Bioorganic & Medicinal Chemistry 18 (2010) 6813–6821

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Cytotoxicity of new pyrazino[1,2-*b*]isoquinoline and 6,15-iminoisoquino[3,2-*b*]3-benzazocine compounds

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ARTICLE INFO

Article history:
Received 8 March 2010
Revised 15 July 2010
Accepted 20 July 2010
Available online 25 July 2010

Keywords:
Cytotoxicity
Structure–activity relationships
Pyrazinoisoquinolines
Isoquinobenzazocines

ABSTRACT

Looking for optimised analogues of compound **2** that might be useful in colon cancer therapy, we here explore the in vitro cytotoxicity against MDA-MB 231 human breast carcinoma, A-549 human lung carcinoma and HT-29 human colon carcinoma cell lines of several analogues and derivatives. The effect of the R_2-substituent and/or the introduction of an arylmethyl side-chain at C-3, as well as the presence of a double bond in the skeleton or a methoxy group at C-1 have been investigated. New 6,15-iminoisoquino[3,2-*b*]3-benzazocine compounds, related to the saframycin family, in which the C(7)–N(8)–C(9)-substructure contains a lactam function, a fused oxazolidine or an aminonitrile function were also studied, and many of them showed low micromolar GI_{50} values.

1. Introduction

We have recently discovered that pyrazino[1,2-*b*]isoquinoline-4-ones **1** and **2** are able to induce apoptosis triggered from the G2/M phase of cell cycle, without DNA damage. Compound **2**, which was highly toxic and selective for HT-29 human colon cancer cells (CI_{50} value of 1.95×10^{-7} M),[1] showed a novel mechanism of cytotoxicity. It promotes the degradation of components of the G2/M checkpoint machinery, in particular cdc2, Cyclin B1 and Wee1.[2] Optimised analogues of **2** might be useful in colon cancer monotherapy, or in combination therapies with anticancer drugs like cisplatin, because these agents would increase the apoptosis induction when tumour cells were arrested in G2/M. However, as the target molecule is still unknown, structure modification of the prototype is the only way to find them. We previously studied the activity of epimers at C-11a of compound **2**, and that of analogues in which the benzyl group at the C(6)-side-chain was eliminated or this chain was replaced by partially reduced phthalimidomethyl or acyloxymethyl groups. However, all these compounds showed lower activity than the lead compound.[1] Then, we decide to investigate the effect of the R_2-substituent and/or the introduction of an arylmethyl side-chain at C-3, as well as the presence of a double bond in the skeleton or a methoxy group at C-1. Here we report the activity of analogues and derivatives shown in Figure 1, in which the A-ring substitution pattern of **2** has been maintained.

In our approach to 11,11a-dehydro-6,15-iminoisoquino[3,2-*b*]-3-benzazocine compounds related to the saframycins family,[3] we found that the in vitro micromolar cytotoxicity found in many compounds of this series is apparently uninfluenced by the ability to generate DNA-alkylating electrophilic C(7)-iminium ions. In fact, structures without the characteristic N(8)–C(7) hemiaminal or aminonitrile motifs that have been presumed to be essential to cytotoxic activity, showed values very similar to those of their N(8)–C(7)–lactam precursors.[4] We consider relevant to extend this study to pentacyclic saturated analogues in which the C(7)–N(8)–C(9)-substructure contains a lactam function (compounds **22**–**35**), a fused oxazolidine (compounds **36** and **37**), or an aminonitrile function (compounds **38**–**41**) (Fig. 2). Compound **41** is structurally related to Zalypsis®,[5] a drug currently in clinical development for the treatment of solid tumours and haematological malignancies.[6,7]

2. Results and discussion

2.1. Chemistry

Synthesis of compounds **3**–**6**, **10**–**16** and **19**–**21** has been previously described.[1,8,9] *N*-Alkyl derivatives **7**–**9** were obtained from (6*R**,11a*S**)-6-benzyloxymethyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one[1] through addition of the corresponding aldehyde in the presence of $NaCNBH_3$[10] (Scheme 1).

Compound **18** was obtained by alkylation of the **3**-enolate with 2,4,5-trimethoxy-3-methyl-phenylmethyl chloride,[11] followed by equilibration of the first obtained C(3)-epimer **17** with 2,6-di-*tert*-butyl-4-methylphenol (BHT) as a hindered proton source[12] (Scheme 2).

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doi:10.1016/j.bmc.2010.07.049

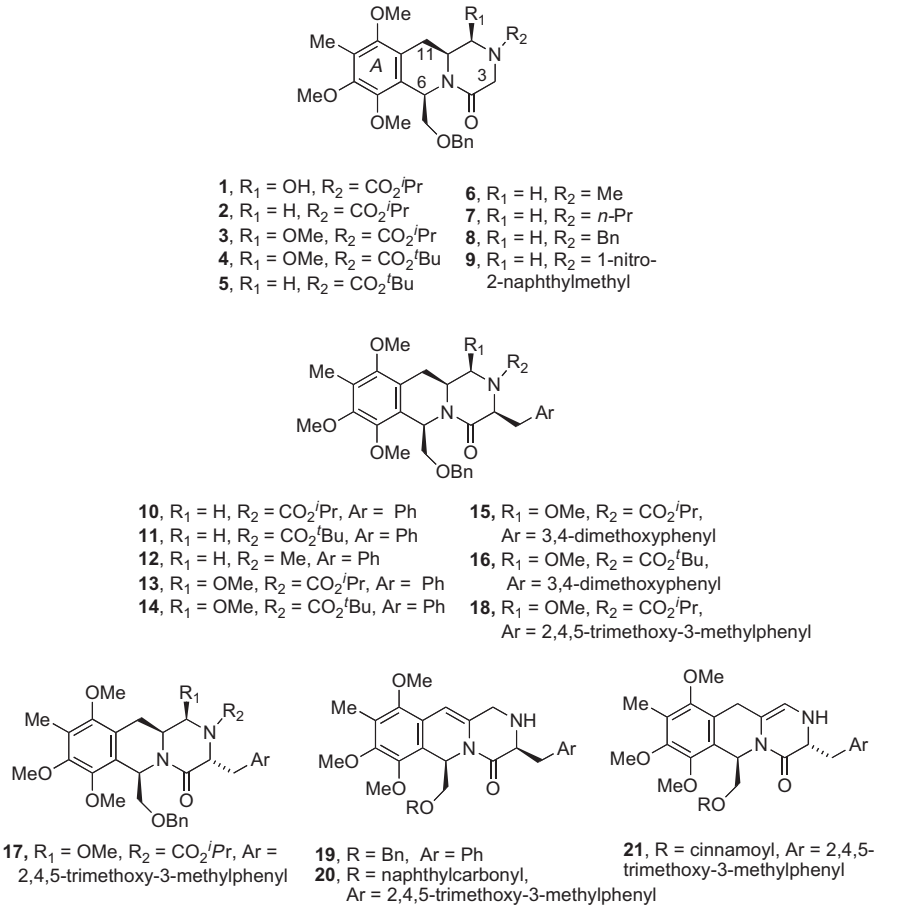


Figure 1.

The relative stereochemistry of both epimers was determined by conclusive NOE experiments. The chemical shifts of the H-1, H-6 and H-11a protons are lower in **17** than in **18** because, in the first compound, the benzene ring present in the C(3)-side-chain produces an anisotropic effect on those protons. The ^{1}H NMR spectrum of compound **17**, in which the 3,11a-protons are in an *anti*-relationship, are simple in comparison to that of compound **18**, in which the conformational freedom of the carbamate function is restringed due to unfavoured steric interactions with the substituent at C-3 and C-6. Figure 3 shows the conformations of minimum energy calculated with the MM2 Chem3D programme for both epimers.

Compound **17** could not be purified because it is very instable in contrast to compound **18**. This fact can be explained taking into account the probable elimination of the C(1)-methoxy group in the first compound to generate a very reactive acyliminium cation involving the C(1)–N(2) positions. This group is antiperiplanar to the unshared N(2)-electron pair in the conformation of minimum energy calculated for **17** (Fig. 4).

