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Synthesis and biological evaluation of B-ring analogues of (−)-rhazinilam

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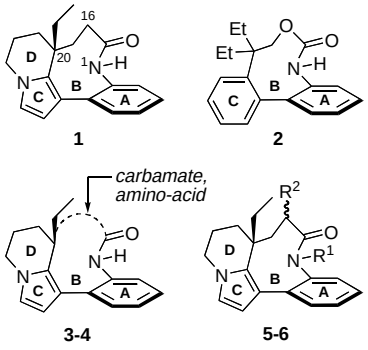
Abstract—Three macrocyclic analogues of rhazinilam **1** having a 11- or 12-membered B-ring with an endocyclic carbamate group or an amino-acid residue were synthesized from the natural product. These analogues **3** and **4** displayed a very low activity on tubulin. Thirty N-1 and C-16 substituted analogues of rhazinilam were also synthesized regioselectively from rhazinilam. Stereochemical analyses showed that N-1 and C-16α analogues have the same conformation as rhazinilam, whereas C-16β analogues adopt a different conformation for rings B and D. All N-1 and C-16 analogues were less active than rhazinilam on tubulin, though analogues **5a**, **6a**α, **6b**α, and **6f** having the less bulky substituents retained close affinities. A few analogues either active (like **6f**) or inactive (like **5o**) on tubulin showed significant inhibition of the growth of KB cancer cells.

1. Introduction

In the past decades, the mitotic spindle has been an attractive target for the development of anticancer agents. 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, as, for instance vinca alkaloids and taxoids, are successfully used in cancer therapy.[1] The search for antimitotic compounds having a novel mode of tubulin binding is still highly relevant, in order to solve issues of selectivity and drug resistance. (−)-Rhazinilam **1** is a natural compound isolated from various *Apocynaceae*, whose tetracyclic structure possesses an axially chiral phenyl–pyrrole subunit bridged by a nine-membered lactam ring.[2] It was found to have unique antimitotic properties, with in vitro inhibition of both microtubule assembly and disassembly, and the formation of abnormal tubulin spirals.[3] As a consequence of these tubulin-binding properties, rhazinilam showed in vitro cytotoxicity toward various cancer cell lines in the low micromolar range, however no activity was found in vivo.



Driven by the original in vitro properties of rhazinilam, several groups have undertaken structure–activity relationships (SAR) aimed at increasing the in vitro activity and solving the problem of in vivo inactivity. In the absence of structural data on the rhazinilam binding site in tubulin, a number of observations were made through the testing of analogues obtained by semi-synthesis[4,5] or total synthesis.[6–10] In particular, it was shown that the introduction of substituents on rings A, C, and D has a negative effect on the activity, suggesting a narrow tubulin binding pocket. In addition, these data suggest that the conservation of the boat–chair conformation of B-ring, imposed by the *cis*-amide group and the quaternary C-20 carbon, is important for preserving the

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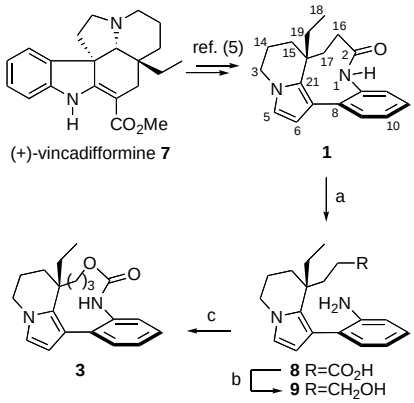
affinity for tubulin. Indeed, analogues with a secondary or tertiary C-20 as well as analogues with a smaller B-ring were less active than rhazinilam. Biphenyl-carbamate **2** was found to be the most active analogue of rhazinilam so far, with a 2-fold activity on tubulin compared to rhazinilam, however its in vitro cytotoxicity is still in the low micromolar range, very close to that of the natural compound.[7b,10] In three dimensions, compound **2** is almost superimposable to rhazinilam, and its improved activity originates mainly in the replacement of the amide group by a carbamate. These results prompted us to perform further modifications of B-ring by semi- and total syntheses.

In this paper, we describe the synthesis, structural analysis and biological activity of three analogues **3** and **4** with an enlarged (11- and 12-membered) B-ring as well as thirty rhazinilam analogues **5** and **6** substituted in the N-1 and C-16 positions of B-ring. The first series of compounds **3** and **4** were obtained by opening of the lactam ring of (−)-rhazinilam, functionalization and ring re-closure. These first analogues of rhazinilam with a larger B-ring, containing either an additional C–O bond (compound **3**) or an amino-acid residue (compounds **4a** and **4b**) compared to rhazinilam, were first designed to provide further information on the role of B-ring conformation and flexibility. In addition, the presence of the additional hydrogen-bond donor/acceptor amide group in compounds **4** was thought to be of potential benefit for the interaction with tubulin. The second series of analogues **5** and **6** were synthesized by regioselective direct functionalization of (−)-rhazinilam **1** and were designed to analyze further the influence of the substitution of the amide group nitrogen and α-carbon on the biological activity.

2. Results and discussion

2.1. Analogues 3 and 4 with a macrocyclic B-ring

(−)-Rhazinilam **1** was synthesized in two steps from the alkaloid (+)-vincadifformine **7** by a recently optimized procedure (Scheme 1).[5b] Macrocycle **3** was obtained in three steps from rhazinilam as follows. The hydrolysis of the lactam group of rhazinilam was first performed in 82% yield under relatively harsh conditions, using excess KOH in refluxing ethanol and water for 23 h. Alternatively, the reaction time could be substantially shortened under microwave heating (120 °C, 100 W, sealed tube), giving amino-acid **8** in 85% yield in only 30 min. Reduction of the carboxylic acid with excess $LiAlH_4$ in refluxing THF provided amino-alcohol **9** in 69% yield. The ^1H NMR spectra of compounds **8** and **9** showed that these compounds occur as a mixture of two conformers (87/13 for **8** and 80/20 for **9** in $CDCl_3$) arising from probable atropisomerization around the phenyl–pyrrole bond. These two conformers interconvert rapidly as shown by the presence of exchange correlations in NOESY spectra recorded at 295 K. A number of reagents and reaction conditions were tested for the macrocyclization of amino-alcohol **9** to give 11-membered carbamate **3**. The best yield (21%) was obtained



Scheme 1. Reagents and conditions: (a) KOH (42 equiv), EtOH/H_2O 1:1, microwave heating (120 °C, sealed tube), 30 min, 85%; (b) $LiAlH_4$ (20 equiv), THF, reflux, 22 h, 69%; (c) NaHMDS (2 equiv), $(Cl_3CO)_2C{=}O$ (1 equiv), THF, 0 °C, 10 min, 21%. NaHMDS, sodium bis(trimethylsilyl)amide.

by deprotonation of **9** with 2 equiv of NaHMDS, followed by reaction with diluted triphosgene in THF at 0 °C.[11]

By contrast with intermediates **8** and **9** the ^1H NMR spectrum ($CDCl_3$) of compound **3** showed the presence of only one conformer, as the cyclization is much more sterically favored with the phenyl–pyrrole axis of **9** in the same (a*R*) configuration as rhazinilam. Molecular modeling experiments (Monte-Carlo random search) indicated that the amide group of **3** adopts the more stable *trans* conformation (Fig. 1), whereas that of rhazinilam is fixed in the *cis* conformation by the more rigid nine-membered ring (see Fig. 3A). The plane angle between the phenyl and pyrrole rings is similar in **3** and in rhazinilam, with respective values of 87° and 89°, indicating that the enlargement of B-ring does not significantly affect the ACD ring system.

The synthesis of macrocycles **4a** and **4b** incorporating a glycine or L-valine in their B-ring was performed as follows (Scheme 2). Initially, the carboxylic acid group of **8** was converted to the methyl ester and the resulting compound coupled with Fmoc-glycine. However, the hydrolysis of the ester group and subsequent removal of the Fmoc group were low yielding. A more straightforward approach consisted of reacting directly amino-acid **8**

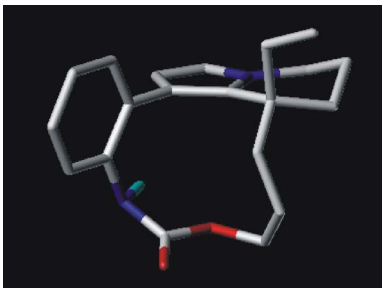
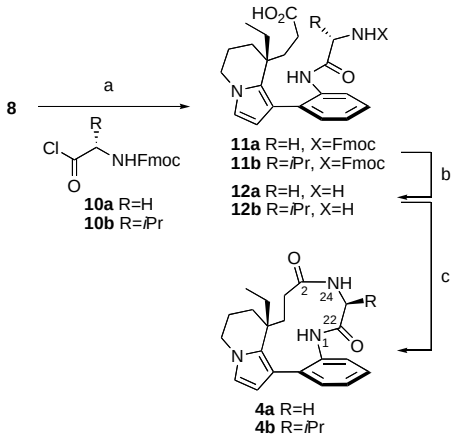


Figure 1. Lowest energy conformer of macrocycle **3** obtained by molecular modeling (all hydrogen atoms, except for H-1, were omitted for clarity).



Scheme 2. Reagents and conditions: (a) CH_2Cl_2/10% aq $NaHCO_3$ 1/1, **10a** or **10b** (1 equiv), 20 °C, 90 min, 46% (**11a**) or 66% (**11b**); (b) THF/piperidine 9:1, 20 °C, 88% (R=H) or 92% (R=*i*Pr); (c) TBTU (3 equiv), HOBT (3 equiv), $EtN(iPr)_2$ (4 equiv), DMF (c = 1 mM), 20 °C, 24 h, 30% (**4a**) or 10% (**4b**). Fmoc, 9-fluorenylmethoxycarbonyl; TBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; HOBT, 1-hydroxybenzotriazole.

with the acid chloride **10a**, obtained in high yield from Fmoc-glycine and thionyl chloride.[12] This method afforded the coupled product **11a** in 46% yield. After Fmoc group removal with piperidine (88% yield of amino-acid **12a**), the macrocyclization occurred in the presence of TBTU and HOBT as coupling reagents to give macrocycle **4a** in 30% yield.[13,14] The compound was purified twice, by reverse-phase column chromatography followed by HPLC in order to remove traces of the peptide coupling reagents, which in part is responsible for the low yield of isolated compound. Other peptide coupling reagents such as BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexa-fluorophosphate) did not afford improved yields.

The same strategy was applied for the synthesis of macrocycle **4b** containing a L-valine (Scheme 2). This other amino-acid was chosen in order to study the influence of a bulky Cα-substituent on the conformation and biological activity of the macrocyclic rhazinilam analogue. Thus, compound **4b** was obtained in three steps from amino-acid **8** and Fmoc-valine acid chloride **10b**. A difficult purification again explains in part the low macrocyclization yield. Similar to acyclic intermediates **8** and **9**, NMR experiments indicated that intermediates **11a**,**b** and **12a**,**b** exist as a mixture of conformers interconverting at room temperature. However, by contrast to macrocycle **3** which showed only one conformer, macrocycles **4a** and **4b** both exist as a mixture of two conformers (named **4ax**/**4ay** and **4bx**/**4by**) in acetone-d_6 at 295 K, in a ratio of, respectively, 1/1 and 85/15. These conformers interconvert at 295 K, as shown by correlations observed on the NOESY spectrum recorded at this temperature. These correlations disappeared on NOESY spectrum recorded at 233 K, which shows that the interconversion is blocked at this temperature. The two conformers most probably arise from classical *cis*–*trans* isomerization of one or both amide groups. It seems that the presence of the *iso*propyl group in macrocycle **4b** energetically favors one conformer compared to macrocycle **4a**. Molecular modeling calculations (Monte-Carlo random search) were performed with compounds **4a** and **4b**. For each compound, four conformers of similar energies were obtained, in which the N(1)–C(22) amide is always *trans*, whereas the N(24)–C(2) amide is either *trans* or *cis* (with two different orientations in each case). Amongst these four conformers, only one could be fitted with the NMR dataset of one of the conformers of **4a**, **4ax**, as well as of the major conformer of **4b**, **4bx** (Fig. 2).

The second conformers **4ay** and **4by** could not be assigned unambiguously. The NMR data arguing for the proposed structure **4ax** (Fig. 2A), where both amide groups have *trans* conformations, are the following: (1) observed NOESY correlations (Fig. 2A); (2) deshielding of proton H-12, resoning at 8.41 ppm (compared to 7.20 ppm for rhazinilam), due to the proximity of the anisotropy cone of the C-22 carbonyl group; (3) torsion angles of 26° for H(23a)–C(23)–N(24)–H(24) and 142° for H(23b)–C(23)–N(24)–H(24), calculated from the coupling constants ^3J(H-23a–H-24) = 5.8 Hz and ^3J(H-23b–H-24) = 7.0 Hz.[15] Similarly, the NMR data arguing for the proposed structure **4bx** (Fig. 2B), where the N(1)–C(22) amide is *trans* and the N(24)–C(2) amide is *cis*, are the following: (1) observed NOESY correlations (Fig. 2B); (2) deshielding of proton H-12, resoning at 8.14 ppm, less pronounced than for **4ax** probably because H-12 is farther away from the C-22 carbonyl; (3) torsion angle of 153° for H(23)–C(23)–N(24)–H(24), calculated from the coupling constant ^3J(H-23–H-24) = 8.3 Hz. In this structure, the C-23 *iso*propyl group lies in a pseudo-equatorial position which probably stabilizes this particular conformation and may explain the preponderance of conformer **4bx**, whereas the glycine conformers **4ax** and **4ay** are equally abundant. The plane angle between phenyl and pyrrole rings is 91° for both conformers **4ax** and **4bx**, and is again very similar to that of rhazinilam (89°), indicating the conservation of the three-dimensional structure of the ACD ring system.

In summary, the three-dimensional structures proposed for macrocycle **3**, for one conformer of macrocycle **4a**,

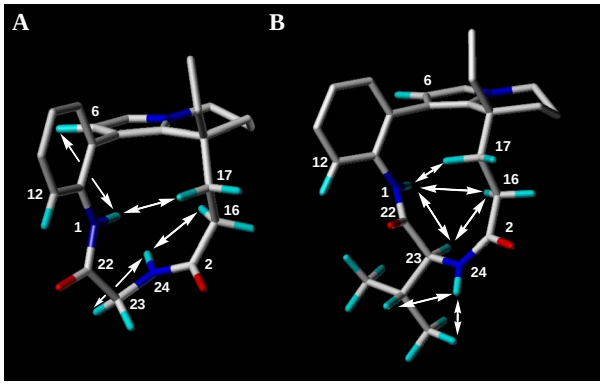


Figure 2. Molecular modeling structure of macrocycles **4a**–**b** with selected NOESY correlations. (A) Conformer **4ax**. (B) Major conformer **4bx** (some hydrogen atoms were omitted for clarity).

and the major conformer of macrocycle **4b** show a very different B-ring conformation compared to rhazinilam, with a *trans* N(1)–C(22) amide, whereas the amide group of rhazinilam is *cis*. In addition, the presence of two interconverting conformers for **4a** and **4b** shows that the 12-membered B-ring of these compounds is flexible, by contrast to the rigid nine-membered B-ring of rhazinilam.

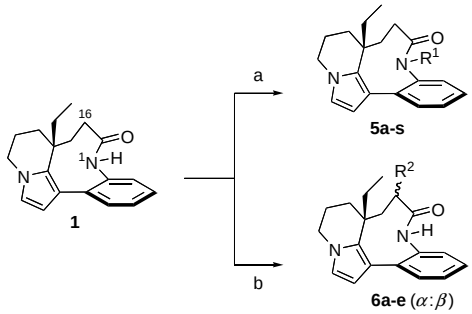
The 11- and 12-membered macrocycles **3**, **4a**, and **4b** showed a very weak activity on the assembly and disassembly of microtubules.[16] In addition, these compounds showed no cytotoxicity toward KB cancer cells. Acyclic intermediates **8**, **9** and **11a**,**11b** were also evaluated and showed no activity on tubulin and on KB cells. The low activity of macrocycles **3** and **4** might be imputed to the excessive flexibility of B-ring compared to that of rhazinilam, which enables the N-1 amide group of the analogues to adopt the more stable *trans* conformation. The importance of the *cis* amide conformation for the binding to tubulin could suggest that H-1 amide proton is engaged in a hydrogen-bond with the protein and has to remain intact and in the right orientation in order to preserve the activity.

2.2. N-1 and C-16 analogues 5 and 6

In consequence of the above results showing a loss of tubulin-binding activity upon enlargement of rhazinilam B-ring, it was decided to maintain the nine-membered rigid structure of rhazinilam and to perform direct modifications around the amide group on the N1 or C16 position. The improved activity of carbamate **2** compared to that of the corresponding amide also encouraged us in this way.[7b] The introduction of three substituents (Me, CH_2CO_2Ph, and *t*Boc) on the N-1 amide nitrogen of rhazinilam as well as the biological properties of these analogues were briefly described in a previous report.[5a] N-1-methylrhazinilam was the most active N-1 analogue reported, with a four times lower activity compared to that of rhazinilam on the inhibition of microtubule disassembly. In continuation to this work, we describe the introduction of a larger number of chemically diverse substituents on the amide group at N-1 and C-16.

Deprotonation of the amide proton of (−)-rhazinilam **1** by 1 equiv *n*-butyllithium at 0 °C followed by quenching with an electrophile afforded N-substituted analogues **5a**–**s** regioselectively and in moderate to good yield (Scheme 3 and Table 1, entries 1–19). On the other hand, using 2 equiv *n*BuLi generated the amide dianion which reacted regioselectively on C-16 with electrophiles to give analogues **6a**–**e** (Scheme 3 and Table 1, entries 20–24). In case of C-16 ethyl (**6b**, entry 21) and benzoyl (**6d**, entry 23) analogues, the corresponding N-substituted compounds **5a** and **5k** were also isolated in 33% and 25% yield, respectively. In both N- and C-substitutions, unreacted rhazinilam was recovered in substantial amounts. Among the strong bases screened for this functionalization, *n*BuLi gave the highest yields.

