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Synthesis and antitumoral evaluation of indole alkaloid analogues containing an hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indole skeleton

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ABSTRACT

The scope of acid-mediated cyclative additions of electrophiles to tryptophan-derived α-amino nitriles for the synthesis of 10*b*-substituted-1,2,4,5,10*b*,10*c*-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indoles analogues of indole alkaloids has been studied. The results demonstrate the high potential of the methodology for the synthesis of 10*b*-bromo-derivatives, by bromination with NBS, 10*b*-allyl-derivatives, by bromo-allyl exchange, and 10*b*-prenyl-derivatives, by reaction with prenyl bromide in the presence of $Mg(NO_3)_2{\cdot}6H_20$. Some of the new pyrroloimidazoindole derivatives displayed moderate μM cytotoxicities in human cancer cell lines and at 10 μg/mL inhibited more than 50% EGFR or HIF-1α.

1. Introduction

The novel ring system 1,2,4,5,10*b*,10*c*-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indole (Fig. 1, **A**) can be considered as a constrained hybrid of 1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indole (**B**) and 2,3,9,9*a*-tetrahydroimidazo[1,2-*a*]indole (**C**), both present in a growing number of indole alkaloids with a wide range of biological activities.[1] Thus, the hexahydropyrrolo[2,3-*b*]indole is present in the acetylcholinesterase inhibitors physostigmine (**1**) and phenserine[2] (**2**), in the antibacterials flustramines[3] and mollenines,[4] in the numerous group of fused piperazine derivatives [among others: the multidrug resistance reversal agents ardeemins[5] (**3**), the mycotoxin brevianamide E,[6] the vasodilator amauromine,[7] roquefortines,[8] okaramines,[9] gypsetin,[10] or the immunomodulators sporidesmins[11]], in dimeric and polymeric indole alkaloids such as the somatostatin antagonists psycholeine and quadrigemine C,[12] and as a modified tryptophan residue in several peptides such as himastatin,[13] chloptosin,[14] or the *Bacillus subtilis* pheromone ComX[15] (**4**).

On the other hand, the tetrahydroimidazo[1,2-*a*]indole is present in the cholecystokinin CCK_1 receptor antagonist asperlicin (**5**),[16] the substance P antagonist fiscalin A[17] (**6**), the antifungic fumiquinazolines,[18] tryptoquivalines,[19] and in chaetominine (**7**)[20] or kapakahines,[21] which contain an additional *peri*-fused piperidone ring.

In the context of a research program focused on the synthesis of privileged scaffolds, we discovered that 10b-unsubstituted-1,2,4,5,10*b*,10*c*-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indoles are easily and efficiently obtained with high stereoselectivity by acid-promoted domino tautomerization of tryptophan-derived α-amino nitriles.[1,22] To approach the synthesis of higher substituted analogues of the mentioned indole alkaloids, we have recently explored the access to 10*b*-substituted-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indoles by acid-promoted electrophile cyclative additions in the cyclohexanone-derived α-amino nitrile **8a** (Scheme 1).[23,24] These preliminary studies demonstrated the possibility of introducing halogens (Cl and Br) and the prenyl and hydroxyl groups into the 10*b* position of the pyrroloimidazoindole skeleton by acid-promoted ring-closing halogenation, prenylation and oxidation. On the other hand, a bromo-allyl exchange gave access to the corresponding 10*b*-allyl derivative. The resulting 10*b*-substituted-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*] indoles were evaluated as antitumorals in human cancer cell lines. The 9,10*b*-dibromo and the 10*b*-allyl derivatives **11a** and **15a**, shown in Scheme 1, displayed micromolar cytotoxicity in human lung carcinoma (A549) and colon carcinoma (HT-29) cell lines.[23] Based on these preliminary results, now we have studied and communicate herein the versatility of cyclative electrophile additions to other tryptophan-derived α-amino nitriles and the antitumoral evaluation of the resulting products. Amino nitriles derived from acetone (**b**), *N*-benzyl-4-piperidone (**c**), phenylacetaldehyde (**d**), benzaldehyde (**e**) and pivalaldehyde (**f**) were selected as starting materials for this study.

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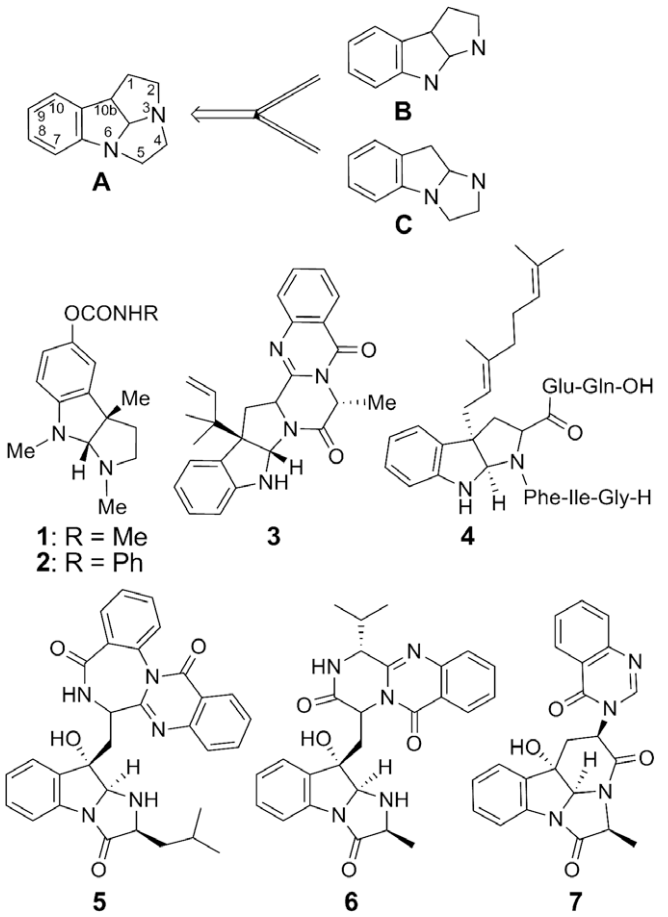


Figure 1. The pyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indole ring system and related indole alkaloids.

2. Results and discussion

2.1. Chemistry

As shown in Scheme 1, we first studied the bromination of α-amino nitriles **8b-f**, by applying the methodology previously developed for **8a**,[23] consisting of treatment with *N*-bromosuccinimide (NBS, 1 equiv for 10*b*-monobromation and 2 equiv for 9,10*b*-dibromination) in the presence of 10% TFA in CH_2Cl_2 solution at 40 °C. Under these reaction conditions, the acetone-derived amino nitrile **8b** gave very similar results to those described for the cyclohexanone derivative **8a**, except that three equiv. of NBS were required for its complete dibromination, obtaining exclusively the respective 2-*exo*-diastereoisomers **9b** and **11b** in high yields (Table 1).