The pentacyclic compounds **22**–**27** and **34** have been previously described.[8,9] Formation of D-ring from compound **18** to obtain compound **28** required the use of trifluoromethanesulfonic acid (triflic acid) to avoid the deprotonation of acyliminium ions **I** to give enamides. This elimination was observed in reactions promoted with trifluoroacetic acid that attempted a similar cyclisation.[9] It is probable that superacids make possible the cyclisation through the generation of *C,N*-biscationic intermediates **II**, which are more reactive than intermediates **I**. The *O*-benzyl group and the carbamate function were hydrolysed under these reaction conditions (Scheme 3).

Compounds **30**–**32** and **35**, that were supposed to be more active than their NH-precursors **26**–**28** and **34**,[13] were obtained from these precursors by reductive methylation, while O-acylation of **26** afforded compounds **29** and **33** (Scheme 4).

Substitution of the lactam function of pentacyclic compounds in these series by a hemiaminal or α-amino nitrile function, that may be precursors of alkylating iminium intermediates, usually increases the cytotoxic activity. Because of hemiaminals are too unstable, α-amino nitriles have been widely studied. These compounds are obtained by reductive cyanation of their lactam precursors with DIBAL-H, DIBAL-H and *n*-BuLi, Red-Al or LAH, among other reducing agents, followed by cyanation with KCN or TMSCN. Reduction of 9-hydroxymethyl derivatives usually give fused oxazolidine compounds through capture of the C(7)-iminium intermediates by the C(9)-side-chain, and over reduction to amines have been observed in certain cases.[14] Oxazolidine derivatives **36** and **37** were obtained in excellent yields from the C(9)-hydroxymethyl compounds **30** and **31**, respectively, but, due to their instability, they were not purified by column chromatography and were immediately submitted to cyanation with trimethylsilyl cyanide in the presence of $F_3B{\cdot}Et_2O$ as a Lewis acid to give amino nitriles **38** and **39** as the only diastereoisomers. In the case of compound **32** the oxazolidine compound was not isolated, giving in the same treatment the amino nitrile **40**. Compound **38** was transformed into the *O*-acyl derivative **41** (Scheme 5).

2.2. Antiproliferative activity

In collaboration with the biopharmaceutical company PharmaMar, the antiproliferative activity was evaluated using a panel of three human cell lines: MDA-MB 231 (human breast carcinoma), A-549 (human lung carcinoma) and HT-29 (human colon carcinoma) to determine the values GI_{50} (the drug concentration inhib-

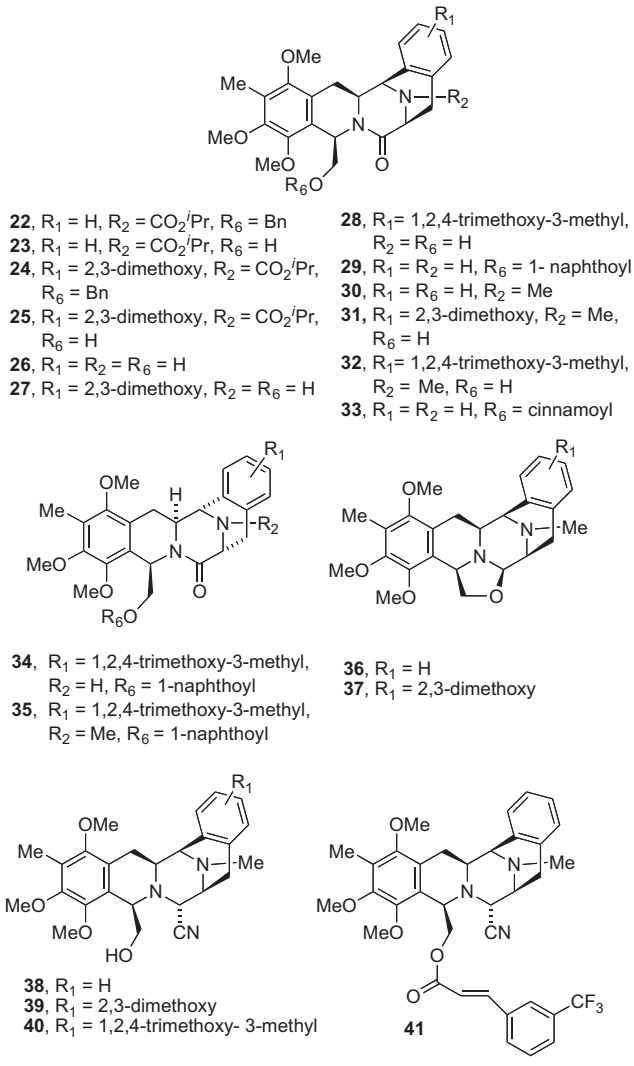
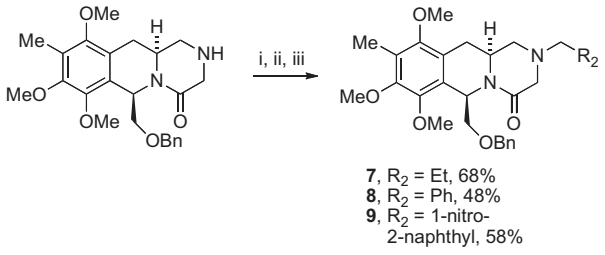


Figure 2.



Scheme 1. Reagents and conditions: (i) R_2CHO (5 equiv), CH_3CN, rt, 2 h; (ii) HCl (2 mL), rt, 30 min; (iii) $NaCNBH_3$ (2.7 equiv), rt, 2 h.

iting the growth of cell lines by 50%), TGI (total growth inhibition) and DL_{50} (the half of the lethal concentration).[15] Compounds that showed GI_{50} values <20 μM for some of the cell lines have been included in Table 1, as well as data for ET-743 and compound **2** in the same assay for comparison.

Many of the studied tricyclic compounds showed GI_{50} values at the low micromolar range, but none of them was more active than compound **2**. Thus compound **3**, whose hemiaminal motif could generate DNA-alkylating electrophilic C(1)-iminium ions[16] was less active, although it was also selective against HT-29 cells. If we look at the effect of the N-substituent, we see that the substitution of the $CO_2{}^iPr$ by $CO_2{}^tBu$ in compound **5** implied also a different selectivity. Among N-alkylated compounds, **7** and **9** were 100-fold less active than compound **2**, although **9** retained the activity against the MDA-MB cell line, and **8** was 10-fold less active. The effect of incorporation of a 3-arylmethyl chain in 1-methoxy compounds was rather independent of their relative stereochemistry. The activity of compounds **3** and **15** against colon cancer was similar while **16** was more active than **4**. In contrast, introduction of a 3-benzyl chain decreased the activity of **2** (compare their values with those of compounds **10**–**12**). The cytotoxicity of enamides **19**–**21** was also lower than that of compound **2**.

Respect to the pentacyclic compounds here studied, many of them showed low micromolar GI_{50} values. The substitution pattern of E-ring has not much influence in the cytotoxicity. *O*-Benzyl (**22** and **24**) and *O*-acyl derivatives (**29** and **33**) were more active against some of the tumour cell lines that their 9-hydroxymethyl analogues (**23**, **25**–**28**). Compounds **30**–**32** were even less active. The 7-cyano compounds are more active that their lactam precursors (compare compounds **30** and **38**). The activity *O*-1-naphthoyl **34** and **35** was similar to that of diastereoisomers **29** and **33**. Acylation of the 9-hydroxymethyl side-chain in 7-cyanoderivative **38** did not affect the GI_{50} value (see compound **41**), but decreases the TGI and DL_{50} values.

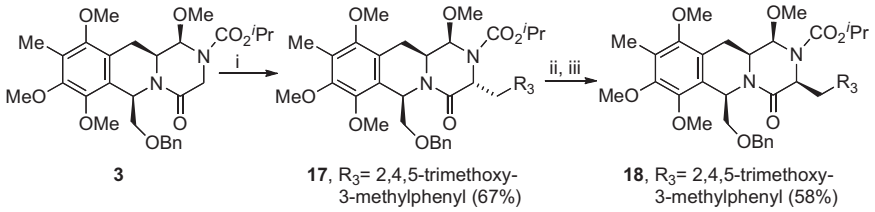
3. Conclusions

In conclusion, although the structure modifications of compound **2** studied in this work did not increase its antitumor potential, we have got a lot of information about structure–activity relationships, and we did not discard that some optimised analogues will be found in the future. We have also found that the saturated pentacyclic derivatives here studied have a biological activity very similar to that of the previously studied 14,14a-unsaturated compounds.

