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Synthesis and Biological Evaluation of A-Ring Biaryl-Carbamate Analogues of Rhazinilam

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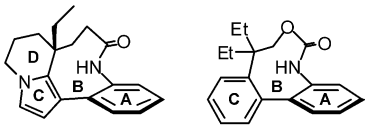
Abstract—An improvement of the synthesis of biphenyl-carbamate **2a**, the most active analogue of rhazinilam **1** so far, was performed using the Pd-catalyzed borylation/Suzuki coupling (BSC) method developed in our laboratories. The preparation of A-ring analogues of **2a** bearing electron-withdrawing or donating groups is reported according to this new synthetic scheme. The antitubulin properties as well as the cytotoxicity of these compounds toward human cancer cell lines were evaluated in comparison with rhazinilam and **2a**.

Introduction



1 **2a**

(−)-Rhazinilam **1** is a tetracyclic alkaloid isolated from various *Apocynaceae*,[1] possessing an axially chiral phenyl-pyrrole subunit and a 9-membered median lactam ring. It was found to inhibit in vitro both microtubules disassembly and assembly, the latter phenomenon being imputable to the formation of abnormal tubulin spirals.[2] As a consequence to these unique antitubulin properties, rhazinilam showed significant in vitro cytotoxicity toward various cancer cell lines,[2] but no activity was found in vivo. As part of a program directed toward the semi- and total synthesis of **1** and analogues,[3] we showed that biphenyl-carbamate analogue (−)-**2a** was the most active analogue so far, with a 2-fold activity on microtubules disassembly compared to **1** and a similar cytotoxicity.[4] In continuation with this study, we wanted to analyze the influence of the A-ring substitution on the biological activity. Indeed, among the numerous rhazinilam analogues tested, only two possessed one A-ring substituent (in the position *para* to the nitrogen).[5] We report therein an improved synthesis of racemic **2a** as well as the use of this new synthetic pathway to obtain A-ring analogues of **2a** in a straightforward fashion. These analogues were biologically evaluated and compared with **1** and **2a**.

Results and Discussion

Chemistry

Our previous synthesis of racemic **2a** was based on a Stille coupling between Boc-protected stannane **3** and triethylsilyl (TES)-protected iodophenyl alcohol **4a**, furnishing biphenyl **5** in 49% yield (Scheme 1). Stannane **3** was obtained by directed *ortho*-metalation of Boc-protected aniline followed by transmetalation with Bu_3SnCl.[6] Iodide **4a** was obtained in four steps, 40% overall yield, from commercially available material.[4] After removal of the protecting groups with TFA, cyclization occured smoothly in the presence of triphosgene, giving racemic **2a** in 88% yield. The active atropisomer (−)-**2a** was obtained after chiral column HPLC separation. The atropisomer (+)-**2a**, like (+)-rhazinilam,[5] is biologically inactive.[4]

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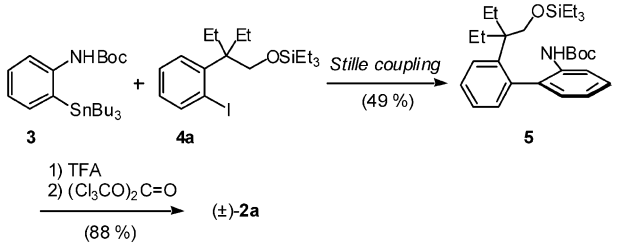
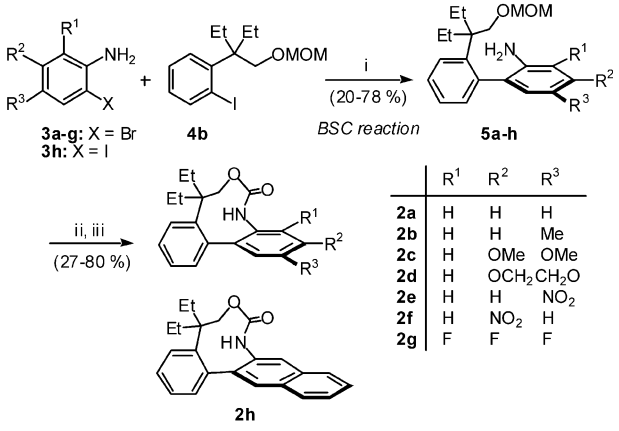