The C-substitution products **6a**–**e** were obtained as mixtures of two diastereoisomers α and β.[17] The α:β



Scheme 3. Reagents and conditions: (a) *n*BuLi (1.05 equiv), THF, 0 °C, 30 min, then electrophile (1.0 equiv); (b) *n*BuLi (2.5 equiv), THF, 0 °C, 30 min, then electrophile (1.0 equiv).

ratio was measured from ^{1}H NMR and HPLC analysis of the mixture, then the two diastereoisomers were separated by preparative HPLC for further stereochemical analysis and biological testing. The perfluorinated *N*-*t*Boc analogue **5p** (entry 16) was synthesized by a different procedure, by reacting (−)-rhazinilam with perfluoro-*tert*-butyl alcohol, triphosgene, and pyridine in CH_2Cl_2 at 0 °C.[11] Finally, 16-hydroxyrhazinilam **6f** (entry 25) was obtained as a by-product during the large-scale conversion of (+)-vincadifformine into (−)-rhazinilam.[5] It was converted to its acetate **6g** (entry 26) using Ac_2O/Et_3N/DMAP in dichloromethane.

The three-dimensional structure of (−)-rhazinilam **1** was solved in 1972 by X-ray analysis.[18] We have again solved this structure with a better resolution for the purpose of the present study (Fig. 3A). The nine-membered lactam B-ring of rhazinilam adopts a boat–chair conformation. In addition, the C-14 atom on piperidine D-ring points down, toward B-ring. This conformation is also preferred in solution ($CDCl_3$), as shown by NOESY experiments (Fig. 3A). The X-ray crystal structure of *N*-*t*Boc-rhazinilam **5o** (Fig. 3B) shows that the introduction of the large *t*Boc group does not modify the conformation of rings B and D.

The absolute configuration of the major and minor C-16 diastereoisomers of analogues **6a**–**g** was determined by X-ray analysis as well as NMR experiments. First, the X-ray crystal structure of the major diastereoisomer of 16-ethylrhazinilam **6b** was solved (Fig. 3C), showing that this diastereoisomer has the α configuration at C-16. Rings B and D of this diastereoisomer **6b**α adopt the same conformation as rhazinilam. By extension, the minor diastereoisomer of compound **6b** has the β configuration at C-16.

The configuration of other C-16 analogues was determined indirectly, as no other X-ray structure could be obtained. It was noticed that all major diastereoisomers of C-16 analogues **6a**–**e** have similar chemical shifts of ^{1}H and ^{13}C atoms located on D-ring and to a lesser extent on B-ring (Table 2, entries 2–6). These chemical shifts are also similar to those of rhazinilam (entry 1). This suggests that all major diastereoisomers have the α configuration at C-16 and adopt the same conforma-

Table 1. Synthesis, cytotoxicity, and antitubulin activity of N-1 and C-16 analogues of (−)-rhazinilam produced via Scheme 3

Entry	Compound	R^1 or R^{2a}	Electrophile	Yield[b] (%)	Ratio α:β	Inhibition of microtubule assembly IC_{50} (**1**)/ IC_{50}[c]	Inhibition of microtubule disassembly IC_{50} (**1**)/ IC_{50}[c]	Cytotoxicity KB cell line IC_{50} (**1**)/ IC_{50}[d]
1	**5a**	Et	EtI	66		1/11	1/2	In
2	**5b**	*n*Pr	*n*-PrI	32		1/22	In	1/33
3	**5c**	Allyl	(Allyl)Cl	46		In	In	In
4	**5d**	CH_2CN	$BrCH_2CN$	81		1/22	In	1/65
5	**5e**	MOM	MOMBr	76		In	In	In
6	**5f**	MEM	MEMCl	62		In	1/23	In
7	**5g**	SEM	SEMCl	71		In	In	1/9
8	**5h**	Bn	BnBr	53		In	In	In
9	**5i**	Ac	AcBr	50		In	In	1/13
10	**5j**	Piv	PivCl	66		In	In	1/58
11	**5k**	Bz	BzCl	59		1/6	In	1/4
12	**5l**	(*p*OMe)Bz	(*p*OMe)BzCl	62		1/6	In	1/33
13	**5m**	(pCF_3)Bz	(pCF_3)BzCl	53		1/9	In	1/8
14	**5n**	C(=O)OEt	ClC(=O)OEt	50		1/22	In	In
15	**5o**	*t*Boc	Boc_2O	85		In	In	1/2
16	**5p**	$C(=O)OC(CF_3)_3$	—[e]	12		In	In	1/2
17	**5q**	C(=O)OPh	ClC(=O)OPh	61		1/18	In	1/5
18	**5r**	Ms	MsCl	46		In	In	In
19	**5s**	Ts	TsCl	67		In	In	1/11
20	**6a**α	α-Me (*S*)	MeI	43	80:20	2	1/2	1/7
	6aβ	β-Me (*R*)				1/5	In	In
21	**6b**α	α-Et (*S*)	EtI	56	79:21	1/14	1/3	1/7
	6bβ	β-Et (*R*)				1/3	1/15	In
22	**6c**α	α-Bn (*S*)	BnBr	52	62:38	1/11	In	1/5
	6cβ	β-Bn (*R*)				In	In	1/5
23	**6d**α	α-Bz (*R*)	BzCl	30	82:18	1/8	In	In
	6dβ	β-Bz (*S*)				1/14	In	In
24	**6e**α	α-*t*Boc (*S*)	Boc_2O	10	98:2	1/22	In	1/25
25	**6f**	α-OH (*S*)	—[f]			1/4	1/7	1/2
26	**6g**	α-OAc (*S*)	—[g]			In	In	In

In = inactive (or IC_{50} not measurable).

[a] Abbreviations: MOM, methoxymethyl; MEM, 2-methoxyethoxymethyl; SEM, 2-(trimethylsilyl)ethoxymethyl; Piv, pivaloyl; Bz, benzoyl; Ms, methanesulfonyl; Ts, 4-toluenesulfonyl; for compounds **6a**–**e**, the absolute [(*R*) or (*S*)] configuration at C-16 is indicated.

[b] Isolated yields (for compounds **6a**–**e**, combined yields after separation of the diastereoisomers by HPLC).

[c] IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubule assembly or disassembly, average of three experiments; IC_{50} (**1**) = 7 μM for assembly and 3 μM for disassembly.

[d] IC_{50} is the concentration of compound corresponding to 50% growth inhibition after 72 h incubation, average of three experiments; IC_{50} (**1**) = 0.5 μM.

[e] Obtained from $(CF_3)_3COH$, $(Cl_3CO)_2C{=}O$, pyridine, CH_2Cl_2, 0 °C.

[f] Obtained from **7** by semi-synthesis.

[g] Obtained from **6f**.

tion as rhazinilam. In addition, large coupling constants observed for H-16 and H-17 indicate that H-16 is in the axial position in these molecules (see Fig. 3C). For instance in compounds **6d**α and **6e**α (which have a more simple coupling pattern for H-16 compared to other analogues), H-16 appears as a doublet with $J = 11$ Hz. The same observations apply to the minor diastereoisomers, which have similar ^1H and ^{13}C chemical shifts for B and D rings (Table 2, entries 9–12), whereas these signals differ markedly in each couple of diastereoisomers (see values under brackets). This suggests that all minor diastereoisomers have the β configuration at C-16, and that B and D-rings of these diastereoisomers adopt a different conformation than rhazinilam and the α diastereoisomers. In this case, one H-17 proton also shows large coupling constants pointing at an axial position of H-16 in the β diastereoisomers. By contrast, the coupling pattern of H-16 is more complex than in the α diastereoisomers. For instance, in benzoyl analogue **6d**β, in addition to the axial–axial and axial–equatorial couplings with H-17 ($J = 13$ and 5 Hz, respectively), H-16 shows a 1.7 Hz coupling constant corresponding to a W-coupling with the amide proton.

A more precise picture of the conformation of the β diastereoisomers was obtained by NOESY experiments performed on both diastereoisomers of the benzoyl analogue **6d** (Fig. 4). Whereas the NOE correlations for the major **6d**α diastereoisomer (Fig. 4A) were identical to those obtained with rhazinilam (Fig. 3A), the correlations observed for the minor **6d**β diastereoisomer are consistent with ring B adopting a boat–boat conformation, whereas C-14 on ring D now points up, opposite to ring B (Fig. 4B). In both diastereoisomers, the H-16 proton is axial and gives the large coupling constant with the axial H-17, whereas in **6d**β the H–N(1)–C(2)–C(16)–H bond system is planar and gives the W-coupling on the ^1H spectrum.

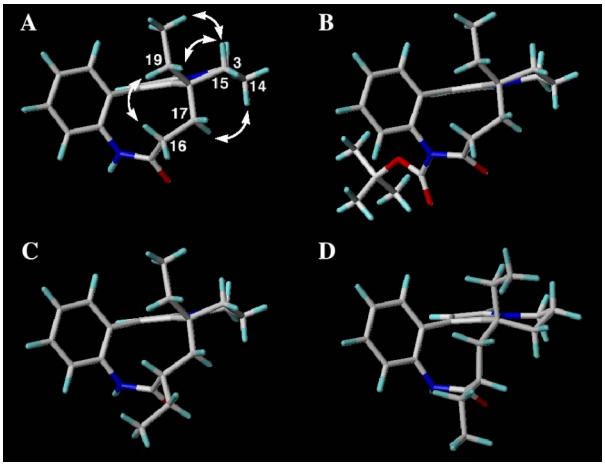


Figure 3. (A) X-ray crystal structure of (−)-rhazinilam **1** and selected NOESY correlations observed in $CDCl_3$ (white arrows). (B) X-ray crystal structure of N-*t*Boc-rhazinilam **5o**. (C) X-ray crystal structure of 16 α-ethylrhazinilam **6b**α. (D) Molecular modeling structure of 16 β-ethylrhazinilam **6b**β.

In order to study these conformational changes into more detail, molecular modeling experiments were conducted, first with rhazinilam. The steric energies of the two possible conformers of ring B [boat–chair (BC) and boat–boat (BB)] and ring D [C-14 down (D) or up (U)] were computed (MMFF94 force field). The four conformers could be classified as follows by decreasing stability: BC-D > BB-U > BC-U > BB-D. The most stable BC-D conformation is identical to that of rhazinilam in solution and in the crystal. It also corresponds to the conformation of the major α C-16 diastereoisomers. The BB-U conformation, which comes next in order of stability, corresponds to the minor β C-16 diastereoisomer. Complementary conclusions were drawn from modeling the two 16-ethyl diastereoisomers **6b**α and **6b**β: the lowest energy conformer of diastereoisomer **6b**α has the BC-D conformation and is superimposable to the X-ray structure of the molecule (Fig. 3C). The lowest energy conformer of diastereoisomer **6b**β has the BB-U conformation and is identical to the related structure of compound **6d**β deduced by NOESY experiments (Fig. 3D and Fig. 4B). The BC-D and the BB-U conformations adopted by the two diastereoisomers are those giving

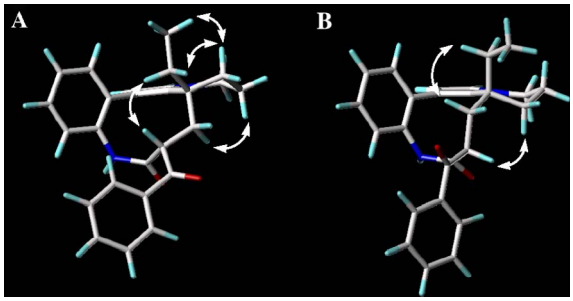


Figure 4. Selected NOESY correlations observed in $CDCl_3$ with (A) diastereoisomer **6d**α and (B) diastereoisomer **6d**β of 16-benzoylrhazinilam. The 3D structures were obtained from molecular modeling.

less unfavorable steric interactions between the C-16 substituent and the rest of the molecule. The stereochemical outcome of the substitution of rhazinilam at C-16 could be explained by the attack of the electrophilic reagent by the amide dianion formed from rhazinilam preferentially from the α position, as the β position is shielded both by the phenyl A-ring and the C-20 ethyl group. With this explanation larger electrophiles would tend to give a higher diastereoisomeric ratio, which is particularly obvious in the case of the *t*Boc group (Table 1, entry 24), for which almost only the α diastereoisomer was observed. However, an accurate relationship between the size of the substituent and the diastereoisomeric ratio is not clearly demonstrated by the present data (entries 20–24) and a number of other stereo-electronic effects could be operating (such as π-stacking interactions between aromatic electrophiles and rhazinilam A-ring). The major α diastereoisomer stays in the stable BC-D conformation of rhazinilam, with the C-16 substituent in the equatorial position pointing away from other residues (Fig. 3C). By contrast, the minor β diastereoisomer interconverts to the more stable BB-U conformation where the C-16 substituent is again equatorial and pointing away from other residues (Fig. 3D).

Finally, the configuration of the 16-hydroxy and 16-acetoxy analogues **6f** and **6g**, synthesized by a different route than the other C-16 analogues, was determined by similar NMR experiments. First, the chemical shifts

Table 2. Selected ^1H and ^{13}C chemical shifts for α and β C-16 analogues[a]

Entry	Compound	Δδ(H-3)	δ(C-3)	Δδ(H-14)	δ(C-14)	Δδ(H-15)	δ(C-15)	δ(C-16)	Δδ(H-17)	δ(C-17)	δ(C-19)
1	**1**	−0.23	46.1	0.39	19.4	0.15	33.1	28.1	1.00	36.6	30.1
2	**6a**α	−0.23	46.1	0.41	19.4	0.17	33.3	31.9	1.31	45.8	30.6
3	**6b**α	−0.22	46.1	0.40	19.4	0.17	33.5	39.2	1.26	44.7	30.6
4	**6c**α	−0.23	46.1	0.40	19.4	0.14	33.4	40.1	1.22	43.3	30.5
5	**6d**α	−0.20	46.1	0.46	19.4	0.09	33.6	47.1	0.91	40.7	30.4
6	**6e**α	−0.22	46.2	0.39	19.4	0.10	33.5	44.9	0.68	39.0	30.4
7	**6f**	−0.16	45.9	0.19	19.4	0.00	33.0	65.8	0.77	45.9	31.6
8	**6g**	−0.20	46.1	0.32	19.5	0.09	33.9	67.7	1.09	43.0	30.8
9	**6a**β	−0.08 (−0.15)	45.7 (0.4)	0.23 (0.18)	20.0 (−0.6)	0.38 (−0.21)	28.8 (4.5)	42.3 (−10.4)	0.28 (1.03)	41.7 (4.1)	36.0 (−5.4)
10	**6b**β	−0.09 (−0.13)	45.7 (0.4)	0.22 (0.18)	20.0 (−0.6)	0.51 (−0.34)	28.9 (4.6)	49.1 (−9.9)	0.21 (1.05)	40.4 (4.3)	36.1 (−5.5)
11	**6c**β	−0.09 (−0.14)	45.6 (0.5)	0.21 (0.19)	20.0 (−0.6)	0.53 (−0.39)	28.8 (4.6)	49.0 (−8.9)	0.26 (0.96)	39.4 (3.9)	36.1 (−5.6)
12	**6d**β	−0.08 (−0.12)	45.7 (0.4)	0.22 (0.24)	20.0 (−0.6)	0.50 (−0.41)	28.8 (4.8)	55.7 (−8.6)	0.66 (0.25)	36.2 (4.5)	36.2 (−5.8)

[a] $\Delta\delta = \delta$(axial proton) − δ(equatorial proton). Values under brackets refer to δ(α diastereoisomer) − δ(β diastereoisomer). All values in ppm. Attributions were made on the basis of usual 2D NMR experiments.

of B and D-rings atoms of **6f** and **6g** have close values to those of the **6a–e** α-diastereoisomers (Table 2, entries 7 and 8), although this effect is more pronounced with **6g** than with **6f**. In addition, NOESY correlations similar to those observed with rhazinilam (Fig. 3A) and 16 α-benzoylrhazinilam (Fig. 4A) were observed with compound **6f**. These experiments show that **6f** and **6g** have the α configuration at C-16 and also adopt the BC-D conformation.

The antitubulin activity and the cytotoxicity of analogues **5** and **6** were evaluated and compared to those of rhazinilam **1** (Table 1). All N-1 analogues (entries 1–19) were less active than rhazinilam on the inhibition of both microtubule assembly and disassembly. The most active analogue was the N-ethyl analogue **5a** (entry 1), which was twice less active than **1** on the inhibition of microtubule disassembly. However, there was a rather poor correlation between the tubulin-binding effect and the cytotoxicity. For instance, compound **5a** was inactive on KB cells. Conversely, several cytotoxic N-1 analogues and in particular carbamates **5o–q** (entries 15–17) had almost no effect on tubulin.[19] Flow cytometry experiments conducted with compound **5o** indicated that this compound is active during the G2/M phase of the cell cycle, like rhazinilam, however it was not possible to determine if tubulin was the actual intracellular target of this compound. Control experiments indicated that the deprotection of the *t*Boc group to release free rhazinilam did not occur in the cell culture medium. Available NMR and X-ray diffraction data for N-1 analogues (see Fig. 3B for **5o**) show that they adopt the same overall conformation as rhazinilam. In consequence, the decrease in the binding to tubulin observed for these compounds seems to reflect mostly steric repulsions between the N-1 substituent and the protein. The difference of polarity and lipophilicity of the substituent may explain the discrepancies observed among the different analogues. A second explanation could put forward the possibility that the H-1 proton of rhazinilam is engaged in an hydrogen-bond with tubulin, in what case the presence of any substituent at N-1 would lower the affinity.