4. Experimental section

4.1. General experimental information

All reagents were of commercial quality and were used as received. Solvents were dried and purified using standard techniques. 'Petroleum ether' refers to the fraction boiling at 40–60 °C. Reactions were monitored by thin layer chromatography,



Scheme 2. Reagents and conditions: (i) LHMDS (1.2 equiv), THF, −78 °C, 2,4,5-trimethoxy-3-methylphenylmethyl chloride (2 equiv), 45 min; (ii) *t*BuLi (2.5 equiv), THF, −78 °C, 10 s; (iii) BHT (5.8 equiv), THF, −78 °C, 1 min.

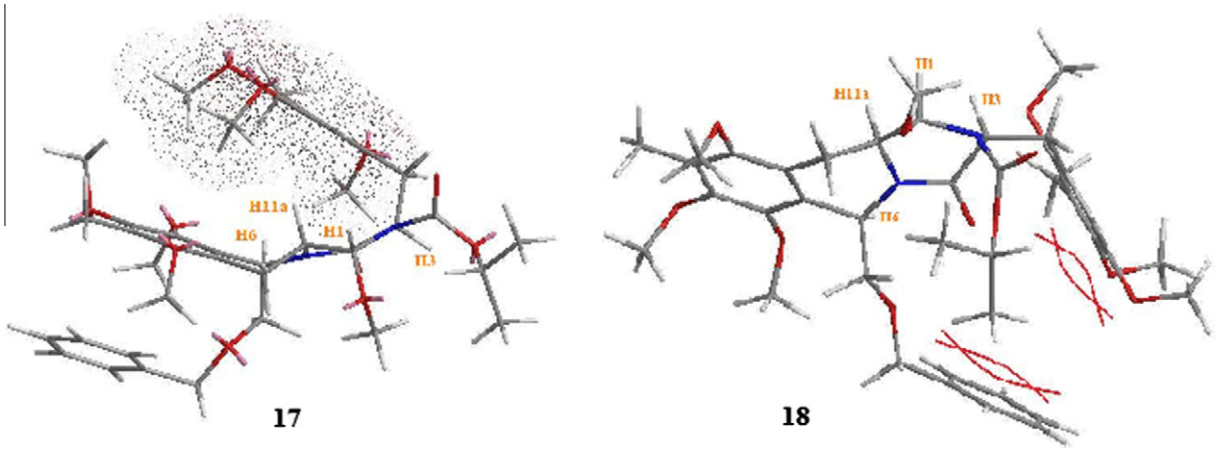


Figure 3.

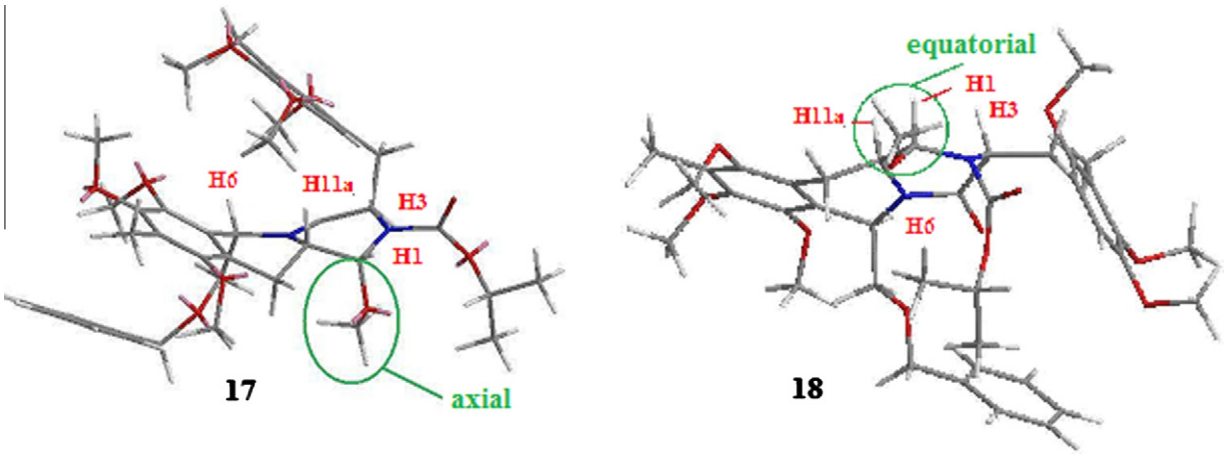


Figure 4.

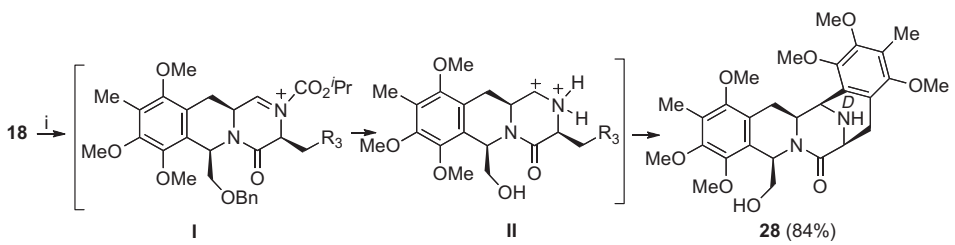
on aluminium plates coated with silica gel with fluorescent indicator. Separations by flash chromatography were performed on silica gel with 40–63 μm particle size. Melting points were measured in a hot stage microscope, and are uncorrected. Infra-red spectra were recorded on a FT-IR spectrophotometer, with solid compounds compressed into KBr pellets and liquid compounds examined as films on NaCl discs. NMR spectra were obtained at 250 MHz for ^{1}H and 63 MHz for ^{13}C, with $CDCl_3$ as solvent (Servicio de Resonancia Magnética Nuclear, Universidad Complutense). Elemental analyses were determined by the Servicio de Microanálisis Elemental, Universidad Complutense.

4.2. N-alkylation: general procedure to obtain compounds 7–9

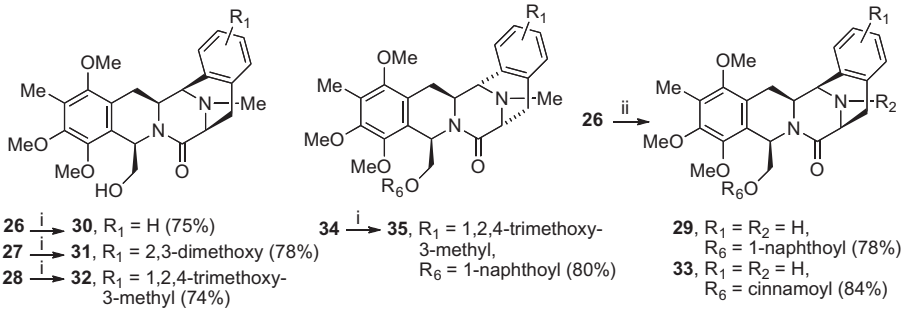
A solution of compound **4**[1] (212 mg, 0.50 mmol) in acetonitrile (20 mL) and the corresponding aldehyde (0.20 mL, 2.5 mmol) was stirred at room temperature for 2 h, then a 1 N HCl aqueous solution (2 mL) was added and the mixture was stirred for 30 min. After addition of $NaCNBH_3$ (85 mg, 1.35 mmol), stirring was continued for 2 h. The reaction mixture was poured into ice-water (2 mL). The solution was extracted with ethyl acetate (20 mL × 3), and the organic layer was washed with a saturated NaCl solution, dried over Na_2SO_4 anhydrous, filtered and concentrated in vacuo. The residue was purified by flash column chromatography.