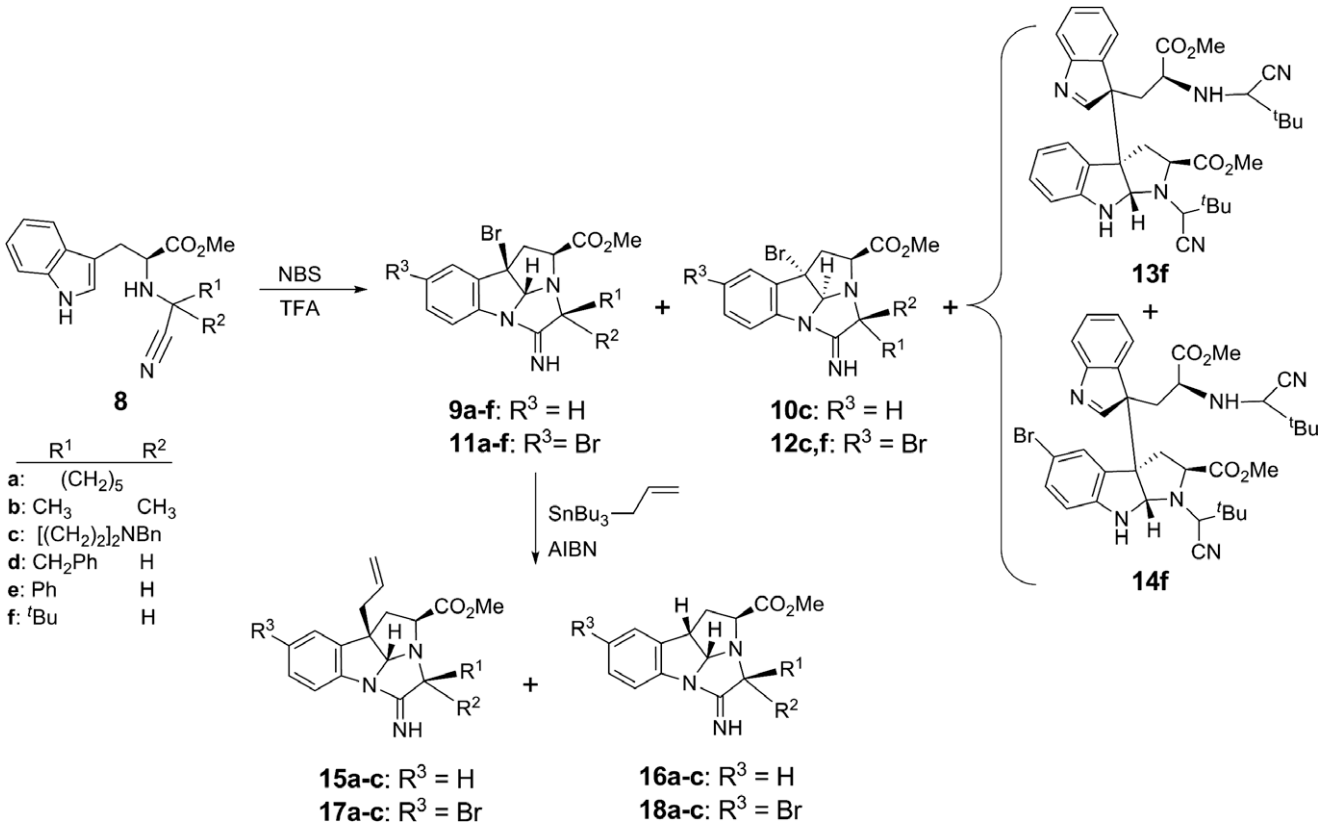
As described for the acid-promoted tautomerization,[22] mixtures of 2-*exo*- and 2-*endo*-pyrroloimidazoindoles were obtained both in the mono- and dibromination of the more constrained amino nitrile derived from 4-piperidone **8c**. As shown in Table 1, the diastereomeric ratio was dependent on the reaction time and temperature. Thus, the 2-*exo*-isomers **9c** and **11c** were the kinetic controlled products that slowly isomerized to the more stable 2-*endo* isomers **10c** and **12c**, respectively.

Aldehyde-derived amino nitriles **8d**–**f** were much more reactive toward NBS and complex reaction mixtures of decomposition products were obtained under the reaction conditions developed for the ketone derivatives **8a–c**. Trying to avoid this decomposition, the influence of the TFA concentration, temperature and time of monobromination on the reaction products was studied in the (*R*)-epimer of the phenylacetaldehyde-derived amino nitrile **(*R*)-8d**. The HPLC analysis of the crude reaction mixtures showed a significant increase in the yield of the 10*b*-bromo-pyrroloimidazoindole **9d** with the decrease of the TFA concentration from 10 to 1% and with the reduction of the reaction time from 2 h to 15 min, but **9d** was unstable and could not be isolated for its complete characterization. A similar treatment of the benzaldehyde-derived amino nitriles **8e** led to complex mixtures of decomposition products. Interestingly, in the bromination of the pivalaldehyde-derived amino nitriles **8f** [an inseparable (2:3) epimeric (*R*):(*S*) mixture] with one equiv. of NBS in solution of 1% of TFA in CH_2Cl_2, a low yield (22%) of the 10*b*-bromo-pyrroloimidazoindole **9f** was isolated, along with 21% of an ≈(1:1) mixture of the dimeric compounds **13f** and **14f**, which could not be resolved. A (2.5:1) mixture of 2-*exo*-/2-*endo*-9,10*b*-dibromo-pyrroloimidazoindoles **11f** and **12f** was obtained and chromatographically resolved in low yield from the reaction of **8f** with 2 equiv of NBS in 1% solution of TFA in CH_2Cl_2. Under these conditions, the formation of the dimeric species **13f**/**14f** was not observed. Dimerization was neither observed in the abovementioned reactions of the other amino nitriles **8a–e**.

The HPLC-MS analysis of the **13f** + **14f** mixture showed two peaks in a (1.2:1) area ratio with the respective dimeric mass of 625.70 and 703.52. The latter with the characteristic isotopic mass pattern of brominated compounds, which was confirmed by the Br microanalysis of the sample. The ^{1}H NMR spectrum also showed the presence of two dimeric compounds. Thus, four different singlets appeared, corresponding to the ^{t}Bu groups, and another four for the OMe groups. However, only two doublets appeared at 5.49 and 5.56 ppm for the 8a-H protons of the pyrroloindole moiety, each one coupled with a doublet at 4.50 and 4.63 ppm, respectively, corresponding to the NH at position 8. The 3H-indole moiety was evidenced, in each case, from the presence of one singlet at 6.88 and 6.94 ppm, corresponding to the 2-H protons, and from the absence of additional 8-H and 8*a*-H pairs that should have appeared in the case of a second pyrroloindole unit. The absence of signals for 3*a*-H in the pyrroloindole unit and for 3-H in the 3H-indole one suggested the linkage points between them. This assignment was confirmed by the ^{13}C NMR spectrum and the one and two bonds ^{1}H,^{13}C correlation spectra HSQC and HMBC. These spectra showed the presence of four quaternary carbons for the cyano groups (117.07, 117.27 for the pyrroloindole moiety, and 119.21 and 119.26 for those of the 3H-indole units). The bromination position in **14f** was also assigned based on HMBC ^{1}H,^{13}C correlations.

A (3*aS*)-configuration at the pyrroloindole ring was assigned to both **13f** and **14f** based on the ^{1}H chemical shifts of their methoxycarbonyl groups (3.72 and 3.74 ppm), characteristic of a 2-*exo*-configuration.[22,23] On the other hand, a (3*R*)-configuration was tentatively assigned to the 3H-indole moiety based on the NOEs effects observed in the NOESY spectrum of **13f + 14f**, shown in Scheme 2. Particularly, the NOEs between the pyrroloindole 8*a*-H and the indole 7-H and 2-H, as well as, between this proton and one of the 3-H protons of the pyrroloindole. According to the minimized energy models for both (3*R*)- and (3*S*)-epimers, generated with the Chem3D® program, only the (3*R*)-epimer in a π-stacked conformation would explain the observed NOEs. As the starting α-amino nitrile **8f** was used as an inseparable (*R,S*)-epimeric mixture at C-α, it was not possible to assign the configuration at these carbons in **(13 + 14)f**.

The reaction mechanism shown in Scheme 2 could explain the formation of the dimeric species. As recently reported by Movassaghi et al. for the synthesis of dimeric pyrroloindole alkaloids, such as chimonanthine, from 3*a*-bromo-pyrroloindoles,[25] the reaction with NBS would generate the 3*a*-bromo-pyrroloindoles **A**, where, due to the steric hindrance and the low acidity medium (1% TFA, versus the 10% used for the ketone-derived amino nitriles), the formation of the free radicals **B** would compete

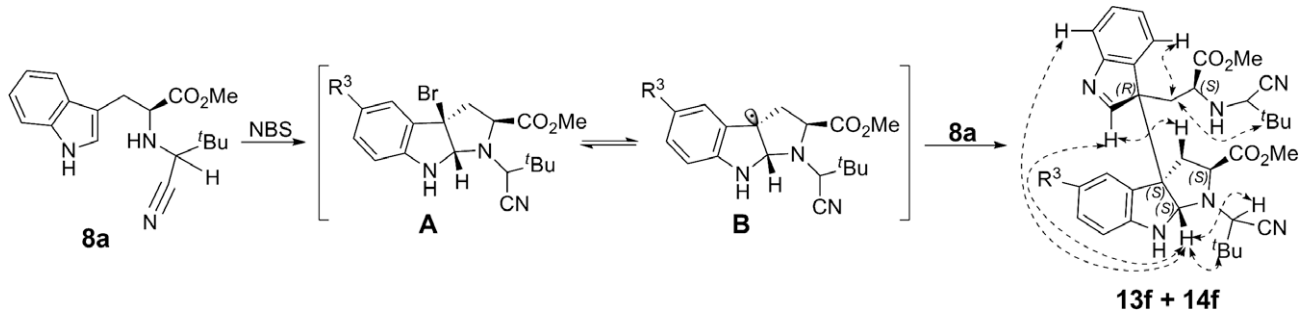


Scheme 1. Synthesis of 10*b*-bromo- and -allyl-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indoles.