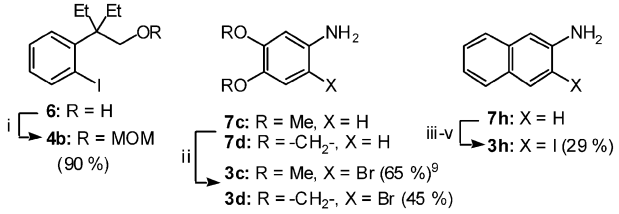
Scheme 1. Original synthesis of biphenyl-carbamate analogue **2a**.[4]

In prospect of synthesizing various A-ring analogues, we looked for a more direct and flexible synthetic method, in particular we were concerned that the *ortho*-metalation approach could not be compatible with a great variety of substituents on the aniline ring. Besides, we wished to replace the Stille coupling by the more biologically-friendly tin-free Suzuki coupling. We recently developed a one-pot palladium-catalyzed borylation/Suzuki coupling (BSC) approach which enables the straightforward and efficient synthesis of 2,2′-disubstituted biaryl compounds from two aryl halides (Scheme 2).[7]

Thus, unprotected 2-bromoaniline **3a** (R = H) was submitted to the Pd-catalyzed borylation with pinacolborane, followed by in situ Suzuki coupling with phenyl iodide **4b**, giving biphenyl **5a** in 78% yield. The smaller MOM group was chosen instead of TES as a protecting group for the primary alcohol **6** (Scheme 3) to decrease unfavorable steric interactions during the coupling.[8] Then, after deprotection of the MOM group with HCl in refluxing methanol, the urethane group was installed as previously with triphosgene, furnishing **2a** in 80% yield (62% from **4b**). This synthetic pathway enables the fast incorporation of A-ring substituents from substituted 2-bromoanilines in three steps, without protection of the amino group. According to this strategy, substituted biaryl-carbamates **2b–h** (Scheme 2) were synthesized from 2-halogenoanilines **3b–h** in satisfying



Scheme 2. Synthesis of rhazinilam A-ring biaryl analogues **2a–h**. Reagents and conditions: (i) **3a–h**, (pin)BH, Et_3N, $Pd(OAc)_2$, PCy_2 (*o*-biph), dioxane, 80 °C, 1 h, then H_2O, **4b**, $Ba(OH)_2$, 100 °C, 1 h; (ii) concd HCl, MeOH, reflux, 1 h; (iii) $(Cl_3CO)_2C{=}O$, pyridine, CH_2Cl_2, −78 °C, 30 min. MOM = methoxymethyl, pin = pinacol, PCy_2 (*o*-biph) = 2-(dicyclohexylphosphino)biphenyl.



Scheme 3. Synthesis of aryl building blocks. Reagents and conditions: (i) MOMCl, *N*,*N*-diisopropylethylamine, CH_2Cl_2, 0 °C to rt, 16 h; (ii) *n*-Bu_4NBr_3, CH_2Cl_2, MeOH, rt, 30 min; (iii) NaHMDS, THF, rt, 30 min, then Boc_2O, 30 min; (iv) *t*-BuLi, THF, −20 °C, 2 h, then ICH_2CH_2I, −78 °C to rt, 3 h; (v) TFA, CH_2Cl_2, 0 °C, 30 min.

yields (10–28% overall, unoptimized). 2-Halogenoanilines **3b–h** were commercially available or prepared in few steps from the corresponding anilines (Scheme 3).

The electron-donating (**3b–d**) or withdrawing (**3e–h**) substituents of these building blocks were deliberately chosen in order to study the effect of the electronic character of ring A on the biological activity. Dimethoxy- and methylenedioxy-substituted bromoanilines **3c** and **3d** were obtained by bromination of the corresponding anilines with tetrabutylammonium tribromide.[9] The known 2-amino-3-iodonaphthalene **3h**[10] was synthesized in a convenient manner from 2-aminonaphthalene by *t*-Boc protection, regioselective directed *ortho*-metallation followed by quenching with diiodoethane (78/22 ratio of 3- and 1-iodo isomers) and cleavage of the *t*-Boc group.