4.2.1. (6*R**,11a*S**)-6-Benzyloxymethyl-7,8,10-trimethoxy-9-methyl-2-propyl-1,2,3,6,11,11a-hexahydropyrazino[1,2-*b*]isoquinolin-4-one (7)

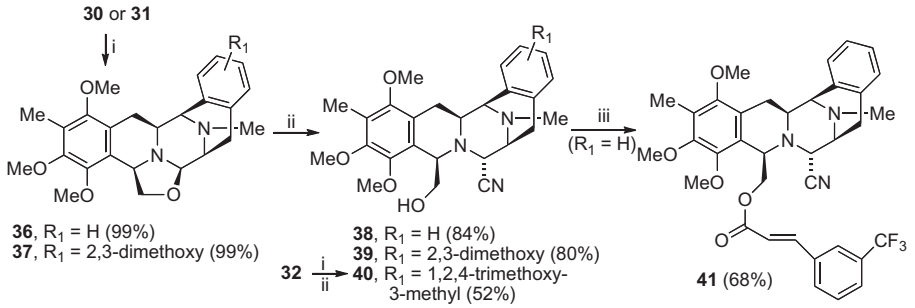
The residue was purified by flash column chromatography (3:7 hexane/EtOAc) to give **7** (160 mg, 68%) as an orange solid: mp 59–60 °C; IR (NaCl) ν_{max} 2937, 1651, 1463 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.27 (m, 5H), 6.13 (dd, J = 6.4 and 3.0 Hz, 1H), 4.76



Scheme 3. Reagents and conditions: (i) F_3CSO_3H (equiv), rt, 1 h.



Scheme 4. Reagents and conditions: (i) HCO_2H (0.15 mL), HCHO (0.06 equiv), in MeOH, reflux, 1 h; (ii) $R(Ar)CO_2H$, EDC (2 equiv), DMAP (1.1 equiv), DM, rt, 21 h.



Scheme 5. Reagents and conditions: (i) $LiALH_4$ (2 equiv), THF, −17 °C, 30 min then 0 °C, 1 h and rt, 15 min; (ii) TMSCN (2.7 equiv), $BF_3{\cdot}OEt_2$ (0.7 equiv), −30 °C, 2 h; (iii) $R(Ar)CO_2H$, EDC (2 equiv); DMAP (1.1 equiv), DCM, rt, 21 h.

(d, *J* = 9.9 Hz, 1H), 4.46 (d, *J* = 9.9 Hz, 1H), 3.93 (m, 1H), 3.86 (s, 3H), 3.82 (m, 2H), 3.79 (s, 3H), 3.68 (s, 3H), 3.40 (d, *J* = 13.4 Hz, 1H), 3.05 (d, *J* = 13.4 Hz, 1H), 3.00 (dd, *J* = 14.2 and 9.6 Hz, 1H), 2.83 (dd, *J* = 14.1 and 3.7 Hz, 1H), 2.77 (m, 2H), 2.40 (t, *J* = 6.0 Hz, 2H), 2.20 (s, 3H), 1.56 (m, 2H), 0.96 (t, *J* = 6.0 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.7, 152.0, 149.6, 146.0, 138.4, 128.2, 127.7, 127.4, 124.5, 124.1, 123.7, 72.4, 70.3, 60.2, 59.9, 59.7, 59.0, 57.8, 54.6, 47.8, 47.5, 28.5, 19.8, 11.6, 9.2. Anal. Calcd for $C_{27}H_{36}N_2O_5$: C, 69.21; H, 7.74; N, 5.98. Found: C, 68.98; H, 7.57; N, 5.86.

4.2.2. (6*R**,11a*S**)-6-Benzyloxymethyl-2-benzyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydropyrazino[1,2-*b*]isoquinolin-4-one (8)

The residue was purified by flash column chromatography (1:1 hexane/EtOAc) to give **8** (100 mg, 48%) as a yellow solid: mp 61–62 °C; IR (NaCl) ν_{max} 2940, 1651, 1531 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.19 (m, 10H), 6.02 (dd, *J* = 6.5 and 3.0 Hz, 1H), 4.65 (d, *J* = 9.9 Hz, 1H), 4.35 (d, *J* = 9.9 Hz, 1H), 3.82 (m, 2H), 3.81 (m, 1H), 3.76 (s, 3H), 3.69 (s, 3H), 3.59 (s, 3H), 3.51 (s, 2H), 3.31 (d, *J* = 13.4 Hz, 1H), 3.00 (d, *J* = 13.4 Hz, 1H), 2.93 (dd, *J* = 13.9 and 9.7 Hz, 1H), 2.68 (d, *J* = 2.9 Hz, 2H), 2.66 (dd, *J* = 13.9 and 3.5 Hz, 1H), 2.10 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.6, 152.1, 149.7, 146.0, 138.4, 137.0, 128.9, 128.4, 128.3, 127.7, 127.4, 124.5, 124.2, 123.7, 72.5, 70.3, 61.5, 60.3, 59.9, 59.7, 57.8, 54.1, 47.8, 47.6, 28.5, 9.3. Anal. Calcd for $C_{31}H_{36}N_2O_5$: C, 72.07; H, 7.02; N, 5.42. Found: C, 71.98; H, 6.87; N, 5.34.

4.2.3. (6*R**,11a*S**)-6-Benzyloxymethyl-2-(1-nitro-2-naphtylmethyl)-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydropyrazino[1,2-*b*]isoquinolin-4-one (9)

The residue was purified by flash column chromatography (1:1 hexane/EtOAc) to give **9** (200 mg, 58%) as a brown solid: mp 69–70 °C; IR (NaCl) ν_{max} 2940, 1651, 1531 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.86 (m, 2H), 7.70 (m, 1H), 7.58 (m, 3H), 7.23 (m, 5H), 6.00 (dd, *J* = 6.6 and 2.9 Hz, 1H), 4.65 (d, *J* = 9.9 Hz, 1H), 4.35 (d, *J* = 9.9 Hz, 1H), 3.80 (m, 1H), 3.77 (s, 3H), 3.71 (m, 2H), 3.68 (s, 3H), 3.59 (m, 2H), 3.54 (s, 3H), 3.32 (d, *J* = 13.3 Hz, 1H), 2.99 (d, *J* = 13.3 Hz, 1H), 2.93 (dd, *J* = 13.9 and 9.9 Hz, 1H), 2.77 (dd, *J* = 13.9 and 3.5 Hz, 1H), 2.72 (d, *J* = 2.8 Hz, 2H), 2.09 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 165.7, 152.2, 149.7, 147.8, 146.0, 138.4, 133.4, 130,7, 128.7, 128.3, 128.0, 127.8, 127.5, 127.2, 127.4, 126.8, 124.6, 124.3, 124.2, 123.9, 121.6, 72.5, 70.3, 60.3, 59.9, 59.8, 57.7, 57.6, 54.3, 48.0, 47.6, 28.4, 9.3. Anal. Calcd for $C_{35}H_{37}N_3O_7$: C, 68.72; H, 6.10; N, 6.87. Found: C, 68.54; H, 5.87; N, 6.49.

4.2.4. (1*R**,3*S**,6*R**,11a*S**)-6-Benzyloxymethyl-2-isopropyloxycarbonyl-1,7,8,10-tetramethoxy-3-(2,4,5-trimethoxy-3-methylbenzyl)-9-methyl-1,2,3,6,11,11a-hexahydropyrazino[1,2-*b*]isoquinolin-4-one (18)

To a stirred solution of compound **17** (0.47 mmol) in dry THF (5 mL), under argon, at −78 °C was added *tert*-Buli (1.7 M in pentane, 1.7 mL, 2.93 mmol). Upon complete addition the solution became deep orange/brown in colour. After 10 s a solution of 2,6-di*tert*-butyl-4-methylphenol (BHT) (600 mg, 2.72 mmol) in dry THF (3 mL) was added in one portion. The brown colour faded pale yellow. After 30 s, to the reaction mixture was added a 1 N HCl aqueous solution (5 mL). The solution was warmed to room temperature and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with water, with saturated aqueous solution of NaCl (5 mL), dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was purified by flash column chromatography (1:1 hexane/EtOAc) to give **18** (200 mg, 58%) as a yellow solid: mp 73–74 °C; IR (NaCl) ν_{max} 2940, 1698, 1652 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.30 (m, 5H), 6.88 (s, 1H), 6.17 (m, 1H), 5.61 (s, 1H), 5.07 (dd, *J* = 8.2 and 4.0 Hz, 1H), 4.75 (sept, *J* = 6.2 Hz, 1H), 4.73 (d, *J* = 12.2 Hz, 1H), 4.54 (d, *J* = 12.2 Hz, 1H), 4.36 (dd, *J* = 12.7 and 6.3 Hz, 1H), 3.80 (m, 15H), 3.78 (m, 2H), 3.66 (s, 3H), 3.63 (s, 3H), 3.35 (m, 1H), 3.10 (m, 1H), 2.97 (dd, *J* = 16.4 and