C-16 analogues were in general less active than rhazinilam both on tubulin and KB cells (entries 20–26), but to a lesser extent than N-1 analogues. In this case, there was also a better correlation between the tubulin-binding effect and the cytotoxicity. Of the two α and β diastereoisomers, the α diastereoisomer was generally the most active, except in the case of benzyl analogue **6c** where both diastereoisomers had, in first approximation, the same level of activity. As we have demonstrated before that α diastereoisomers have the same conformation as rhazinilam, whereas that of β diastereoisomers differs significantly, these data confirm that the conservation of B-ring conformation is an essential feature for the binding to tubulin, in accordance with previous SAR studies. For both N-1 and C-16 analogues, the decrease in the affinity for tubulin seems to arise mainly from the bulkiness of the substituent. Indeed the most active compounds (**5a**, **6a**α, **6b**α, and **6f**) were the ones bearing the less bulky substituents. For instance, compound **6a**α was twice more active than rhazinilam on microtubule assembly. This influence of steric interactions was observed in all previous SAR studies and further indicates that the compounds bind to a narrow tubulin binding pocket.[2] In a number of cases, the analogues which had little or no activity on tubulin were significantly cytotoxic on KB cells, which could arise from a number of phenomena, including the possibility that tubulin is not the sole cellular target of these compounds.

3. Summary and conclusions

This paper describes the synthesis of 11- and 12-membered macrocyclic analogues of rhazinilam **3** and **4**, as well as nine-membered B-ring analogues **5** and **6** substituted in the N-1 or C-16 position. Stereochemical analyses showed that N-1 and C-16α analogues have the same conformation as rhazinilam, whereas C-16β analogues adopt a different conformation for rings B and D. All analogues were less active than rhazinilam on tubulin, though analogues **5a**, **6a**α, **6b**α, and **6f** having the less bulky substituents retained interesting affinities for the protein, comparable to those of the most active analogues of rhazinilam previously described. This poor tolerance to bulky substituents indicates that rhazinilam binds to a very narrow pocket of the protein. The correlation between the affinity for tubulin and the cytotoxicity toward KB cancer cells was not clear for the N-1 analogues (e.g., **5a** and **5o**), whereas it was better for C-16 analogues. The importance of the conservation of the rigid boat–chair conformation of rhazinilam B-ring, with a *cis* N(1)–C(2) amide conformation, was clearly established by this study. In particular, macrocycles **3** and **4** having a flexible B-ring with a *trans* N(1)–C(22) amide group have almost no affinity for tubulin. In consequence, future design of other analogues of rhazinilam should conserve the space occupation and structural integrity of the natural product B-ring. The replacement of C-ring by other aromatic groups, which was initiated by the synthesis of biphenyl and phenyl–pyridine analogues,[7,9,10] could be a privileged option. A metabolization study of rhazinilam by cytochromes P450, mimicking its in vivo transformation and suggesting other research directions, will be reported in due course.

4. Experimental

Reagents were commercially available and used without further purification unless otherwise stated. THF was distilled from sodium/benzophenone and methylene chloride from di-phosphorus pentoxide (P_2O_5) immediately before use. Yields refer to chromatographically and spectroscopically homogeneous materials. Reactions were monitored by thin-layer chromatography carried out on 0.25 mm SDS silica gel coated glass plates (60F254) using UV light as visualizing agent and ethanolic sulfuric molybdate or cerium ammonium sulfate and heat as staining agents. Merck silica gel 60

(particle size 40–63 μM) or Econosil Prep C18 HL (particle size 40–63 μM) was used for flash chromatography. 1 and 2 mm SDS silica gel-coated glass plates (60F254) were used for preparative TLC using UV light as visualizing agent. Microwave heating was performed with a CEM Discover focused reactor in adapted sealed tubes. NMR spectra were recorded on Bruker AMX-300, AMX-400 or AMX-500 instruments at 295 K and calibrated using tetramethylsilane as an internal reference. Attributions were made on the basis of 2D experiments. The following abbreviations were used to designate the multiplicities: s, singlet; d, doublet; t, triplet, q, quartet; m, multiplet; br, broad; ar, aromatic ring; eq, equatorial; ax, axial; and Cq, quaternary carbon. Atom numbering refers to rhazinilam (Scheme 1). IR spectra were recorded on a Perkin Elmer BX FT-IR spectrometer. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded under Electrospray Ionization (ESI) or Matrix-Assisted Laser Desorption Ionization-Time-of-Flight (MALDI-TOF) conditions at the Laboratoire de Spectrométrie de Masse (ICSN). Melting points (mp) are uncorrected and were recorded on a Büchi B-540 capillary melting point apparatus. Optical rotations were recorded on a Jasco P-1010 polarimeter. HPLC analyses and purifications were performed on Waters systems equipped with a photodiode array detector (monitoring at 200–400 nm), using a Hypercarb column (10 cm × 0.46 cm for analytic, 15 cm × 1 cm for preparative), by Thermo Electron, Inc. or a C18 Symmetry column (25 cm × 0.46 cm for analytic, 25 cm × 1.0 cm for semi-preparative), by Waters, Inc. Molecular modeling studies were conducted using Sybyl 6.9.1 software (linux). Conformational searches were performed with the Monte-Carlo random search procedure using MMFF94 force field parameters.

4.1. (−)-Rhazinilam (1)[5,20,21]

Beige crystals; mp 217 °C (lit. 214–216 °C)[21]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (dd, J = 6.6, 2.4 Hz, 1H, H-9), 7.34 (ddd, J = 7.5, 7.5, 2.4 Hz, 1H, H-11), 7.29 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H-10), 7.19 (dd, J = 7.5, 1.8 Hz, 1H, H-12), 6.63 (br s, 1H, H-1), 6.50 (d, J = 2.7 Hz, 1H, H-5), 5.75 (d, J = 3.0 Hz, 1H, H-6), 4.00 (dd, J = 12.0, 4.8 Hz, 1H, H-3eq), 3.77 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 2.46 (dd, J = 12.3, 12.3 Hz, 1H, H-17ax), 2.37 (dd, J = 12.6, 12.6 Hz, 1H, H-16ax), 2.26 (ddddd, J = 12.3, 12.3, 12.3, 5.7, 2.7 Hz, 1H, H-14ax), 1.96 (dd, J = 12.6, 7.8 Hz, 1H, H-16eq), 1.87 (m, 1H, H-14eq), 1.71 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.56 (m, 1H, H-15eq), 1.44 (m, 1H, H-17eq), 1.40 (m, 1H, H-19a), 1.26 (m, 1H, H-19b), 0.71 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.3 (C-2), 140.4 (C-8), 138.1 (C-13), 131.4 (C-9), 130.6 (C-21), 128.0 (C-11), 127.2 (C-10), 126.8 (C-12), 119.1 (C-5), 117.3 (C-7), 109.6 (C-6), 46.1 (C-3), 38.9 (C-20), 36.6 (C-17), 33.1 (C-15), 30.1 (C-19), 28.1 (C-16), 19.4 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{24}$ −432 (c 1.0, $CHCl_3$) (lit. −421, c 1.0, $CHCl_3$)[21]; IR (film) $\tilde{\nu}$ 3225, 1672 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{22}N_2NaO$ [(M+Na)$^+$]: 317.1630, found: 317.1644.

4.2. Amino-acid (8)

In a sealed tube, (−)-rhazinilam **1** (426 mg, 1.45 mmol) was dissolved in water/ethanol 1:1 (3 mL). Powdered KOH (3.44 g, 61.3 mmol) was added, the tube was sealed, and the reaction mixture was heated under microwave irradiation at 120 °C for 30 min. After cooling, the reaction mixture was transferred into an Erlenmeyer, cooled to 0 °C, and treated with a 1 N HCl aqueous solution until pH 4–5. The aqueous solution was extracted with dichloromethane and the organic layer was dried over $MgSO_4$. After evaporation, the crude mixture was purified by flash chromatography (dichloromethane/ethyl acetate 4:1) to give compound **8** (384 mg, 85%) as a white powder; mp 74 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 2 conformers in a 87/13 ratio, *major conformer*: δ 7.20 (m, 2H, H_{ar}), 6.92 (m, 2H, H_{ar}), 6.61 (d, J = 2.7 Hz, 1H, H-5), 5.99 (d, J = 2.7 Hz, 1H, H-6), 3.90 (dd, J = 5.4, 5.4 Hz, 2H, H-3), 2.36 (m, 1H, H-15a), 2.04 (m, 1H, H-15b), 1.97 (m, 1H, H-14a), 1.77 (m, 2H, H-14b and H-16a), 1.73 (m, 1H, H-19a), 1.63 (m, 1H, H-17a), 1.60 (m, 1H, H-16b), 1.57 (m, 1H, H-17b), 1.48 (m, 1H, H-19b), 0.80 (t, 3H, J = 7.2 Hz, 3H, H-18) ppm; *minor conformer* (characteristic signals): δ 7.42 (dd, J = 7.5, 2.1 Hz, 1H, H_{ar}), 7.33 (ddd, J = 7.5, 7.5, 2.1 Hz, 1H, H_{ar}), 7.09 (m, 1H, H_{ar}), 6.69 (m, 1H, H_{ar}), 6.51 (d, J = 2.7 Hz, 1H, H-5), 5.75 (d, J = 2.4 Hz, 1H, H-6), 0.71 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) *major conformer*: δ 175.9 (C-2), 142.7 (C-13), 132.0 (CH_{ar}), 130.8 (C-21), 128.3 (CH_{ar}), 126.6 (C-8), 120.4 (CH_{ar}), 119.3 (C-5), 116.8 (CH_{ar}), 115.2 (C-7), 109.3 (C-6), 46.2 (C-3), 39.6 (C-20), 36.9 (C-19), 35.6 (C-15), 33.2 (C-17), 30.1 (C-16), 21.8 (C-14), 9.1 (C-18) ppm, *minor conformer* (characteristic signals): δ 179.0, 145.4, 131.5, 127.9, 118.9, 117.7, 115.5, 114.7, 110.2, 46.1, 39.2, 30.3, 29.8, 21.3, 9.3 ppm; $[\alpha]_D^{24}$ −9 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3470, 3370, 2960, 2873, 1730 cm^{-1}; HRMS (MALDI-TOF) calcd for $C_{19}H_{25}N_2O_2$ [(M+H)$^+$]: 313.1916, found: 313.1909.

4.3. Amino-alcohol (9)

To a suspension of $LiAlH_4$ (2.06 g, 54.3 mmol) in THF (20 mL) at 0 °C under argon was added dropwise a solution of compound **8** (848 mg, 2.71 mmol) in THF (10 mL). After stirring for 5 min at 0 °C, the mixture was refluxed for 22 h. After cooling to 0 °C, the mixture was treated sequentially with water (2 mL), a 15% NaOH aqueous solution (2 mL), water (6 mL), and filtered through Celite. The solution was dried over $MgSO_4$ and evaporated under vacuum. The residue was purified by flash chromatography (heptane/ethyl acetate 4:1), to give compound **9** as an oil (562 mg, 69%); ^{1}H NMR (300 MHz, $CDCl_3$) 2 conformers in a 4/1 ratio, *major conformer*: δ 7.14 (m, 2H, H_{ar}), 6.76 (m, 2H, H_{ar}), 6.58 (d, J = 1.2 Hz, 1H, H-5), 5.99 (d, J = 1.2 Hz, 1H, H-6), 3.91 (dd, J = 5.5, 5.5 Hz, 2H, H-3), 3.61 (m, 3H, H-1 and H-2a), 3.30 (m, 1H, H-2b), 1.92 (m, 1H, H-14a), 1.80 (m, 1H, H-19a), 1.73 (m, 1H, H-14b), 1.71 (m, 2H, H-15), 1.56 (m, 1H, H-17a), 1.54 (m, 1H, H-16a), 1.49 (m, 1H, H-19b), 1.28 (m, 1H, H-16b), 1.23 (m, 1H, H-17b), 0.84 (t, 3H,

J = 6.9 Hz, 3H, H-18) ppm, *minor conformer* (characteristic signal): δ 0.74 (t, J = 6.9 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) *major conformer*: δ 144.9 ($(Cq)_{ar}$), 132.4 (C-21), 132.2 (CH_{ar}), 128.4 (CH_{ar}), 126.0 ($(Cq)_{ar}$), 119.1 and 119.0 (CH_{ar} and C-5), 115.9 (CH_{ar}), 115.8 (C-7), 109.7 (C-6), 63.3 (C-2), 46.6 (C-3), 39.9 (C-20), 37.8 (C-17), 36.2 (C-19), 31.0 (C-15), 29.0 (C-16), 22.3 (C-14), 9.6 (C-18) ppm, *minor conformer* (characteristic signals): δ 146.0, 132.0, 128.2, 125.1, 117.6, 115.0, 110.4, 63.7, 46.6, 39.7, 37.7, 33.9, 30.4, 28.9, 21.9, 9.8 ppm; $[\alpha]_D^{23}$ −7 (c 0.70, $CHCl_3$); IR (film) $\tilde{\nu}$ 3436, 2962 cm^{-1}; HRMS (MALDI-TOF) calcd for $C_{19}H_{27}N_2O$ [$(M+H)^+$]: 299.2123, found: 299.2122.

4.4. Macrocycle (3)

To a solution of compound **9** (42 mg, 0.14 mmol) in THF (9.5 mL) at 20 °C under argon was added dropwise NaHMDS (2 M in THF, 141 μL, 0.14 mmol). After cooling to 0 °C, a solution of triphosgene (42 mg, 0.14 mmol) in THF (9.5 mL) was added dropwise and the mixture was stirred at 0 °C for 10 min. A saturated aqueous solution of $NaHCO_3$ was added and the aqueous layer was extracted with dichloromethane. After drying over Na_2SO_4, the solvents were removed under vacuum and the residue was purified by preparative TLC (heptane/ethyl acetate 2:3) to give compound **3** as a beige powder (9.5 mg, 21%); mp 190 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 1H, H_{ar}), 7.26 (m, 3H, H_{ar}), 6.54 (d, J = 2.7 Hz, 1H, H-5), 6.03 (br s, 1H, H-1), 5.91 (d, J = 2.7 Hz, 1H, H-6), 4.17 (m, 1H, H-2a), 3.89 (t, J = 5.6 Hz, 1H, H-3), 3.44 (td, J = 10.5, 2.1 Hz 1H, H-2b), 2.00–1.90 (m, 2H, H-14eq and H-19a), 1.83 (m, 1H, H-14ax), 1.75 (m, 1H, H-15ax), 1.67 (m, 1H, H-19b), 1.62 (m, 1H, H-15eq), 1.52 (m, 1H, H-16a), 1.46 (m, 1H, H-17a), 1.34 (m, 1H, H-17b), 1.28 (m, 1H, H-16b), 0.89 (t, J = 7.3 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 156.5 (C=O), 138.6 ($(Cq)_{ar}$), 136.2 ($(Cq)_{ar}$), 132.3 (C-12), 130.6 (C-21), 128.8 (CH_{ar}), 127.3 (CH_{ar}), 126.8 (CH_{ar}), 118.9 (C-5), 116.4 (C-7), 110.4 (C-6), 64.2 (C-2), 46.4 (C-3), 40.0 (C-20), 37.3 (C-19), 35.4 (C-17), 30.7 (C-15), 25.2 (C-16), 21.5 (C-14), 9.5 (C-18) ppm; $[\alpha]_D^{24}$ −90 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3265, 2957, 1721 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{24}N_2NaO_2$ [$(M+Na)^+$]: 347.1735, found: 347.1723.

4.5. Compound (11a)

To a vigorously stirred solution of compound **8** (564 mg, 1.81 mmol) in dichloromethane (12 mL) and 10% aqueous $NaHCO_3$ (12 mL) at 20 °C was added dropwise a solution of Fmoc-glycine acid chloride **10a**[12] (569 mg, 1.81 mmol) in dichloromethane (14 mL). After stirring for 1.5 h, the mixture was diluted with water and dichloromethane, and the two layers were decanted. The aqueous layer was acidified to pH 5 by addition of a 1 N HCl aqueous solution and extracted with dichloromethane. The organic layers were gathered, dried over $MgSO_4$, and evaporated under vacuum. The residue was purified by preparative TLC (dichloromethane/ethyl acetate 7:3), to give compound **11a** as a pale yellow powder (490 mg, 46%); mp 146 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 2 conformers in a 3/1 ratio, *major conformer*: δ 8.47 (d, 1H, J = 7.5 Hz, H_{ar}), 8.11 (br s, 1H, H-1), 7.78 (m, 2H, H_{ar}), 7.61 (m, 1H, H_{ar}), 7.39–7.20 (m, 7H, H_{ar}), 7.07 (m, 1H, H_{ar}), 6.44 (m, 1H, H-5), 6.28 (br s, 1H, N*H*Fmoc), 5.96 (m, 1H, H-6), 4.42 (m, 1H, C(=O)OCH_2), 4.23 (m, 1H, C(=O)OCH$_2$C*H*), 4.17 (m, 1H, C(=O)OCH_2), 4.03 (m, 1H, C(=O)CH_2NH), 3.89 (m, 1H, C(=O)CH_2NH), 3.81 (m, 1H, H-3a), 3.63 (m, 1H, H-3b), 2.25 (m, 2H), 2.05–1.50 (m, 7H), 1.42 (m, 1H, H-19), 0.80 (t, J = 7.5 Hz, 3H, H-18) ppm, *minor conformer* (characteristic signals): δ 8.38 (d, J = 7.5 Hz, 1H, H_{ar}), 5.91 (m, 1H, H-6), 0.65 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) *major conformer*: δ 177.7 (C=O), 168.1 (C=O), 156.7 (*C*(=O)OCH$_2$), 143.8 ($(Cq)_{ar}$), 144.0 ($(Cq)_{ar}$), 141.4 (2$(Cq)_{ar}$), 136.2 ($(Cq)_{ar}$), 131.9 (CH_{ar}), 131.4 (CH_{ar}), 128.2 (CH_{ar}), 127.9 (CH_{ar}), 127.2 (CH_{ar}), 125.3 (CH_{ar}), 123.4 (CH_{ar}), 120.1 (CH_{ar}), 119.7 (CH_{ar}), 113.8 ($(Cq)_{ar}$), 110.0 (C-6), 67.5 (C(=O)O*C*H$_2$), 47.2 (C(=O)OCH$_2$*C*H), 46.2 (C-3), 45.6 (C(=O)*C*H$_2$NH), 39.4 (C-20), 35.4 (*C*H$_2$), 34.0 (*C*H$_2$), 30.1 (*C*H$_2$), 29.5 (*C*H$_2$), 21.1 (*C*H$_2$), 9.4 (C-18) ppm, *minor conformer* (characteristic signals): δ 179.0 (C=O), 166.4 (C=O), 156.4 (C=O), 109.8 (C-6), 68.5 (C(=O)O*C*H$_2$), 46.5 (C(=O)OCH$_2$*C*H), 46.0 (*C*H$_2$), 45.3 (*C*H$_2$), 36.1 (*C*H$_2$), 33.4 (*C*H$_2$), 30.4 (*C*H$_2$), 30.2 (*C*H$_2$), 21.4 (*C*H$_2$) ppm; $[\alpha]_D^{24}$ −27 (c 0.43, $CHCl_3$); IR (film) $\tilde{\nu}$ 3349, 2963, 1719 cm^{-1}; HRMS (ESI) calcd for $C_{37}H_{39}N_3NaO_5$ [$(M+Na)^+$]: 614.2631, found: 614.2638.