Biological evaluation

The cytotoxicity and antitubulin activity of the new analogues **2b–h** were evaluated and compared to those of (−)-**1** and (±)-**2a** (Table 1). It should be noted that compounds **2a–h** tested therein were racemic. It was shown earlier that, as (+)-rhazinilam and (+)-**2a** are inactive, the activity of the racemic mixtures is twice lower than that of the levo atropisomer.[4]

Table 1. Cytotoxicity and antitubulin activity of (−)-rhazinilam and racemic A-ring analogues **2a–h**

Compd	Cytotoxicity KB cell line IC_{50} (μM)[a]	Cytotoxicity MCF7 cell line IC_{50} (μM)[a]	Inhibition of microtubules assembly IC_{50} (μM)[b]	Inhibition of microtubules disassembly IC_{50} (μM)[b]
1	0.6	4.0	6.7	3.7
2a	1.1	6	6.5	3.4
2b	2.9	16	160	na
2c	>20	18	190	—[d]
2d	19	20	40	39
2e	11	6.5	100	200
2f	3.7	9	12	6.3
2g	4.0	8	73	19
2h	5.5	3.0	—[c]	—[c]

[a]IC_{50} is the concentration of compound corresponding to 50% growth inhibition after 72 h incubation.
[b]IC_{50} is the concentration of compound required to inhibit 50% of the rate of microtubules assembly or disassembly (na = not active).
[c]Unable to determine (low solubility).
[d]200 times less active than **1** at 30% inhibition.

The cytotoxicity of **1** and **2a** on human breast adenocarcinoma MCF7 cells as well as the IC_{50} values of these compounds for the inhibition of tubulin polymerization have not been reported earlier.

The IC_{50} values of rhazinilam for tubulin polymerization and depolymerization are comparable and therefore are probably both responsible for the cytotoxicity toward KB and MCF7 cells. It was shown earlier that this unique property is related to the abitily of rhazinilam to form spirallike complexes with tubulin.[2] Racemic unsubstituted biphenyl analogue **2a** was found to be as active as rhazinilam on both microtubules formation and dissociation and twice less cytotoxic towards KB cells, consistently with previous reports.[4] Similarly, **2a** was 1.5 times less cytotoxic than rhazinilam on MCF7 cells.

Racemic A-ring biphenyl-carbamate analogues **2b–h** were markedly less active than unsubstituted **2a** on the tubulin test, except the nitro derivative **2f** which showed two-fold IC_{50} values. Thus, by analogy with **2a**, the (−)-atropisomer of **2f** should be as active as (−)-rhazinilam. Consistently, the IC_{50} values of **2b–h** on KB cells were all smaller (3 to more than 20 times) than that of **2a**, **2f** being again the most active compound. Less difference between **2b–h** and **2a** was observed on MCF7 cells, with IC_{50} values from 0.5 to 3 times that of **2a**. Interestingly, **2h** was slightly more active than **2a** and rhazinilam on this cell line. For low solubility reasons, we were unable to determine if this cytotoxicity originated in the antitubulin properties of **2h**. For other analogues **2b–g**, it appears that the cytotoxicity toward both cancer cell lines can be only partially linked to the antitubulin effect. For instance compounds **2b** and **2e** had a very weak antitubulin effect but were substantially cytotoxic, which suggests that these cytotoxicities arise from other factors. Further investigation is underway to explain these observations.

Finally, the electron-withdrawing or donating character of A-ring substituents seems to have no clear effect on the activity of these analogues. On the other hand, the present results suggest that the increase of the steric hindrance on ring A induced by the addition of substituents is harmful to the antitubulin activity and to the cytotoxicity, as particularly shown with compound **2c**.

In conclusion, racemic biaryl-carbamate analogues of rhazinilam, bearing a variety of electron-withdrawing or donating substituents on ring A, were synthesized in a straightforward fashion by the BSC method. These compounds were all less active on KB cancer cells and tubulin than the unsubstituted analogue **2a**, presumably due to steric factors. However nitro-substituted compound **2f** retained an interesting activity. On MCF7 cancer cells, biphenyls **2b–g** were all less active than rhazinilam and **2a**, except **2h** which was slightly more active. Further biological testing is required to explain the discrepancies observed among the present assays. The present work also suggests future modifications on B and C-rings to increase the antitumor potential of compound **2a**.