Table 1
Results of the NBS-mediated bromination of α-amino nitriles **8**

Amino nitrile	NBS (equiv.)	TFA (%)	*T* (°C)	Time (h)	Products[b]
8a[a]	1	10	−40	3	**9a** (91%)
8a[a]	2	10	−40	3	**11a** (94%)
8b	1	10	−40	3	**9b** (83%)
8b	3	10	−40	2	**11b** (68%)
8c	1	10	−40	0.5	**9c** (95%) + **10c** (5%)[c]
8c	1	10	−40	24	**9c** (82%) + **10c** (8%)[c]
8c	1	10	0	24	**9c** (70%) + **10c** (30%)[c]
8c	1	10	25	24	**9c** (55%) + **10c** (45%)[c]
8c	3	10	−40	2	**11c** (65%) + **12c** (35%)[c]
(***R***)-**8d**	1	10	−40	2	**9d** (19%)[c]
(***R***)-**8d**	1	1	−40	0.25	**9d** (98%)[c]
(***R***)-**8d**	1	1	−40	2	decomposition
8e	1	1	−40	2	decomposition
8f	1	1	−40	2	**9f** (22%) + [**13f** + **14f** (21%)]
8f	2	1	−40	2	**11f** (25%) + **12f** (10%)

[a] Ref. 1.
[b] Isolated yields, except for **8c**.
[c] Yields determined by HPLC.



Scheme 2. Proposed mechanism for the formation of dimmers **13f + 14f** and most significant NOEs observed in their NOESY spectrum.

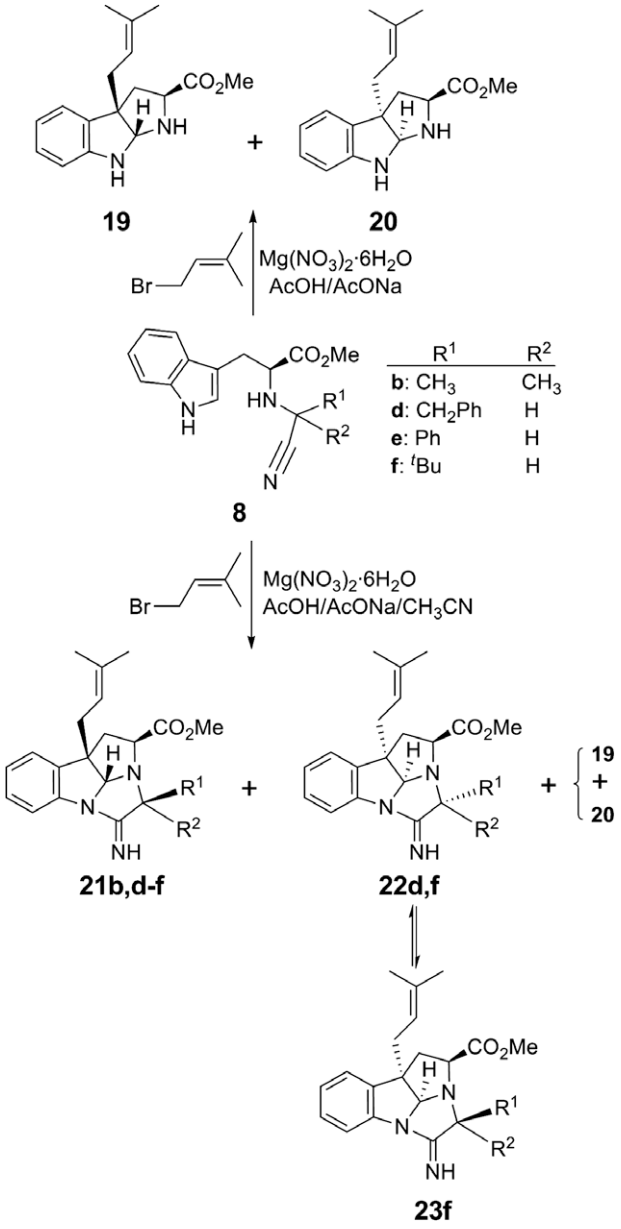
with the cyclization to the pyrroloimidazoindoles **9f**–**12f**. The free radical species **B** would attack to the C-3 of a second amino nitrile molecule to give the dimeric compounds **13f + 14f**. This mixture did not cyclize to pyrroloimidazoindole derivatives after 15 days of treatment with 10% solution of TFA in CH_2Cl_2, being recovered unaltered.

In the ketone-derived 10*b*-bromo-pyrroloimidazoindoles **9b,c** and **11b,c**, the replacement of the bromo by an allyl group was studied by applying the reaction conditions developed for the cyclohexanone-derived analogues **9a** and **11a**, consisting of treatment with allyltributyltin in the presence of a 10% of the free radical initiator AIBN in refluxing xylene[23] (Scheme 1). The new 10*b*-allyl-pyrroloimidazoindoles **15b,c** and **17b,c** were obtained in significant lower yields (26–57%) than those reported for **15a** and **17a** (70 and 65%). These lower yields were consequence of a partial (11–30%) dehalogenation to the corresponding 10*b*-unsubstituted-pyrroloimidazoindoles **16b,c** and **18b,c** and to a higher partial decomposition of the starting materials.

Finally, as the prenyl or reverse prenyl groups are among the most recurrent substituents in indole alkaloids, we studied the scope of the cyclative prenylation of tryptophan-derived α-amino nitriles in ketone (**8b,c**) and aldehyde (**8d–f**) derivatives for the synthesis of 10*b*-prenyl-pyrroloimidazoindoles. Our previously reported prenylation of the cyclohexanone derivative **8a** involved the reaction with prenyl bromide in the presence of $Mg(NO_3)_2{\cdot}6H_2O$ in pH 2.9 AcOH/AcONa buffer at room temperature.[23] This methodology was first applied to the acetone-derived amino nitrile **8b**. Due to the high instability of this amino nitrile in the AcOH/AcONa buffer, only low yields of the 2-*exo*- and 2-*endo*-3a-prenyl-pyrroloindoles **19** and **20** (Scheme 3) could be isolated. The formation of these compounds indicated that, previously to the prenylation, the amino nitrile had reverted to the tryptophan methyl ester. With the aim of decreasing the degradation produced by the aqueous acid media, an heterogeneous CH_2Cl_2/buffer reaction medium was tried, but in this case, the starting amino nitrile was recovered unaltered. Next, we tried to use a miscible organic cosolvent and to change the order of addition of the reagents (addition of the amino nitrile, dissolved into acetonitrile, after the prenyl bromide). In this way, the 2-*exo*-10*b*-prenyl-pyrroloimidazoindole **21b** (41%) was obtained along with a low yield of the pyrroloindoles **19 + 20**. The 4-piperidone-derived amino nitrile **8c** resulted completely degraded even under the new reaction conditions, while, the aldehyde-derived amino nitriles **8d–f** gave low yields of the 10*b*-prenyl-pyrroloimidazoindoles **21**–**22**, along with a variable% of **19 + 20**.

