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Synthesis of 5,6-dihydropyrrolo[2,1-*a*]isoquinolines featuring an intramolecular radical-oxidative cyclization of polysubstituted pyrroles, and evaluation of their cytotoxic activity†

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A three-step protocol for the synthesis of 1,2,3,8,9-pentasubstituted-5,6-dihydropyrrolo[2,1-*a*]-isoquinolines is described, using van Leusen's polysubstituted pyrrole construction followed by intramolecular radical-oxidative cyclization of the isoquinoline system. The cytotoxic activities of the dihydropyrroloisoquinolines were tested on six tumor cell lines. Preliminary structure–activity studies revealed the importance of the identity of the aromatic substituent at the C-2 position, particularly a phenyl, *m*-(amino) phenyl or *m*-(cyclohexylmethylpiperazinamide) phenyl substituent, for cytotoxic activity.

Introduction

The synthesis of pyrrolo[2,1-*a*]isoquinoline (**1**, Fig. 1) has recently become an important goal after the discovery of this tricyclic core in several alkaloids, including (±)-crispine A (**2**)[1] and the lamellarin family (**3**),[2] such as lamellarin D (**4**),[3] which exhibits significant cytotoxic activity. In addition, compounds with a pyrroloisoquinoline frame (**1**) have been described as having phosphodiesterase 10a (PDE 10a) inhibitory activity,[4a,b] antineoplastic activity,[4b–e] and hypotensive, sympatholytic and psychotropic properties,[4f] as well as being useful in the treatment of diseases such as psoriasis.[4g]

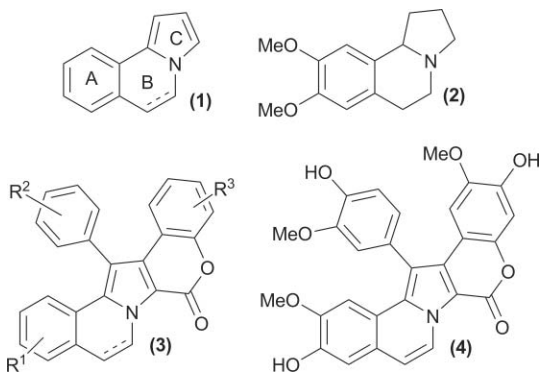
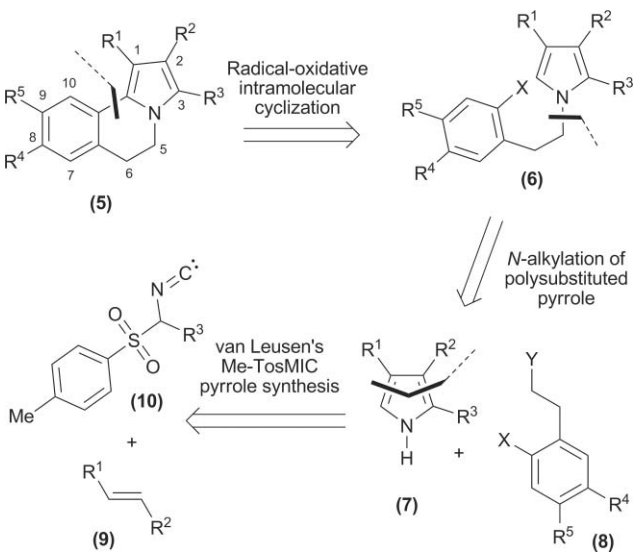


Fig. 1 Alkaloids that contain a pyrrolo[2,1-*a*]isoquinoline core (**1**).

With the discovery of the biological activity of lamellarins,[2j] several routes for the synthesis of the pyrrolo[2,1-*a*]isoquinoline skeleton have been described.[2d,5] The majority of these routes are based on pyrrole construction (ring C, **1**) from 1- and/or *N*-functionalized isoquinolines,[6,7] and the remainder are based on closure of the dihydroisoquinoline system AB (**1**) from the respective pyrrole.[8,9] Here, we report a new and straightforward three-step synthesis of 1,2,3,8,9-pentasubstituted-5,6-dihydropyrrolo[2,1-*a*]isoquinolines **5** (Scheme 1), using readily accessible starting materials, such as 1,2-disubstituted alkenes (**9**) and *p*-toluenesulfonylmethylisocyanide (TosMIC) (**10**), featuring a van Leusen's polysubstituted pyrrole construction and the formation of an aromatic sp^2 C–C bond through an oxidative free radical cyclization. The retrosynthesis for **5** is shown in Scheme 1. Deconstruction of the dihydroisoquinoline system



Scheme 1 Retrosynthesis of 1,2,3,8,9-pentasubstituted-5,6-dihydropyrrolo[2,1-*a*]isoquinolines **5**.

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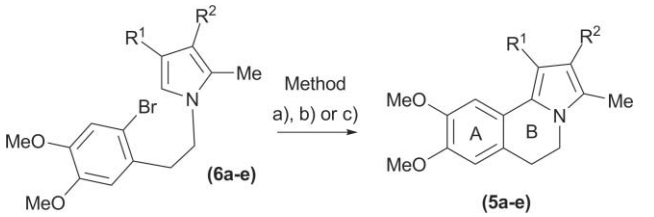
(AB, **5**) *via* a radical-oxidative cyclization leads to precursor **6**, which, in principle, could be obtained through the *N*-alkylation of the pyrrole **7** and the alkylating agent **8**. Finally, the 2,3,4-trisubstituted pyrrole **7** could be constructed using the van Leusen's synthesis of pyrroles, reaction of TosMIC (**10**) with a Michael acceptor **9**.[10] We measured the cytotoxic activity of the products in six cancer cell lines.

Results and discussion

Chemistry

Following our retrosynthetic strategy, we first obtained the alkenes **9b–e** *via* the Heck reaction with a $Pd(OAc)_2$ catalyst. The compounds **9b–d** were obtained exclusively as (*E*)-isomers with moderate (**9d**, 65%) to good yields (**9b**, 97% and **9c**, 80%).[11] The 3-phenylacrylonitrile (**9e**) was isolated as a mixture of *E*/*Z* isomers (65 : 35, determined by ^{1}H-NMR) with 88% yield. In parallel, the commercially available TosMIC was alkylated under phase-transfer conditions, as reported previously by van Leusen,[12] to afford the mono-alkylated TosMIC (**10**, R^3 = Me) in 90% yield. With the alkenes **9a–e** and the Me-TosMIC in hand, our next step was the synthesis of **7** (Scheme 2), following van Lausen's protocol, to obtain the 2,3,4-polysubstituted pyrroles.[10] Pyrrole **7a** was obtained from the commercially available ethylacrylate in 85% yield,[13] whereas the pyrroles **7b–e** were prepared from their respective above-described alkenes, **9b–e**, with moderate (**7c–e** 61–82% yields) to good yields (**7b**, 91%, Scheme 2). It is noteworthy that the C-4 substituent in each pyrrole acted as an electron withdrawing group (R^1 = –CO_2Et or –CN) in the general van Leusen's reaction mechanism. Having prepared the pyrroles **7a–e**, our next step was their *N*-alkylation with 2-(2-bromo-4,5-dimethoxyphenyl)ethyl *p*-toluenesulfonate (**8**, X = Br and Y = OTs, 2.0 eq.), in the presence of NaH (2.5 eq.) in DMSO solvent, to afford the *N*-alkyl-pyrroles **6a–e** with moderate yields (60–87%, Scheme 2). The elimination product from **8** was also observed. In preliminary reactions, the 2-(2-bromo-4,5-dimethoxyphenyl)ethyl bromide compound (**8**, X = Y = Br) was used as an alkylating agent, but the elimination product was afforded as the major product. To avoid the elimination product, Albericio[8b] previously reported *N*-alkylation of the methyl pyrrole-2-carboxylate, using K_2CO_3 as the base instead of a stronger base such as NaH. However, alkylation was only carried out in the absence of methoxy groups on the aryl moiety of **8**.