Experimental Protocols

Chemistry

Reagents were commercially available and used without further purification unless otherwise stated. Solvents were distilled under argon over appropriate drying agents immediately before use. NMR spectra were recorded on Bruker AC-250 or AC-300 instruments and calibrated using tetramethylsilane as an internal reference. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. IR spectra were recorded on a Nicolet FT-IR 205 spectrometer. High resolution mass spectra (HRMS) were recorded under liquid secondary ion (LSIMS), Matrix Assisted Laser Desorption Ionization (MALDI) or Chemical Ionization (CI) conditions at the Laboratoire de Spectrométrie de Masse, ICSN, Gif-sur-Yvette, France or at the Laboratoire Central d'Analyse du CNRS, Vernaison, France. The physical data of compounds **3c**,[9] **3h**,[10] **6**,[4] and **2a**[4] were previously described.

[2-Ethyl-2-(2-iodophenyl)butoxy]methoxymethane (4b). To a stirred solution of alcohol **6** (8.0 g, 26.3 mmol) in dry dichloromethane (130 mL) under argon at 0 °C was added *N*,*N*-diisopropylethylamine (16 mL, 91.9 mmol) and chloromethyl methyl ether (6 mL, 79.0 mmol) dropwise. The temperature was allowed to warm to rt for 16 h, then a saturated aqueous $NaHCO_3$ solution (200 mL) was added and the aqueous phase was extracted with dichloromethane. The organic solution was dried over $MgSO_4$ and evaporated under vaccum. The residue was purified by flash chromatography (silica gel, heptane/ethyl acetate 9/1) to afford **4b** as a colorless oil (8.2 g, 90%); ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.68$ (t, $J = 7.5$ Hz, 6H), 1.93 (dq, $J = 15$, 7.5 Hz, 2H), 2.35 (dq, $J = 15$, 7.5 Hz, 2H), 3.38 (s, 3H), 3.97 (s, 2H), 4.69 (s, 2H), 6.85 (td, $J = 7.4$, 1.8 Hz, 1H), 7.20 (dd, $J = 8.4$, 1.8 Hz, 1H), 7.29 (td, $J = 7.5$, 1.2 Hz, 1H), 8.04 (dd, $J = 7.8$, 1.0 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 8.5$ (*C*H$_3$), 25.4 (*C*H$_2$), 46.8 (*C*q), 55.4 (*C*H$_3$), 69.4 (*C*H$_2$), 94.5 (*C*q), 96.8 (*C*H$_2$), 127.5 (*C*H), 127.6 (*C*H), 130.4 (*C*H), 143.8 (*C*H), 144.4 (*C*q) ppm; HRMS (LSIMS) calcd for $C_{14}H_{21}ILiO_2$ [$(M+Li)^+$]: 355.0746; found: 355.0770.

2-Bromo-4,5-methylenedioxyaniline (3d). *n*-Bu_4NBr_3 (4 g, 8.3 mmol) was added to a solution of 3,4-methylenedioxyaniline (1 g, 7.3 mmol) in dichloromethane (27 mL) and methanol (13 mL). After 20 min stirring at rt, the solution was diluted with diethyl ether (30 mL), the organic solution was washed successively with a saturated aqueous Na_2SO_3 solution (30 mL) and with brine. After drying over $MgSO_4$, the solvents were removed under vacuum and the residue was purified by flash chromatography (silica gel, heptane/ethyl acetate 4/1-7/3), to afford **3d** as an oil (708 mg, 45%); ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 3.74$ (br s, 2H), 5.87 (s, 2H), 6.36 (s, 1H), 6.87 (s, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 97.8$, 98.8, 101.3, 112.0, 138.8, 140.9, 148.1 ppm; IR (film) $\nu = 1636$, 3186, 3297, 3397 cm^{-1}; HRMS (MALDI) calcd for $C_7H_6BrNO_2$ [$M^{+\bullet}$]: 214.9582; found: 214.9609.