4.6. Compound (11b)

Compound **11b** was synthesized in the same manner as for compound **11a** from compound **8** (404 mg, 1.29 mmol) and Fmoc-L-valine acid chloride **10b**[12] (510 mg, 1.29 mmol). The crude mixture was purified by preparative TLC (dichloromethane/ethyl acetate 7:3), to give compound **11b** as a white powder (545 mg, 66%); mp 78 °C; ^{1}H NMR (300 MHz, acetone-d_6) 2 conformers in a 1/1 ratio: δ 8.55 (m, 2H, H_{ar} and H'_{ar}), 8.36 (m, 2H, H_{ar} and H'_{ar}), 7.96 (d, J = 7.2 Hz, 2H, H_{ar} and H'_{ar}), 7.83 (d, J = 7.2Hz, 2H, H_{ar} and H'_{ar}), 7.50 (m, 2H, H_{ar} and H'_{ar}), 7.44 (m, 2H, H_{ar} and H_{ar}), 7.32 (m, 2H, H_{ar} and H'_{ar}), 7.14 (m, 2H, H_{ar} and H'_{ar}), 6.73 (m, 2H, H-5 and H-5′), 5.96 (br s, 2H, N*H*Fmoc and N*H*Fmoc′), 5.96 (d, J = 2.1 Hz, 2H, H-6 and H-6′), 4.65 (m, 4H, C(=O)OCH_2 and C(=O)OCH'_2), 4.32 (m, 1H, C*H*i*P*r), 4.22 (m, 1H, C*H*i*P*r′), 3.91 (m, 4H, H-3 and H-3′), 2.46 (m, 2H, C*H*(CH$_3$)$_2$ and C*H*(CH$_3$)$'_2$), 2.38 (m, 4H), 2.27 (m, 4H), 2.15 (m, 2H, C(=O)OCH$_2$C*H* and C(=O)OCH$_2$C*H*′), 1.93 (m, 4H), 1.78 (m, 1H, H-19a), 1.70 (m, 4H), 1.60 (m, 1H, H-19a′), 1.55 (m, H-19b), 1.31 (m, 1H, H-19b′), 1.04 (m, 12H, CH(CH_3)$_2$ and CH(CH_3)$'_2$), 0.90 (t, J = 6.9 Hz, 3H, H-18), 0.76 (t, J = 6.9 Hz, 3H, H-18′) ppm; ^{13}C NMR (75.5 MHz, acetone-d_6) δ 175.3 (C=O), 174.8 (C=O), 169.4 (C=O), 169.3 (C=O), 157.0 (2C=O) ; 144.5 ($(Cq)_{ar}$), 141.7 ($(Cq)_{ar}$), 137.6 ($(Cq)_{ar}$), 137.4 ($(Cq)_{ar}$), 132.0 ($(Cq)_{ar}$), 131.8 ($(Cq)_{ar}$), 131.5 (CH_{ar}), 129.2 ($(Cq)_{ar}$), 128.9 ($(Cq)_{ar}$), 128.4 (CH_{ar}), 127.9 (CH_{ar}), 127.7 (CH_{ar}), 126.0 (CH_{ar}), 125.7 (CH_{ar}), 123.2 (CH_{ar}), 123.0 (CH_{ar}), 120.9 (CH_{ar}), 120.3 (CH_{ar}), 119.6 (CH_{ar}), 114.2 ($(Cq)_{ar}$),

110.1 and 109.9 (C-6 and C-6′), 67.1 (C(=O)OCH_2 and C(=O)OCH_2'), 61.8 (CH*i*Pr and CH*i*Pr′), 46.5 and 46.4 (C-3 and C-3′), 39.8 (C(=O)OCH_2CH and C(=O)OCH_2CH'), 39.6 (CH_2), 36.6 (CH_2), 36.0 (CH_2), 34.2 (CH_2), 33.3 (CH_2), 30.8 (CH_2), 30.6 ($CH(CH_3)_2$ and $CH(CH_3)_2'$), 30.3 (CH_2), 30.2 (CH_2), 29.0 (C-20 and C-20′), 21.5 (CH_2), 21.3 (CH_2), 17.5 and 17.3 ($CH(CH_3)_2$ and $CH(CH_3)_2'$), 9.33 and 8.96 (C-18 and C-18′) ppm; HRMS (ESI) calcd for $C_{39}H_{43}N_3NaO_5$ [$(M+Na)^+$]: 656.3100, found: 656.3137.

4.7. Compound (12a)

To a solution of compound **11a** (412 mg, 0.70 mmol) in THF (23 mL) at 20 °C was added piperidine (2.5 mL). The mixture was stirred for 2.5 h and evaporated under vacuum. The residue was purified by flash chromatography (C18, ethanol/water 9:1), to give compound **12a** as a white powder (227 mg, 88%); mp 178 °C; ^{1}H NMR (300 MHz, $CDCl_3$) 2 conformers in a 82/18 ratio, *major conformer*: δ 9.27 (m, 1H, H-1), 8.45 (d, J = 7.5 Hz, 1H, H_{ar}), 7.27 (m, 2H, H_{ar}), 7.03 (m, 1H, H_{ar}), 6.59 (m, 1H, H-5), 6.09 (m, 1H, H-6), 4.06 (m, 2H, H-3), 3.35 (m, 2H, C(=O)CH_2NH_2), 2.93 (br s, 2H, NH_2), 2.15 (m, 2H), 1.93–1.59 (m, 6H), 1.41 (m, 1H, H-19a), 0.81 (m, 1H, H-19b), 0.81 (t, J = 6.3 Hz, 3H, H-18) ppm, *minor conformer* (characteristic signals): δ 9.17 (m, H-1), 0.69 (t, J = 6.6 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) *major conformer*: δ 179.9 (C-2), 171.3 (NHC(=O)), 137.1 (CH_{ar}), 132.1 ($(Cq)_{ar}$), 131.7 (CH_{ar}), 128.9 ($(Cq)_{ar}$), 127.7 (CH_{ar}), 126.8 (CH_{ar}), 119.7 (C-5), 114.3 ($(Cq)_{ar}$), 110.1 (C-6), 46.4 (C-3 and C(=O)CH_2NH_2), 39.6 (C-20), 38.1 (CH_2), 30.1 (CH_2), 26.4 (CH_2), 22.9 (CH_2), 21.8 (CH_2), 9.55 (C-18) ppm, $[\alpha]_D^{24}$ −23 (*c* 0.40, $CHCl_3$); IR (film) $\tilde{\nu}$ 3361, 2962, 2360, 1706 cm^{-1}; HRMS (MALDI-TOF) calcd for $C_{21}H_{27}N_3NaO_5$ [$(M+Na)^+$]: 392.1950, found: 392.1947.

4.8. Compound (12b)

Compound **12b** was synthesized in the same manner as for compound **12a** from compound **11b** (491 mg, 0.77 mmol). Yellow powder (294 mg, 92%); mp 100 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 2 conformers in a 4/1 ratio, *major conformer*: δ 8.89 (d, J = 8.1 Hz, 1H, H_{ar}), 7.70 (dd, J = 8.1, 8.1 Hz, 1H, H_{ar}), 7.64 (m, 1H, H_{ar}), 7.41 (m, 1H, H_{ar}), 7.22 (d, J = 2.1 Hz, 1H, H-5), 6.32 (d, J = 2.1 Hz, 1H, H-6), 4.36 (m, 2H, H-3), 4.17 (d, J = 3.3 Hz, 1H, CH*i*Pr), 3.44 (m, 2H), 2.64 (m, 1H), 2.44 (m, 1H, $CH(CH_3)_2$), 2.19–1.94 (m, 7H), 1.68 (m, 1H, H-19a), 1.53 (m, 1H, H-19b), 1.34 (d, J = 6.6 Hz, 3H, $CH(CH_3)_2$), 1.24 (d, J = 6.6 Hz, 3H, $CH(CH_3)_2$), 0.94 (t, J = 7.2 Hz, 3H, H-18) ppm, *minor conformer* (characteristic signals): δ 7.13 (d, J = 2.1 Hz, 1H, H-5), 6.26 (d, J = 2.1 Hz, 1H, H-6) ppm; ^{13}C NMR (75.5 MHz, DMSO-d_6) *major conformer* δ 174.3 (C-2), 169.3 (NHC(=O)), 136.5 ($(Cq)_{ar}$), 130.4 (CH_{ar}), 130.0 ($(Cq)_{ar}$), 127.7 ($(Cq)_{ar}$), 126.8 (CH_{ar}), 121.5 (CH_{ar}), 118.0 (C-5), 117.8 (CH_{ar}), 113.0 ($(Cq)_{ar}$), 108.8 (C-6), 68.1 (CH*i*Pr), 45.1 (C-3), 36.3 (CH_2), 32.6 (CH_2), 32.3 (CH_2), 29.8 (CH_2), 29.1 (CH_2), 29 (C-20), 20.5 (CH_2), 18.3 ($CH(CH_3)_2$), 17.0 ($CH(CH_3)_2$), 7.89 (C-18) ppm; *minor conformer* (characteristic signals): δ 8.0 (C-18) ppm; IR (film) $\tilde{\nu}$ 3363, 2964, 2360, 1710 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{34}N_3O_3$ [$(M+Na)^+$]: 412.2600, found: 412.2605.

4.9. Macrocycle (4a)

To a solution of compound **12a** (31 mg, 0.084 mmol) in dry DMF (83 mL) under argon at 20 °C were added TBTU (80 mg, 0.25 mmol), HOBT (34 mg, 0.25 mmol), and di *iso*propylethylamine (58 μL, 0.34 mmol). After stirring for 24 h, water was added and the aqueous layer was extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude mixture was purified by flash chromatography (C18, ethanol/water 60:40, then 65:35) followed by HPLC (semi-preparative C18 Symmetry, H_2O/CH_3CN 3:2, 4.7 mL min^{-1}) to give compound **4a** as a white film (9 mg, 30%); HPLC analysis (analytic C18 Symmetry, H_2O/CH_3CN 3:2, 1 mL min^{-1}) t_R 11.1 min; ^{1}H NMR (500 MHz, acetone-d_6) 2 conformers in a 1/1 ratio, *conformer* **4ax**: δ 8.41 (d, J = 8.0 Hz, 1H, H-12), 8.34 (dd, J = 7.0, 7.0 Hz, 1H, CH_2NHC(=O)), 8.22 (s, 1H, H-1), 7.26 (dd, J = 7.5, 7.5 Hz, 1H, H-11), 7.10 (m, 1H, H-9), 6.99 (dd, J = 7.5, 7.5 Hz, 1H, H-10), 6.77 (d, J = 2.5 Hz, 1H, H-5), 5.75 (d, J = 2.5 Hz, 1H, H-6), 4.09 (m, 1H, H-3a), 3.89 (m, 1H, H-3b), 3.86 (dd, J = 16.0, 7.0 Hz, 1H, C(=O)CH_2NH), 3.56 (dd, J = 16.0, 5.8 Hz, 1H, C(=O)CH_2NH), 2.42 (m, 1H, H-16a), 2.04 (m, 1H, H-16b), 1.96 (m, 1H, H-14a), 1.89 (m, 1H, H-17a), 1.83 (m, 1H, H-15a), 1.80 (m, 1H, H-14b), 1.69 (m, 1H, H-19a), 1.60 (m, 1H, H-15b), 1.27 (m, 1H, H-19b), 0.80 (t, J = 7.5 Hz, 3H, H-18) ppm, *conformer* **4ay**: δ 8.57 (s, 1H, H-1), 8.34 (dd, J = 7.0, 7.0 Hz, 1H, CH_2NHC(=O)), 7.92 (m, 1H, H-12), 7.30 (dd, J = 7.5, 7.5 Hz, 1H, H-11), 7.11 (m, 1H, H-10), 7.10 (m, 1H, H-9), 6.72 (d, J = 2.3 Hz, 1H, H-5), 5.97 (d, J = 2.3 Hz, 1H, H-6), 4.09 (m, 1H, H-3a), 3.89 (m, 1H, H-3b), 3.86 (dd, J = 16.0, 7.0 Hz, 1H, C(=O)CH_2NH), 3.56 (dd, J = 16.0, 5.8 Hz, 1H, C(=O)CH_2NH), 2.42 (m, 1H, H-16a), 2.04 (m, 1H, H-16b), 1.96 (m, 1H, H-14a), 1.89 (m, 1H, H-17a), 1.83 (m, 1H, H-15a), 1.80 (m, 1H, H-14b), 1.60 (m, 1H, H-15b), 1.36 (m, 1H, H-17b), 1.25 (m, 1H, H-19a), 1.05 (m, 1H, H-19b), 0.60 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (125.8 MHz, acetone-d_6) *conformer* **4ax**: δ 177.3 (C-2), 169.5 (C(=O)CH_2), 138.1 (C-13), 132.0 (C-9), 130.8 (C-21), 128.4 (C-11), 128.2 (C-8), 122.6 (C-10), 120.8 (C-5), 116.7 (C-12), 113.5 (C-7), 111.3 (C-6), 48.0 (C(=O)CH_2NH), 46.5 (C-3), 41.2 (C-20), 39.9 (C-17), 36.9 (C-19), 32.0 (C-15), 29.8 (C-16), 23.2 (C-14), 9.9 (C-18) ppm, *conformer* **4ay**: δ 177.3 (C-2), 169.5 (C(=O)CH_2), 136.9 (C-13), 131.8 (C-9), 129.1 (C-21), 128.2 (C-8), 128.0 (C-11), 124.3 (C-10), 119.7 (C-5), 116.7 (C-12), 114.9 (C-7), 111.3 (C-6), 48.0 (C(=O)CH_2NH), 46.5 (C-3), 39.9 (C-17), 39.8 (C-20), 35.9 (C-19), 32.0 (C-15), 29.8 (C-16), 23.2 (C-14), 9.4 (C-18) ppm; $[\alpha]_D^{23}$ −10 (*c* 0.58, $CHCl_3$); IR (film) $\tilde{\nu}$ 3353, 2963, 1682 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{25}N_3NaO_2$ [$(M+Na)^+$]: 374.1844, found: 374.1836.

4.10. Macrocycle (4b)

Compound **4b** was synthesized in the same manner as for compound **4a** from compound **12b** (61 mg, 0.15 mmol). White film (6 mg, 10%); ^{1}H NMR (500 MHz, acetone-d_6) 2 conformers in a 85/15 ratio, *major conformer*: δ 8.14 (dd, J = 8.2, 1.3 Hz, 2H, H-12 and H-1), 7.30 (ddd, J = 7.7, 7.7, 1.6 Hz, 1H, H_{ar}), 7.17 (dd, J = 7.7, 1.4 Hz, 1H, H-9), 7.09 (ddd, J = 7.5, 7.5, 1.4 Hz, 1H, H_{ar}), 6.70 (d, J = 2.7 Hz, 1H, H-5), 6.47 (d, J = 8.3 Hz, 1H, CHN*H*C(=O)), 5.86 (d, J = 2.7 Hz, 1H, H-6), 3.93 (dd, J = 5.5, 5.5 Hz, 2H, H-3), 3.82 (dd, J = 9.8, 8.0 Hz, 1H, C*H*iPr), 2.48 (m, 1H, H-16a), 2.16 (m, 1H, H-16b), 2.00 (m, 1H, H-14a), 1.87 (m, 1H, H-14b), 1.79 (m, 2H, H-15), 1.58 (m, 1H, C*H*$(CH_3)_2$), 1.52 (m, 2H, H-17), 1.34 (m, 1H, H-19a), 1.13 (m, 1H, H-19b), 1.01 (d, J = 7.4 Hz, 3H, CH(CH_3)$_2$), 0.95 (d, J = 7.4 Hz, 3H, CH(CH_3)$_2$), 0.65 (t, J = 7.4 Hz, 3H, H-18) ppm, *minor conformer* (characteristic signals): δ 7.47 (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, H-12), 7.24 (ddd, J = 7.5, 7.5, 2.0 Hz, 1H, H_{ar}), 6.96 (dd, J = 7.5, 7.5 Hz, 1H, H_{ar}), 6.66 (d, J = 2.5 Hz, 1H, H-5), 5.67 (d, J = 2.5 Hz, 1H, H-6), 0.79 (t, J = 7.3 Hz, 3H, H-18) ppm; ^{13}C NMR (125.8 MHz, acetone-d_6) *major conformer* (characteristic signals): δ 132.0 (CH_{ar}), 128.1 (CH_{ar}), 124.0 (CH_{ar}), 122.1 (CH_{ar}), 120.2 (C-5), 114.4 (C-6), 64.6 (*C*H*i*Pr), 46.4 (C-3), 41.5 (C-17), 36.3 (C-19), 32.1 (C-15), 30.7 (C-16), 30.3 (*C*H$(CH_3)_2$), 23.9 (C-14), 19.3 (CH(CH$_3$)$_2$), 9.3 (C-18) ppm; HRMS (ESI) calcd for $C_{24}H_{31}N_3NaO_2$ [(M+Na)$^+$]: 416.2314, found: 416.2300.