Interestingly, the pure pivalaldehyde-derived (4*R*)-2-*endo*-isomer **22f** in the $CDCl_3$ solution of the ^1H NMR sample slowly epimerized at C-4 to give **23f**, achieving the equilibrium at an (1:1) epimeric proportion after 60 days in solution at room temperature. As we have previously suggested,[22] this epimerization at C-4 must proceed through the enamine tautomer of the endocyclic amidine group.

In all new pyrroloimidazoindoles herein described, the absolute configuration at C-10*b*, C-10*c*, and C-4 was assigned based on the ^1H NMR chemical shift of the methoxycarbonyl group [in 2-*endo*-isomers this group appears at (3.00–3.36 ppm), ≈0.5 ppm upfield with respect to the 2-*exo*-isomers (3.60–3.75 ppm)] and on the NOE effects observed in the respective 1D NOESY spectra.[21–24]



Scheme 3. Prenylation of α-amino nitriles **8b,d–f**.

2.2. Antitumoral evaluation

The new pyrroloimidazoindole derivatives herein described were evaluated as antitumorals in HTS programmes, which included in parallel screening of cytotoxicity and of inhibition of several molecular targets involved in tumor growth, such as mitosis, epidermal growth factor receptor (EGFR), β-catenin, hypoxia-inducible factor 1α (HIF-1α), and histone deacetylases. The cytotoxicity was evaluated on three representative human cancer cell lines: breast (MDA-MB-231), lung (A549), and colon (HT-29), according to the National Cancer Institute (NCI) protocols. The three cell growth parameters: GI_{50} (concentration that produces 50% growth inhibition), TGI (concentration that produces total growth inhibition), and LC_{50} (concentration that produces 50% of cellular death) were determined from the data analysis, automatically generated by the HTS laboratory information management system. The results of the compounds that displayed GI_{50} values lower than the highest 100 μM concentration are shown in Table 2. The cyclohexanone-derived 9,10*b*-dibromo-pyrroloimidazoindole **11a**, the piperidone-derived 10*b*-allyl derivatives **16c** and **17c**, and the acetone-derived 10*b*-prenyl derivative **21b** were the most active compounds, displaying moderate cytotoxicities, in the μM range, in the three tested cell lines. The 10*b*-bromo derivative **9c** showed selectivity for A549 and HT-29 cells, while, **9f** and **21f** showed selectivity for breast MDA-MB-231 cells. These results did not allow us to establish structure–activity relationships.

Table 2
Results of the antitumoral evaluation of pyrroloimidazoindole derivatives

Compound	Cytotoxicity (μM)									% Inhibition[a]	
	MDA-MB-231			A549			HT-29				
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	EGFR	HIF-1α
9c	>100	>100	>100	17.6	20.2	20.2	17.6	20.2	20.2	31	75
9f	17.6	25.5	25.5	>100	>100	>100	>100	>100	>100	0	70
10c	>100	>100	>100	>100	>100	>100	18.8	20.2	20.2	27	73
11a	6.21	8.49	>100	7.24	10.8	16.1	7.45	9.31	14.1	83	ND
11b	>100	>100	>100	>100	>100	>100	>100	>100	>100	45	55
11c	11.7	17.4	17.4	10.8	17.4	17.4	7.66	14.6	17.4	24	94
11f	>100	>100	>100	>100	>100	>100	>100	>100	>100	0	73
13f + 14f	>100	>100	>100	>100	>100	>100	>100	>100	>100	40	65
15a	23.5	24.6	25.7	23.3	24.1	25.2	22.4	23.5	25.2	52	ND
16a	ND	ND	ND	9.23	9.23	9.23	9.23	9.23	9.23	ND	ND
16b	ND	ND	ND	10.5	10.5	10.5	10.5	10.5	10.5	ND	ND
16c	ND	ND	ND	7.21	7.21	7.21	7.21	7.21	7.21	ND	ND
17c	8.22	18.7	18.7	8.22	12.1	17.2	7.47	7.84	8.40	15	99
21b	11.0	28.3	28.3	5.66	28.3	28.3	4.24	28.3	28.3	0	73
21d	>100	>100	>100	22.1	24.1	24.1	20.2	24.1	24.1	20	78
21e	>100	>100	>100	>100	>100	>100	>100	>100	>100	13	78
21f	15.2	26.2	26.2	>100	>100	>100	>100	>100	>100	0	83
22d	9.39	24.1	24.1	12.0	24.1	24.1	>100	>100	>100	0	61

[a] Inhibition produced by a 10 μg/mL concentration.

The activity on molecular targets was determined in 96-well microplates at five fixed concentrations (10, 2, 0.4, 0.08, and 0.016 μg/mL), using established fluorescence cell-based bioassays. Data are expressed as inhibition and cell survival percentages of the controls. Active compounds were considered those that displayed inhibition values higher than 50% and cell survival higher than 70%. An ELISA immunoassay in HeLa cells was used for the evaluation of mitosis inhibition,[26] using paclitaxel (1 μg/mL) and vinblastine (1 μg/mL) as control inhibitors. The inhibition of EGFR, β-catenin, and hypoxia-inducible factor 1α (HIF-1α) was evaluated in tumoral cells stably transfected with a luciferase (luc) reporter gene under the control of a specific promoter of the signalling target.[27] HeLa cells transfected with the AP1-luc reporter gene were used for screening on the EGFR signalling pathway, using EGF as activator and the selective inhibitor AG-1478[28] as control inhibitor. SW480 cells of human colorectal adenocarcinoma transfected with the TCF4-luc promoter gene, which confer constitutive activation of the β-catenin signalling pathway, were used for the screening on this target. HeLa cells transfected with the HIF1-luc reporter gene were used for the screening on HIF-1α, using the iron chelator deferoxamine (50 μM) as HIF-1α inductor.[29] MDA-MB231 (human breast carcinoma) cell extracts were used for the evaluation of activity on histone deacetylases, using trichostatin A[30] (250 nM) as specific inhibitor. None of the compounds displayed inhibition of mitosis, β-catenin, and histone deacetylases. However, as shown in Table 2, the cyclohexanone-derived 9,10*b*-dibromo and the 10*b*-allyl derivatives **11a** and **15a** inhibited EGFR more than 50% at the highest tested concentration (10 μM) and, at this concentration, several compounds displayed high inhibition percentages of HIF-1α. In view of the overall antitumoral evaluation results, **11a**, **17c**, and **21b** could represent interesting hints for the search of novel antitumoral agents.

3. Conclusions

In summary, the studies herein described confirm the potential of tryptophan-derived α-amino nitriles for the highly stereoselective generation of diversely substituted 1,2,4,5,10*b*,10*c*-hexahydropyrrolo[1′,2′,3′:1,9*a*,9]imidazo[1,2-*a*]indoles by cyclative addition of electrophiles. This potential is limited in aldehyde-derivedα-amino nitriles by their high reactivity toward NBS and the instability of the corresponding brominated pyrroloimidazoindole derivatives. On the other hand, the instability of α-amino nitriles in aqueous AcOH/AcONa buffer is in part responsible of the low yield of the cyclative prenylation reaction. The moderated cytotoxic activity and inhibition of EGFR and HIF-1α herein demonstrated by some pyrroloimidazoindole derivatives could be a good indication for the search of novel antitumoral agents.

4. Experimental

4.1. General methods

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2mm layer of silica gel 60 F254. Silica gel 60 (230–400 mesh) was used for flash chromatography. Preparative radial chromatography was performed on 20 cm diameter glass plates coated with a 1-mm layer of silica gel PF254. Analytical RP-HPLC was performed on a Novapak C_{18} (3.9 × 150 mm, 4 μm) column, with a flow rate of 1 mL/min, and using a tunable UV detector set at 214 nm. Mixtures of CH_3CN (solvent A) and 0.05% TFA in H_2O (solvent B) were used as mobile phases. HPLC-EMS was performed on an Atlantis T3 C_{18} (2.1 × 100 mm, 3 μm) column at 30 °C, with a flow rate of 0.25 mL/min. Gradients (5–80%) of CH_3CN with 0.08% of formic acid (solvent A) in 0.1% of formic acid in H_2O (solvent B) were used as mobile phases. ^1H NMR spectra were recorded at 300, 400, or 500 MHz, using TMS as reference, and ^{13}C NMR spectra were recorded at 75, 100, or 125 MHz. The NMR spectra assignment was based on COSY, HSQC, and HMBC spectra. ESI-MS spectra were performed, in positive mode, using MeOH as solvent.