Table 1
In vitro cytotoxicity (*M* values) against human cancer cell lines

Compound	Values	Breast MDA-MB 231	Lung A-549	Colon HT-29
Ecteinascidin 743	GI_{50}	3.96×10^{-10}	2.81×10^{-9}	4.42×10^{-10}
	TGI	1.01×10^{-9}	3.15×10^{-9}	4.88×10^{-10}
	LC_{50}	1.70×10^{-9}	3.89×10^{-9}	$>1.31 \times 10^{-6}$
1	GI_{50}	9.27×10^{-6}	5.68×10^{-6}	8.32×10^{-6}
	TGI	1.53×10^{-5}	1.17×10^{-5}	9.84×10^{-6}
	LC_{50}	n.d.	1.74×10^{-5}	n.d.
2	GI_{50}	1.09×10^{-6}	4.49×10^{-7}	1.95×10^{-7}
	TGI	n.d.	1.89×10^{-6}	2.73×10^{-7}
	LC_{50}	n.d.	n.d.	5.27×10^{-7}
3	GI_{50}	1.23×10^{-5}	1.12×10^{-5}	8.66×10^{-6}
	TGI	$>1.84 \times 10^{-5}$	$>1.84 \times 10^{-5}$	$>1.84 \times 10^{-5}$
	LC_{50}	$>1.84 \times 10^{-5}$	$>1.84 \times 10^{-5}$	$>1.84 \times 10^{-5}$
5	GI_{50}	1.41×10^{-5}	8.82×10^{-6}	1.12×10^{-5}
	TGI	$>1.90 \times 10^{-5}$	$>1.90 \times 10^{-5}$	$>1.90 \times 10^{-5}$
	LC_{50}	$>1.90 \times 10^{-5}$	$>1.90 \times 10^{-5}$	$>1.90 \times 10^{-5}$
7	GI_{50}	1.66×10^{-5}	1.00×10^{-5}	1.20×10^{-5}
	TGI	$>2.13 \times 10^{-5}$	$>2.13 \times 10^{-5}$	$>2.13 \times 10^{-5}$
	LC_{50}	$>2.13 \times 10^{-5}$	$>2.13 \times 10^{-5}$	$>2.13 \times 10^{-5}$
8	GI_{50}	1.12×10^{-5}	7.16×10^{-6}	4.06×10^{-6}
	TGI	$>1.94 \times 10^{-5}$	1.61×10^{-5}	9.48×10^{-6}
	LC_{50}	$>1.94 \times 10^{-5}$	$>1.94 \times 10^{-5}$	1.78×10^{-5}
9	GI_{50}	8.01×10^{-6}	$>1.63 \times 10^{-5}$	1.63×10^{-5}
	TGI	$>1.63 \times 10^{-5}$	$>1.63 \times 10^{-5}$	$>1.63 \times 10^{-5}$
	LC_{50}	$>1.63 \times 10^{-5}$	$>1.63 \times 10^{-5}$	$>1.63 \times 10^{-5}$
10	GI_{50}	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$	9.13×10^{-6}
	TGI	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$
	LC_{50}	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$
11	GI_{50}	7.13×10^{-6}	7.30×10^{-6}	1.30×10^{-5}
	TGI	1.05×10^{-5}	$>1.62 \times 10^{-5}$	$>1.62 \times 10^{-5}$
	LC_{50}	$>1.62 \times 10^{-5}$	$>1.62 \times 10^{-5}$	$>1.62 \times 10^{-5}$
12	GI_{50}	$>1.88 \times 10^{-5}$	1.22×10^{-5}	1.13×10^{-5}
	TGI	$>1.88 \times 10^{-5}$	$>1.88 \times 10^{-5}$	$>1.88 \times 10^{-5}$
	LC_{50}	$>1.88 \times 10^{-5}$	$>1.88 \times 10^{-5}$	$>1.88 \times 10^{-5}$
13	GI_{50}	1.22×10^{-5}	1.11×10^{-5}	1.23×10^{-5}
	TGI	$>1.58 \times 10^{-5}$	$>1.58 \times 10^{-5}$	$>1.58 \times 10^{-5}$
	LC_{50}	$>1.58 \times 10^{-5}$	$>1.58 \times 10^{-5}$	$>1.58 \times 10^{-5}$
15	GI_{50}	9.53×10^{-6}	6.21×10^{-6}	9.38×10^{-6}
	TGI	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$
	LC_{50}	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$
16	GI_{50}	$>1.41 \times 10^{-5}$	9.20×10^{-6}	7.64×10^{-6}
	TGI	$>1.41 \times 10^{-5}$	$>1.41 \times 10^{-5}$	$>1.41 \times 10^{-5}$
	LC_{50}	$>1.41 \times 10^{-5}$	$>1.41 \times 10^{-5}$	$>1.41 \times 10^{-5}$
20	GI_{50}	8.79×10^{-6}	2.78×10^{-6}	1.11×10^{-5}
	TGI	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$
	LC_{50}	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$
21	GI_{50}	1.06×10^{-5}	6.53×10^{-6}	1.23×10^{-5}
	TGI	$>1.52 \times 10^{-5}$	$>1.52 \times 10^{-5}$	$>1.52 \times 10^{-5}$
	LC_{50}	$>1.52 \times 10^{-5}$	$>1.52 \times 10^{-5}$	$>1.52 \times 10^{-5}$
22	GI_{50}	8.32×10^{-6}	$>1.66 \times 10^{-5}$	1.40×10^{-5}
	TGI	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$
	LC_{50}	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$	$>1.66 \times 10^{-5}$
23	GI_{50}	1.76×10^{-5}	$>1.96 \times 10^{-5}$	$>1.96 \times 10^{-5}$
	TGI	$>1.96 \times 10^{-5}$	$>1.96 \times 10^{-5}$	$>1.96 \times 10^{-5}$
	LC_{50}	$>1.96 \times 10^{-5}$	$>1.96 \times 10^{-5}$	$>1.96 \times 10^{-5}$
24	GI_{50}	6.05×10^{-6}	7.11×10^{-6}	1.23×10^{-5}
	TGI	$>1.51 \times 10^{-5}$	$>1.51 \times 10^{-5}$	$>1.51 \times 10^{-5}$
	LC_{50}	$>1.51 \times 10^{-5}$	$>1.51 \times 10^{-5}$	$>1.51 \times 10^{-5}$
28	GI_{50}	1.61×10^{-5}	$>1.89 \times 10^{-5}$	$>1.89 \times 10^{-5}$
	TGI	$>1.89 \times 10^{-5}$	$>1.89 \times 10^{-5}$	$>1.89 \times 10^{-5}$
	LC_{50}	$>1.89 \times 10^{-5}$	$>1.89 \times 10^{-5}$	$>1.89 \times 10^{-5}$
29	GI_{50}	8.64×10^{-6}	1.33×10^{-5}	6.22×10^{-6}
	TGI	$>1.73 \times 10^{-5}$	$>1.73 \times 10^{-5}$	$>1.73 \times 10^{-5}$
	LC_{50}	$>1.73 \times 10^{-5}$	$>1.73 \times 10^{-5}$	$>1.73 \times 10^{-5}$
33	GI_{50}	1.50×10^{-5}	1.05×10^{-6}	6.67×10^{-6}
	TGI	$>1.80 \times 10^{-5}$	$>1.80 \times 10^{-5}$	$>1.80 \times 10^{-5}$
	LC_{50}	$>1.80 \times 10^{-5}$	$>1.80 \times 10^{-5}$	$>1.80 \times 10^{-5}$

Table 1 (*continued*)

Compound	Values	Breast MDA-MB 231	Lung A-549	Colon HT-29
34	GI_{50}	1.03×10^{-5}	7.32×10^{-6}	6.88×10^{-6}
	TGI	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$
	LC_{50}	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$	$>1.46 \times 10^{-5}$
35	GI_{50}	7.46×10^{-6}	6.17×10^{-6}	5.17×10^{-6}
	TGI	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$
	LC_{50}	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$	$>1.44 \times 10^{-5}$
38	GI_{50}	7.65×10^{-6}	5.78×10^{-6}	7.12×10^{-6}
	TGI	1.29×10^{-5}	1.20×10^{-5}	1.05×10^{-5}
	LC_{50}	1.87×10^{-5}	1.87×10^{-5}	1.51×10^{-5}
40	GI_{50}	4.70×10^{-6}	3.43×10^{-6}	3.25×10^{-6}
	TGI	9.03×10^{-6}	8.13×10^{-6}	4.52×10^{-6}
	LC_{50}	1.41×10^{-5}	1.32×10^{-5}	6.50×10^{-6}
41	GI_{50}	2.47×10^{-6}	2.32×10^{-6}	2.47×10^{-6}
	TGI	2.62×10^{-6}	2.93×10^{-6}	2.93×10^{-6}
	LC_{50}	2.78×10^{-6}	3.86×10^{-6}	3.55×10^{-6}