4.11. General procedure for the N-1 and C-16 substitution of (−)-rhazinilam (1)

To a solution of (−)-rhazinilam **1** (100 mg, 0.34 mmol) in THF (2.5 mL) at 0 °C under argon was added dropwise *n*-butyllithium (1.6 M in hexane, 212 μL, 0.34 mmol for N-1 substitutions or 424 μL, 0.68 mmol for C-16 substitutions). After stirring the mixture for 30 min at 0 °C, the electrophilic reagent (see Table 1, 0.34 mmol) was added dropwise and the mixture was allowed to warm to 20 °C. The evolution of the reaction was monitored by TLC. When no more evolution was observed, the reaction was quenched with a saturated aq solution of NH_4Cl and the aqueous layer was extracted with dichloromethane. After drying over $MgSO_4$, the solution was evaporated under reduced pressure and the residue was purified by flash or thin layer chromatography. For compounds **6a**–**d**, the diastereoisomeric mixture obtained after purification on silica gel was separated by preparative HPLC.

4.12. 1-Ethylrhazinilam (5a)

White powder (65 mg, 66% from 89 mg rhazinilam); mp 169 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (dd, J = 7.3Hz, J = 1.1Hz, 1H, H_{ar}), 7.34 (m, 2H, H_{ar}), 7.22 (dd, J = 8.5Hz, J = 1.5Hz, 1H, H_{ar}), 6.45 (d, J = 2.8Hz, 1H, H-5), 5.81 (d, J = 3.2 Hz, 1H, H-6), 4.02 (dd, J = 12.0, 5.2Hz, 1H, H-3eq), 3.79 (ddd, J = 12.0, 12.0, 4.7 Hz, 1H, H-3ax), 3.69 (m, 1H, NCH_2), 3.14 (dq, J = 13.5, 7.2Hz, 1H, NCH_2), 2.49 (dd, J = 12.7, 12.7 Hz, 1H, H-17ax), 2.37 (dd, J = 13.2, 13.2 Hz, 1H, H-16ax), 2.24 (ddddd, J = 12.8, 12.8, 12.8, 5.5, 2.7 Hz, 1H, H-14ax), 1.98 (dd, J = 13.2, 7.5 Hz, 1H, H-16eq), 1.84 (m, 1H, H-14eq), 1.70 (ddd, J = 13.0, 13.0, 2.8Hz, 1H, H-15ax), 1.54 (m, 1H, H-15eq), 1.43 (m, 1H, H-17eq), 1.38 (m, 1H, H-19a), 1.18 (m, 1H, H-19b), 1.03 (m, J = 7.0 Hz, 3H, NCH_2CH_3), 0.68 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.6 (C-2), 143.6 (C-13), 139.4 ($(Cq)_{ar}$), 131.8 (CH_{ar}), 130.7 (C-21), 128.0 (CH_{ar}), 126.6 (CH_{ar}), 126.5 (CH_{ar}), 118.2 (C-5), 116.5 ($(Cq)_{ar}$), 110.3 (C-6), 46.2 (C-3), 43.8 (N*C*H$_2$), 38.9 (C-20), 36.6 (C-17), 33.3 (C-15), 30.2 (C-16 or C-19), 29.7 (C-19 or C-16), 19.5 (C-14), 13.7 (NCH$_2$*C*H$_3$), 8.2 (C-18) ppm; $[\alpha]_D^{25}$ −338 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2962, 1651 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{26}N_2NaO$ [(M+Na)$^+$]: 345.1943, found: 345.1942.

4.13. 1-Propylrhazinilam (5b)

White powder (26 mg, 32% from 71 mg rhazinilam); mp 165 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (dd, J = 6.9, 1.8Hz, 1H, H_{ar}), 7.38 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H_{ar}), 7.29 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H_{ar}), 7.21 (dd, J = 7.5, 1.2Hz, 1H, H_{ar}), 6.47 (d, J = 2.7 Hz, 1H, H-5), 5.83 (d, J = 2.7 Hz, 1H, H-6), 4.03 (dd, J = 12.0, 5.1Hz, 1H, H-3eq), 3.81 (ddd, J = 12.0, 12.0, 4.5Hz, 1H, H-3ax), 3.68 (ddd, J = 13.5, 9.3, 6.6Hz, 1H, NCH_2), 2.93 (ddd, J = 13.2, 9.6, 6.2 Hz, 1H, NCH_2), 2.50 (dd, J = 12.3, 12.3, 1H, H-17ax), 2.38 (dd, J = 12.9, 12.9 Hz, 1H, H-16ax), 2.22 (ddddd, J = 12.3, 12.3, 12.3, 5.4, 3.0 Hz, 1H, H-14ax), 1.99 (dd, J = 13.2, 7.8 Hz, 1H, H-16eq), 1.84 (m, 1H, H-14eq), 1.72 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.52 (m, 4H, H-15eq and NCH$_2$CH_2), 1.42 (m, 1H, H-17eq), 1.37 (m, 1H, H-19a), 1.16 (m, 1H, H-19b), 0.84 (t, J = 7.5 Hz, 3H, N(CH$_2$)$_2$CH_3), 0.70 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.8 (C-2), 144.2 (C-13), 139.3 (C-8), 131.8 (CH_{ar}), 130.7 (C-21), 128.0 (CH_{ar}), 126.6 (CH_{ar}), 126.5 (CH_{ar}), 118.3 (C-5), 116.5 (C-7), 110.2 (C-6), 51.1 (N*C*H$_2$), 46.2 (C-3), 38.9 (C-20), 36.6 (C-17), 33.3 (C-15), 30.2 (C-19), 29.7 (C-16), 21.5 (NCH$_2$*C*H$_2$), 19.5 (C-14), 11.5 (N(CH$_2$)$_2$*C*H$_3$), 8.2 (C-18) ppm; $[\alpha]_D^{23}$ −332 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2963, 1648 cm^{-1}; HRMS (ESI) calcd for $C_{22}H_{28}N_2NaO$ [(M+Na)$^+$]: 359.2099, found: 359.2105.

4.14. 1-Allylrhazinilam (5c)

White powder (58 mg, 46% from 112 mg rhazinilam); mp 158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (dd, J = 7.2, 1.8 Hz, 1H, H_{ar}), 7.31 (ddd, J = 7.7, 7.7, 1.8 Hz, 1H, H_{ar}), 7.25 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H_{ar}), 7.18 (dd, J = 7.5, 1.5Hz, 1H, H_{ar}), 6.44 (d, J = 2.4 Hz, 1H, H-5), 5.77 (d, J = 3.0 Hz, 1H, H-6), 5.74 (m, 1H, NCH$_2$C*H*), 5.03 (m, 2H, NCH$_2$CHCH_2), 4.41 (dd, J = 14.7, 6.0H z, 1H, NCH_2), 4.00 (dd, J = 12.0, 5.1 Hz, 1H, H-3eq), 3.77 (ddd, J = 12.0, 12.0, 4.5 Hz, 1H, H-3ax), 3.49 (dd, J = 14.7, 6.9 Hz, 1H, NCH_2), 2.47 (dd, J = 12.6, 12.6 Hz, 1H, H-17ax), 2.38 (dd, J = 12.8, 12.8 Hz, 1H, H-16ax), 2.22 (ddddd, J = 13.0, 13.0, 13.0, 5.4, 2.7 Hz, 1H, H-14ax), 1.98 (dd, J = 13.2, 7.8 Hz, 1H, H-16eq), 1.84 (m, 1H, H-14eq),

1.69 (ddd, J = 13.5, 13.5, 3.0 Hz, 1H, H-15ax), 1.51 (m, 1H, H-15eq), 1.44 (m, 1H, H-17eq), 1.38 (m, 1H, H-19a), 1.17 (m, 1H, H-19b), 0.66 (t, J = 7.4Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.7 (C-2), 143.8 (C-13), 139.4 (C-8), 134.5 ($NCH_2\mathit{C}H$), 131.8 (CH_{ar}), 130.7 (C-21), 128.0 (CH_{ar}), 126.9 (CH_{ar}), 126.8 (CH_{ar}), 118.5 (C-5), 117.2 ($NCH_2CH\mathit{C}H_2$), 116.6 (C-7), 110.3 (C-6), 52.1 ($N\mathit{C}H_2$), 46.3 (C-3), 39.0 (C-20), 36.7 (C-17), 33.3 (C-15), 30.3 (C-19), 29.7 (C-16), 19.6 (C-14), 8.3 (C-18) ppm; $[\alpha]_D^{24}$ −302 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2964, 1651 cm^{-1}; HRMS (ESI) calcd for $C_{22}H_{26}N_2NaO$ [(M+Na)$^+$]: 357.1943, found: 357.1953.

4.15. 1-Cyanomethylrhazinilam (5d)

White powder (70 mg, 81% from 76 mg rhazinilam); mp 138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47–7.34 (m, 4H, H_{ar}), 6.50 (d, J = 3.0 Hz, 1H, H-5), 5.82 (d, J = 2.1Hz, 1H, H-6), 4.95 (d, J = 17.4 Hz, 1H, $NC\mathit{H}_2$), 4.02 (dd, J = 11.4, 5.1Hz, 1H, H-3eq), 3.77 (ddd, J = 12.0, 12.0, 4.2 Hz, 1H, H-3ax), 3.62 (d, J = 17.4 Hz, 1H, $NC\mathit{H}_2$), 2.44 (m, 1H, H-17a), 2.42 (m, 1H, H-16a), 2.18 (ddddd, J = 13.2, 13.2, 13.2, 5.4, 2.7 Hz, 1H, H-14ax), 2.04 (m, 1H, H-16b), 1.88 (m, 1H, H-14eq), 1.71 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.54 (m, 1H, H-15eq), 1.50 (m, 1H, H-17b), 1.37 (m, 1H, H-19a), 1.13 (m, 1H, H-19b), 0.69 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 175.1 (C-2), 141.9 (C-13), 139.4 ($(Cq)_{ar}$), 132.1 (CH_{ar}), 130.7 (C-21), 128.9 (CH_{ar}), 128.4 (CH_{ar}), 127.0 (CH_{ar}), 119.2 ($(Cq)_{ar}$), 116.1 (C-5), 115.7 ($NCH_2\mathit{C}N$), 109.7 (C-6), 46.2 (C-3), 39.0 (C-20), 36.7 ($N\mathit{C}H_2$), 36.5 (C-17), 33.0 (C-15), 30.2 (C-19), 29.0 (C-16), 19.5 (C-14), 8.3 (C-18) ppm; $[\alpha]_D^{24}$ −321 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2943, 2250, 1669 cm^{-1}; HRMS (ESI) calcd for $C_{22}H_{26}N_2NaO$ [(M+Na)$^+$]: 356.1739, found: 356.1719.

4.16. 1-Methoxymethylrhazinilam (5e)

White powder (53 mg, 76% from 60 mg rhazinilam); mp 131 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (m, 1H, H_{ar}), 7.36 (m, 1H, H_{ar}), 7.31 (m, 2H, H_{ar}), 6.48 (d, J = 2.7 Hz, 1H, H-5), 5.78 (d, J = 2.7 Hz, 1H, H-6), 5.30 (d, J = 10.2 Hz, 1H, $NC\mathit{H}_2$), 4.18 (d, J = 10.2 Hz, 1H, $NC\mathit{H}_2$), 4.02 (dd, J = 12.0, 5.1 Hz, 1H, H-3eq), 3.79 (ddd, J = 12.0, 12.0, 4.5 Hz, 1H, H-3ax), 3.33 (s, 1H, $OC\mathit{H}_3$), 2.48 (m, 1H, H-17a), 2.44 (m, 1H, H-16a), 2.22 (ddddd, J = 13.2, 13.2, 13.2, 5.1, 2.7 Hz, 1H, H-14ax), 2.04 (m, 1H, H-16b), 1.86 (m, 1H, H-14eq), 1.73 (ddd, J = 13.5, 13.5, 3.0 Hz, 1H, H-15ax), 1.54 (m, 1H, H-15eq), 1.47 (m, 1H, H-17b), 1.38 (m, 1H, H-19a), 1.21 (m, 1H, H-19b), 0.71 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.0 (C-2), 143.1 (C-13), 139.0 (C-8), 131.5 (CH_{ar}), 130.4 ($(Cq)_{ar}$), 128.2 (CH_{ar}), 127.1 (CH_{ar}), 126.8 (CH_{ar}), 118.7 (C-5), 116.7 ($(Cq)_{ar}$), 109.7 (C-6), 79.0 ($N\mathit{C}H_2$), 56.4 ($O\mathit{C}H_3$), 46.1 (C-3), 38.9 (C-20), 36.7 (C-17), 33.1 (C-15), 30.2 (C-19), 29.5 (C-16), 19.5 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{22}$ −317 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2937, 1669 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{26}N_2NaO_2$ [(M+Na)$^+$]: 361.1892, found: 361.1869.

4.17. 1-(2-Methoxy)ethoxymethylrhazinilam (5f)

Oil (243 mg, 62% from 300 mg rhazinilam); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, J = 7.5 Hz, 1H, H_{ar}), 7.34 (m, 3H, H_{ar}), 6.45 (d, J = 2.7 Hz, 1H, H-5), 5.75 (d, J = 2.4 Hz, 1H, H-6), 5.39 (d, J = 10.2 Hz, 1H, $NC\mathit{H}_2$), 4.28 (d, J = 10.5 Hz, 1H, $NC\mathit{H}_2$), 4.00 (dd, J = 12.1, 5.3 Hz, 1H, H-3eq), 3.79 (m, 1H, H-3ax), 3.74 (m, 1H, $CH_2OC\mathit{H}_2$), 3.58–3.49 (m, 3H, $NCH_2OC\mathit{H}_2$ and $C\mathit{H}_2OCH_3$), 3.38 (s, 3H, $OC\mathit{H}_3$), 2.46 (m, 1H, H-17a), 2.42 (m, 1H, H-16a), 2.18 (ddddd, J = 13.2, 13.2, 13.2, 5.4, 2.7 Hz, 1H, H-14ax), 1.96 (m, 1H, H-16b), 1.83 (m, 1H, H-14eq), 1.70 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.52 (m, 1H, H-15eq), 1.47 (m, 1H, H-17b), 1.40 (m, 1H, H-19a), 1.20 (dq, J = 14.5, 7.3 Hz, 1H, H-19b), 0.70 (t, J = 7.3 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.0 (C-2), 143.1 (C-13), 138.9 (C-8), 131.4 (CH_{ar}), 130.4 (C-21), 128.2 (CH_{ar}), 127.1 (CH_{ar}), 126.9 (CH_{ar}), 118.7 (C-5), 116.8 (C-7), 109.8 (C-6), 77.7 ($N\mathit{C}H_2$), 71.7 ($\mathit{C}H_2OCH_3$), 67.9 ($NCH_2O\mathit{C}H_2$), 59.0 ($O\mathit{C}H_3$), 46.1 (C-3), 38.9 (C-20), 36.7 (C-17), 33.1 (C-15), 30.2 (C-19), 29.5 (C-16), 19.5 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{25}$ −312 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2941, 1667 cm^{-1}; HRMS (ESI) calcd for $C_{23}H_{30}N_2NaO_3$ [(M+Na)$^+$]: 405.2154, found: 405.2156.

4.18. 1-(2-Trimethylsilyl)ethoxymethylrhazinilam (5g)

Oil (399 mg, 71% from 399 mg rhazinilam); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, J = 7.5 Hz, 1H, H_{ar}), 7.34 (dd,J = 6.3, 1.2 Hz, 1H, H_{ar}), 7.29 (m, 2H, H_{ar}), 6.46 (d, J = 2.7 Hz, 1H, H-5), 5.76 (d, J = 2.7 Hz, 1H, H-6), 5.37 (d, J = 10.1 Hz, 1H, $NC\mathit{H}_2$), 4.14 (d, J = 10.1 Hz, 1H, $NC\mathit{H}_2$), 3.98 (dd, J = 12.0, 5.1 Hz, 1H, H-3eq), 3.78 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 3.62 (td, J = 9.9, 6.4 Hz, 1H, $NCH_2OC\mathit{H}_2$), 3.47 (td, J = 9.9, 6.4 Hz, 1H, $NCH_2OC\mathit{H}_2$), 2.47 (m, 1H, H-17a), 2.41 (m, 1H, H-16a), 2.19 (ddddd, J = 12.9, 12.9, 12.9, 5.6, 2.7 Hz, 1H, H-14ax), 2.01 (m, 1H, H-16b), 1.84 (m, 1H, H-14eq), 1.71 (ddd, J = 12.9, 12.9, 3.0 Hz, 1H, H-15ax), 1.53 (m, 1H, H-15eq), 1.47 (m, 1H, H-19a), 1.43 (m, 1H, H-17b), 1.20 (dq, J = 14.4, 7.4 Hz, 1H, H-19b), 0.96 (m, 1H, $C\mathit{H}_2Si$), 0.92 (m, 1H, $C\mathit{H}_2Si$), 0.70 (t,J = 7.4 Hz, 3H, H-18), 0.00 (s, 9H, $Si(C\mathit{H}_3)_3$) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.1 (C-2), 144.6 (C-13), 140.4 (C-8), 132.8 (CH_{ar}), 131.8 (C-21), 129.5 (CH_{ar}), 128.4 (CH_{ar}), 128.3 (CH_{ar}), 120.1 (C-5), 118.2 (C-7), 111.1 (C-6), 78.3 ($N\mathit{C}H_2$), 67.2 ($NCH_2O\mathit{C}H_2$), 47.5 (C-3), 40.3 (C-20), 38.1 (C-17), 34.5 (C-15), 31.6 (C-19), 30.9 (C-16), 20.9 (C-14), 19.5 ($\mathit{C}H_2Si$), 9.6 (C-18), 0.0 ($Si(\mathit{C}H_3)_3$) ppm; $[\alpha]_D^{22}$ −183 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2950, 1668 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{36}N_2NaO_2Si$ [(M+Na)$^+$]: 447.2444, found: 447.2418.