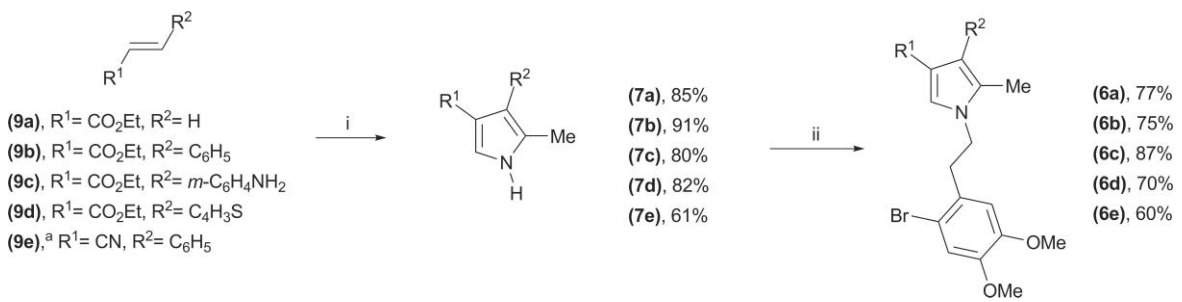
Table 1 Cyclization of the isoquinoline system (AB)



Entry	Product	R^1	R^2	Method[a]	Yield (%)
1	**5a**	CO_2Et	H	a	40
2	**5a**	CO_2Et	H	b	53
3	**5a**	CO_2Et	H	c	70
4	**5b**	CO_2Et	C_6H_5	c	80
5	**5c**	CO_2Et	*m*-$C_6H_4NH_2$	c	85
6	**5d**	CO_2Et	C_4H_3S	c	90
7	**5e**	CN	C_6H_5	c	75

[a] Method: (a) $Pd(OAc)_2$ (0.33 eq.), PPh_3 (0.33 eq.), nBu$_4$NBr (0.1 eq.), K_2CO_3 (2 eq.), DMF, 120° C, 12 h; (b) AIBN (0.3 eq.), nBu$_3$SnH (1.2 eq.), refluxing toluene, 2.0 h; and method (c) nBu$_3$SnH (2.5 eq)/toluene (0.75 M), DLP (2.5 eq.), reflux, 7.0 h.

In preliminary reactions, the *N*-alkyl-pyrrole **6a** was cyclized to the corresponding tetrasubstituted 5,6-dihydro-pyrroloisoquinoline **5a** using either palladium-catalyzed cyclization with a 40% yield (entry 1, Table 1)[14] or using n-Bu$_3$SnH–AIBN conditions, after which the product **5a** was isolated with 53% yield (entry 2). Even though the yield of the tin-mediated radical reaction was moderate (entry 2), intramolecular radical cyclization has rarely been used to construct a pyrroloisoquinoline frame.[15] With this strategy, our next challenge was to improve the yield of the ring closure process by performing a free radical reaction. Miranda[16] previously reported an oxidative-radical cyclization in enamide systems using a mixture of n-Bu$_3$SnH and dilauroyl peroxide (DLP) as the initiator, rather than AIBN, and the oxidized products were obtained. The implementation of this strategy afforded compound **5a** with 70% yield instead of the 40% yield achieved using n-Bu$_3$SnH–AIBN (entry 3, Table 1), giving a 46% overall yield. In applying this optimization procedure, the 5,6-dihydropyrroloisoquinoline **5b** was obtained from the *N*-alkylpyrrole **6b** with 80% yield and 55% overall yield (entry 4),[17a] **6c** was cyclized to the C-2 *m*-aniline pyrroloisoquinoline **5c** with 85% yield and 60% overall yield (entry 5, 63% overall yield previously reported),[4a] and the C-2 thiophene compound **5d**



Scheme 2 Synthesis of the *N*-alkylated pyrroles **6a–e**. Conditions: (i) NaH (2.0 eq.)/Et_2O; **9a–e** (1.0 eq.) and **10** (R^3 = Me, 1.1 eq.)/Et_2O–DMSO (2 : 1), RT, 1.0 h; (ii) **8** (X = Br, Y = OTs, 2.0 eq.), NaH (2.5 eq.), DMSO, RT, 2.0 h. [a] These compounds were isolated and used as a *cis*–*trans* mixture (*cis* : *trans*, 65 : 35).

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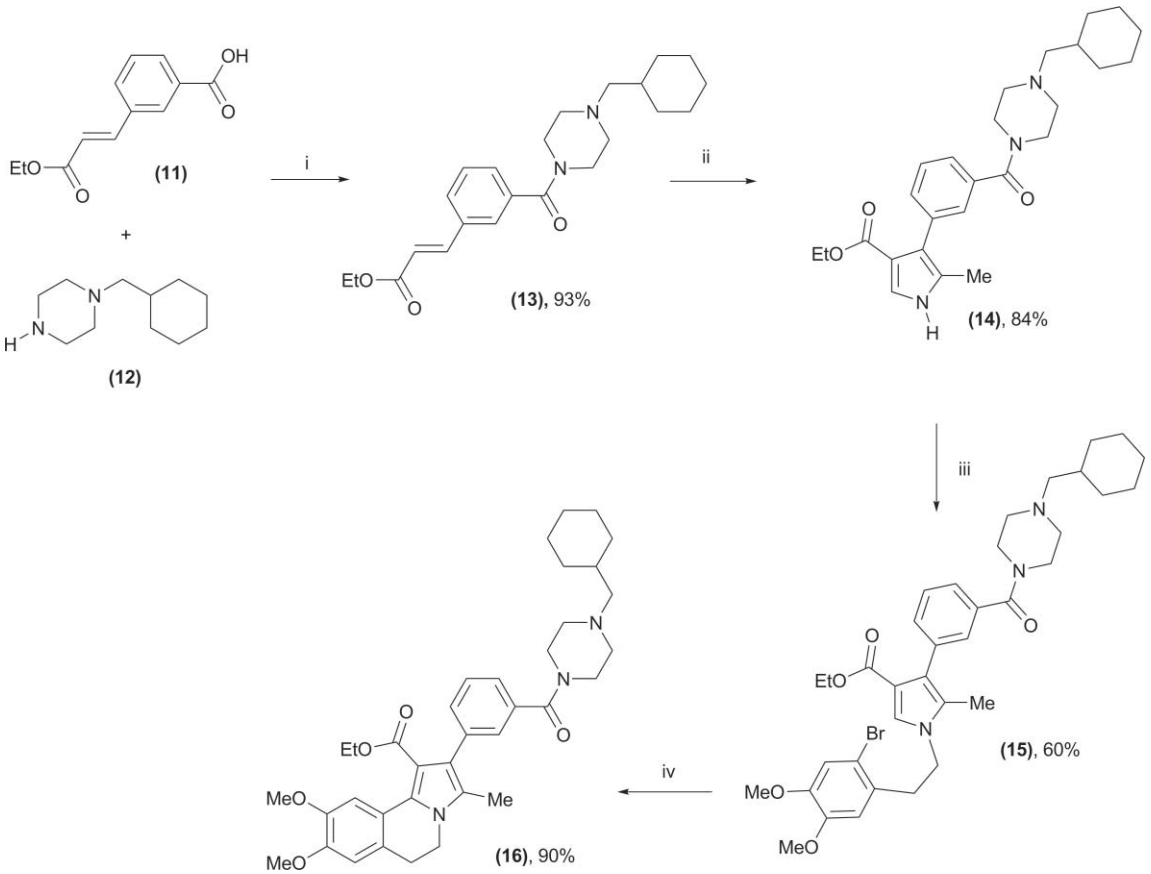

Scheme 3 Synthesis of the pyrrolo[2,1-*a*]isoquinoline **16**. Conditions: (i) DCC (1.1 eq.), CH_2Cl_2, 0° C, 30 min, then RT, 4.0 h. (ii) NaH (2.0 eq.)/Et_2O; **13** (1.0 eq.) and **10** (R^3 = Me, 1.1 eq.)/Et_2O–DMSO (2 : 1), RT, 1.0 h; (iii) **8** (X = Br, Y = OTs, 2.0 eq.), NaH (2.5 eq.), DMSO, RT, 2.0 h; (iv) $n$$Bu_3SnH$ (2.5 eq.)/toluene (0.75 M), DLP (2.5 eq.), reflux, 7.0 h.

was isolated with 90% yield and 52% overall yield (entry 6, 42% overall yield previously reported).[17b] Finally, pyrroloisoquinoline **5e**, which contains the nitrile substituent at the C-1 position in place of the ethyl ester (**5b**), was afforded with 75% yield and 28% overall yield (entry 7).