6.3 Hz, 1H), 2.46 (dd, *J* = 16.4 and 12.7 Hz, 1H), 2.23 (s, 3H), 2.18 (s, 3H), 1.07 (d, *J* = 6.2 Hz, 3H), 0.84 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.1, 155.6, 152.2, 151.2, 150.0, 148.8, 146.1, 145.7, 138.5, 128.1, 127.5, 127.3, 125.2, 124.9, 124.4, 122.6, 111.5, 82.3, 72.6, 70.4, 69.6, 60.4, 60.2, 60.1, 59.9, 59.8, 56.8, 55.8, 53.0, 48.9, 35.7, 27.6, 21.7, 20.9, 9.6, 9.3. Anal. Calcd for $C_{40}H_{52}N_2O_{11}$: C, 65.20; H, 7.11; N, 3.80. Found: C, 64.91; H, 6.96; N, 3.59.

4.2.5. (6*S**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-1,2,4,10,11,13-hexamethoxy-3,12-dimethyl-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-one (28)

To a solution of compound **18** (0.136 mmol) was added trifluoromethanesulfonic acid (2 mL, 13.6 mmol) in one portion, and the mixture was stirred for 1 h at room temperature under argon atmosphere. Then, the reaction mixture was poured into ice-water (3 mL), basified with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate. The organic extracts were washed with saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (9:1 EtOAc/methanol) to give **28** (60 mg, 84%) as a brown solid: mp 97–98 °C; IR (NaCl) ν_{max} 3411, 2939, 1634 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.91 (dd, *J* = 8.8 and 3.8 Hz, 1H), 4.41 (s, 1H), 4.05 (m, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (dd, *J* = 10.6 and 3.8 Hz, 1H), 3.80 (m, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.76 (s, 3H), 3.69 (s, 3H), 3.43 (dd, *J* = 10.6 and 8.8 Hz, 1H), 3.12 (m, 1H), 3.11 (m, 2H), 2.97 (dd, *J* = 17.3 and 6.4 Hz, 1H), 2.23 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.7, 152.5, 152.3, 149.9, 149.8, 146.4, 145.3, 127.9, 124.7, 124.5, 124.1, 123.7, 121.9, 64.5, 60.3, 60.2, 60.1, 60.0, 59.9, 59.8, 55.3, 53.0, 51.6, 49.1, 28.6, 28.0, 9.3. Anal. Calcd for $C_{28}H_{36}N_2O_8$: C, 63.62; H, 6.86; N, 5.30. Found: C, 63.25; H, 6.37; N, 5.09.

4.3. O-acylation general procedure to obtain compounds 29, 33 and 41

A 0.1 M solution of the hydroxyl derivative **26**[9] or **38** (0.4 mmol) in dry DCM, EDC 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.81 mmol), DMAP (0.44 mmol) and the corresponding acid compound (0.44 mmol) was stirred under argon atmosphere at room temperature for 21 h. Then, the solvent was evaporated and the residue was solved in EtOAc. The organic solution was washed with 0.1 N HCl solution, with 1 N aqueous solution of $NaHCO_3$, water and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo.

4.3.1. (6*S**,9*R**,14a*S**,15*R**)-10,11,13-Trimethoxy-12-methyl-9-(1-naphthoyloxymethyl)-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-one (29)

The reaction was carried out with compound **26** (57 mg, 0.134 mmol). The residue was purified by flash column chromatography (EtOAc) to give **29** (60 mg, 78%) as a brown solid: mp 110–111 °C; IR (NaCl) ν_{max} 2936, 1716, 1644 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.75 (d, *J* = 8.4 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 7.4 Hz, 1H), 7.56 (m, 3H), 7.23 (m, 1H), 6.98 (d, *J* = 7.4 Hz, 1H), 6.84 (d, *J* = 7.1 Hz, 1H), 6.36 (dd, *J* = 8.4 and 5.7 Hz, 1H), 6.13 (m, 2H), 4.41 (m, 2H), 4.11 (s, 1H), 3.99 (dd, *J* = 18.8 and 5.9 Hz, 1H), 3.97 (s, 3H), 3.83 (s, 3H), 3.79 (m, 1H), 3.76 (s, 3H), 3.20 (m, 2H), 3.05 (m, 2H), 2.25 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.8, 165.9, 152.5, 150.0, 146.4, 136.1, 133.6, 133.3, 132.1, 131.7, 131.1, 128.4, 128.2, 127.4, 126.8, 126.2, 126.1, 125.9, 125.7, 124.9, 123.7, 123.5, 62.3, 60.5, 60.0, 59.9, 55.8, 53.9, 53.8, 47.6, 32.8, 29.1, 9.4. Anal. Calcd for $C_{35}H_{34}N_2O_6$: C, 72.65; H, 5.92; N, 4.84. Found: C, 72.31; H, 5.57; N, 4.63.

4.3.2. (6*S**,9*R**,14a*S**,15*R**)-9-Cinnamoyloxymehyl-10,11,13-trimethoxy-12-methyl-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-one (33)

The reaction was carried out with compound **26** (64 mg, 0.151 mmol). The residue was purified by flash column chromatography (EtOAc) to give **33** (70 mg, 84%) as a white solid: mp 159–160 °C; IR (NaCl) ν_{max} 2936, 1713, 1644 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.50 (m, 5H), 7.32 (d, *J* = 16.1 Hz, 1H), 7.17 (d, *J* = 6.9 Hz, 1H), 7.14 (m, 1H), 7.00 (m, 1H), 6.83 (d, *J* = 7.5 Hz, 1H), 6.21 (dd, *J* = 10.3 and 4.5 Hz, 1H), 5.90 (d, *J* = 16.1 Hz, 1H), 4.29 (dd, *J* = 11.4 and 4.5 Hz, 1H), 4.22 (dd, *J* = 11.4 and 10.3 Hz, 1H), 4.14 (s, 1H), 4.05 (d, *J* = 5.5 Hz, 1H), 3.92 (s, 3H), 3.80 (s, 3H), 3.79 (m, 1H), 3.75 (s, 3H), 3.23 (m, 2H), 3.10 (m, 2H), 2.23 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.8, 165.8, 152.4, 150.0, 146.3, 144.4, 136.6, 134.5, 132.8, 130.1, 128.9, 128.8, 127.9, 127.3, 126.6, 126.4, 124.9, 123.6, 123.5, 117.4, 62.1, 60.4, 60.0, 59.9, 55.7, 53.9, 53.8, 47.9, 33.0, 29.1, 9.3. Anal. Calcd for $C_{33}H_{34}N_2O_6$: C, 71.46; H, 6.18; N, 5.05. Found: C, 71.12; H, 5.93; N, 4.88.

4.3.3. (6*S**,7*R**,9*R**,14a*S**,15*R**)-9-(*m*-Trifluoromethylcinnamoyloxymethyl)-12,16-dimethyl-10,11,13-trimethoxy-6,7,9,14,14a,15-hexahydro-5*H*-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-carbonitrile (41)

According to the general procedure for O-acylation, the reaction was carried out with compound **38** (60 mg, 0.13 mmol). The

residue was purified by flash column chromatography (7:3 hexane/EtOAc) to give **41** (57 mg, 68%) as a yellow solid: mp 77–78 °C; IR (NaCl) ν_{max} 2937, 1714, 1644, 1462 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.71 (m, 2H), 7.60 (m, 1H), 7.38 (d, J = 16.1 Hz, 1H), 7.20 (m, 2H), 7.09 (m, 2H), 6.05 (d, J = 16.1 Hz, 1H), 4.23 (d, J = 7.5 Hz, 1H), 4.09 (d, J = 7.5 Hz, 2H), 3.91 (s, 3H), 3.83 (s, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 3.67 (s, 1H), 3.63 (dd, J = 17.5 and 1.5 Hz, 1H), 3.39 (d, J = 7.9 Hz, 1H), 3.30 (dd, J = 5.0 and 1.5 Hz, 1H), 3.22 (m, 1H), 2.77 (dd, J = 17.5 and 5.0 Hz, 1H), 2.56 (d, J = 17.9 Hz, 1H), 2.43 (s, 3H), 2.23 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 165.3, 152.4, 149.4, 146.4, 142.0, 135.3, 135.2, 133.5, 131.7, 131.0, 129.5, 127.5, 126.9, 126.5, 126.3, 126.2, 125.2, 124.7, 124.2, 122.6, 121.7, 120.7, 119.8, 62.7, 61.5, 60.4, 60.0, 59.6, 58.4, 57.7, 56.5, 52.5, 42.2, 26.1, 22.3, 9.3. Anal. Calcd for $C_{36}H_{36}F_3N_3O_5$: C, 66.76; H, 5.60; N, 6.49. Found: C, 66.52; H, 5.38; N, 6.23.