2-Amino-3-iodonaphthalene (3h). To a solution of 2-aminonaphthalene (1.025 g, 7.2 mmol) in dry THF (20 mL) under argon at rt was added dropwise sodium bis(trimethylsilyl)amide (2 M solution in THF, 7.5 mL, 15.0 mmol). After 30 min stirring, a solution of di-*tert*-butyl dicarbonate (1.640 g, 7.5 mmol) in 2 mL dry THF was added dropwise and the resulting mixture was stirred for 30 min. A saturated aqueous NH_4Cl solution was added, and the solution was extracted with dichloromethane. After drying over $MgSO_4$, the solvents were removed under vacuum and the residue was purified by flash chromatography (silica gel, heptane/ethyl acetate 4/1) to afford *t*-Boc-protected 2-aminonaphthalene as pale cristals (1.443 g, 83%); ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 1.53$ (s, 9H), 6.78 (br s, 1H), 7.35 (m, 3H), 7.69 (m, 3H), 7.96 (s, 1H) ppm; ^{13}C NMR (62.5 MHz, $CDCl_3$) $\delta = 28.3$, 80.5, 114.5, 119.1, 124.3, 126.3, 127.3, 127.4, 128.6, 129.9, 133.9, 135.8, 152.8 ppm. To a solution of this compound (500 mg, 2.1 mmol) in 10 mL dry THF under argon at −20 °C was added dropwise *tert*-butyl lithium (1.5 M solution in pentane, 3.43 mL, 5.1 mmol) and the resulting solution was stirred for 2 h at −20 °C. After cooling at −78 °C, a solution of diiodoethane (1.448 g, 5.1 mmol) in 5 mL dry THF was added dropwise and the temperature was allowed to warm to rt for 3 h. A saturated aqueous NH_4Cl solution was added, and the solution was extracted with diethyl ether. The resulting organic solution was washed with a saturated aqueous Na_2SO_3 solution and dried over $MgSO_4$. The solvents were evaporated under vacuum and the residue was purified by flash chromatography (silica gel, heptane/ethyl acetate 95/5) to afford 471 mg of a 78/22 mixture (NMR) of regioisomeric 3-iodo and 1-iodo *t*-Boc-protected 2-aminonaphthalene. This mixture (440 mg) was dissolved in dichloromethane (5 mL) and trifluoroacetic acid (5 mL) was added dropwise at 0 °C. After 30 min stirring at 0 °C, the solution was neutralized with a concentrated NaOH solution and the aqueous layer was extracted with dichloromethane. The solution was dried over $MgSO_4$, evaporated and the resulting solid was washed with heptane, yielding **3h** as a white powder (184 mg, 35%), which had identical physical properties to the previously described compound.[10]

General procedure for the synthesis of biphenylcarbamates 2a–h from anilines 3a–h and iodide 4b

2-(2′-Aminobiphenyl-2-yl)-2-ethylbutoxymethoxy methane (5a). To a solution of 2-bromoaniline **3a** (296 mg, 1.7 mmol) in dry dioxane (5 mL) at 25 °C under argon were successively added dry Et_3N (0.96 mL, 6.9 mmol), $Pd(OAc)_2$ (19 mg, 0.086 mmol), PCy_2(*o*-biph) (121 mg, 0.34 mmol), and pinacolborane (0.75 mL, 5.2 mmol) dropwise. The solution was heated to 80 °C for 1 h. After cooling, degased water (1.5 mL) was added dropwise, then **4b** (400 mg, 1.1 mmol) in degased dioxane (1 mL), and $Ba(OH)_2 \cdot 8H_2O$ (1.63 g, 5.2 mmol) were added and the mixture was heated to 100 °C for 1 h. After cooling, the solution was filtered through Celite, brine was added and the aqueous layer was extracted with dichloromethane. After drying over $MgSO_4$, the solvents were evaporated under vacuum and the residue was purified by flash chromatography (silica gel, heptane/ethyl acetate 95/5–9/1) to afford **5a** as an oil (282 mg, 78% from **4b**); ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.70$ (2t, $J = 7.5$ Hz, 6H), 1.70 (m, 4H), 3.28 (s, 3H), 3.47 (br s, 2H), 3.59 (d, $J = 9.6$ Hz, 1H), 3.63 (d, $J = 9.6$ Hz, 1H), 4.49 (d, $J = 6.6$ Hz, 1H), 4.53 (d, $J = 6.3$ Hz, 1H), 6.73 (m, 2H), 7.02 (dd, $J = 7.7$, 2.2 Hz, 1H), 7.03 (dd, $J = 7.5$, 1.5 Hz, 1H), 7.15 (td, $J = 7.5$, 1.8 Hz, 1H), 7.25 (td, $J = 7.5$, 1.5 Hz, 1H), 7.33 (td, $J = 7.2$, 1.7 Hz, 1H), 7.42 (m, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 8.8$, 28.0, 47.5, 55.6, 70.0, 96.9, 115.1, 117.4, 126.2, 127.6, 128.3, 129.4, 130.3, 130.5, 133.3, 138.6, 143.3, 144.3 ppm; IR (film) $\nu = 1615$, 2962, 3369, 3469 cm^{-1}; HRMS (LSIMS) calcd for $C_{20}H_{27}LiNO_2$ $[(M+Li)^+]$: 320.2202; found: 320.2209.

