compound (ca. 100 mg) suitable for further biological testing.



Scheme 6. Asymmetric synthesis of analogue (–)-(**34f**) [45]. (a) Pd_2dba_3 (5 mol%), **40** (6 mol%), $Ba(OH)_2$, dioxane-H_2O, 80°C; (b) concd. HCl, MeOH; (c) $(Cl_3CO)_2C{=}O$, py, CH_2Cl_2, –78°C. MOM = methoxymethyl; pin = pinacol.

CONCLUSION AND PERSPECTIVES

Rhazinilam (**1**) is a natural substance showing unique tubulin-binding properties. For this reason and due to its intriguing molecular structure, this molecule has raised a renewed interest in the scientific community for the last ten years. Indeed a number of synthetic approaches to the natural product and analogues have been reported. Extensive SAR studies were conducted in order to improve the antitubulin activity of (**1**), as well as its *in vitro* cytotoxicity towards cancer cells. These studies have shown that the rigid biaryl/nine-membered lactam structure of rhazinilam, adopting a boat-chair conformation and containing a quaternary carbon atom, is an essential feature for tubulin binding. Moreover, the absolute (a*R*) configuration of the biaryl axis is required for the biological activity. Most structural changes performed on the rhazinilam skeleton affect negatively its antimitotic properties, except the replacement of both the pyrrole C-ring by a phenyl ring and the amide group by a carbamate, leading to compound (**34f**), twice more active than (**1**) on tubulin. The bioactive (a*R*)-configured atropisomer of (**34f**) was recently synthesised in an enantiomerically enriched form.

The search for more cytotoxic rhazinilam analogues seems to rely on the fine-tuning of the drug-tubulin interactions. In the absence of structural data regarding the active binding-site, it is rather hard to predict what modification could lead to improved activity. Further analogue synthesis based on lead compounds (**1**) and (**34f**) could focus on further substitutions on B ring and would certainly benefit from a better understanding of the *in vivo* inactivity of rhazinilam.

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