4.4. *N*-Methylation: general procedure to obtain compounds 30, 31, 32 and 35

Formaldehyde (37%) solution in methanol (0.06 mL, 0.71 mmol) was added to a stirred solution of **26**,[9] **27**,[9] **28** or **34**[8] (0.47 mmol) in formic acid (0.15 mL). The mixture was heated at 100 °C for 1 h, then poured into ice/water (200 mL) and extracted with ethyl acetate (3 × 20 mL). The extracts were washed with 10% aqueous solution of $NaHCO_3$, H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated to give a crude product.

4.4.1. (6*S**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-10,11,13-trimethoxy-12,16-dimethyl-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-one (30)

The reaction was carried out with compound **26** (195 mg, 0.46 mmol). The residue was purified by flash column chromatography (EtOAc) to give **30** (150 mg, 75%) as an orange solid: mp 101–102 °C; IR (NaCl) ν_{max} 3410, 2938, 1634 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 6.95 (m, 2H), 6.93 (m, 2H), 5.66 (dd, J = 8.8 and 4.1 Hz, 1H), 3.65 (s, 3H), 3.58 (m, 1H), 3.56 (s, 3H), 3.54 (s, 1H), 3.52 (s, 3H), 3.45 (dd, J = 11.9 and 4.0 Hz, 1H), 3.37 (m, 2H), 3.05 (dd, J = 16.3 and 11.9 Hz, 1H), 3.03 (m, 1H), 2.85 (dd, J = 16.3 and 4.0 Hz, 1H), 2.67 (dd, J = 17.0 and 4.3 Hz, 1H), 2.51 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.1, 152.2, 149.8, 146.2, 134.0, 132.1, 128.9, 127.5, 127.1, 124.4, 124.1, 123.9, 63.8, 60.6, 60.3, 59.9, 59.8, 59.7, 57.0, 51.0, 39.6, 29.1, 24.7, 9.3. Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.06; H, 6.59; N, 6.03.

4.4.2. (6*S**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-2,3,10,11,13-pentamethoxy-12,16-dimethyl-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-one (31)

The reaction was carried out with compound **27** (350 mg, 0.72 mmol). The residue was purified by flash column chromatography (9:1 EtOAc/methanol) to give **31** (280 mg, 78%) as a yellow solid: mp 113–114 °C; IR (NaCl) ν_{max} 3425, 2939, 1636 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 6.61 (s, 1H), 6.60 (s, 1H), 5.88 (dd, J = 8.6 and 3.9 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H), 3.87 (s, 1H), 3.86 (dd, J = 11.0 and 3.9 Hz, 1H), 3.85 (s, 3H), 3.78 (s, 3H), 3.74 (s, 3H), 3.63 (m, 1H), 3.48 (dd, J = 11.7 and 4.0 Hz, 1H), 3.40 (dd, J = 11.0 and 8.6 Hz, 1H), 3.04 (m, 1H), 3.03 (dd, J = 16.3 and 11.7 Hz, 1H), 2.88 (dd, J = 16.3 and 4.0 Hz, 1H), 2.61 (d, J = 17.1 Hz, 1H), 2.49 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.3, 152.2, 149.8, 148.4, 148.2, 146.3, 125.3, 124.4, 124.2, 124.1, 123.9, 111.1, 109.7, 64.2, 60.5, 60.3, 60.0, 59.9, 59.7, 58.8, 55.9, 55.8, 51.3, 39.5, 29.1, 24.5, 9.3. Anal. Calcd for $C_{27}H_{34}N_2O_7$: C, 65.04; H, 6.87; N, 5.62. Found: C, 64.87; H, 6.43; N, 5.28.

4.4.3. (6*S**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-3,12,16-trimethyl-1,2,4,10,11,13-hexamethoxy-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]3-benzazocin-7-one (32)

The reaction was carried out with compound **28** (40 mg, 0.075 mmol). The residue was purified by flash column chromatography (9:1 EtOAc/methanol) to give **32** (30 mg, 74%) as a yellow solid: mp 57–58 °C; IR (NaCl) ν_{max} 3924, 3444, 1727 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 5.89 (dd, J = 8.8 and 3.8 Hz, 1H), 4.04 (s, 1H), 3.89 (s, 3H), 3.88 (m, 1H), 3.87 (s, 3H), 3.79 (s, 6H), 3.75 (s, 3H), 3.70 (s, 3H), 3.55 (m, 1H), 3.42 (m, 1H), 3.38 (dd, J = 9.1 and 3.8 Hz, 1H), 3.00 (m, 1H), 2.85 (m, 1H), 2.80 (m, 1H), 2.64 (dd, J = 17.9 and 0.9 Hz, 1H), 2.45 (s, 3H), 2.22 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.6, 152.3, 152.2, 150.0, 149.8, 146.6, 146.3, 125.1, 124.6, 124.4, 124.2, 124.1, 121.2, 64.4, 60.4, 60.3, 60.2, 60.1, 60.0, 59.9, 59.0, 55.7, 55.5, 51.5, 39.7, 28.7, 19.7, 9.3. Anal. Calcd for $C_{29}H_{38}N_2O_8$: C, 64.19; H, 7.06; N, 5.16. Found: C, 63.92; H, 6.92; N, 4.87.

4.4.4. (6*R**,9*R**,14a*S**,15*R**)-1,2,4,10,11,13-Hexamethoxy-3,12,16-trimethyl-9-naphthylcarbonyl-oxymethyl-5,6,9,14,14a,15-hexahydro-6,15-iminoisoquino[3,2-*b*]-3-benzazocin-7-one (35)

The residue was purified by flash column chromatography (3:7 hexane/EtOAc) to give **35** (80%) as a white solid: mp 134–135 °C; IR (NaCl) ν_{max} 2937, 1715, 1651 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$) δ 8.64 (dd, J = 6.6 and 2.5 Hz, 1H), 7.87 (d, J = 8.2 Hz, 1H), 7.77 (d, J = 7.1 Hz, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.43 (m, 3H), 6.21 (dd, J = 10.3 and 3.4 Hz, 1H), 4.50 (m, 1H), 4.20 (dd, J = 11.5 and 3.4 Hz, 1H), 3.85 (s, 3H), 3.79 (m, 1H), 3.73 (s, 3H), 3.72 (m, 1H), 3.71 (s, 3H), 3.68 (s, 3H), 3.67 (m, 1H), 3.52 (s, 3H), 3.22 (dd, J = 15.9 and 4.4 Hz, 1H), 3.00 (dd, J = 15.9 and 4.1 Hz, 1H), 2.98 (s, 3H), 2.86 (dd, J = 18.0 and 6.6 Hz, 1H), 2.65 (d, J = 18.0 Hz, 1H), 2.32 (s, 3H), 2.15 (s, 3H), 1.34 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 170.0, 166.1, 152.4, 151.6, 149.7, 149.6, 146.5, 146.3, 133.6, 133.0, 131.2, 130.9, 128.2, 127.4, 125.7, 125.6, 125.5, 125.1, 124.7, 124.6, 124.2, 124.0, 123.6, 120.9, 62.7, 60.5, 60.1, 60.0, 59.9, 59.8, 59.1, 58.8, 55.6, 54.3, 47.8, 39.5, 29.0, 20.4, 9.3, 8.4. Anal. Calcd for $C_{40}H_{44}N_2O_9$: C, 68.95; H, 6.36; N, 4.02. Found: C, 68.75; H, 6.16; N, 4.06.