4.2. General procedure for the acid-promoted cyclative monobromination of ketone-derived α-amino nitriles. Synthesis of 9b,c and 10c

NBS (282 mg, 1.58 mmol) and TFA (500 μL) were added to a −40 °C cooled solution of the corresponding α-amino nitrile **8b,c** (1.58 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at that temperature for 3 h. Then, the reaction mixture was poured into ice (≈10 g), neutralized with concentrated ammonium hydroxide

and extracted with CH_2Cl_2 (20 mL). The organic extracts were successively washed with H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by circular chromatography, using 30–65% gradient of EtOAc in hexane as eluant, to give the corresponding 10*b*-bromo derivatives **9b,c** and **10c**, whose more significant analytical and spectroscopic data are shown in Table 3.

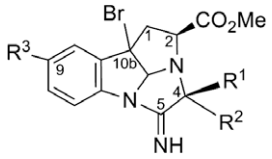
4.3. General procedure for the acid-promoted cyclative monobromination of aldehyde-derived α-amino nitriles. Synthesis of 9d–f and (13 + 14)f

NBS (282 mg, 1.58 mmol) and TFA (50 μL) were added to a −40 °C cooled solution of the corresponding α-amino nitrile **8d–f** (1.58 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at that temperature for 3 h. Afterwards, the reaction mixture was processed as above. In the case of the phenylacetaldehyde and benzaldehyde derivatives **8d** and **8e**, the reaction products were unstable and could not be isolated. In the case of the pivalaldehyde derivative **8f**, the 10*b*-bromo-derivative **9f** (22%), whose analytical and spectroscopic data are summarized in Table 3, and the dimeric mixture of **(13 + 14)f** (21%) were obtained.

4.3.1. Dimeric mixture (13 + 14)f

Foam (21%). HPLC-MS (ES) [Atlantis T3 C_{18} (2.1 × 100 mm, 3 μm), 5–80% gradient of solvent A in 20 min] t_R 13.0 [55%, *m/z* 625.70, (M+1)] and 13.4 [45%, *m/z* 703.52, (M+1)]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91, 0.93, 1.06, 1.10 (4s, 36H, ^{t}Bu), 1.80, 1.95

Table 3
Analytical and spectroscopic data of 10*b*-bromo-pyrroloimidazoindole derivatives **9–12**[a]



	9b	9c	9f	10c	11b	11c	11f	12c	12f
R^1	CH_3	$[(CH_2)_2]_2NBn$	^{t}Bu	$[(CH_2)_2]_2NBn$	CH_3	$[(CH_2)_2]_2NBn$	^{t}Bu	$[(CH_2)_2]_2NBn$	H
R^2	CH_3		H		CH_3		H		^{t}Bu
R^3	H	H	H	H	Br	Br	Br	Br	Br
Config.	2*S*,10*bR*,10*cR*	2*S*,10*bR*,10*cR*	2*S*,10*bR*,10*cR*,4*S*	2*S*,10*bS*,10*cS*	2*S*,10*bR*,10*cR*	2*S*,10*bR*,10*cR*	2*S*,10*bR*,10*cR*,4*S*	2*S*,10*bS*,10*cS*	2*S*,10*bS*,10*cS*,4*R*
Yield (%)	83	55	22	45	68	65	25	35	10
Formula	$C_{16}H_{18}BrN_3O_2$	$C_{25}H_{27}BrN_4O_2$	$C_{18}H_{22}BrN_3O_2$	$C_{25}H_{27}BrN_4O_2$	$C_{16}H_{17}Br_2N_3O_2$	$C_{25}H_{26}Br_2N_4O_2$	$C_{18}H_{21}Br_2N_3O_2$	$C_{25}H_{26}Br_2N_4O_2$	$C_{18}H_{21}Br_2N_3O_2$
ES-MS 471	$[M+1]^+$	364	495	392	495	441	575	471	575
$[\alpha]^{20}_D$	−63.0	−71.8	−38.6	−24.5	−30	−60.8	−42	ND[b]	+38.6
	(*c* 1, MeOH)	(*c* 1.1, MeOH)	(*c* 1.5, MeOH)	(*c* 0.9, MeOH)	(*c* 1, MeOH)	(*c* 1.2, MeOH)	(*c* 1.5, MeOH)		(*c* 1.5, MeOH)
HPLC t_R	5.3	11.8	2.2	10.0	14.1	2.7	2.9	1.8	3.2
(A:B)[c]	(25:75)	(25:75)	(50:50)	(25:75)	(25:75)	(50:50)	(50:50)	(50:50)	(50:50)
^{1}H NMR[d]									
1-H	2.86, 2.94	2.82, 3.03	2.81,3.07	2.81;1.99	2.84, 2.92	2.80, 3.01	2.73;2.99	2.85,3.37	2.80;3.11
2-H	3.17	3.17	3.11	3.99	3.15	3.15	3.03	4.06	4.24
7-H	7.43	7.40	7.42	7.20	7.35	7.25–7.33	7.37	7.30–7.34	7.42
8-H	7.33	7.24–7.34	7.32	7.24	7.43	7.42	7.37	7.40	7.42
9-H	7.16	7.15	7.15	6.98	—	—	—	—	—
10-H	7.43	7.41	7.48	7.40	7.54	7,53	7.46	7.44	7.48
10c-H	6.02	5.99	5.89	5.78	5.99	5.97	5.81	5.82	5.81
OCH_3	3.72	3.68	3.71	2.99	3.72	3.69	3.65	3.17	3.36
R^1	1.56	1.71–2.77, 3.54,	1.14	1.43–2.90,	1.54	1.70–2.72, 3.53,	1.07	1.42–2.75,	1.11
R^2	1.27	7.15–7.41	3.31	3.46, 7.26	1.26	7.29–7.39	3.29	3.48, 7.32	3.46
$J_{1,1}$	12.5	12	12	12	12	12	12	14	14
$J_{1,2}$	6, 12.5	5, 12	4,12	0, 9	5, 12	5, 12	4, 12	0, 9	4, 8
^{13}C NMR[e]									
C_1	50.3	50.7	47.9	50.1	50.1	50.4	47.8	50.4	48.2
C_2	61.7	61.9	66.4	62.9	61.7	61.8	66.3	63.0	68.0
C_4	69.0	70.6	81.4	67.8	69.0	70.6	81.4	68.1	77.2
C_5	174.0	174.0	169.9	175.2	174.0	174.0	169.9	178.9	170.5
C_{6a}	143.5	142.9	114.0	146.8	142.7	142.1	137.7	146.3	117.3
C_7	115.1	115.4	115.5	124.8	116.8	117.0	117.1	128.6	128.0
C_8	130.4	130.4	130.3	130.3	133.4	133.3	128.1	137.8	133.2
C_9	125.0	125.1	125.0	123.8	117.3	117.3	117.4	128.2	128.0
C_{10}	125.3	125.2	125.1	114.3	128.3	128.2	133.3	116.2	133.2
C_{10a}	135.4	135.9	135.4	135.3	137.5	138.0	137.5	137.8	137.9
C_{10b}	61.1	61.4	62.5	63.0	59.9	60.1	61.2	63.12	64.1
C_{10c}	95.2	95.5	98.7	94.7	95.3	95.6	98.8	95.3	99.7
OCH_3	52.5	52.5	52.2	51.5	52.6	52.6	52.3	52.2	52.0
R^1	25.9	29.0, 33.6, 49.1,	27.3, 35.1	29.4, 34.0,	25.9	28.9, 33.4, 49.1,	27.3, 35.1	29.6, 29.9,	26.9, 36.2
R^2	20.7	49.4	—	49.5, 49.7	20.6	49.4	—	49.7, 49.9,	—
CO_2	171.6	171.8	170.1	172.6	171.4	171.5	169.9	172.9	170.9

[a] Foams with satisfactory analysis for C, H, N.
[b] Not determined.
[c] Novapack C_{18} (3.9 × 150 mm, 4 μm). A = CH_3CN, B = 0.05% TFA in H_2O.
[d] Spectra registered at 300 or 400 MHz in $CDCl_3$, assigned with the help of COSY spectra.
[e] Spectra registered at 75 or 100 MHz in $CDCl_3$, assigned with the help of HSQC and HMBC spectra.