9,9-Diethyl-8,9-dihydro-5*H*-7-oxa-5-azadibenzo[a,c]cyclononen-6-one (2a). To a solution of compound **5a** (280 mg, 0.89 mmol) in methanol (4 mL) at rt was added 36% aqueous HCl (1 mL) and the mixture was refluxed for 1 h. After cooling to 0 °C, the solution was neutralized with concd. aqueous NaOH and extracted with dichloromethane. The organic solution was dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by flash chromatography (silica gel, heptane/ethyl acetate 7/3) to afford 2-(2′-aminobiphenyl-2-yl)-2-ethylbutan-1-ol[4] as an oil (208 mg, 86%). The conversion of this amino-alcohol to carbamate **2a** using triphosgene has been previously described (93% yield).[4]

Carbamate 2b. General procedure from 2-bromo-4-methylaniline (**3b**), 10% yield for three steps; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 0.67$ (t, $J = 7.7$ Hz, 3H), 0.95 (t, $J = 7.5$ Hz, 3H), 1.50–1.91 (m, 4H), 2.35 (s, 3H), 3.82 (d, $J = 10.5$ Hz, 1H), 4.24 (d, $J = 11$ Hz, 1H), 5.97 (br s, 1H), 6.66 (dd, $J = 7.7$, 1.5 Hz, 1H), 7.00 (d, $J = 8.8$ Hz, 1H), 7.13 (m, 2H), 7.19 (td, $J = 7.3$, 1.0 Hz, 1H), 7.35 (td, $J = 8.0$, 1.3 Hz, 1H), 7.49 (dd, $J = 8.5$, 1.2 Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 8.1$, 8.3, 21.0, 23.6, 25.0, 48.3, 73.8, 124.9, 126.1, 128.0, 128.3, 128.4, 130.5, 132.7, 133.9, 135.2, 139.6, 141.8, 144.4, 156.9 ppm; IR (film) $\nu = 1721$, 2964, 3256 cm^{-1}; HRMS (CI) calcd for $C_{20}H_{24}NO_2$ $[(M+H)^+]$: 310.1807; found: 310.1821.

Carbamate 2c. General procedure from 2-bromo-4,5-dimethoxyaniline (**3c**),[9] 11% yield for three steps; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 0.70$ (t, $J = 7.2$ Hz, 3H), 0.96 (t, $J = 7.2$ Hz, 3H), 1.5–1.9 (m, 4H), 3.86 (s, 3H), 3.86 (d, $J = 11$ Hz, 1H), 3.94 (s, 3H), 4.27 (d, $J = 11$ Hz, 1H), 5.84 (br s, 1H), 6.71 (s, 1H), 6.83 (s, 1H), 6.91 (dd, $J = 7.5$, 1.5 Hz, 1H), 7.23 (td, $J = 7.5$, 1.3 Hz, 1H), 7.35 (td, $J = 7.3$, 1.8 Hz, 1H), 7.5 (d, $J = 7.5$ Hz, 1H) ppm; ^{13}C NMR (62.5 MHz, $CDCl_3$) $\delta = 8.1$, 8.5, 23.4, 25.1, 48.3, 55.9, 56.1, 74.0, 108.9, 112.7, 126.3, 128.1, 128.6, 129.4, 133.0, 136.4, 139.2, 142.1, 146.2, 148.1, 157.2 ppm; IR (film) $\nu = 1732$, 2962, 3332 cm^{-1}; HRMS (CI) calcd for $C_{21}H_{26}NO_4$ $[(M+H)^+]$: 356.1862; found: 356.1840.