4.19. 1-Benzylrhazinilam (5h)

Oil (41 mg, 53% from 60 mg rhazinilam); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, J = 7.5, 1.5 Hz, 1H, H_{ar}), 7.30–7.15 (m, 7H, H_{ar}), 6.81 (dd, J = 7.5, 1.5 Hz, 1H, H_{ar}), 6.48 (d, J = 2.7 Hz, 1H, H-5) ; 5.77 (d, J = 2.7 Hz, 1H, H-6), 5.33 (d, J = 14.6 Hz, 1H,

NCH_2), 4.05 (dd, J = 11.7, 5.1 Hz 1H, H-3eq), 3.81 (ddd, J = 12.0, 12.0, 4.5 Hz, 1H, H-3ax), 3.70 (d, J = 14.6 Hz, 1H, NCH_2), 2.54 (m, 1H, H-17a), 2.43 (m, 1H, H-16a), 2.30 (ddddd, J = 12.9, 12.9, 12.9, 5.4, 2.7 Hz, 1H, H-14ax), 2.05 (m, 1H, H-16b), 1.86 (m, 1H, H-14eq), 1.73 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.55 (m, 1H, H-15eq), 1.48 (m, 1H, H-17b), 1.41 (m, 1H, H-19a), 1.19 (m, 1H, H-19b), 0.70 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.9 (C-2), 144.0 ((Cq)$_{ar}$), 139.2 ((Cq)$_{ar}$), 138.3 ((Cq)$_{ar}$), 131.7 (CH$_{ar}$), 130.5 (C-21), 129.0 (CH$_{ar}$), 128.3 (CH$_{ar}$), 127.8 (CH$_{ar}$), 127.1 (CH$_{ar}$), 126.8 (CH$_{ar}$), 126.7 (CH$_{ar}$), 118.5 (C-5), 116.5 ((Cq)$_{ar}$), 109.8 (C-6), 52.7 (NCH_2), 46.2 (C-3), 39.0 (C-20), 36.6 (C-17), 33.2 (C-15), 30.2 (C-19), 29.6 (C-16), 19.5 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{25}$ −293 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2962, 1652 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{28}N_2NaO$ [(M+Na)$^+$]: 407.2058, found: 407.2099.

4.20. 1-Acetylrhazinilam (5i)

White powder (32 mg, 50% from 56 mg rhazinilam); mp 118 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (dd, J = 7.6, 1.8 Hz, 1H, H$_{ar}$), 7.35 (ddd, J = 7.8, 7.8, 1.2 Hz, 1H, H$_{ar}$), 7.28 (ddd, J = 7.2, 7.2, 1.5 Hz, 1H, H$_{ar}$), 7.13 (dd, J = 7.5, 1.5 Hz, 1H, H$_{ar}$), 6.43 (d, J = 2.1 Hz, 1H, H-5), 5.71 (d, J = 2.7 Hz, 1H, H-6), 3.94 (dd, J = 11.4, 5.4 Hz 1H, H-3eq), 3.70 (ddd, J = 12.0, 12.0, 4.5 Hz, 1H, H-3ax), 2.48 (m, 1H, H-16a), 2.41 (m, 1H, H-17a), 2.34 (s, 3H, NCOCH_3), 2.17 (ddddd, J = 12.9, 12.9, 12.9, 5.1, 2.7 Hz, 1H, H-14ax), 1.98 (m, 1H, H-16b), 1.82 (m, 1H, H-14eq), 1.70 (ddd, J = 13.2, 13.2, 2.7 Hz, 1H, H-15ax), 1.45 (m, 1H, H-15eq), 1.41 (m, 1H, H-19a), 1.35 (m, 1H, H-17b), 1.12 (m, 1H, H-19b), 0.70 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.4 (C-2), 173.8 (NCO), 140.4 ((Cq)$_{ar}$), 138.5 ((Cq)$_{ar}$), 131.6 (CH$_{ar}$), 130.1 (C-21), 128.3 (CH$_{ar}$), 127.6 (CH$_{ar}$), 127.5 (CH$_{ar}$), 119.7 (C-5), 117.0 (C-7), 109.4 (C-6), 46.2 (C-3), 39.1 (C-20), 37.5 (C-17), 32.5 (C-15), 32.0 (C-16), 30.3 (C-19), 26.8 (NCOCH_3), 19.7 (C-14), 8.5 (C-18) ppm; $[\alpha]_D^{24}$ −345 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2941, 1705 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{24}N_2NaO_2$ [(M+Na)$^+$]: 359.1735, found: 359.1722.

4.21. 1-Pivaloylrhazinilam (5j)

White powder (52 mg, 66% from 61 mg rhazinilam); mp 90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (dd, J = 7.5, 1.8 Hz, 1H, H$_{ar}$), 7.27 (ddd, J = 7.7, 7.7, 1.8 Hz, 1H, H$_{ar}$), 7.20 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, H$_{ar}$), 6.90 (dd, J = 7.8, 1.5 Hz, 1H, H$_{ar}$), 6.39 (d, J = 3.0 Hz, 1H, H-5), 5.84 (d, J = 2.7 Hz, 1H, H-6), 3.93 (dd, J = 12.0, 5.0 Hz, 1H, H-3eq), 3.68 (ddd, J = 12.3, 12.3, 4.8 Hz, 1H, H-3ax), 2.44 (m, 1H, H-16a), 2.39 (m, 1H, H-17a), 2.18 (ddddd, J = 13.1, 13.1, 13.1, 5.4, 2.7 Hz, 1H, H-14ax), 1.99 (m, 1H, H-16b), 1.77 (m, 1H, H-14eq), 1.69 (ddd, J = 13.4, 13.4, 3.0 Hz, 1H, H-15ax), 1.46 (m, 1H, H-15eq), 1.38 (m, 1H, H-19a), 1.30 (m, 1H, H-17b), 1.21 (s, 9H, NCOC(CH_3)$_3$), 1.09 (m, 1H, H-19b), 0.68 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 188.2 (NCO), 176.5 (C-2), 142.0 (C-13), 139.9 (C-8), 131.9 (CH$_{ar}$), 130.4 (C-21), 128.5 (CH$_{ar}$), 127.2 (CH$_{ar}$), 126.2 (CH$_{ar}$), 119.0 (C-5), 117.1 (C-7), 110.8 (C-6), 46.1 (C-3), 44.0 (NCOC(CH$_3$)$_3$), 39.1 (C-20), 36.4 (C-17), 32.7 (C-15), 31.1 (C-16), 30.0 (C-19), 28.4 (2 NCOC(CH$_3$)$_3$), 27.2 (NCOC(CH$_3$)$_3$), 19.7 (C-14), 8.4 (C-18) ppm; $[\alpha]_D^{24}$ −491 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2961, 1693 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{30}N_2NaO_2$ [(M+Na)$^+$]: 402.2239, found: 402.2274.

4.22. 1-Benzoylrhazinilam (5k)

Pale yellow solid (66 mg, 59% from 82 mg rhazinilam); mp 90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 6.3 Hz, 1H, H$_{ar}$), 7.52 (m, 2H, H$_{ar}$), 7.46–7.24 (m, 5H, H$_{ar}$), 7.16 (d, J = 7.2 Hz, 1H, H$_{ar}$), 6.40 (d, J = 2.7 Hz, 1H, H-5), 5.87 (d, J = 3.0 Hz, 1H, H-6), 3.86 (dd, J = 12.3, 5.1 Hz 1H, H-3eq), 3.66 (ddd, J = 12.3, 12.3, 4.6 Hz, 1H, H-3ax), 2.58 (m, 1H, H-16a), 2.36 (ddd, J = 12.9, 12.9, 1.5 Hz, 1H, H-17ax), 2.11 (m, 1H, H-16b), 2.02 (m, 1H, H-14a), 1.86 (m, 1H, H-14b), 1.70 (m, 1H, H-15a), 1.47 (m, 1H, H-19a), 1.40 (m, 1H, H-15b), 1.32 (m, 1H, H-17eq), 1.17 (m, 1H, H-19b), 0.71 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.6 (C-2), 173.6 (NCO), 141.0 ((Cq)$_{ar}$), 139.4 ((Cq)$_{ar}$), 136.1 ((Cq)$_{ar}$), 133.8 (CH$_{ar}$), 131.7 (CH$_{ar}$), 131.6 (CH$_{ar}$), 130.2 (CH$_{ar}$), 130.1 (C-21), 128.6 (CH$_{ar}$), 128.5 (CH$_{ar}$), 128.2 (CH$_{ar}$), 127.6 (CH$_{ar}$), 126.7 (CH$_{ar}$), 119.6 (C-5), 116.9 ((Cq)$_{ar}$), 110.1 (C-6), 46.0 (C-3), 39.1 (C-20), 36.6 (C-17), 32.4 (C-15), 31.5 (C-16), 30.1 (C-19), 19.5 (C-14), 8.5 (C-18) ppm; $[\alpha]_D^{23}$ −381 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2956, 1689 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{26}N_2NaO_2$ [(M+Na)$^+$]: 421.1892, found: 421.1898.

4.23. 1-(*p*-Methoxybenzoyl)rhazinilam (5l)

White powder (81 mg, 62% from 89 mg rhazinilam); mp 169 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 8.7 Hz, 2H, H$_{ar}$), 7.43 (dd, J = 7.5, 1.8 Hz, 1H, H$_{ar}$), 7.33 (ddd, J = 7.5, 7.5, 2.1 Hz, 1H, H$_{ar}$), 7.27 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, H$_{ar}$), 7.13 (dd, J = 7.5, 1.5 Hz, 1H, H$_{ar}$), 6.79 (d, J = 9.0 Hz, 2H, H$_{ar}$), 6.40 (d, J = 3.0 Hz, 1H, H-5), 5.88 (d, J = 3.0 Hz, 1H, H-6), 3.85 (dd, J = 12.0, 5.1 Hz, 1H, H-3eq), 3.73 (s, 3H, OCH_3), 3.66 (ddd, J = 12.0, 12.0, 4.2 Hz, 1H, H-3ax), 2.58 (dd, J = 13.5, 13.5 Hz, 1H, H-16ax), 2.39 (ddd, J = 13.5, 13.5, 1.5 Hz, 1H, H-17ax), 2.12 (m, 1H, H-16eq), 2.04 (m, 1H, H-14a), 1.72 (m, 1H, H-14b), 1.69 (m, 1H, H-15a), 1.48 (m, 1H, H-19a), 1.44 (m, 1H, H-15b), 1.30 (m, 1H, H-17eq), 1.18 (m, 1H, H-19b), 0.72 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.6 (C-2), 172.6 (NCO), 162.5 ((Car)OCH$_3$), 141.3 ((Cq)$_{ar}$), 139.5 (C-8), 131.6 (CH$_{ar}$), 130.7 (CH$_{ar}$), 130.1 (C-21), 128.5 (CH$_{ar}$), 128.1 ((Cq)$_{ar}$), 127.3 (CH$_{ar}$), 126.5 (CH$_{ar}$), 119.4 (C-5), 116.9 (C-7), 113.5 (CH$_{ar}$), 110.2 (C-6), 55.4 (OCH_3), 46.0 (C-3), 39.1 (C-20), 36.4 (C-17), 32.4 (C-15), 31.5 (C-16), 30.1 (C-19), 19.5 (C-14), 8.4 (C-18) ppm; $[\alpha]_D^{24}$ −413 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2961, 1686 cm^{-1}; HRMS (ESI) calcd for $C_{27}H_{28}N_2NaO_3$ [(M+Na)$^+$]: 452.2031, found: 452.2051.

4.24. 1-(*p*-Trifluoromethylbenzoyl)rhazinilam (5m)

White powder (80 mg, 53% from 94 mg rhazinilam); mp 86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (m, 3H, H_{ar}), 7.55 (dd, J = 7.5, 2.0 Hz, 1H, H_{ar}), 7.46 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H_{ar}), 7.39 (ddd, J = 7.5, 7.5Hz, 1.8 Hz, 1H, H_{ar}), 7.24 (m, 2H, H_{ar}), 6.49 (d, J = 2.4 Hz, 1H, H-5), 5.91 (d, J = 2.7 Hz, 1H, H-6), 3.96 (dd, J = 12.0, 5.1 Hz 1H, H-3eq), 3.73 (ddd, J = 12.0, 12.0, 4.8 Hz 1H, H-3ax), 2.65 (dd$J$ = 13.9, 13.9 Hz, 1H, H-16ax), 2.42 (ddd, J = 13.4, 13.4, 1.8 Hz, 1H, H-17ax), 2.16 (m,1H, H-16eq), 2.08 (m, 1H, H-14a), 1.84 (m, 1H, H-14b), 1.77 (ddd, J = 13.5, 13.5, 3.3 Hz, 1H, H-15ax), 1.50 (m, 1H, H-19a), 1.45 (m, 1H, H-15eq), 1.40 (m, 1H, H-17eq), 1.24 (m, 1H, H-19b), 0.80 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 176.5 (C-2), 172.3 (N*C*O), 140.3 ((Cq)$_{ar}$), 139.7 ((Cq)$_{ar}$), 139.3 ((Cq)$_{ar}$), 133.3 (q, $^2J_{C,F}$ = 32 Hz, (*C*ar)CF_3), 131.8 (CH_{ar}), 130.2 (C-21), 129.2 (q, $^1J_{C,F}$ = 272 Hz, *C*F_3), 128.8 (CH_{ar}), 128.1 (CH_{ar}), 127.9 (CH_{ar}), 126.7 (CH_{ar}), 125.3 (m, *C*H$_{ar}$(C-q)$_{ar}CF_3$), 119.7 (C-5), 116.8 (C-7), 109.9 (C-6), 46.1 (C-3), 39.1 (C-20), 36.8 (C-17), 32.3 (C-15), 31.5 (C-16), 30.1 (C-19), 19.5 (C-14), 8.5 (C-18) ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ −63.0 ppm; $[\alpha]_D^{24}$ −390 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2946, 1694, 1682, 1327 cm^{-1}; HRMS (ESI) calcd for $C_{27}H_{25}F_3N_2NaO_2$ [(M+Na)$^+$]: 489.1766, found: 489.1763.

4.25. 1-Ethoxycarbonylrhazinilam (5n)

White crystals (36 mg, 50% from 61 mg rhazinilam); mp 137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (dd, J = 7.5, 1.4 Hz, 1H, H_{ar}), 7.34 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, H_{ar}), 7.28 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H_{ar}), 7.18 (dd,J = 7.4, 1.6 Hz, 1H, H_{ar}), 6.45 (d, J = 3.0 Hz, 1H, H-5), 5.76 (d, J = 2.1 Hz, 1H, H-6), 4.14 (m, 2H, O*C*H_2), 3.95 (dd, J = 12.0, 4.8 Hz, 1H, H-3eq), 3.71 (ddd, J = 12.0, 12.0, 4.2 Hz, 1H, H-3ax), 2.48 (dd, J = 13.5, 13.5 Hz, 1H, H-17ax), 2.37 (dd, J = 13.8, 13.8 Hz, 1H, H-16ax), 2.17 (ddddd, J = 12.9, 12.9, 12.9, 5.1, 3.0 Hz, 1H, H-14ax), 2.00 (dd, J = 13.8, 6.3 Hz, 1H, H-16eq), 1.82 (m, 1H, H-14eq), 1.70 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.43 (m, 1H, H-15eq), 1.39 (m, 1H, H-19a), 1.31 (m, 1H, H-17eq), 1.17 (t, J = 7.5 Hz, 3H, OCH$_2$C*H*$_3$), 1.12 (m, 1H, H-19b), 0.71 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.3 (C-2), 153.2 (N*C*O), 140.4 (C-13), 138.6 (C-8), 131.4 (CH_{ar}), 130.1 (C-21), 128.2 (CH_{ar}), 127.3 (CH_{ar}), 126.9 (CH_{ar}), 119.6 (C-5), 116.6 (C-7), 109.2 (C-6), 62.7 (O*C*H$_2$), 46.0 (C-3), 39.0 (C-20), 37.0 (C-17), 32.4 (C-15), 31.3 (C-16), 30.2 (C-19), 19.5 (C-14), 14.1 (OCH$_2$*C*H$_3$), 8.4 (C-18) ppm; $[\alpha]_D^{24}$ −427 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2963, 1771, 1724 cm^{-1}; HRMS (ESI) calcd for $C_{22}H_{26}N_2NaO_3$ [(M+Na)$^+$]: 389.1841, found: 389.1818.

4.26. 1-*tert*-Butoxycarbonylrhazinilam (5o)

White crystals (250 mg, 85% from 219 mg rhazinilam); mp 197 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (dd, J = 7.4, 1.7 Hz, 1H, H_{ar}), 7.33 (ddd, J = 7.2, 7.2, 1.2 Hz, 1H, H_{ar}), 7.25 (dd, J = 7.2, 7.2 Hz, 1H, H_{ar}), 7.14 (dd, J = 7.9, 1.6 Hz, 1H, H_{ar}), 6.46 (d, J = 2.4 Hz, 1H, H-5), 5.78 (d, J = 1.8 Hz, 1H, H-6), 3.95 (dd, J = 12.3, 4.8 Hz, 1H, H-3eq), 3.73 (ddd, J = 12.3, 12.3, 4.2 Hz, 1H, H-3ax), 2.48 (dd, J = 13.8, 13.8 Hz, 1H, H-17ax), 2.37 (dd, J = 13.8, 13.8 Hz, 1H, H-16ax), 2.15 (ddddd, J = 13.2, 13.2, 13.2, 5.1, 3.0 Hz, 1H, H-14ax), 1.99 (dd, J = 14.4, 6.3 Hz, 1H, H-16eq), 1.82 (m, 1H, H-14eq), 1.71 (ddd, J = 13.2, 13.2, 2.7 Hz, 1H, H-15ax), 1.48 (m, 1H, H-15eq), 1.43 (m, 1H, H-19a), 1.35 (m, 1H, H-17eq), 1.35 (s, 9H, C(C*H*$_3$)$_3$), 1.14 (m, 1H, H-19b) ; 0.71 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.2 (C-2), 151.6 (N*C*O), 140.8 (C-13), 138.5 (C-8), 131.3 (CH_{ar}), 130.0 (C-21), 128.0 (CH_{ar}), 127.0 (CH_{ar}), 126.9 (CH_{ar}), 119.4 (C-5), 116.7 (C-7), 109.2 (C-6), 82.2 (*C*(CH$_3$)$_3$), 46.0 (C-3), 39.0 (C-20), 37.0 (C-17), 32.5 (C-15), 31.4 (C-16), 30.2 (C-19), 27.9 (C(*C*H$_3$)$_3$), 19.5 (C-14), 8.4 (C-18) ppm; $[\alpha]_D^{24}$ −385 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2967, 1760, 1687 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{30}N_2NaO_3$ [(M+Na)$^+$]: 417.2154, found: 417.2152.