Having demonstrated the viability of the intramolecular radical-oxidative cyclization strategy, we next demonstrated the scope of the methodology through the synthesis of the 5,6-dihydropyrroloisoquinoline **16** (Scheme 3), which is an inhibitor of PDE 10a.[4b] First, we prepared the alkene **11** by performing a Heck reaction between 3-iodobenzoic acid and ethylacrylate, with 66% yield.[18] In parallel, the 1-(cyclohexylmethyl)piperazine (**12**) was obtained with 62% yield from *N*-formylpiperazine[19a] and (bromomethyl)cyclohexane, as previously reported by Meanwell.[19b] The coupling reaction between **11** and *N*-alkyl piperazine **12** (Scheme 3) was achieved in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 at 0° C, to afford **13** with 93% yield. With the alkene **13** in hand, our next step was the synthesis of the 2,3,4-trisubstituted-pyrrole **14**, obtained with 84% yield through a mediated van Leusen's reaction. Finally, *N*-alkylation of **14** afforded the pyrroloisoquinoline precursor **15** (60% yield), which was radical-cyclized to **16** with 90% yield and 42% overall yield from **13** (Scheme 3, ref. 4b 36% overall yield). The structure of the pyrroloisoquinoline **16** was confirmed by crystallographic analysis (Fig. 2).

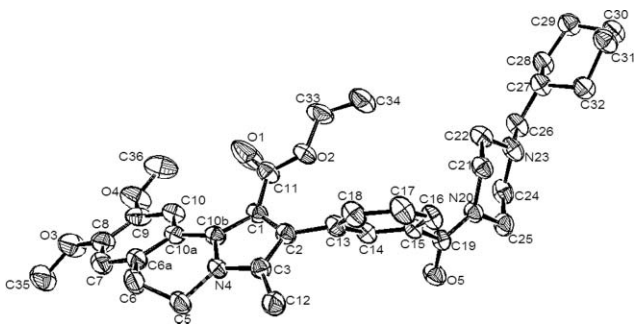

Fig. 2 ORTEP drawing of **16** (30% probability level). The cyclohexyl-methyl group at the piperazine moiety is modeled as disordered over two positions.

Biological activity

We evaluated the inhibitory activity of compounds **5a–e**, **16**, and **16·HCl**[20] toward the growth of PC-3 prostate cancer, U-251 central nervous system cancer, K-562 leukemia, HCT-15 colon cancer, MFC-7 breast cancer and SKUL-1 lung cancer cells (Table 2). Compound **5a** (R^1 = CO_2Et and R^2 = H) moderately inhibited the growth of two cancer cell lines, with IC_{50} values in the micromolar range (U-251, IC_{50} = 33.43 ± 4.500 μM; and K-562, IC_{50} = 48.11 ± 0.600 μM) and did not show activity toward the SKLU-1 cancer

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Table 2 The IC_{50} values (μM) of compounds **5a–e**, **16** and **16·HCl** in the six cancer cell lines.[a]

Comp.	PC-3 (prostate)	U-251 (CNS)	K-562 (leukemia)	HCT-15 (colon)	MCF-7 (breast)	SKLU-1 (lung)
5a	68.17 ± 5.300	33.43 ± 4.500	48.11 ± 0.600	54.07 ± 4.400	68.51 ± 4.70	100
5b	18.15 ± 0.600	4.86 ± 0.600	76.78 ± 7.300	0.14 ± 0.060	25.2 ± 2.000	0.59 ± 0.005[b]
5c	21.2 ± 1.200	5.96 ± 0.500	2.5 ± 0.800	0.01 ± 0.003[b]	1.3 ± 0.100	0.10 ± 0.010[b]
5d	8.47 ± 0.230	6.99 ± 0.670	4.07 ± 0.490	0.59 ± 0.050[b]	7.41 ± 0.090	2.13 ± 0.030
5e	>100	>100	>100	>100	>100	>100
16	0.16 ± 0.010[b]	0.05 ± 0.009[b]	0.16 ± 0.010[b]	0.02 ± 0.010[b]	5.58 ± 0.040	0.02 ± 0.001[b]
16·HCl	3.58 ± 0.100	0.16 ± 0.020[b]	1.15 ± 0.050	0.05 ± 0.003[b]	1.06 ± 0.040	0.44 ± 0.010[b]
CPT[c]	0.12 ± 0.010 [b]	0.024 ± 0.005 [b]	0.59 ± 0.020 [b]	0.13 ± 0.005 [b]	0.16 ± 0.01 [b]	0.15 ± 0.009 [b]

[a] Values are means of three experiments, (>100, not active). [b] IC_{50} values can be expressed in the nanomolar range (nM). [c] Camptothecin (CPT) was purchased from Sigma-Aldrich Chemical Co.

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cell line. Substitution of the R^2 = H substituent at the C-2 position with a benzene ring (compound **5b**, $R^1 = CO_2Et$ and $R^2 = C_6H_5$) produced an increase in the growth inhibitory activity in five cell lines, U-251 (IC_{50} = 4.86 ± 0.600 μM), HCT-15 (IC_{50} = 0.14 ± 0.060 μM), and SKLU-1 (IC_{50} = 0.59 ± 0.005 μM), with the exception of K-562 (IC_{50} = 76.78 ± 7.300 μM). In contrast to **5b**, when the ethyl ester substituent at the C-1 position was changed to a cyano group (compound **5e**, R^1 = CN and $R^2 = C_6H_5$), the resulting compound did not inhibit proliferation of any of the six cancer cell lines tested. Based on this last result, the ethyl ester substituent was fixed at the C-1 position, and only the aromatic moiety at the C-2 position was modified (**5c–d**). In a previous report,[4a] compound **5c** ($R^1 = CO_2Et$ and $R^2 = m\text{-}C_6H_4\text{–}NH_2$) was described as having PDE 10a inhibitory activity (IC_{50}= 110 nM) and was assessed *in vitro* on a MDA-MB-231 human breast carcinoma proliferation assay (% inhibition at 10 μM = 93.0). Here, we evaluated the ability of compound **5c** to inhibit growth of six cancer cell lines. Surprisingly, incorporation of the amine group at the *meta* position of the benzene ring in **5c** significantly increased the inhibitory activity relative to **5b**: U-251 (IC_{50} = 5.96 ± 0.500 μM), K-562 (IC_{50} = 2.5 ± 0.800 μM), MCF-7 (IC_{50} = 1.3 ± 0.100 μM), and SKLU-1 (IC_{50} = 0.10 ± 0.010 μM). Furthermore, the IC_{50} of the HCT-15 cell line was measured to be in the nanomolar range (10.0 ± 0.003 nM). When the benzene ring was changed to a thiophene ring (compound **5d**, $R^1 = CO_2Et$ and $R^2 = C_4H_3S$), growth inhibition of three cell lines surpassed the inhibitory activity of **5b**: PC-3 (IC_{50} = 8.47 ± 0.230 μM), K-562 (IC_{50} = 4.07 ± 0.490 μM), and MCF-7 (IC_{50} = 7.41 ± 0.090 μM), but was not better than **5c** in any cell lines except the PC-3 prostate cell line. These preliminary results demonstrated the positive effect of the ethyl ester substituent at the C-1 position on the growth inhibitory activity of **5a–d**, showing better results than compounds containing a nitrile substituent (**5e**). Additionally, the aromatic moiety at the C-2 position plays an important role (**5b–d**), specifically when this substituent is a benzene ring (**5b–c**), and the *meta*-substituted amine group (**5c**) is even more significant.

In addition, we evaluated the activity of compound **16**, which has a cyclohexylmethyl piperazinamide substituent at the *meta* position of the benzene. The piperazinamide moiety was shown to have an important role in the biological activity of some compounds, such as dihydroimidazo[4,5-b]quinolinones, which have been reported to show blood platelet cAMP PDE inhibitory activity and ADP-induced platelet aggregation.[19a] Furthermore, the hydrochloric salt of **16** (**16·HCl**) was described as a PDE 10a inhibitor (IC_{50} = 410 nM).[4b] These reports suggest that the cyclohexylmethyl piperazinamide substituent in **16** played an important role in the cytotoxic activity of compounds with IC_{50} values in the nanomolar range: U-251 (IC_{50} = 50 ± 0.009 nM), HCT-15 (IC_{50} = 20 ± 0.010 nM), and SKLU-1 (IC_{50} = 20 ± 0.001 nM). The inhibitory growth activity of the hydrochloric salt **16·HCl** was evaluated, but the activity was not higher than that of the neutral compound (**16**). It is noteworthy that even when the activity of **16** was higher in three cell lines than the activity of the *m*-amine compound **5c**, the latter compound had the smallest IC_{50} value in the HCT-15 cell line (IC_{50} = 10.0 ± 0.003 nM).