(2bs, 2H, α-NH), 2.80, 3.40 [2m, 4H, 3-H (pyrroloindole)], 2.95, 2.98 [2d, 2H, *J* = 7 Hz, CN-*CH* (3H-indole unit)], 2.96, 3.05 (2m, 4H, β-H), 3.55 [m, 2H, α-H (3H-indole unit)], 3.63, 3.67, 3.72, 3.74 (4s, 12H, OCH_3), 3.95 [dd, 2H, *J* = 7 and 14 Hz, 2-H (pyrroloindole)], 4.02, 4.20 [2s, 2H, CN-*CH* (pyrroloindole unit)], 4.50, 4.63 [2d, 2H, *J* = 3.5 Hz, 8-NH (pyrroloindole)], 5.49, 5.56 [2d, 2H, *J* = 3.5 Hz, 8*a*-H (pyrroloindole)], 6.72, 6.84 [2s, 2H, 2-H (3H-indole)], 6.96 [t, 1H, *J* = 7 Hz, 5-H (pyrroloindole)], 7.16 [t, 2H, *J* = 7 Hz, 5-H (3H-indole)], 7.19, 7.20 [2t, 2H, *J* = 7 Hz, 6-H (3H-indole)], 7.28, 7.34 [2d, 2H, *J* = 7 Hz, 4-H (pyrroloindole)], 7.53, 7.72 [2d, 2H, *J* = 7 Hz, 7-H (3H-indole)], 7.56 [d, 2H, *J* = 7 Hz, 4-H (3H-indole)]. ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.0, 26.8, 27.0 [$C(CH_3)_3$], 29.0 [C_β (3H-indole unit)], 34.8, 37.8, 37.2 [$C(CH_3)_3$], 40.9, 40.7 [C_3 (pyrroloindole)], 52.3, 52.41, 52.0, 52.0 (OCH_3), 61.5, 60.5 [CN-*CH* (3H-indole unit)], 61.6, 61.6 [C_α (3H-indole unit)], 64.0, 64.3 [C_2 (pyrroloindole)], 73.9, 74.2 [C_{3a} (pyrroloindole)], 85.4, 85.8 [C_{8a} (pyrroloindole)], 109.6, 110.3 [C_3 (3H-indole)], 111.8, 112.0 [C_7 (3H-indole)], 112.3 [C_5-Br (pyrroloindole)], 112.7, 113.6 [C_7 (pyrroloindole)], 117.1, 117.3, 119.2, 119.3 (CN), 119.2, 119.3 [C_4 (3H-indole)], 119.3, 119.7 [C_5 (3H-indole)], 121.1 [C_5 (pyrroloindole)], 122.1, 122.3 [C_6 (3H-indole)], 124.6, 125.1 [C_2 (3H-indole)], 125.4, 127.7 [C_4 (pyrroloindole)], 129.9 [C_{3a} (3H-indole)], 129.7, 132.0 [C_{3b} (pyrroloindole)], 130.8, 133.5 [C_6 (pyrroloindole)], 134.6, 134.8 [C_{7a} (3H-indole)], 147.3, 148.8 [C_{7a} (pyrroloindole)], 172.9, 173.2, 174.1 (CO_2).

4.4. General procedure for the acid-promoted cyclative dibromination of ketone-derived α-amino nitriles. Synthesis of 11b,c and 12c

NBS (846 mg, 4.74 mmol) and TFA (500 μL) were added to a −40 °C cooled solution of the corresponding α-amino nitrile **8b,c** (1.58 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at that temperature for 3 h. Afterwards, the reaction mixture was processed as above. Significant analytical and spectroscopic data of the resulting 9,10*b*-dibromo derivatives **(11–12)b,c** are summarized in Table 3.

4.5. Acid-promoted cyclative dibromination of the aldehyde-derived α-amino nitrile 8f. Synthesis of 11f and 12f

NBS (846 mg, 4.74 mmol) and TFA (50 μL) were added to a −40 °C cooled solution of the α-amino nitrile **8f** (1.58 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at that temperature for 3 h. Afterwards, the reaction mixture was processed as above. Significant analytical and spectroscopic data of the resulting 9,10*b*-dibromo derivatives **11f** and **12f** are summarized in Table 3.

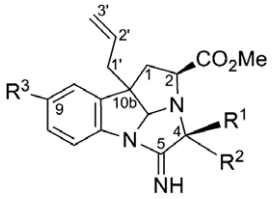
4.6. General procedure for the exchange of 10*b*-bromo to 10*b*-allyl. Synthesis of (15–18)b,c

AIBN (115 mg, 0.7 mmol) and allyltributyltin (445 μL, 2 mmol) were added to a solution of the corresponding 10*b*-bromo-pyrroloimidazoindole **9b,c** and **11b,c** (1 mmol) in xylene (5 mL), and the mixture was stirred under reflux for 2 h. Afterwards, the solvent was removed under reduced pressure and the residue was dissolved in CH_3CN (10 mL). This solution was washed with hexane (2 × 20 mL). The CH_3CN phase was evaporated to dryness and the residue was purified by circular chromatography, using 15–45% gradient of EtOAc in hexane as eluant, to give the 10*b*-allyl derivatives **15b,c** and **17b,c** (foams), along with 10*b*-unsubstituted analogues **16b,c**[22] and **18b,c**. Most significant analytical and spectroscopic data of allyl derivatives **15b,c** and **17b,c** are shown in Table 4.

4.7. Synthesis of (2*S*,3*aS*,8*aR*)- and (2*S*,3*aR*,8*aS*)-2-methoxycarbonyl-3*a*-prenyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indoles (19 and 20)

Prenyl bromide (267 μL, 2.28 mmol) was dropwise added to a vigorously stirred solution of the α-amino nitrile **8b** (108.4 mg, 0.38 mmol) and magnesium nitrate hexahydrate (489 mg, 1.9 mmol) in acetic acid/sodium acetate buffer (pH 2.9, prepared from 8 g of sodium acetate, 100 mL of acetic acid, and 20 mL of

Table 4
Analytical and spectroscopic data of 10*b*-allyl-pyrroloimidazoindole derivatives **15** and **17**[a]