4.27. 1-Phenoxycarbonylrhazinilam (5q)

Oil (51 mg, 61% from 60 mg rhazinilam); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (m, 1H, H_{ar}), 7.42–7.27 (m, 5H, H_{ar}), 7.20 (dd, J = 7.2, 7.2 Hz, 1H, H_{ar}), 7.11 (d, J = 8.1 Hz, 2H, H_{ar}), 6.59 (d, J = 2.7 Hz, 1H, H-5), 5.93 (d, J = 2.7 Hz, 1H, H-6), 4.06 (dd, J = 12.0, 4.8 Hz, 1H, H-3eq), 3.82 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 2.58 (dd, J = 12.6, 12.6 Hz, 1H, H-17ax), 2.50 (dd, J = 12.6, 12.6 Hz, 1H, H-16ax), 2.26 (ddddd, J = 12.9, 12.9, 12.9, 5.1, 2.7 Hz, 1H, H-14ax), 2.13 (m, 1H, H-16eq), 1.88 (m, 1H, H-14eq), 1.79 (ddd, J = 13.5, 13.5, 3.0 Hz, 1H, H-15ax), 1.53 (m, 2H, H-15eq and H-19a), 1.44 (m, 1H, H-17b), 1.27 (m, 1H, H-19b), 0.79 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 174.3 (C-2), 151.4, 150.8, 140.2 (N*C*O, CO_2*C*ar and C-13), 138.7 (C-8), 131.6 (CH_{ar}), 130.3 (C-21), 129.3 (CH_{ar}), 128.5 (CH_{ar}), 127.8 (CH_{ar}), 127.1 (CH_{ar}), 125.9 (CH_{ar}), 121.4 (CH_{ar}), 120.0 (C-5), 116.6 (C-7), 109.3 (C-6), 46.2 (C-3), 39.2 (C-20), 37.3 (C-17), 32.5 (C-15), 31.4 (C-16), 30.4 (C-19), 19.6 (C-14), 8.5 (C-18) ppm; $[\alpha]_D^{24}$ −459 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2943, 1784, 1725 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{26}N_2NaO_3$ [(M+Na)$^+$]: 437.1841, found: 437.1852.

4.28. 1-Methanesulfonylrhazinilam (5r)

White powder (36 mg, 46% from 61 mg rhazinilam); mp 171 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.36 (m, 4H, H_{ar}), 6.52 (d, J = 2.7 Hz, 1H, H-5), 5.96 (d, J = 2.7 Hz, 1H, H-6), 4.04 (dd, J = 12.0, 4.8 Hz, 1H, H-3eq), 3.82 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 3.14 (s, 3H, SO_2C*H*$_3$), 2.49 (m, 1H, H-17a), 2.45 (m, 1H, H-16a), 2.20 (m, 2H, H-14a and H-16b), 1.91 (m, 1H, H-14b), 1.73 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.59 (m, 1H, H-15eq), 1.50 (m, 1H, H-17b), 1.38 (m, 1H, H-19a), 1.16 (m, 1H, H-19b), 0.68 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 175.3 (C-2), 138.9 (C-8), 136.7 (C-13), 131.8 (CH_{ar}), 130.8 (C-21), 130.0 (CH_{ar}), 128.7 (CH_{ar}), 128.2 (CH_{ar}), 119.0 (C-5), 116.0 (C-7), 110.9 (C-6), 46.3 (C-3), 42.3 (SO_2*C*H$_3$), 39.1 (C-20), 37.1 (C-17), 32.8

(C-15), 31.4 (C-16, 30.4 (C-19), 19.4 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{24}$ −255 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2962, 1685, 1348 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{24}N_2NaO_3S$ $[(M+Na)^+]$: 395.1405, found: 395.1397.

4.29. 1-(*p*-Toluenesulfonyl)rhazinilam (5s)

White powder (62 mg, 67% from 61 mg rhazinilam); mp 196 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, J = 9.0 Hz, 2H, H_{ar}), 7.60 (dd, J = 7.8, 0.9 Hz, 1H, H_{ar}), 7.48–7.35 (m, 3H, H_{ar}), 7.26 (d, J = 9.0 Hz, 2H, H_{ar}), 6.25 (d, J = 2.6 Hz, 1H, H-5), 5.45 (d, J = 2.6 Hz, 1H, H-6), 3.94 (dd, J = 12.0, 5.1 Hz, 1H, H-3eq), 3.71 (ddd, J = 12.0, 12.0, 4.5 Hz, 1H, H-3ax), 2.41 (s, 3H, (Car)CH_3), 2.36 (m, 2H, H-16a and H-17a), 2.15 (m, 1H, H-14a), 2.05 (m, 2H, H-16b), 1.82 (m, 1H, H-14b), 1.67 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.47 (m, 1H, H-15eq), 1.40 (m, 1H, H-17b), 1.29 (m, 1H, H-19a), 1.09 (m, 1H, H-19b), 0.66 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.9 (C-2), 144.3 (Cq(ar)), 139.9 (C-8), 136.9 (C-13), 136.1 (Cq(ar)), 132.0 (CH_{ar}), 130.2 (C-21), 129.5 (2 CH_{ar}), 129.0 (CH_{ar}), 128.2 (CH_{ar}), 128.1 (CH_{ar}), 118.5 (C-5), 115.7 (C-7), 110.8 (C-6), 46.1 (C-3), 38.9 (C-20), 36.7 (C-17), 32.8 (C-15), 31.5 (C-16), 30.1 (C-19), 21.7 ((Car)CH_3), 19.4 (C-14), 8.3 (C-18) ppm; $[\alpha]_D^{24}$ −259 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 2963, 1709, 1360 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{28}N_2NaO_3S$ $[(M+Na)^+]$: 471.1718, found: 471.1715.

4.30. 16(*S*)-methylrhazinilam (6aα)

White powder (43 mg, 34% from 120 mg rhazinilam); HPLC separation (preparative Hypercarb column, MeOH/CH_2Cl_2 4:1, 3.0 mL/min) t_R 17.6 min; mp 238 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (dd, J = 6.9, 1.8 Hz, 1H, H-12), 7.37–7.26 (m, 2H, H_{ar}), 7.21 (dd, J = 7.4, 1.7 Hz, 1H, H_{ar}), 6.68 (br s, 1H, H-1), 6.49 (d, J = 2.7 Hz, 1H, H-5), 5.75 (d, J = 2.7 Hz, 1H, H-6), 3.99 (dd, J = 12, 5.6 Hz, 1H, H-3eq), 3.76 (ddd, J = 12.3, 12.3, 5.0 Hz, 1H, H-3ax), 2.56 (m, 1H, H-16), 2.47 (dd, J = 13.3, 13.3 Hz, 1H, H-17ax), 2.24 (ddddd, J = 13.2, 13.2, 13.2, 5.7, 3.0 Hz, 1H, H-14ax), 1.83 (m, 1H, H-14eq), 1.68 (ddd, J = 13.5, 13.5, 3.0 Hz, 1H, H-15ax), 1.51 (m, 1H, H-15eq), 1.41 (m, 1H, H-19a), 1.28 (m, 1H, H-19b), 1.16 (d, J = 13.3 Hz, 1H, H-17eq), 1.06 (d, J = 6.3 Hz, 3H, CH_3), 0.70 (t, J = 7.3 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 181.1 (C-2), 140.4 (C-8), 137.9 (C-13), 131.4 (C-12), 130.7 (C-21), 128.1 (CH_{ar}), 127.3 (CH_{ar}), 127.0 (CH_{ar}), 119.1 (C-5), 117.2 (C-7), 109.5 (C-6), 46.1 (C-3), 45.8 (C-17), 38.8 (C-20), 33.3 (C-15), 31.9 (C-16), 30.6 (C-19), 21.0 (CH_3), 19.4 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{23}$ −203 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3194, 2962, 1669 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{24}N_2NaO$ $[(M+Na)^+]$: 331.1786, found: 331.1780.

4.31. 16(*R*)-methylrhazinilam (6aβ)

White powder (11 mg, 9% from 120 mg rhazinilam); HPLC separation (preparative Hypercarb column, MeOH/CH_2Cl_2 4:1, 3.0 mL/min) t_R 26.2 min; mp 195 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40 (m, 1H, H_{ar}), 7.34 (m, 2H, H_{ar}), 7.29 (m, 1H, H_{ar}), 6.54 (d, J = 2.7 Hz, 1H, H-5), 6.28 (br s, 1H, H-1), 5.86 (d, J = 2.7 Hz, 1H, H-6), 3.96 (m, 1H, H-3a), 3.88 (m, 1H, H-3b), 2.80 (m, 1H, H-16), 2.13 (m, 1H, H-14a), 2.06 (ddd, J = 12.3, 12.3, 2.4 Hz, 1H, H-15ax), 1.90 (m, 1H, H-14b), 1.65 (m, 1H, H-15eq), 1.56 (dd, J = 14.1, 14.1 Hz, 1H, H-17ax), 1.53 (m, 1H, H-19a), 1.35 (m, 1H, H-19b), 1.28 (m, 1H, H-17eq), 1.20 (d, 3H, J = 6.9 Hz, CH_3), 0.64 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.7 (C-2), 140.7 (C-8), 136.8 (C-13), 132.7 (C-21), 131.2 (CH_{ar}), 128.3 (CH_{ar}), 128.0 (CH_{ar}), 127.8 (CH_{ar}), 119.6 (C-5), 116.9 (C-7), 108.5 (C-6), 45.7 (C-3), 42.3 (C-17), 41.7 (C-16), 38.6 (C-20), 36.0 (C-19), 28.8 (C-15), 20.2 (CH_3), 20.0 (C-14), 8.6 (C-18) ppm; $[\alpha]_D^{23}$ −24 (*c* 0.50, $CHCl_3$); IR (film) $\tilde{\nu}$ 3195, 2852, 1664 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{24}N_2NaO$ $[(M+Na)^+]$: 331.1786, found: 331.1783.

4.32. 16(*S*)-Ethylrhazinilam (6bα)

White powder (46 mg, 45% from 94 mg rhazinilam); HPLC (preparative Hypercarb column, MeOH, 3.0 mL/min) t_R 14.0 min; mp 142 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, J = 7.1, 2.0 Hz, 1H, H-12), 7.35 (ddd, J = 7.5, 7.5, 1.8 Hz, 1H, H_{ar}), 7.30 (ddd, J = 7.5, 7.5, 1.5 Hz, 1H, H_{ar}), 7.18 (dd, J = 7.2, 1.8 Hz, 1H, H-9), 6.59 (br s, 1H, H-1), 6.49 (d, J = 2.7 Hz, 1H, H-5), 5.76 (d, J = 2.4 Hz, 1H, H-6), 3.99 (dd, J = 11.7, 5.4 Hz, 1H, H-3eq), 3.77 (ddd, J = 12.3, 12.3, 4.8 Hz,1H, H-3ax), 2.46 (dd, J = 11.7, 11.7 Hz, 1H, H-17ax), 2.41 (m, 1H, H-16), 2.24 (ddddd, J = 12.7, 12.7, 12.7, 5.4, 2.9 Hz, 1H, H-14ax), 1.84 (m, 1H, H-14eq), 1.69 (m, 2H, H-15a and CH_2CH$_3$), 1.52 (m, 1H, H-15b), 1.41 (m, 1H, H-19a), 1.28 (m, 2H, CH_2CH$_3$ and H-19b), 1.20 (d, J = 11.7 Hz, 1H, H-17eq), 0.86 (t, J = 7.4 Hz, 3H, CH$_2$CH_3), 0.70 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 179.7 (C-2), 140.7 (C-8), 137.9 (C-13), 131.4 (C-12), 130.9 (C-21), 128.1 (CH_{ar}), 127.5 (CH_{ar}), 127.2 (CH_{ar}), 118.9 (C-5), 117.2 (C-7), 109.5 (C-6), 46.1 (C-3), 44.7 (C-17), 39.2 (C-16), 38.7 (C-20), 33.5 (C-15), 30.6 (C-19), 29.1 (CH_2CH_3), 19.4 (C-14), 11.9 (CH_2CH_3), 8.2. (C-18) ppm; $[\alpha]_D^{23}$ −254 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3190, 2961, 1666 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{26}N_2NaO$ $[(M+Na)^+]$: 345.1943, found: 345.1935.

4.33. 16(*R*)-Ethylrhazinilam (6bβ)

White powder (12 mg, 12% from 94 mg rhazinilam); HPLC (preparative Hypercarb column, MeOH, 3.0 mL/min) t_R 18.5 min; mp 171 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 1H, H_{ar}), 7.30 (m, 2H, H_{ar}), 7.28 (m, 1H, H_{ar}), 6.54 (d, J = 2.7 Hz, 1H, H-5), 6.27 (br s, 1H, H-1), 5.85 (d, J = 2.4 Hz, 1H, H-6), 3.96 (m, 1H, H-3a), 3.87 (m, 1H, H-3b), 2.54 (m, 1H, H-16), 2.10 (m, 2H, H-14a and H-15a), 1.88 (m, 1H, H-14b), 1.64 (m, 1H, CH_2CH$_3$), 1.59 (m, 1H, H-15b), 1.49 (m, 2H, H-17a and H-19a), 1.36 (m, 1H, H-19b), 1.28 (m, 2H, H-17b and CH_2CH$_3$), 1.00 (t, J = 7.3 Hz, 3H, CH$_2$CH_3), 0.64 (t, J = 7.3 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.5 (C-2), 140.7 (C-8), 136.8 (C-13), 132.8 (C-21), 131.0 (CH_{ar}), 128.2 (CH_{ar}),

128.0 (CH_{ar}), 127.7 (CH_{ar}), 119.6 (C-5), 116.9 (C-7), 108.6 (C-6), 49.1 (C-16), 45.7 (C-3), 40.4 (C-17), 38.5 (C-20), 36.1 (C-19), 28.9 (C-15), 27.2 (CH_2CH_3), 20.0 (C-14), 12.1 (CH_2CH_3), 8.6 (C-18) ppm; $[\alpha]_D^{23}$ −59 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3188, 2960, 1653 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{26}N_2NaO$ [$(M+Na)^+$]: 345.1943, found: 345.1935.

4.34. 16(*S*)-Benzylrhazinilam (6cα)

Beige powder (69 mg, 32% from 163 mg rhazinilam); HPLC (preparative Hypercarb column, $MeOH/CH_2Cl_2$ 3:2, 3.0 mL/min) t_R 9.8 min; mp 177 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (dd, J = 7.1, 2.0 Hz, 1H, H_{ar}), 7.30–7.18 (m, 5H, H_{ar}), 7.03 (m, 2H, H_{ar}), 6.56 (dd, J = 8.3, 2.0 Hz, 1H, H_{ar}), 6.49 (d, J = 2.4 Hz, 1H, H-5), 6.39 (br s, 1H, H-1), 5.73 (d, J = 2.7 Hz, 1H, H-6), 4.00 (dd, J = 12.0, 5.4 Hz, 1H, H-3eq), 3.77 (ddd, J = 12.3, 12.3, 4.8 Hz, 1H, H-3ax), 2.91 (dd, J = 12.6, 8.7 Hz, 1H, CH_2Ph), 2.74 (ddd, J = 10.2, 6.0, 6.0 Hz, 1H, H-16), 2.55 (m, 1H, CH_2Ph), 2.51 (dd, J = 10.8, 10.8 Hz, 1H, H-17ax), 2.24 (ddddd, J = 12.9, 12.9, 12.9, 5.4, 2.7 Hz, 1H, H-14ax), 1.84 (m, 1H, H-14eq), 1.66 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.52 (m, 1H, H-15eq), 1.38 (m, 1H, H-19a), 1.29 (d, J = 10.8 Hz, 1H, H-17eq), 1.18 (m, 1H, H-19b), 0.57 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 178.7 (C-2), 140.4 (C-8), 139.4 (C-23), 137.5 (C-13), 131.2 (CH_{ar}), 130.8 (C-21), 129.2 (CH_{ar}), 128.2 (CH_{ar}), 127.8 (CH_{ar}), 127.3 (CH_{ar}), 127.2 (CH_{ar}), 126.2 (CH_{ar}), 119.0 (C-5), 117.3 (C-7), 109.5 (C-6), 46.1 (C-3), 43.3 (C-17), 41.5 (CH_2Ph), 40.1 (C-16), 38.7 (C-20), 33.4 (C-15), 30.5 (C-19), 19.4 (C-14), 7.9 (C-18) ppm; $[\alpha]_D^{24}$ −423 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3201, 2963, 1673 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{28}N_2NaO$ [$(M+Na)^+$]: 407.2099, found: 407.2077.

4.35. 16(*R*)-Benzylrhazinilam (6cβ)

Beige powder (42 mg, 20% from 163 mg rhazinilam); HPLC (preparative Hypercarb column, $MeOH/CH_2Cl_2$ 3:2, 3.0 mL/min) t_R 13.5 min; mp 178 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (m, 4H, H_{ar}), 7.24 (m, 2H, H_{ar}), 7.17 (m, 3H, H_{ar}), 6.54 (d, J = 3.0 Hz, 1H, H-5), 6.33 (br s, 1H, H-1), 5.86 (d, J = 2.4 Hz, 1H, H-6), 3.94 (m, 1H, H-3a), 3.85 (m, 1H, H-3b), 3.09 (dd, J = 13.2, 6.3 Hz, 1H, CH_2Ph), 2.97 (m, 1H, H-16), 2.60 (dd, J = 13.2, 9.0 Hz, 1H, CH_2Ph), 2.04 (m, 1H, H-14a), 1.95 (ddd, J = 12.9, 12.9, 2.6 Hz, 1H, H-15ax), 1.83 (m, 1H, H-14b), 1.52 (dd, J = 13.5, 13.5 Hz, 1H, H-17ax), 1.42 (m, 2H, H-15eq and H-19a), 1.33 (m, 1H, H-19b), 1.26 (dd, J = 12.0, 4.1 Hz, 1H, H-17eq), 0.55 (t, J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.2 (C-2), 140.7 (C-8), 139.3 ($CH_2(Cq)_{ar}$), 136.9 (C-13), 132.6 (C-21), 131.2 (CH_{ar}), 129.0 (CH_{ar}), 128.5 (CH_{ar}), 128.2 (CH_{ar}), 128.1 (CH_{ar}), 127.8 (CH_{ar}), 126.4 (CH_{ar}), 119.6 (C-5), 116.8 (C-7), 108.5 (C-6), 49.0 (C-16), 45.6 (C-3), 40.1 (CH_2Ph), 39.4 (C-17), 38.4 (C-20), 36.1 (C-19), 28.8 (C-15), 20.0 (C-14), 8.6 (C-18) ppm; $[\alpha]_D^{24}$ −18 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3436, 2961, 1651 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{28}N_2NaO$ [$(M+Na)^+$]: 407.2099, found: 407.2084.