Nowadays, an important group of lamellarins are described as inhibitors of topoisomerase I (Top I-DNA), including the lamelarin D (**4**).[2j,3d,21] With this idea, the cytotoxic activity of the synthesized compounds **5a–e**, **16** and **16·HCl** was compared with a commercially available inhibitor of Top I-DNA, camptothecin (CPT).[22] As is shown in Table 2, we observed that CPT was more active than all the synthesized pyrrolo[2,1-*a*]isoquinolines in the prostate PC-3 cell line (IC_{50} = 0.12 ± 0.010 μM), CNS U-251 cell line (IC_{50} = 0.024 ± 0.005 μM) and breast MCF-7 cell line (IC_{50} = 0.16 ± 0.010 μM). On the other hand, the synthesized compound **16** showed more activity in the leukemia K-562 cell line (IC_{50} = 0.16 ± 0.010 μM) and lung SKLU-1 cell line (IC_{50} = 0.02 ± 0.001 μM) compared with the reference CPT (IC_{50} = 0.59 ± 0.020 μM and 0.15 ± 0.009 μM, respectively). Finally, compound **5c** showed a higher activity than CPT (IC_{50} = 0.13 ± 0.005 μM) in the colon HCT-15 cell line (IC_{50} = 0.01 ± 0.003 μM) and a similar activity in the lung SKLU-1 cell line (IC_{50} = 0.10 ± 0.010 μM).

Conclusion

In summary, a short and practical route to the C-1 and C-2 functionalized 5,6-dihydropyrrolo[2,1-*a*]isoquinolines, involving a van Leusen's polysubstituted pyrrole construction and an intramolecular radical-oxidative cyclization of the AB isoquinoline system, is described. The expedient synthesis of a more complex structure that contains the *m*-(cyclohexylmethylpiperazinamide) phenyl substituent at the C-2 position of the pyrroloisoquinoline frame (**16**) underscores the scope of the structural diversity of this methodology. We evaluated the inhibitory activities of the 5,6-dihydropyrroloisoquinolines **5a–b**, **16**, and **16·HCl** toward the growth of six cancer cell lines. We demonstrated that the aromatic moiety at the C-2 position has a significant influence on the cytotoxic activity (**5b–d**), particularly if this moiety is a benzene ring (**5b–c**) or, even more significantly, if this moiety is an amine group, *i.e.* **5c**, which showed the lowest IC_{50} value, in the nanomolar

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range (HCT-15 cell line). We also observed that a cyclohexylmethyl piperazinamide substituent at the *meta* position of the benzene ring in **16** increased the activity relative to the compound containing a simple benzene ring at the C-2 position (**5b**), with IC_{50} values in the nanomolar range (HCT-15 and SKLU-1 cell lines). The cytotoxic activity of the synthesized compounds was compared with the Top I-DNA inhibitor camptothecin (CPT). The compounds **16** and **5c** were more active than CPT in the K-562, HCT-15 and SKLU-1 cell lines. In view of these preliminary structure–activity relationship studies, we are currently continuing to synthesize new 1,2,3,8,9-pentasubstituted-5,6-dihydropyrrolo[2,1-*a*]isoquinolines, applying the synthetic methodology described here toward development of novel anticancer agents.

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Experimental

General details

^{1}H NMR spectra were recorded on Varian Gemini-200 MHz and Eclipse 300 MHz JEOL spectrometers in deuterated chloroform ($CDCl_3$) solutions with internal standard TMS (0 ppm), or in deuterated dimethyl sulfoxide (DMSO-d_6). The chemical shifts are reported in parts per million (δ (ppm)). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The coupling constants (J) are reported in Hertz (Hz). ^{13}C NMR spectra were recorded at 50 MHz and 75 MHz on the same instruments. Assignments of ^{13}C spectra were performed by distortionless enhancement by polarization transfer (DEPT) experiments. IR spectra were collected on a FT-IR Tensor 27 Bruker spectrometer. Mass spectra were recorded on a JEOL JEM-AX505HA spectrometer by electronic impact (EI) at 70 eV for low resolution. Elemental analysis was performed on a CE-440 elemental analyzer (Exeter Analytical, Inc). X-Ray crystallography was carried out on a Bruker Smart Apex CCD diffractometer. Flash column chromatography was carried out on silica gel 60 (230–400 mesh ASTM) from Macherey-Nagel GmbH & Co.

(*E*)-Ethyl 3-(3-(4-(cyclohexylmethyl)piperazine-1-carbonyl)-phenyl)acrylate (13)

To a solution of (*E*)-3-(2-ethoxycarbonyl-vinyl)benzoic acid (**11**, 1.0 g, 4.5 mmol) in CH_2Cl_2 (4.5 mL), 1-(cyclohexylmethyl)piperazine (**12**, 0.83 g, 4.5 mmol) was added and cooled to 0° C. *N*,*N*′-Dicyclohexylcarbodiimide (1.0 g, 5 mmol) was then added, and the mixture was stirred for 30 min. After that time, the reaction was warmed at room temperature and stirred for an additional 4.0 h. The *N*,*N*′-dicyclohexylurea was eliminated by filtration and washed with CH_2Cl_2. The solvent was evaporated *in vacuo*, and the residue was purified by flash column chromatography on silica gel, TLC (EtOAc–Et_3N, 80 : 20 v/v): R_f 0.71, to furnish **13** (1.97 g) as a brown solid, m.p. 58 °C, yield 93%. ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 0.79–0.95 (m, 2H), 1.11–1.25 (m, 3H), 1.34 (t, J = 7.2, 3H), 1.45–1.80 (m, 6H), 2.17 (d, J = 7.2 Hz, 2H), 2.36 (brs, 2H), 2.47 (brs, 2H), 3.42 (brs, 2H), 3.80 (brs, 2H), 4.27 (q, J = 7.0 Hz, 2H), 6.46 (d, J = 16.0 Hz, 1H), 7.40–7.43 (m, 2H), 7.55–7.57 (m, 2H), 7.67 (d, J = 16.0 Hz, 1H); ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 169.4, 166.7, 143.5, 136.7, 134.8, 129.0, 128.6, 126.5, 119.4, 65.3, 60.6, 53.9, 53.2, 47.6, 42.1, 34.9, 31.7, 26.7, 26.0, 14.3; IR (Film, cm^{-1}) 3411, 3059, 2981, 2923, 2851, 2806, 2771, 1713, 1638, 1443; MS (EI) m/z 384 (M^+, 15%), 301 (M^+–83, 100%); Found: C, 71.90; H, 8.32; N, 7.32. Calc. for $C_{23}H_{32}N_2O_3$: C, 71.84; H, 8.39; N, 7.29%.

Experimental procedure for the pyrroles

A solution of the alkene (2.6 mmol) and 1-(1-isocyanoethylsulfonyl)-4-methylbenzene (**10**, 0.6 g, 2.9 mmol) in Et_2O–DMSO (2 : 1, 15 mL) was added dropwise to a suspension of NaH (0.23 g, 5.7 mmol, 60% dispersion in mineral oil and previously washed with dry hexane) in dry ether (5 mL). The mixture was stirred at room temperature for 1.0 h, then H_2O (15 mL) was added dropwise and the product was extracted with EtOAc (3 × 30 mL). The organic layer was washed with H_2O and brine (3 × 10 mL), and dried with Na_2SO_4, then evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel to furnish the respective pyrrole.

Ethyl 5-methyl-1*H*-pyrrole-3-carboxylate (7a). From commercially available ethylacrylate (0.28 mL, 2.6 mmol), purified by flash column chromatography on silica gel, to furnish 0.34 g of **7a** as a white solid, m.p. 65° C (lit.[ref. 13] 70° C), yield 85%. TLC (hexane–EtOAc–Et_3N, 45 : 50 : 5 v/v): R_f 0.69, ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.33 (t, J = 7.2 Hz, 3H), 2.23 (s, 3H), 4.26 (q, J = 7.2 Hz, 2H), 6.28–6.30 (m, 1H), 7.26–7.28 (m, 1H), 8.75 (brs, 1H, –N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 165.5, 128.8, 122.5, 116.3, 106.9, 59.6, 14.4, 12.7; IR (KBr, cm^{-1}) 3276, 3169, 2983, 2928, 2867, 2685, 2577, 1676, 1586, 1517, 1441; MS (EI) m/z 153 (M^+, 80%), 108 (M^+–45, 100%); Found: C, 62.76; H, 7.22; N, 9.22. Calc. for $C_8H_{11}NO_2$: C, 62.73; H, 7.24; N, 9.14%.