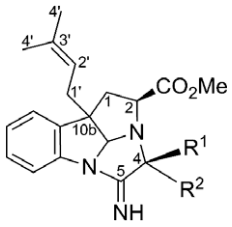


	15b	15c	17b	17c
R^1	CH_3	$[(CH_2)_2]_2NBn$	CH_3	$[(CH_2)_2]_2NBn$
R^2	CH_3		CH_3	
R^3	H	H	Br	Br
Config.	2*S*,10*bS*,10*cR*	2*S*,10*bS*,10*cR*	2*S*,10*bS*,10*cR*	2*S*,10*bS*,10*cR*
Yield (%)	36	57	26	28
Formula	$C_{19}H_{23}N_3O_2$	$C_{28}H_{32}N_4O_2$	$C_{19}H_{22}BrN_3O_2$	$C_{28}H_{31}BrN_4O_2$
ES-MS $[M+1]^+$	326	457	404	532
$[\alpha]^{20}_D$	−89	−45	−145	−29
	(*c* 1, MeOH)	(*c* 0.8, MeOH)	(*c* 0.8, MeOH)	(*c* 0.9, MeOH)
HPLC t_R (A:B)[b]	6.2 (25:75)	7.2 (25:75)	17.6 (25:75)	11.7 (25:75)
^1H NMR[c]				
1-H	2.22, 2.24	2.22, 2.26	2.22, 2.23	2.21, 2.3
2-H	3.24	3.22	3.20	3.20
7-H	7.42	7.38	7.37	7.30–7.34
8-H	7.22	7.25	7.27	7.30–7.34
9-H	7.09	7.07	—	—
10-H	7.17	7.14	7.34	7.30–7.34
10*c*-H	5.50	5.46	5.49	5.46
OCH_3	3.69	3.65	3.69	3.67
1′-H	2.61, 2.78	2.64, 2.78	2.75	2.64,2.77
2′-H	5.63	5.58	5.62	5.61
3′-H	5.08, 5.15	5.05, 5.13	5.12 5.17	5.11, 5.17
R^1	1.55	1.69–2.69	1.53	1.71–2.69,
R^2	1.26	3.51, 7.3	1.24	3.52, 7.32
^{13}C NMR[d]				
C_1	44.8	45.3	44.7	49.3
C_2	61.8	61.8	61.7	61.8
C_4	69.1	70.6	69.0	70.6
C_5	174.0	174.0	175.1	175.0
C_{6a}	144.8	144.4	144.1	143.7
C_7	114.5	114.8	116.1	116.6
C_8	128.6	127.0	131.5	133.1
C_9	124.2	124.2	116.5	116.5
C_{10}	123.4	123.3	126.4	127.1
C_{10a}	136.8	137.2	139.1	139.5
C_{10b}	52.4	53.1	53.3	53.2
C_{10c}	90.1	90.4	90.2	90.5
OCH_3	52.3	52.3	52.4	53.2
$C_{1'}$	42.5	42.4	42.2	49.3
$C_{2'}$	133.5	133.67	132.9	131.5
$C_{3'}$	119.2	119.0	119.7	119.5
R^1	26.0	29.3, 30.9,	25.9	29.7, 33.4,
R^2	20.9	49.4, 49.7	20.9	42.1, 45.2
CO_2	173.2	173.5	172.9	173.2

[a] Foams with satisfactory analysis for C, H, N.
[b] Novapack C_{18} (3.9 × 150 mm, 4 μm). A = CH_3CN, B = 0.05% TFA in H_2O.
[c] Spectra registered at 300 or 400 MHz in $CDCl_3$, assigned with the help of COSY spectra.
[d] Spectra registered at 75 or 100 MHz in $CDCl_3$, assigned with the help of HSQC and HMBC spectra.

H_2O, 15 mL) under argon. After 2 h of stirring at room temperature, the reaction mixture was sequentially neutralized with Na_2CO_3 and extracted with CH_2Cl_2 (20 mL). The organic extracts were successively washed with H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by circular chromatography, using 12–50% gradient of EtOAc in hexane as eluant, to give 3*a*-prenyl-pyrroloindole derivatives **19** (10.9 mg, 10%) and **20** (6.1 mg, 5%) as foams.

Table 5
Analytical and spectroscopic data of 10*b*-prenyl-pyrroloimidazoindole derivatives **21**-**23**[a]



	21b	21d	21e	21f	22d	22f	23f
R^1	CH_3	CH_2Ph	Ph	^tBu	CH_2Ph	^tBu	^tBu
R^2	CH_3	H	H	H	H	H	H
Config.	2*S*,10*bS*,10*cR*	2*S*,10*bS*,10*cR*,4*S* 2*S*,10*bR*,10*cS*,4*R*	2*S*,10*bS*,10*cR*,4*S* 2*S*,10*bR*,10*cS*,4*R*		2*S*,10*bS*,10*cR*,4*S* 2*S*,10*bR*,10*cS*,4*S*		
Yield (%)	41	22	11	10	22	24	
Formula	$C_{21}H_{27}N_3O_2$	$C_{26}H_{29}N_3O_2$	$C_{25}H_{27}N_3O_2$	$C_{23}H_{31}N_3O_2$	$C_{26}H_{29}N_3O_2$	$C_{23}H_{31}N_3O_2$	$C_{23}H_{31}N_3O_2$
ES-MS $[M+1]^+$	354	416	402	382	416	382	
$[\alpha]^{20}_D$	−131.7 (*c* 0.6, MeOH)	−70.0 (*c* 1, MeOH)	−88.6 (*c* 1.5, MeOH)	−88 (*c* 1.5, MeOH	+65.0 (*c* 0.6, MeOH)		
HPLC t_R (A:B)[b]	20.8 (25:75)	5.7 (50:50)	10.1 (40:60)	2.3 (50:50)	5.5 (50:50)	27.9 (50:50)	24.7 (50:50)
^1H NMR[c]							
1-H	2.24, 2.28	2.19, 2.34	2.30, 2.38	2.16, 2.35	2.23, 2.39	2.21, 2.43	2.41
2-H	3.26	3.17	3.42	3.17	3.90	4.04	3.86
7-H	7.18	7.45	7.53	7.49	7.33	7.41	7.09
8-H	7.33	7.25	7.30	7.26	7.13	7.16	6.98
9-H	7.14	7.07	7.12	7.07	6.92	6.94	6.57
10-H	7.15	7.15	7.19	7.14	7.01	7.09	6.67
10c-H	5.48	5.17	5.28	5.29	5.02	5.13	4.71
OCH_3	3.70	3.56	3.75	3.65	3.03	3.10	3.25
1′-H	2.64	2.59	2.56	2.58	2.42	2.44	2.51
2′-H	4.95	4.98	4.95	5.00	4.91	4.98	5.15
4′-H	1.62,1.65	1.62, 1.67	1.55, 1.62	1.61,1.66	1.50;1.60	1.53,1.61	1.56,1.71
R^1	1.61	3.06, 3.22, 7.14–7.33	7.65, 7.41, 7.34	1.14	2.94, 3.02, 720–7.34	1.04	3.64
R^2	1.25	3.90	4.91	3.26	4.07	3.28	1.06
$J_{1,1}$	11.5	12	12	12	13.5	13.5	13
$J_{1,2}$	6, 11.5	6, 12	6, 12	6, 12	1.5, 8.5	3, 9	3, 7
^{13}C NMR[d]							
C_1	44.2	42.6	42.9	42.2	42.7	42.0	40.8
C_2	62.2	64.7	64.3	66.7	66.1	68.6	65.1
C_4	68.8	73.5	74.9	81.7	69.1	78.1	65.5
C_5	174.0	171.6	170.2	171.3	172.5	171.7	171.7
C_{6a}	144.0	144.9	144.6	145.4	146.3	145.8	149.7
C_7	123.8	114.2	114.5	114.7	113.4	115.4	125.5
C_8	129.5	128.2	128.5	128.3	128.4	128.9	128.3
C_9	123.8	124.1	124.4	123.1	123.5	123.4	109.2
C_{10}	123.8	123.2	123.4	124.1	123.3	123.5	119.5
C_{10a}	138.3	137.5	137.7	137.5	137.6	138.6	135.1
C_{10b}	53.1	52.0	53.7	54.4	54.9	55.1	57.6
C_{10c}	89.4	92.6	92.7	94.1	93.2	96.5	90.8
OCH_3	52.5	54.0	52.2	51.9	51.4	51.7	51.9
$C_{1'}$	36.6	26.5	36.5	37.1	37.1	36.5	37.0
$C_{2'}$	118.4	119.1	119.0	117.3	119.0	118.7	118.6
$C_{3'}$	136.0	135.1	136.2	135.1	135.2	134.9	135.8
$C_{4'}$	18.4, 25.9	18.3, 25.9	18.2, 25.9	25.9, 18.2	18.2;256.0	18.0, 25.9	18.2, 25.9
R^1	21.0	28.2, 126.7, 128.4, 129.5, 137.5	127.6, 128.1, 128.8, 135.1	24.6, 34.9	—	—	27.3, 36.5
R^2	25.2	—	—	—	38.7, 126.6, 128.4,129.5, 138.1	26.3, 36.7	—
CO_2	172.5	171.6	171.9	171.9	173.8	173.7	172.1

[a] Foams with satisfactory analysis for C, H, N.
[b] Novapack C_{18} (3.9 × 150 mm, 4 μm). A = CH_3CN, B = 0.05% TFA in H_2O.
[d] Spectra registered at 300 or 400 MHz in $CDCl_3$, assigned with the help of COSY spectra.
[e] Spectra registered at 75 or 100 MHz in $CDCl_3$, assigned with the help of HSQC and HMBC spectra.