Carbamate 2d. General procedure from 2-bromo-4,5-methylenedioxyaniline (**3d**), 28% yield for three steps;

^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 0.70$ (t, $J = 7.2$ Hz, 3H), 0.93 (t, $J = 7.0$ Hz, 3H), 1.55–1.90 (m, 4H), 3.84 (d, $J = 11.5$ Hz, 1H), 4.25 (d, $J = 11.0$ Hz, 1H), 5.83 (br s, 1H), 6.03 (s, 2H), 6.64 (s, 1H), 6.77 (s, 1H), 6.85 (dd, $J = 7.5$, 1.5 Hz, 1H), 7.20 (td, $J = 7.3$, 1.3 Hz, 1H), 7.36 (td, $J = 7.3$, 1.8 Hz, 1H), 7.48 (d, $J = 8.7$ Hz, 1H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 8.1$, 8.4, 23.7, 24.8, 48.5, 73.9, 101.7, 106.5, 109.6, 126.4, 128.2, 128.6, 130.1, 133.0, 137.8, 139.3, 142.2, 145.2, 146.8, 157.1 ppm; IR (film) $\nu = 1717$, 2964, 3256 cm^{-1}; HRMS (LSIMS) calcd for $C_{20}H_{22}NO_4$ [$M^{+\bullet}$]: 339.1471; found: 339.1485.

Carbamate 2e. General procedure from 2-bromo-4-nitroaniline (**3e**), 19% yield for three steps; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.72$ (t, $J = 7.4$ Hz, 3H), 0.98 (t, $J = 7.5$ Hz, 3H), 1.65 (m, 2H), 1.81 (m, 1H), 1.91 (m, 1H), 3.84 (d, $J = 10.2$ Hz, 1H), 4.22 (d, $J = 10.2$ Hz, 1H), 6.70 (br s, 1H), 6.77 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.20 (d, $J = 8.1$ Hz, 1H), 7.25 (td, $J = 7.3$, 1.2 Hz, 1H), 7.43 (td, $J = 8.1$, 2.1 Hz, 1H), 7.55 (d, $J = 8.1$ Hz, 1H), 8.18 (s, 1H), 8.20 (dd, $J = 8.4$, 3.0 Hz) ppm; ^{13}C NMR (62.5 MHz, $CDCl_3$) $\delta = 8.1$, 23.9, 24.7, 48.4, 73.6, 123.1, 124.9, 125.1, 126.0, 128.2, 129.3, 132.5, 137.4, 141.8, 142.4, 144.7, 145.7, 155.7 ppm; IR (film) $\nu = 1736$, 2987, 3054 cm^{-1}; HRMS (LSIMS) calcd for $C_{19}H_{21}N_2O_4$ [$(M+H)^+$]: 341.1501; found: 341.1510.