Ethyl 5-methyl-4-phenyl-1*H*-pyrrole-3-carboxylate (7b). From ethyl cinnamate (**9b**, 0.45 g, 2.6 mmol), purified by flash column chromatography on silica gel, to furnish 0.54 g of **7b** as a yellow solid, m.p. 136 °C, yield 91%. TLC (hexane–EtOAc–Et_3N, 45 : 50 : 5 v/v): R_f 0.66, ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.17 (t, J = 7.2 Hz, 3H), 2.13 (s, 3H), 4.14 (q, J = 7.2 Hz, 2H), 7.23–7.37 (m, 6H), 8.59 (brs, 1H, –N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 165.1, 135.2, 130.5, 127.4, 126.6, 126.1, 122.9, 122.1, 114.5, 59.3, 14.1, 11.4; IR (KBr, cm^{-1}) 3277, 2986, 2942, 2896, 2819, 1952, 1884, 1811, 1682, 1606, 1518, 1494, 1447, 1409, 1383; MS (EI) m/z 229 (M^+, 100%), 184 (M^+–45, 90%); Found: C, 73.32; H, 6.63; N, 6.08. Calc. for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11%.

Ethyl 4-(3-aminophenyl)-5-methyl-1*H*-pyrrole-3-carboxylate (7c). From (*E*)-ethyl 3-(3-aminophenyl)acrylate (**9c**, 0.50 g, 2.6 mmol), purified by flash column chromatography on silica gel, to furnish 0.51 g of **7c** as a white solid, m.p. 48 °C, yield 80%. TLC (hexane–EtOAc–Et_3N, 45 : 50 : 5 v/v): R_f 0.28; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.19 (t, J = 7.2 Hz, 3H), 2.14 (s, 3H), 3.61 (brs, 2H, –NH_2), 4.15 (q, J = 7.2 Hz, 2H), 6.59–6.72 (m, 3H), 7.13 (t, J = 8.1 Hz, 1H), 7.30–7.31 (m, 1H), 8.47 (brs, 1H, –N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 165.0, 145.5, 136.2, 128.3, 126.5, 122.7, 122.2, 121.2, 117.6, 114.6, 113.3, 59.3, 14.2, 11.5; IR (KBr, cm^{-1}) 3459, 3369, 3288, 2983, 2942, 2900, 1684, 1615, 1519, 1491, 1464, 1409, 1382; MS (EI) m/z 244 (M^+, 100%), 198 (M^+ –46, 100%); Found: C, 68.92; H, 6.76; N, 11.39. Calc. for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47%.

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Ethyl 5-methyl-4-(thiophen-2-yl)-1*H*-pyrrole-3-carboxylate (7d). From (*E*)-ethyl 3-(thiophen-3-yl)acrylate (**9d**, 0.47 g, 2.6 mmol), purified by flash column chromatography on silica gel, to furnish 0.50 g of **7d** as yellow solid mp 108 °C, yield 82%. TLC (hexane–EtOAc–Et_3N, 45 : 50 : 5 v/v): R_f 0.64; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.22 (t, J = 7.2 Hz, 3H), 2.21 (s, 3H), 4.18 (q, J = 7.2 Hz, 2H), 6.98–7.05 (m, 2H), 7.27–7.31 (m, 2H), 8.60 (brs, 1H, –N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 164.8, 136.0, 128.4, 127.2, 126.4, 124.7, 123.0, 115.3, 114.3, 59.5, 14.2, 11.7; IR (KBr, cm^{-1}) 3259, 3139, 2984, 2927, 2866, 1674, 1578, 1506, 1468, 1410, 1329; MS (EI) m/z 235 (M$^+$, 100%), 190 (M$^+$–45, 80%); Found: C, 61.28; H, 5.60; N, 5.93. Calc. for $C_{12}H_{13}NO_2S$: C, 61.25; H, 5.57; N, 5.95%.

5-Methyl-4-phenyl-1*H*-pyrrole-3-carbonitrile (7e). From (*E*)/(*Z*)-3-phenylacrylonitrile (**9e**, 65 : 35, 0.34 g, 2.6 mmol), purified by flash column chromatography on silica gel, to furnish 0.29 g of **7e** as a white solid, m.p. 142 °C, yield 61%. TLC (hexane–EtOAc–Et_3N, 45 : 50 : 5 v/v): R_f 0.53; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.28 (s, 3H), 7.17–7.18 (d, J = 3.0 Hz, 1H), 7.25–7.34 (m, 1H), 7.38–7.42 (m, 4H), 8.74 (brs, 1H, –N*H*); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 133.1, 128.7, 128.5, 126.8, 125.9, 124.7, 123.1, 117.2, 92.9, 11.7; IR (KBr, cm^{-1}) 3301, 3141, 3051, 2925, 2856, 2219, 1603, 1584, 1519, 1496, 1455; MS (EI) m/z 182 (M$^+$, 100%); Found: C, 79.02; H, 5.60; N, 15.39. Calc. for $C_{12}H_{10}N_2$: C, 79.10; H, 5.53; N, 15.37%.

Ethyl 4-(3-(4-(cyclohexylmethyl)piperazine-1-carbonyl)phenyl)-5-methyl-1*H*-pyrrole-3-carboxylate (14). From (*E*)-ethyl 3-(3-(4-(cyclohexylmethyl)piperazine-1-carbonyl)phenyl)acrylate (**13**, 1.0 g, 2.6 mmol), purified by flash column chromatography on silica gel, to furnish 0.96 g of **14** as a yellow solid, m.p. 137 °C, yield 84%. TLC (hexane–EtOAc–Et_3N, 45 : 50 : 5 v/v): R_f 0.28; ^{1}H-NMR (200 MHz, $CDCl_3$) δ (ppm): 0.83–0.94 (m, 2H), 1.12–1.23 (m, 6H), 1.42–1.49 (m, 1H), 1.68–1.80 (m, 5H), 2.04 (s, 3H), 2.14 (d, J = 7.2 Hz, 2H), 2.35 (brs, 2H), 2.45 (brs, 2H), 3.53 (brs, 2H), 3.77 (brs, 2H), 4.12 (q, J = 7.2 Hz, 2H), 7.27–7.40 (m, 5H), 9.3 (brs, 1H, –N*H*); ^{13}C-NMR (50 MHz, $CDCl_3$) δ (ppm): 170.8, 164.8, 135.4, 134.8, 131.7, 129.1, 127.7, 127.0, 124.9, 123.1, 121.2, 114.0, 65.5, 59.2, 54.2, 53.4, 47.8, 42.2, 34.9, 31.8, 26.7, 26.0, 14.3, 14.2, 11.3, 11.2; IR (KBr, cm^{-1}) 3411, 3181, 3123, 2922, 2014, 1899, 1710, 1600, 1519, 1491, 1451; MS (EI) m/z 437 (M$^+$, 10%), 354 (M$^+$–83, 100%); Found: C, 71.26; H, 8.19; N, 9.57. Calc. for $C_{26}H_{35}N_3O_3$: C, 71.37; H, 8.06; N, 9.60%.

Experimental procedure for the *N*-alkylpyrroles

NaH (0.2 g, 5.7 mmol, 60% dispersion in mineral oil and previously washed with dry hexane) was added portionwise to a solution of the pyrrole (2.3 mmol) and 2-bromo-4,5-dimethoxyphenethyl 4-methylbenzenesulfonate (**8**, 1.9 g, 4.5 mmol) in dry dimethyl sulfoxide (10 mL). The mixture was stirred at room temperature for 2.0 h, and EtOAc (20 mL) was added followed by washing with H_2O and brine (3 × 10 mL). The organic layer was dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel to furnish the respective *N*-alkylpyrrole.