4.5. Reductive cyanation: general procedure to obtain compounds 38, 39 and 40

$LiAlH_4$ 2.0 M in dry THF (0.32 mmol) was added to a solution of compound **30, 31** or **32** (0.16 mmol) in dry THF (1 mL) under argon atmosphere. The mixture was stirring at −17 °C for 30 min, and continued during 1 h at 0 °C and 15 min at room temperature. Then the solution was poured into ice and extracted with ethyl acetate (20 mL × 3). The organic layer was washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give the oxazolidine derivatives **36** or **37**. To a stirred solution of this crude compounds in dry DCM (5 mL) under argon atmosphere was added trimethylsilylcyanide (0.43 mmol) and trifluoroboroetherate (0.112 mmol). After stirring at −30 °C for 2 h, the reaction mixture was quenched by addition of 10 mL of 10% aqueous solution of $NaHCO_3$ and extracted with DCM (30 mL × 3). The extracts were washed with H_2O and with a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude was purified by flash column chromatography.

4.5.1. (6*S**,7*R**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-10,11,13-trimethoxy-12,16-dimethyl-6,7,9,14,14a,15-hexahydro-5*H*-6,15-imino- isoquino[3,2-*b*]3-benzazocine-7-carbonitrile (38)

The reaction was carried out with compound **36** (67 mg, 0.158 mmol). The residue was purified by flash column chromatography (EtOAc) to give **38** (60 mg, 84%) as an orange solid: mp 60–61 °C; IR (NaCl) ν_{max} 3448, 2936, 1462 cm^{-1}. ^1H NMR (250 MHz,

$CDCl_3$) δ 7.26 (m, 2H), 7.12 (m, 2H), 3.91 (dd, J = 10.1 and 5.2 Hz, 1H), 3.86 (d, J = 1.8 Hz, 1H), 3.84 (s, 3H), 3.79 (s, 3H), 3.77 (s, 1H), 3.76 (s, 3H), 3.73 (m, 1H), 3.62 (m, 1H), 3.46 (m, 1H), 3.33 (m, 1H), 3.01 (m, 1H), 3.00 (m, 1H), 2.79 (dd, J = 17.6 and 5.0 Hz, 1H), 2.68 (d, J = 17.7 Hz, 1H), 2.55 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 152.3, 149.5, 146.5, 135.6, 133.1, 127.3, 127.2, 127.1, 126.8, 124.5, 124.4, 123.3, 120.3, 61.2, 61.0, 60.9, 60.4, 60.0, 59.6, 57.3, 56.7, 53.0, 42.2, 26.0, 22.4, 9.3. Anal. Calcd for $C_{26}H_{31}N_3O_4$: C, 69.47; H, 6.95; N, 9.35. Found: C, 69.12; H, 6.72; N, 8.98.

4.5.2. (6*S**,7*R**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-2,3,10,11,13-pentamethoxy-12,16-dimethyl-6,7,9,14,14a,15-hexahydro-5*H*-6,15-iminoisoquino[3,2-*b*]3-benzazocine-7-carbonitrile (39)

The reaction was carried out with compound **37** (60 mg, 0.124 mmol). The residue was purified by flash column chromatography (1:1 hexane/EtOAc) to give **39** (50 mg, 80%) as a yellow solid: mp 60–61 °C; IR (NaCl) ν_{max} 3462, 2929, 1515 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.60 (s, 1H), 6.50 (s, 1H), 3.90 (m, 1H), 3.89 (m, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H), 3.82 (m, 1H), 3.79 (s, 3H), 3.75 (s, 3H), 3.70 (m, 1H), 3.60 (m, 1H), 3.41 (d, J = 7.7 Hz, 1H), 3.25 (dd, J = 17.7 and 7.7 Hz, 1H), 3.01 (m, 1H), 3.00 (m, 1H), 2.77 (dd, J = 17.5 and 4.9 Hz, 1H), 2.59 (d, J = 17.7 Hz, 1H), 2.51 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 152.3, 149.5, 148.2, 147.8, 146.5, 126.6, 124.8, 124.4, 124.3, 123.3, 120.3, 109.8, 61.1, 61.0, 61.0, 60.3, 60.0, 59.6, 57.5, 56.5, 55.9, 55.8, 52.5, 42.2, 25.6, 22.3, 9.3. Anal. Calcd for $C_{28}H_{35}N_3O_6$: C, 65.99; H, 6.92; N, 8.25. Found: C, 65.47; H, 6.63; N, 8.01.

4.5.3. (6*S**,7*R**,9*R**,14a*S**,15*R**)-9-Hydroxymethyl-1,2,4,10,11,13-hexamethoxy-3,12,16-trimethyl-6,7,9,14,14a,15-hexahydro-5*H*-6,15-iminoisoquino[3,2-*b*]3-benzazocine-7-carbonitrile (40)

The reaction was carried out with compound **32** (10 mg, 0.018 mmol). The residue was purified by flash column chromatography (7:3 hexane/EtOAc) to give **40** (5 mg, 52%) as a yellow solid: mp 94–95 °C; IR (NaCl) ν_{max} 3335, 2931, 1693 cm^{-1}; ^{1}H NMR (700 MHz, $CDCl_3$) δ 4.20 (m, 1H), 3.91 (dd, J = 10.7 and 5.2 Hz, 1H), 3.87 (m, 1H), 3.84 (s, 6H), 3.82 (m, 1H), 3.81 (s, 3H), 3.80 (s, 6H), 3.73 (s, 3H), 3.68 (dd, J = 11.3 and 5.2 Hz, 1H), 3.57 (m, 1H), 3.18 (dd, J = 17.9 and 4.4 Hz, 1H), 3.06 (m, 1H), 3.05 (m, 1H), 2.85 (dd, J = 17.6 and 4.4 Hz, 1H), 2.66 (d, J = 17.9 Hz, 1H), 2.56 (s, 3H), 2.22 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (175 MHz, $CDCl_3$) δ 152.4, 151.2, 150.2, 149.6, 146.5, 146.2, 124.6, 124.4, 123.1, 96.1, 61.0, 60.6, 60.4, 60.3, 60.1, 60.0, 59.8, 57.2, 55.9, 55.6, 51.7, 42.3, 22.1, 9.4, 9.3. Anal. Calcd for $C_{30}H_{39}N_3O_7$: C, 65.08; H, 7.10; N, 7.59. Found: C, 64.93; H, 6.89; N, 7.43.

4.6. Cell proliferation assay

Cells were plated in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. After that, cells were treated with vehicle alone (control) or compounds at the concentrations indicated. One plate from each different cell line was fixed and stained, and used for Tz reference (see next paragraph). Treated cells were further incubated for 48 h. To quantify the cytotoxic potential of compounds the sulforhodamine B (SRB) protein stain method was used as follows: cells were washed twice with phosphate-buffered saline (PBS), fixed for 15 min in 1% glutaraldehyde solution, rinsed twice in PBS and stained in 0.4% (SRB) solution for 30 min at room temperature. Cells were then rinsed several times in 1% acetic acid solution and air-dried. SRB was then extracted in 10 mM trizma base solution and the absorbance measured at 490 nm. Cell survival is expressed as percentage of control cell growth. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used. The assay is dependent on the reduction of MTT by mitochondrial dehydrogenases of viable cell to a blue formazan product, which comes be measured spectrophotometrically. Tumour cells were incubated in each well with serial dilutions (5, 2.5, 1, 0.5, 0.1, 0.05, 0.01, and 0.005 μg/mL) of the tested compounds. After 2 days of incubation (37 °C, 5% CO_2 in a humid atmosphere) 50 μL of MTT (5 mg/mL in PBS) was added to each well and the plate was incubated for a further 2 h (37 °C). The resulting formazan was dissolved in 100 μL DMSO and read at 490 nm. All determinations were carried out in triplicate.

Acknowledgements

This work was supported by CICYT CTQ2006-10930/BQU and Comunidad Autónoma de Madrid (Group 920234 Grant). A FPI fellowship to I. Ortín is also acknowledged. The authors thank PharmaMar for cytotoxicity determinations on MDA-MB 231, A-549 and HT-29 cell lines.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.049.

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