4.36. 16(*R*)-Benzoylrhazinilam (6dα)

White powder (30 mg, 25% from 91 mg rhazinilam); HPLC (preparative Hypercarb column, $MeOH/CH_2Cl_2$ 3:2, 3.0 mL/min) t_R 10.0 min; mp 186 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.71 (m, 2H, H_{ar}), 7.58–7.47 (m, 3H, H_{ar}), 7.42 (m, 3H, H_{ar}), 7.27 (m, 1H, H_{ar}), 6.60 (br s, 1H, H-1), 6.49 (d, J = 2.7 Hz, 1H, H-5), 5.73 (d, J = 2.7 Hz, 1H, H-6), 4.43 (d, J = 10.7 Hz, 1H, H-16), 4.00 (dd, J = 12.0, 5.3 Hz, 1H, H-3eq), 3.80 (ddd, J = 12.3, 12.3, 4.5 Hz, 1H, H-3ax), 2.79 (dd, J = 14.4, 10.7 Hz, 1H, H-17ax), 2.34 (ddddd, J = 13.2, 13.2, 13.2, 5.4, 3.3 Hz, 1H, H-14ax), 1.88 (m, 2H, H-14eq and H-17eq), 1.76 (ddd, J = 13.5, 13.5, 3.3 Hz, 1H, H-15ax), 1.67 (m, 1H, H-15eq), 1.52 (m, 1H, H-19a), 1.33 (m, 1H, H-19b), 0.70 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 196.0 (*C*OPh), 172.1 (C-2), 141.1 (C-8), 136.8 ($(Cq)_{ar}$), 136.5 ($(Cq)_{ar}$), 133.1 (CH_{ar}), 132.0 (CH_{ar}), 130.5 (C-21), 128.5 (CH_{ar}), 128.4 (CH_{ar}), 128.1 (CH_{ar}), 128.0 (CH_{ar}), 127.4 (CH_{ar}), 119.3 (C-5), 116.8 (C-7), 109.7 (C-6), 47.1 (C-16), 46.1 (C-3), 40.7 (C-17), 38.2 (C-20), 33.6 (C-15), 30.4 (C-19), 19.4 (C-14), 8.2. (C-18) ppm; $[\alpha]_D^{24}$ −108 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3307, 2961, 1699, 1651 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{26}N_2NaO_2$ [$(M+Na)^+$]: 421.1892, found: 421.1874.

4.37. (*S*)-Benzoylrhazinilam (6dβ)

White powder (7 mg, 5% from 91 mg rhazinilam); HPLC (preparative Hypercarb column, $MeOH/CH_2Cl_2$ 3:2, 3.0 mL/min) t_R 22.3 min; mp 186 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.09 (dd, J = 8.3, 1.2 Hz, 2H, CO(*o*-H_{ar})), 7.55 (dd, J = 7.4, 7.4 Hz, 1H, CO(*p*-H_{ar})), 7.45 (dd, J = 7.7, 7.7 Hz, 2H, CO(*m*-H_{ar})), 7.37 (m, 2H, H_{ar}), 7.31 (ddd, J = 7.5, 7.5, 1.9 Hz, 1H, H_{ar}), 7.07 (d, J = 7.8 Hz, 1H, H_{ar}), 6.56 (d, J = 2.7 Hz, 1H, H-5), 6.29 (br s, 1H, H-1), 5.84 (d, J = 2.7 Hz, 1H, H-6), 4.57 (ddd, J = 13.1, 4.8, 1.7 Hz, 1H, H-16), 4.00 (ddd, J = 13.7, 2.9, 2.9 Hz, 1H, H-3eq), 3.92 (ddd, J = 11.9, 11.9, 5.0 Hz, 1H, H-3ax), 2.18 (m, 1H, H-17ax), 2.16 (m, 2H, H-14a and H-15a), 1.94 (m, 1H, H-14b), 1.66 (dd, J = 9.9, 5.4 Hz, 1H, H-15b), 1.58 (m, 1H, H-19a), 1.52 (dd, J = 14.2, 4.5 Hz, 1H, H-17eq), 1.47 (m, 1H, H-19b), 0.67 (t,J = 7.5 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 195.5 (*C*OPh), 170.9 (C-2), 140.1 (C-8), 136.5 ($(Cq)_{ar}$), 136.1 ($(Cq)_{ar}$), 133.4 (CH_{ar}), 132.3 (C-21), 130.7 (CH_{ar}), 129.0 (CH_{ar}), 128.8 (CH_{ar}), 128.6 (CH_{ar}), 128.5 (CH_{ar}), 127.9 (CH_{ar}), 119.9 (C-5), 117.1 (C-7), 108.7 (C-6), 55.7 (C-16), 45.7 (C-3), 38.2 (C-20), 36.2 (C-17 and C-19), 28.8 (C-15), 20.0 (C-14), 8.6 (C-18) ppm; $[\alpha]_D^{24}$ −99 (*c* 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3241, 2925, 1651 cm^{-1}; HRMS (ESI) calcd for $C_{26}H_{26}N_2NaO_2$ [$(M+Na)^+$]: 421.1892, found: 421.1892.

4.38. 16(*S*)-*tert*-Butoxycarbonylrhazinilam (6eα)

Oil (45 mg, 10% from 319 mg rhazinilam); ^1H NMR (300 MHz, $CDCl_3$) δ 7.42 (m, 1H, H_{ar}), 7.38–7.28 (m, 2H, H_{ar}), 7.23 (m, 1H, H_{ar}), 6.66 (br s, 1H, H-1), 6.48 (d, J = 2.7 Hz, 1H, H-5), 5.75 (d, J = 2.4 Hz, 1H, H-6), 3.99 (dd, J = 11.7, 5.1 Hz, 1H, H-3eq), 3.77 (ddd,

J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 3.39 (d, J = 11.1 Hz, 1H, H16), 2.53 (dd, J = 14.4, 11.1 Hz, 1H, H-17ax), 2.24 (ddddd, J = 13.0, 13.0, 13.0, 5.4, 3.3 Hz, 1H, H-14ax), 1.85 (m, 2H, H-14eq and H-17eq), 1.71 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.61 (m, 1H, H-15eq), 1.42 (s, 9H, C(CH_3)$_3$), 1.33 (m, 1H, H-19a), 1.25 (m, 1H, H-19b), 0.69 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.5 (C-2), 169.0 ($CO_2C(CH_3)_3$), 140.7 (C-8), 137.5 (C-13), 131.5 (CH_{ar}), 130.4 (C-21), 128.2 (CH_{ar}), 127.7 (CH_{ar}), 127.4 (CH_{ar}), 119.1 (C-5), 116.9 (C-7), 109.6 (C-6), 81.3 ($C(CH_3)_3$), 46.2 (C-3), 44.9 (C-16), 39.0 (C-17), 38.2 (C-20), 33.5 (C-15), 30.4 (C-19), 27.8 (C(CH_3)$_3$), 19.4 (C-14), 8.1 (C-18) ppm; $[\alpha]_D^{24}$ −148 (c 0.35, $CHCl_3$); IR (film) $\tilde{\nu}$ 3315, 2965, 1732, 1681 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{30}N_2NaO_3$ [(M+Na)$^+$]: 417.2154, found: 417.2126.

4.39. 1-Perfluoro-*tert*-butoxycarbonylrhazinilam (5p)

To a solution of perfluoro- *tert*-butyl alcohol (47 μL, 0.34 mmol) in dichloromethane (1 mL) under argon at 0 °C were added a solution of triphosgene (50 mg, 0.17 mmol) in dichloromethane (2 mL) and dry pyridine (136 μL, 1.70 mmol). The mixture was stirred for 30 min at 0 °C and for 4.5 h at 20 °C, then a solution of (−)-rhazinilam **1** (100 mg, 0.34 mmol) in dichloromethane (2 mL) was added dropwise. After stirring for 45 min at 20 °C, a saturated aq solution of $NaHCO_3$ was added and the aqueous layer was extracted with dichloromethane. The organic layer was washed with a 1 M aq solution of $CuSO_4$, dried over $MgSO_4$, and the solvent was evaporated under vacuum. The residue was purified by preparative TLC (heptane/ethyl acetate 1:1) to give compound **5p** as an oil (23 mg, 12%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (dd, J = 6.9, 2.1 Hz, 1H, H_{ar}), 7.42 (ddd, J = 7.5, 7.5, 2.1 Hz, 1H, H_{ar}), 7.37 (ddd, J = 7.2, 7.2, 1.8 Hz, 1H, H_{ar}), 7.21 (dd, J = 7.2, 2.0 Hz, 1H, H_{ar}), 6.53 (d, J = 3.0 Hz, 1H, H-5), 5.75 (d, J = 2.7 Hz, 1H, H-6), 4.01 (dd, J = 12.0, 4.8 Hz, 1H, H-3eq), 3.77 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 2.49 (dd, J = 12.1, 12.1 Hz, 1H, H-17ax), 2.41 (dd, J = 12.6, 12.6 Hz, 1H, H-16ax), 2.22 (ddddd, J = 13.5, 13.5, 13.5, 5.4, 3.0 Hz, 1H, H-14ax), 2.07 (dd, J = 12.6, 6.6 Hz, 1H, H-16eq), 1.88 (m, 1H, H-14eq), 1.75 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.46 (m, 1H, H-17eq), 1.40 (m, 1H, H-19a), 1.16 (m, 1H, H-19b), 0.75 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 173.4 (C-2), 152.3 ((Cq)$_{ar}$), 138.5 ((Cq)$_{ar}$), 131.7 (CH_{ar}), 130.2 ((Cq)$_{ar}$), 128.7 (CH_{ar}), 128.4 (CH_{ar}), 126.6 (CH_{ar}), 120.3 (C-5), 118.0 ((Cq)$_{ar}$), 108.9 (C-6), 46.2 (C-3), 39.1 (C-20), 37.5 (C-17), 32.4 (C-15), 32.2 (C-16), 30.4 (C-19), 19.5 (C-14), 8.5 (C-18) ppm; ^{19}F NMR (282 MHz, $CDCl_3$) δ −69.5 ppm; $[\alpha]_D^{24}$ −382 (c 0.64, $CHCl_3$); IR (film) $\tilde{\nu}$ 2921, 1822, 1687 cm^{-1}; HRMS (ESI) calcd for $C_{24}H_{21}F_9N_2NaO_3$ [(M+Na)$^+$]: 579.1306, found: 579.1312.

4.40. 16(*S*)-Hydroxyrhazinilam (6f)

This compound was obtained as a side-product during the conversion of (+)-vincadifformine **7** into (−)-rhazinilam **1**;[5] beige powder; mp 228 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.32 (m, 3H, H_{ar}), 7.22 (m, 1H, H_{ar}), 7.08 (br s, 1H, H-1), 6.48 (d, J = 2.7 Hz, 1H, H-5), 5.82 (d, J = 2.7 Hz, 1H, H-6), 4.13 (m, 1H, H-16), 3.96 (ddd, J = 12.0, 4.2, 4.2 Hz, 1H, H-3eq), 3.83 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 3.30 (d, J = 7.5 Hz, OH), 2.39 (dd, J = 11.4, 11.4 Hz, 1H, H-17ax), 2.09 (m, 1H, H-14ax), 1.62 (dd, J = 11.4, 2.4 Hz, 1H, H-17eq), 1.90 (m, 1H, H-14eq), 1.71 (m, 2H, H-15ax and H-15eq), 1.41 (m, 1H, H-19a), 1.32 (m, 1H, H-19b), 0.70 (t, J = 7.2 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 177.5 (C-2), 140.2 (C-8), 135.4 (C-13), 131.3 (CH_{ar}), 130.8 (C-21), 128.3 (CH_{ar}), 128.2 (CH_{ar}), 127.7 (CH_{ar}), 119.2 (C-5), 116.8 (C-7), 109.1 (C-6), 65.7 (C-16), 45.9 (C-3 and C-17), 37.6 (C-20), 33.0 (C-15), 31.6 (C-19), 19.4 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{23}$ −168 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3386, 3062, 2934, 2873, 1680 cm^{-1}; HRMS (ESI) calcd for $C_{19}H_{22}N_2NaO_2$ [(M+Na)$^+$]: 333.1579, found: 333.1585.

4.41. 16(*S*)-Acetoxyrhazinilam (6g)

To a solution of compound **6f** (57 mg, 0.18 mmol) in dry pyridine (2.2 mL) under argon at 20 °C was added acetic anhydride (1.1 mL, 11.8 mmol). After stirring for 24 h, dichloromethane and water were added and the aqueous layer was extracted with dichloromethane. The organic layer was washed with a 1 M aq solution of $CuSO_4$, dried over $MgSO_4$, and the solvent was evaporated under vacuum. The crude mixture was purified by preparative TLC (heptane/ethyl acetate 1:4), to give compound **6g** (42 mg, 58%) as a beige solid, and 1-acetyl-16(*S*)-acetoxyrhazinilam (29 mg, 32%); mp 230 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.33 (m, 4H, H_{ar}), 6.68 (br s, 1H, H-1), 6.50 (d, J = 3.0 Hz, 1H, H-5), 5.80 (d, J = 2.7 Hz, 1H, H-6), 4.96 (dd, J = 11.1, 1.5 Hz, 1H, H-16), 4.00 (dd, J = 12.0, 4.8 Hz, 1H, H-3eq), 3.80 (ddd, J = 12.0, 12.0, 4.8 Hz, 1H, H-3ax), 2.72 (dd, J = 13.8, 11.1 Hz, 1H, H-17ax), 2.18 (ddddd, J = 13.2, 13.2, 13.2, 5.4, 3.0 Hz, 1H, H-14ax), 2.06 (s, 3H, COCH_3), 1.86 (m, 1H, H-14eq), 1.72 (ddd, J = 13.2, 13.2, 3.0 Hz, 1H, H-15ax), 1.63 (m, 2H, H-15eq and H-17eq), 1.46 (m, 1H, H-19a), 1.33 (m, 1H, H-19b), 0.70 (t, J = 7.4 Hz, 3H, H-18) ppm; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 172.8, 170.3 (C-2 and COCH$_3$), 140.1, 135.6 (C-8 and C-13), 131.1 (CH_{ar}), 129.8 (C-21), 128.3 (3 CH_{ar}), 119.3 (C-5), 117.4 (C-7), 109.6 (C-6), 67.7 (C-16), 46.1 (C-3), 43.0 (C-17), 37.3 (C-20), 33.9 (C-15), 30.8 (C-19), 20.9 (COCH_3), 19.5 (C-14), 8.2 (C-18) ppm; $[\alpha]_D^{24}$ −288 (c 1.0, $CHCl_3$); IR (film) $\tilde{\nu}$ 3471, 2962, 1741, 1681 cm^{-1}; HRMS (ESI) calcd for $C_{21}H_{24}N_2NaO_3$ [(M+Na)$^+$]: 375.1685, found: 375.1683.

4.42. X-ray diffraction analysis for compounds 1, 5o, and 6bα

For all structures, data were collected with a Nonius Kappa-CCD diffractometer (Mo Kα, graphite monochromator).[22a] The absolute configuration of (−)-rhazilinam **1** was assigned to each compound. The structures have been solved by direct methods using program *SHELXS86*[22b] and refined by program *SHELXL93*[22c] using full matrix least-squares based on F^2. All details are listed in the experimental section. Refinement was

disturbed only in compound **6bα** by the presence of one desordered solvent molecule (ethanol) in the unit cell. CCDC253697 (compound **1**), CCDC253698 (compound **5o**), and CCDC253699 (compound **6bα**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB 1EZ, UK; fax/(+44)1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

4.43. Inhibition of microtubule disassembly

The drug, dissolved in DMSO at different concentrations, was added to a solution of microtubules (tubulin concentration ca. 20 μM, freshly prepared from sheep brain) at 37 °C. Then the solution was placed in a temperature-controlled cell at 9 °C (microtubule disassembly) and the decrease of the optical density was monitored in a UV spectrophotometer at 350 nm for 1 min. The maximum rate of disassembly was recorded and compared to that of a sample without drug. The IC_{50} of the compound was calculated from the effect of several concentrations and compared to the IC_{50} of rhazinilam obtained within the same day with the same tubulin preparation.

4.44. Inhibition of tubulin assembly

The assay was conducted in a reverse manner as above: the drug, dissolved in DMSO at different concentrations, was added to a solution of free tubulin at 0 °C. Then the solution was placed in a temperature-controlled cell at 37 °C (microtubule assembly) and the increase of the optical density was monitored in a UV spectrophotometer at 350 nm for 1 min. The maximum rate of assembly was recorded and compared to a sample without drug. The IC_{50} of the compound was calculated from the effect of several concentrations and compared to the IC_{50} of rhazinilam obtained within the same day with the same tubulin preparation.

4.45. Cytotoxicity assays

The effect of the drugs on the growth of KB human cell lines was monitored at the Laboratoire de Cultures Cellulaires, ICSN, Gif-sur-Yvette, France. The IC_{50} value refers to the concentration of drug corresponding to 50% growth inhibition after 72 h incubation.[23]

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