Ethyl 1-(2-bromo-4,5-dimethoxyphenethyl)-5-methyl-1*H*-pyrrole-3-carboxylate (6a). From **7a** (0.35 g, 2.3 mmol), purified by flash column chromatography on silica gel, to furnish. 0.7 g of **6a** as a yellow solid, m.p. 48 °C, yield 77%. TLC (hexane–EtOAc–Et_3N, 60 : 30 : 10 v/v): R_f 0.52; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.31 (t, J = 7.2 Hz, 3H), 2.0 (s, 3H), 3.04 (t, J = 6.9 Hz, 2H), 3.69 (s, 3H), 3.85 (s, 3H), 4.01 (t, J = 6.9 Hz, 2H), 4.24 (q, J = 6.9 Hz, 2H), 6.23–6.24 (m, 1H), 6.27 (s, 1H), 7.01 (s, 1H), 7.19 (d, J = 1.8 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 164.9, 148.6, 148.4, 129.9, 128.8, 125.3, 115.4, 114.9, 113.9, 113.4, 107.9, 59.4, 56.1, 55.9, 46.6, 37.9, 14.5, 11.6; IR (Film, cm^{-1}) 3596, 3522, 3128, 3080, 2975, 2938, 2846, 1702, 1603, 1569, 1507, 1460, 1447, 1382; MS (EI) m/z 350 (M$^+$–45, 5%), 352 ([M$^+$+2]–45, 5%), 316 (M$^+$–79, 100%); Found: C, 54.60; H, 5.57; N, 3.55. Calc. for $C_{18}H_{22}BrNO_4$: C, 54.56; H, 5.60; N, 3.53%.

Ethyl 1-(2-bromo-4,5-dimethoxyphenethyl)-5-methyl-4-phenyl-1*H*-pyrrole-3-carboxylate (6b). From **7b** (0.52 g, 2.3 mmol), purified by flash column chromatography on silica gel, to furnish 0.8 g of **6b** as a white solid, m.p. 128 °C, yield 75%. TLC (hexane–EtOAc–Et_3N, 60 : 30 : 10 v/v): R_f 0.52; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.14 (t, J = 7.2 Hz, 3H), 1.92 (s, 3H), 3.1 (t, J = 7.2 Hz, 2H), 3.72 (s, 3H), 3.86 (s, 3H), 4.07 (t, J = 6.9 Hz, 2H), 4.11 (q, J = 7.2 Hz, 2H), 6.33 (s, 1H), 7.02 (s, 1H), 7.2–7.28 (m, 3H), 7.29–7.36 (m, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 164.6, 149.2, 149.0, 135.8, 130.6, 129.1, 127.7, 127.4, 126.1, 125.8, 123.4, 116.3, 114.2, 59.1, 56.4, 56.2, 47.0, 37.8, 14.1, 9.9; IR (KBr, cm^{-1}) 3134, 3046, 3013, 2981, 2958, 2932, 2907, 2865, 2838, 2603, 1954, 1895, 1814, 1761, 1679, 1603, 1533, 1509, 1458, 1439, 1385; MS (EI) m/z 471 (M$^+$, 2%), 473 (M$^+$ +2, 2%), 426 (M$^+$ –45, 5%), 428 ([M$^+$ +2]–45, 5%), 392 (M$^+$–79, 100%); Found: C, 61.14; H, 5.63; N, 2.90. Calc. for $C_{24}H_{26}BrNO_4$: C, 61.02; H, 5.55; N, 2.97%.

Ethyl 4-(3-aminophenyl)-1-(2-bromo-4,5-dimethoxyphenethyl)-5-methyl-1*H*-pyrrole-3-carboxylate (6c). From **7c** (0.56 g, 2.3 mmol), purified by flash column chromatography on silica gel, to furnish 0.97 g of **6c** as a yellow solid, m.p. 54 °C, yield 87%. TLC (hexane–EtOAc–Et_3N, 40 : 50 : 10 v/v): R_f 0.26; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.16 (t, J = 7.2 Hz, 3H), 1.93 (s, 3H), 3.09 (t, J = 7.2 Hz, 2H), 3.61 (brs, 1H, –NH_2), 3.72 (s, 3H), 3.86 (s, 3H), 4.05 (t, J = 6.9 Hz, 2H), 4.12 (q, J = 6.9 Hz, 2H), 6.34 (s, 1H), 6.55–6.64 (m, 3H), 7.02 (s, 1H), 7.12 (t, J = 7.8 Hz, 1H), 7.26 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 164.7, 148.7, 148.5, 145.5, 136.6, 128.8, 128.3, 127.8, 125.7, 121.3, 117.7, 115.4, 114.0, 113.4, 113.3, 59.2, 56.2, 56.0, 46.9, 37.9, 14.2, 10.0; IR (KBr, cm^{-1}) 3454, 3367, 3233, 3128, 2974, 2938, 2844, 1705, 1607, 1508, 1451, 1382; MS (EI) m/z 407 (M$^+$–79, 5%), 30 (M$^+$–456, 100%); Found: C, 59.24; H, 5.62; N, 5.72. Calc. for $C_{24}H_{27}BrN_2O_4$: C, 59.14; H, 5.58; N, 5.75%.

Ethyl 1-(2-bromo-4,5-dimethoxyphenethyl)-5-methyl-4-(thiophen-2-yl)-1*H*-pyrrole-3-carboxylate (6d). From **7d** (0.54 g, 2.3 mmol), purified by flash column chromatography on silica gel, to furnish 0.77 g of **6d** as a yellow oil, yield 70%. TLC (hexane–EtOAc–Et_3N, 60 : 30 : 10 v/v): R_f 0.35; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.18 (t, J = 7.2 Hz, 3H), 1.97 (s, 3H), 3.08 (t, J = 7.2 Hz, 2H), 3.72 (s, 3H), 3.85 (s, 3H), 4.06 (t, J = 6.9 Hz, 2H), 4.14 (q, J = 7.2 Hz, 2H), 6.3 (s, 1H), 6.87 (dd, J= 3.3 y 0.9 Hz, 1H), 6.99–7.02 (m, 2H), 7.24–7.27 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 164.3, 148.8, 148.6, 136.4, 129.7, 128.7, 127.3, 126.3, 125.9, 124.8, 115.6, 114.9, 114.4, 114.0, 113.4, 59.3, 56.2, 56.0, 47.1, 37.8, 14.2, 10.0; IR (Film, cm^{-1}) 3127, 3072, 2976, 2936, 2843, 1709, 1602, 1534, 1508, 1461, 1443; MS (EI) m/z

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398 (M^+–79, 100%); Found: C, 55.18; H, 5.17; N, 2.95. Calc. for $C_{22}H_{24}BrNO_4S$: C, 55.23; H, 5.06; N, 2.93%.

1-(2-Bromo-4,5-dimethoxyphenethyl)-5-methyl-4-phenyl-1*H*-pyrrole-3-carbonitrile (6e). From **7e** (0.42 g, 2.3 mmol), purified by flash column chromatography on silica gel, to furnish 0.58 g of **6e** as a yellow solid, m.p. 160 °C, yield 60%. TLC (hexane–EtOAc–Et_3N, 60 : 30 : 10 v/v): R_f 0.35; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.06 (s, 3H), 3.08 (t, J = 6.9 Hz, 2H), 3.7 (s, 3H), 3.86 (s, 3H), 4.09 (t, J = 6.9 Hz, 2H), 6.3 (s, 1H), 7.02 (s, 1H), 7.08 (s, 1H), 7.26–7.43 (m, 5H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 148.9, 148.6, 133.4, 128.9, 128.5, 128.3, 127.3, 127.0, 126.8, 124.2, 116.7, 115.6, 114.1, 113.4, 92.1, 56.2, 56.0, 47.2, 37.7, 10.1; IR (KBr, cm^{-1}) 3125, 3080, 3058, 2994, 2959, 2936, 2908, 2838, 2215, 1736, 1604, 1506, 1461, 1440, 1390, 1347; MS (EI) m/z 345 (M^+–79, 100%); Found: C, 62.10; H, 5.00; N, 6.58. Calc. for $C_{22}H_{21}BrN_2O_2$: C, 62.13; H, 4.98; N, 6.59%.