Carbamate 2f. General procedure from 2-bromo-5-nitroaniline (**3f**), 15% yield for three steps; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.70$ (t, $J = 7.2$ Hz, 3H), 0.96 (t, $J = 7.2$ Hz, 3H), 1.57 (m, 2H), 1.81 (m, 1H), 1.92 (m, 1H), 3.83 (d, $J = 10.8$ Hz, 1H), 4.24 (d, $J = 10.8$ Hz, 1H), 6.53 (br s, 1H), 6.75 (dd, $J = 7.5$, 1.2 Hz, 1H), 7.24 (td, $J = 7.5$, 1.2 Hz, 1H), 7.44 (td, $J = 8.1$, 1.8 Hz, 1H), 7.46 (d, $J = 9.0$ Hz, 1H), 7.55 (d, $J = 8.1$ Hz, 1H), 7.97 (d, $J = 2.4$ Hz, 1H), 8.10 (dd, $J = 8.7$, 2.4 Hz) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) $\delta = 8.0$, 8.1, 24.1, 24.7, 48.4, 73.6, 120.1, 120.4, 126.6, 128.4, 129.3, 130.6, 131.8, 137.6, 137.7, 141.6, 147.2, 152.0, 155.9 ppm; IR (film) $\nu = 1728$, 2966, 3256 cm^{-1}; HRMS (CI) calcd for $C_{19}H_{21}N_2O_4$ [$(M+H)^+$]: 341.1501; found: 341.1505.

Carbamate 2g. General procedure from 2-bromo-4,5,6-trifluoroaniline (**3g**), 27% yield for three steps; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 0.71$ (t, $J = 7.2$ Hz, 3H), 0.96 (t, $J = 7.2$ Hz, 3H), 1.60 (m, 2H), 1.83 (m, 2H), 3.86 (d, $J = 10.5$ Hz, 1H), 4.23 (d, $J = 11.4$ Hz, 1H), 5.78 (br s, 1H), 6.80 (dd, $J = 7.8$, 2.2 Hz, 1H), 6.96 (ddd, $J_{HF} = 10.2$, 7.8, 1.8 Hz, 1H), 7.24 (td, $J = 7.8$, 0.9 Hz, 1H), 7.41 (td, $J = 8.1$, 1.5 Hz, 1H), 7.52 (d, $J = 8.1$ Hz, 1H) ppm; ^{13}C NMR (62.5 MHz, CD_3OD) $\delta = 8.3$, 8.5, 25.7, 26.6, 49.6, 74.7, 113.8 (dd, $J_{CF=}19$, 3 Hz), 124.9 (dd, $J_{CF} = 11$, 3 Hz), 127.6, 129.5, 130.3, 133.7, 138.2–142.2 (ddd, $J^1 = 247$ Hz), 138.2, 142.4, 143.4, 145.0–149.0 (ddd, $J^1 = 248$ Hz), 146.9–150.7 (ddd, $J^1 = 246$ Hz), 157.9 ppm; IR (film) $\nu = 1713$, 2964, 3233 cm^{-1}; HRMS (CI) calcd for $C_{19}H_{19}F_3NO_2$ [$(M+H)^+$]: 350.1368; found: 350.1378.

Carbamate 2h. General procedure from 2-amino-3-iodonaphthalene (**3h**), 28% yield for three steps; ^{1}H NMR (250 MHz, $CDCl_3$) $\delta = 0.68$ (t, $J = 7.2$ Hz, 3H), 0.95 (t, $J = 7.2$ Hz, 3H), 1.55 (m, 1H), 1.84 (m, 3H), 3.80 (d, $J = 11.2$ Hz, 1H), 4.27 (d, $J = 11.2$ Hz, 1H), 6.67 (br s, 1H), 6.85 (dd, $J = 7.5$, 1.2 Hz, 1H), 7.19 (td, $J = 7.2$, 1.0 Hz, 1H), 7.39 (td, $J = 7.6$, 1.5 Hz, 1H), 7.50 (m, 4H), 7.79 (m, 3H) ppm; ^{13}C NMR (62.5 MHz, $CDCl_3$) $\delta = 8.1$, 8.3, 24.0, 25.0, 48.2, 73.7, 122.3, 126.1, 126.2, 126.2, 127.2, 127.6, 128.0, 128.4, 128.5, 131.1, 132.7, 133.4, 135.1, 139.5, 141.7, 143.2, 156.7 ppm; IR (film) $\nu = 1720$, 2963, 3247 cm^{-1}; HRMS (LSIMS) calcd for $C_{23}H_{23}NO_2$ [$M^{+\bullet}$]: 345.1729; found: 345.1738.

Biological assays[2,5]

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

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

Cytotoxicity assays. the effect of the drugs on the growth of KB and MCF7 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. Docetaxel IC_{50} values: 4 nM for KB cells and 3.5 nM for MCF7 cells.

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