4.7.1. (2*S*,3*aS*,8*aR*)-2-Methoxycarbonyl-3*a*-prenyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indole (19)

HPLC [Novapak C_{18} (3.9 × 150 mm, 4 μm), (A:B, 50:50)] t_R 2.1 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H, CH_3), 1.68 (d, 3H, CH_3), 1.74 (bs, 1H, 1-NH), 2.01 (dd, 1H, J = 11 and 12 Hz, 3-H), 2.38 (dd, 1H, J = 6 and 12 Hz, 3-H), 2.44 (m, 2H, 1′-H), 3.71 (dd, 1H, J = 6 and 11, 2-H), 3.71 (s, 3H, OCH_3), 4.91 (s, 1H, 8*a*-H), 5.13 (m, 1H, 2′-H), 6.57 (dd, 1H, J = 8 Hz, 7-H), 6.73 (t, 1H, J = 7.5 Hz, 5-H), 7.04 (dd, 1H, J = 7.5 and 8 Hz, 6-H), 7.04 (d, 1H, J = 7.5 Hz, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 and 26.0 (CH_3), 36.9 ($C_{1'}$), 44.1 (C_2), 52.1 (OCH_3), 58.7 (C_{3a}), 59.4 (C_3), 82.1 (C_{8a}), 109.0 (C_7), 118.8 (C_5), 119.9 ($C_{2'}$), 123.6 (C_6), 128.1 (C_4), 133.1 (C_{3b}), 134.6 ($C_{3'}$), 149.9 (C_{7a}), 174.3 (CO_2). ES-MS m/z 286 $[M+1]^+$. Anal. Calcd. for $C_{17}H_{22}N_2O_2$: C, 71.30; H, 7.74; N, 9.78. Found: C, 71.54; H, 7.89; N, 9.57.

4.7.2. (2*S*,3*aR*,8*aS*)-2-Methoxycarbonyl-3*a*-prenyl-1,2,3,3*a*,8,8*a*-hexahydropyrrolo[2,3-*b*]indole (20)

HPLC [Novapak C_{18} (3.9 × 150 mm, 4 μm), (A:B, 50:50)] t_R 1.9 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.55 (s, 3H, CH_3), 1.68 (d, 3H, CH_3), 1.74 (br s, 1H, 1-NH), 2.37 (dd, 1H, J = 8 and 13 Hz, 3-H), 2.48 (dd, 1H, J = 4 and 13 Hz, 3-H), 2.53 (m, 2H, 1′-H), 3.34 (s, 3H, OCH_3), 3.88 (dd, 1H, J = 4 and 8, 2-H), 4.86 (s, 1H, 8*a*-H), 5.71 (m, 1H, 2′-H), 6.55 (dd, 1H, J = 7Hz, 7-H), 6.71 (t, 1H, J = 7 Hz, 5-H), 7.02 (m, 2H,4-H and 6-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.0 and 26.0 (CH_3), 41.5 ($C_{1'}$), 43.0 (C_2), 51.9 (OCH_3), 57.1 (C_{3a}), 50.1 (C_3), 82.4 (C_{8a}), 109.6 (C_7), 118.1 (C_5), 118.9 ($C_{2'}$), 123.8 (C_6), 128.3 (C_4), 132.6 (C_{3b}), 134.0 ($C_{3'}$), 149.5 (C_{7a}), 173.9 (CO_2). ES-MS m/z 286 $[M+1]^+$.

4.8. General procedure of cyclative prenylation of tryptophan-derived α-amino nitriles. Synthesis of 21(b,d–f), 22d and 22f

Prenyl bromide (267 μL, 2.28 mmol) was dropwise added to a vigorously stirred suspension of the corresponding α-amino nitrile **8b–f** (0.38 mmol) dissolved in CH_3CN (2 mL) in a solution of magnesium nitrate hexahydrate (489 mg, 1.9 mmol) in acetic acid/sodium acetate buffer (pH 2.9, prepared from 8 g of sodium acetate, 100 mL of acetic acid, and 20 mL of H_2O, 15 mL) under argon. After 2 h of stirring at room temperature, the reaction mixture was sequentially neutralized with Na_2CO_3 and extracted with CH_2Cl_2 (20 mL). The organic extracts were successively washed with H_2O (5 mL) and brine (5 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was purified by circular chromatography, using 12–50% gradient of EtOAc in hexane as eluant, to give the corresponding 10*b*-prenyl-pyrroloimidazoindoles **21**–**22** as foams, whose significant analytical and spectroscopic data are summarized in Table 5.

4.9. Evaluation of cytotoxicity

A colorimetric assay, using the sulforhodamine B (SRB) reaction, was adapted for a quantitative measurement of cell growth and viability, following the technique described by Skehan et al.[31] Cells (MDA-MB-231, A549 and HT-29) were seeded in 96-well microtiter plates, at 5 × 10^3 cells per well in aliquots of 195 μL of RPMI medium, and they were allowed to attach to the plate surface by growing in drug free medium for 18 h. Afterwards, samples were added in aliquots of 5 μL [dissolved in (3:7) DMSO/H_2O]. After 48 h exposure, cells were fixed by adding 50 μL of cold 50% (wt/vol) trichloroacetic acid, and incubating at 4 °C for 60 min. Then, the plates were washed with deionized H_2O and dried. 100 microliters of SRB solution (0.4% wt/vol in 1% acetic acid) was added to each microtiter well and these were incubated at room temperature for 10 min. Unbound SRB was removed by washing with 1% acetic acid, the plates were air dried, and the bound stain was solubilized with Tris buffer. Optical densities were read on an automated spectrophotometer plate reader at a single wavelength of 490 nm. Data analysis was automatically generated by the high throughput screening LIMS implemented at the laboratory. The three response parameters GI_{50} (50% cell growth inhibition), LC_{50} (50% lethal concentration), and TGI (total growth inhibition) were extracted from concentration-response curves by linear interpolation, according to the National Cancer Institute (NCI) protocols.[32]

4.10. Evaluation of EGFR signalling pathway inhibition

HeLa cells stably transfected with the AP1-luc reporter gene [maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and 100 U/mL of penicillin and streptomycin at 37 °C and 5% of CO_2] were plated at a density of 20,000 cells/well in white opaque 96-well microplates and let to set for 24 h. Afterwards, prior to stimulation with EGF (25 ng/mL), cells were treated with vehicle alone (1% DMSO), test compounds at five different concentrations (10, 2, 0.4, 0.08, and 0.016 μg/mL) or the EGFR inhibitor AG-1478 (20 μM). To evaluate first the potential cytotoxic effects of compounds, cells were incubated with calcein-AM (0.1 μM) for 30 min at 37 °C, and the cell survival was quantified using a microplate fluorometer(ex-485 nm/em-535 nm, green fluorescence). Cells were washed with phosphate buffered saline (PBS) to remove culture medium and excess of calcein-AM. Then, luciferase activity was assessed in a microplate luminometer, using the Promega Bright-Glo Luciferase Assay System. Results were expressed as percentage of control values (survival and reporter activity).

4.11. Evaluation of inhibition of HIF-1α

HeLa cells stably transfected with the HIF1-luc reporter gene (maintained in DMEM, supplemented with 10% FBS, 1% L-glutamine and 100 U/mL of penicillin and streptomycin at 37 °C and 5% of CO_2) were plated at a density of 20,000 cells/well in white opaque 96-well microplates and let to set for 24 h. Established cultures were pre-treated with vehicle alone (1% DMSO) or test compounds at the defined final concentrations for 30 min and then treated with 50 μM deferoxamine (hypoxia mimetic) and incubated for 24 h. To evaluate first the potential cytotoxic effects of the compounds, cells were incubated with 0.1 μM calcein-AM for 30 min at 37 °C, and cell survival quantified using a microplate fluorometer (ex-485 nm/em-535 nm, green fluorescence). Cells were washed in PBS to remove culture medium and excess calcein-AM, and then, luciferase activity was assessed in a microplate luminometer using the Promega Bright-Glo Luciferase Assay System. Results were expressed as percentage of control values (survival and reporter activity).

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