Ethyl 1-(2-bromo-4,5-dimethoxyphenethyl)-4-(3-(4-(cyclohexylmethyl)piperazine-1-carbonyl)-5-methyl-1*H*-pyrrole-3-carboxylate (15). From **14** (1.0 g, 2.3 mmol), purified by flash column chromatography on silica gel, to furnish 0.94 g of **15** as a white solid, m.p. 158 °C, yield 60%. TLC (hexane–EtOAc–Et_3N, 60 : 30 : 10 v/v): R_f 0.26; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.81–0.92 (m, 2H), 1.13–1.28 (m, 6H), 1.38–1.48 (m, 1H), 1.69–1.79 (m, 5H), 1.96 (s, 3H), 2.14 (d, J = 6.9 Hz, 2H), 2.34 (brs, 2H), 2.45 (brs, 2H), 3.08 (t, J = 7.2 Hz, 2H), 3.53 (brs, 2H), 3.73 (s, 3H), 3.77 (brs, 2H), 3.87 (s, 3H), 4.07 (t, J = 7.2 Hz, 2H), 4.12 (q, J = 7.2 Hz, 2H), 6.37 (s, 1H), 7.03 (s, 1H), 7.25–7.41 (m, 5H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 170.5, 164.3, 148.9, 148.6, 135.5, 135.1, 131.6, 129.3, 128.8, 128.0, 127.7, 126.0, 125.1, 122.5, 115.8, 114.1, 113.6, 113.2, 65.5, 59.1, 56.2, 56.0, 54.0, 47.8, 47.0, 45.8, 42.2, 37.8, 35.0, 31.8, 26.7, 26.1, 14.3, 14.2, 9.9; IR (KBr, cm^{-1}) 3121, 3054, 2979, 2921, 2845, 2804, 2767, 1704, 1624, 1509, 1445, 1405; MS (EI) m/z 679 (M^+, 10%), 681 (M^++2, 10%), 596 (M^+–83, 100%); Found: C, 63.54; H, 6.83; N, 6.10. Calc. for $C_{36}H_{46}BrN_3O_5$: C, 63.52; H, 6.81; N, 6.17%.

Experimental procedure for the 5,6-dihydropyrrolo[2,1-*a*]-isoquinolines

Ethyl 8,9-dimethoxy-3-metyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-1-carboxylate (5a).

Method a). To a solution of *N*-alkylpyrrole **6a** (0.67 g, 1.7 mmol) in dry DMF (10 mL) was added $Pd(OAc)_2$ (0.125 g, 0.33 mmol), PPh_3 (0.089 g, 0.33 eq.), n-Bu_4NBr (0.55 g, 1.7 mmol), and K_2CO_3 (0.47 g, 3.4 mmol). The resulting suspension was stirred for 12 h at 120 °C. Subsequently, the reaction was cooled to room temperature, and H_2O was added. The solution was extracted with EtOAc (3 × 10 mL). The organic layer was washed with H_2O and brine (3 × 10 mL), dried with Na_2SO_4, and then evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel, to furnish the pyrroloisoquinoline **5a** (0.21 g) as a white solid, m.p. 130° C, yield 40%. TLC (hexane–AcOEt–Et_3N, 50 : 45 : 5 v/v): R_f 0.54; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.35 (t, J = 7.2 Hz, 3H), 2.23 (d, J = 0.9 Hz, 3H), 2.94 (t, J = 6.6 Hz, 2H), 3.87 (t, J = 6.9 Hz, 2H), 3.90 (s, 3H), 3.96 (s, 3H), 4.29 (q, J = 7.2 Hz, 2H), 6.43 (d, J = 0.9 Hz, 1H), 6.70 (s, 1H), 8.44 (s, 1H) ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 165.5, 148.0, 147.6, 132.9, 127.2, 125.2, 121.6, 111.5, 110.4, 110.3, 110.0, 59.5, 56.1, 55.9, 41.0, 29.3, 14.5, 11.9; IR (KBr, cm^{-1}) 3432, 3107, 3013, 2977, 2935, 2843, 2677, 1685, 1609, 1581, 1529, 1468; MS (EI) m/z 315 (M^+, 100%); Found: C, 68.50; H, 6.75; N, 4.47. Calc. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44%.

Method b). A solution of *N*-alkylpyrrole **6a** (0.59 g, 1.5 mmol) and AIBN (0.07 g, 0.45 mmol) in degassed dry toluene (10 mL) was heated at refluxing temperature, and n-Bu_3SnH (0.48 mL, 1.8 mmol) was added dropwise. The reaction was stirred for 2 h. After that time, the reaction was cooled to room temperature, the solvent was removed under reduced pressure, and the crude residue was purified by flash column chromatography on silica gel. Hexane was first added to remove the n-Bu_3SnBr, then hexane–EtOAc–Et_3N (70 : 25 : 5 to 50 : 45 : 5) was added to furnish the pyrroloisoquinoline **5a** (0.25 g), with a 53% yield.

Method c). To a refluxing solution of the *N*-alkylpyrrole **6a** (0.59 g, 1.5 mmol) in degassed dry toluene (10 mL), a solution of n-Bu_3SnH (1.0 mL, 3.7 mmol) in toluene (5 mL) was added dropwise (syringe pump) over 7 h. During that time, solid dilauroyl peroxide (DLP) was added portionwise (1.49 g, 3.7 mmol, 0.11 g/30 min). The solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography on silica gel. Hexane was first added to remove the n-Bu_3SnBr, then hexane–EtOAc–Et_3N (70 : 25 : 5 to 50 : 45 : 5) was added to furnish the desired pyrroloisoquinoline **5a** (0.33 g), with a 70% yield.

Ethyl 8,9-dimethoxy-3-metyl-2-phenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-1-carboxylate (5b). From the *N*-alkylpyrrole **6b** (0.71 g, 1.5 mmol), purified by flash column chromatography on silica gel, to furnish 0.47 g (yield 80%) of **5b** as a white solid, m.p. 170° C (lit.[ref. 17a] 167–168° C), following the method *c* described above. TLC (hexane–AcOEt:Et_3N, 50 : 45 : 5 v/v): R_f 0.54; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.80 (t, J = 7.2 Hz, 3H), 2.09 (s, 3H), 2.92 (t, J = 6.9 Hz, 2H), 3.82 (s, 3H), 3.84 (s, 3H), 3.84 (t, 2H), 3.94 (q, J = 7.2 Hz, 2H), 6.63 (s, 1H), 7.16–7.31 (m, 5H), 7.9 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 167.0, 148.3, 148.2, 136.8, 130.8, 130.1, 127.6, 125.9, 125.5, 125.2, 123.9, 121.8, 111.3, 111.0, 59.7, 56.1, 41.2, 29.2, 13.6, 10.1, 1.0; IR (KBr, cm^{-1}) 3093, 3048, 2985, 2958, 2904, 2873, 2833, 2049, 1957, 1895, 1835, 1765, 1691, 1604, 1533, 1498, 1459, 1389; MS (EI) m/z 391 (M^+, 100%); Found: C, 73.66; H, 6.45; N, 3.58. Calc. for $C_{24}H_{25}NO_4$: C, 73.64; H, 6.44; N, 3.58%.

Ethyl 2-(3-aminophenyl)-8,9-dimethoxy-3-methyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-1-carboxylate (5c). From the *N*-alkylpyrrole **6c** (0.73 g, 1.5 mmol), purified by flash column chromatography on silica gel, to furnish 0.52 g (yield 85%) of **5c** as a yellow solid, m.p. 168 °C (lit.[ref. 4a] 170–172 °C), following the method *c* described above. TLC (hexane–AcOEt:Et_3N, 50 : 45 : 5 v/v): R_f 0.25; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.96 (t, J = 7.2 Hz, 3H), 2.18 (s, 3H), 2.98 (t, J = 6.6 Hz, 2H), 3.46 (brs, 2H, –NH_2), 3.90 (s, 3H), 3.91 (s, 3H, overlapping a *triplet*, 2H), 4.05 (q, J = 7.2 Hz, 2H), 6.6–6.71 (m, 4H), 7.1–7.15 (m, 1H), 7.95 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 167.2, 147.9, 147.7, 145.7, 137.5, 130.4, 128.5, 125.4, 125.1, 123.6, 121.4, 120.7, 116.9, 112.9, 110.6, 110.2, 59.9, 56.0, 55.9, 41.1, 29.1, 13.7, 10.3; IR (KBr, cm^{-1}) 3455, 3369, 3232, 2933, 2842, 1691, 1608, 1531, 1494, 1467; MS (EI) m/z 406 (M^+, 100%); Found: C, 70.97; H, 6.48; N, 6.87. Calc. for $C_{24}H_{26}N_2O_4$: C, 70.92; H, 6.45; N, 6.89%.

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Ethyl 8,9-dimethoxy-3-methyl-2-(thiophen-2-yl)-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-1-carboxylate (5d). From the *N*-alkylpyrrole **6d** (0.72 g, 1.5 mmol), purified by flash column chromatography on silica gel, to furnish 0.54 g (yield 90%) of **5d** as a yellow solid, m.p. 114 °C, following the method *c* described above. TLC (hexane–AcOEt–Et_3N, 50 : 45 : 5 v/v): R_f 0.51; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.01 (t, J = 7.2 Hz, 3H), 2.22 (s, 3H), 2.98 (t, J = 6.6 Hz, 2H), 3.90 (s, 3H), 3.91 (s, 3H), 3.92 (t, J = 6.6 Hz, 2H), 4.90 (q, J = 7.2 Hz, 2H), 6.71 (s, 1H), 6.89 (dd, J= 3.6 y 1.2 Hz, 1H), 7.04 (dd, J= 5.1 y 3.3 Hz, 1H), 7.28 (dd, J= 5.4 y 1.2 Hz, 1H), 7.92 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 166.7, 148.1, 147.8, 137.5, 130.8, 127.4, 126.6, 126.5, 125.1, 124.6, 121.2, 115.3, 111.1, 110.6, 110.3, 60.1, 56.0, 55.9, 41.3, 29.1, 13.7, 10.4; IR (KBr, cm^{-1}) 3096, 2979, 2921, 2874, 2850, 1691, 1609, 1584, 1541, 1518, 1458, 1388; MS (EI) m/z 397 (M^+, 100%); Found: C, 66.46; H, 5.80; N, 3.48. Calc. for $C_{22}H_{23}NO_4S$: C, 66.48; H, 5.83; N, 3.52%.

8,9-Dimethoxy-3-methyl-2-phenyl-5,6-dihydropyrrolo[2,1-*a*]isoquinolne-1-carbonitrile (5e). From the *N*-alkylpyrrole **6e** (0.64 g, 1.5 mmol), purified by flash column chromatography on silica gel, to furnish 0.39 g (yield 75%) of **5e** as a white solid, m.p. 233 °C, following the method *c* described above. TLC (hexane–AcOEt–Et_3N, 50 : 45 : 5 v/v): R_f 0.34; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 2.29 (s, 3H), 3.04 (t, J = 6.6 Hz, 2H), 3.91 (s, 3H), 3.96 (s, 3H), 3.98 (t, J = 6.6 Hz, 2H), 6.74 (s, 1H), 7.27–7.44 (m, 5H), 7.82 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 148.8, 148.5, 135.2, 133.3, 129.1, 128.5, 126.8, 125.7, 124.6, 124.0, 119.9, 118.4, 110.9, 106.9, 86.2, 56.1, 56.0, 41.2, 28.4, 10.3; IR (KBr, cm^{-1}) 3047, 2998, 2945, 2908, 2839, 2206, 1734, 1606, 1536, 1498, 1481, 1454, 1436; MS (EI) m/z 344 (M^+, 100%); Found: C, 76.69; H, 5.88; N, 8.10. Calc. for $C_{22}H_{20}N_2O_2$: C, 76.72; H, 5.85; N, 8.13%.

Ethyl 2-(3-(4-(cyclohexylmethyl)piperazine-1-carbonyl)phenyl)-8,9-dimethoxy-3-methyl-5,6-dihydropyrrolo[2,1-*a*]isoquinoline-1-carboxylate (16). From the *N*-alkylpyrrole **15** (1.0 g, 1.5 mmol), purified by flash column chromatography on silica gel, to furnish 0.81 g (yield 90%) of **16** as a white solid, m.p. 160 °C, following the method *c* described above. TLC (hexane–AcOEt–Et_3N, 50 : 45 : 5 v/v): R_f 0.28; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 0.85–0.95 (m, 2H), 0.92 (t, J = 6.9 Hz, 3H), 1.13–1.28 (m, 3H), 1.44–1.50 (m, 1H), 1.68–1.79 (m, 5H), 2.14–2.15 (m, 5H), 2.36 (brs, 2H), 2.44 (brs, 2H), 2.99 (t, J = 6.6 Hz, 2H), 3.5 (brs, 2H), 3.79 (brs, 2H), 3.90 (s, 3H), 3.92 (s, 3H), 3.92 (t, 2H), 4.03 (q, J = 7.2 Hz, 2H), 6.73 (s, 1H), 7.29–7.42 (m, 4H), 7.97 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 170.3, 166.5, 148.2, 147.8, 136.8, 135.4, 131.3, 131.1, 128.5, 127.8, 125.7, 125.3, 124.8, 122.9, 121.2, 110.7 (2C), 65.4, 59.7, 56.0, 55.9, 53.6, 47.6, 42.1, 41.2, 34.9, 31.8, 29.1, 26.7, 26.0, 13.7, 10.2; IR (KBr, cm^{-1}) 3102, 2980, 2929, 2849, 2808, 2770, 1737, 1691, 1618, 1578, 1528, 1467, 1441; MS (EI) m/z 599 (M^+, 80%), 516 (M^+–83, 100%); Found: C, 72.02; H, 7.53; N, 7.00. Calc. for $C_{36}H_{45}N_3O_5$: C, 72.09; H, 7.56; N, 7.01%.

Crystallographic data

Crystal structure analysis of **16** (solved by R. A. Toscano): $C_{36}H_{45}N_3O_5$, FW = 599.75, colorless, 0.428 × 0.162 × 0.162 mm^3, monoclinic, $P2_1/n$, a = 10.400(1), b = 11.217(1), c = 27.800(2) Å, β = 96.122(1)°, V = 3224.6(5) Å^3, Z = 4, D_x = 1.235 Mg m^{-3}, μ = 0.082 mm^{-1}, 26547 reflections were measured on a Bruker Smart Apex CCD diffractometer with area detector (graphite monochromator, λ = 0.71073 Å) with a resolution of $(\sin\theta/\lambda)_{max}$ = 0.83 Å^{-1} at a temperature of 21° C. The reflections were corrected for absorption and scaled on the basis of multiple measured reflections using an empirical method. (0.9832–0.9566 correction range) 5912 reflections were unique (R_{int} = 0.0581). The structure was solved using Direct Methods (program SHELXS-97) and refined with SHELXL-97 against F^2 from all reflections. Non hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were located in difference Fourier maps. Methyl and phenyl hydrogen atoms were refined with a riding model; all other hydrogen atoms were refined freely with isotropic displacement parameters. 171 parameters were refined with no restraints. $R1/wR2[I > 2\sigma(I)]$: 0.0658/0.1377. $R1/wR2$[all refl.]: 0.1118/0.1587, S = 0.917. The maximum residual electron density peak had a height of 0.230 and –0.143 Å^{-3}.

Cell lines and culture medium

The compounds were screened *in vitro* against six human cancer cell lines: HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), K-562 (human chronic myelogenous leukemia), U-251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), and SKLU-1 (human lung adenocarcinoma) cell lines, supplied by the National Cancer Institute (NCI, USA). The human tumor cytotoxicities were determined following protocols established by the NCI.[23] The cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 10000 units/mL penicillin G sodium, 10000 µg mL^{-1} streptomycin sulfate, 25 µg mL^{-1} amphotericin B (Gibco), and 1% non-essential amino acids (Gibco). Cells were maintained at 37° C in a humidified atmosphere with 5% CO_2. The viability of the cells used in the experiments exceeded 95%, as determined with a trypan blue assay.

Cytotoxicity assay. Cytotoxicity, after treatment of the tumor cells with the test compounds, was determined using the protein binding dye, sulforhodamine B (SRB), in a microculture assay to measure cell viability and cell growth.[23] The cells were removed from the tissue culture flasks by treatment with trypsin and diluted with fresh media. Of the cell suspension, 100 µL, containing 5000 or 7500 cells per well, were pipetted into 96 well microtiter plates (Costar), and the material was incubated at 37° C for 24 h in a 5% CO_2 atmosphere. Subsequently, a 100 µL aliquot of the test compounds in solution, obtained by dilution of the stocks, was added to each well. The cultures were exposed for 48 h to the drug at concentrations ranging from 1 × 10^{-3} to 10 µM. After the incubation period, cells were fixed to the plastic substratum by the addition of 50 µL cold 50% aqueous trichloroacetic acid. The plates were incubated at 4° C for 1 h, washed with tap H_2O, and air-dried. The trichloroacetic acid-fixed cells were stained by the addition of 0.4% SRB. Free SRB solution was then removed by washing with 1% aqueous acetic acid. The plates were then air-dried, and the bound dye was solubilized by the addition of 10 mM unbuffered Tris base (100 µL). The plates were placed on a shaker for 5 min, and the absorption was determined at 515 nm using an ELISA plates reader (Bio-Tex Instruments).

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