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Pyrazino[1,2-*b*]isoquinolines: Synthesis and study of their cytostatic and cytotoxic properties

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ABSTRACT

The in vitro antitumor potential of novel pyrazino[1,2-*b*]-isoquinoline-4-ones that contain a half portion of significant natural products was explored in three cancer cell lines: MDA-MB 231 human breast carcinoma, A-549 human lung carcinoma, and HT-29 human colon carcinoma. In general, these compounds show mid to low μM GI_{50}s, but LC_{50}s over 100 μM with the exceptions of compounds **3b** and **31** that are moderately toxic in all cell lines, while compound **4a** is highly toxic and selective for HT-29 cells with LC_{50} values in the high nanomolar range. Experiments directed to elucidate possible mechanisms of action with compounds **3a**, **29**, and **31** showed that compound **3a** is able to efficiently induce apoptosis triggered directly from the G2/M phase of cell cycle, while compounds **29** and **31** are potentially cytostatic agents that induce the G1/S arrest of cell cycle. All three compounds do not act through DNA damage, since they do not activate this signaling at the level of sensors, transducers, and executers. Furthermore, the apoptosis induction of **3a** is not mediated by activation of pro-apoptotic kinases JNK and p38 or by activation of AKT.

1. Introduction

In spite of the impressive progress in diagnosis, surgery, and therapy, the overall cancer mortality is still high, the medical need is largely unmet, and the therapeutic role of innovative drugs addressing molecular targets is not yet well defined. Therefore, cytotoxic drugs will continue to represent a large part of the cancer treatments in combination with those new agents. Since the beginning of chemotherapy, plants, microorganisms, and more recently, marine organisms have been a main source of cytotoxic anticancer agents, and their role in providing new active compounds should not be disregarded for the future.[1,2] Activity of antitumor natural products belonging to the tetrahydroisoquinoline family, from which representative structures are shown in Figure 1, has been under study for the last 30 years,[3–14] but their development has been limited by their natural scarcity and the complexity of their synthesis or semisynthesis.[15–25]

Some members of these series have been structurally modified, but structure–activity relationships are relatively unexplored and have been restricted to the derivatization of the natural products or to late-stage modification of advanced synthetic intermediates.[26–28] In this context it is relevant that most of the biological activity of ecteinascidin 743 (ET-743, trabectedin) is maintained in its simpler analogue phthalascidin.[29] Ecteinascidin 743, which is the most active member of the group, was first granted the status of orphan drug for treatment of soft tissue sarcoma and ovarian cancer, and it has been approved in Europe for the first indication as Yondelis®.[30–33] Regarding the mechanism of action, although certain compounds in this family may generate cytotoxic oxygen radicals after their interaction with DNA, most of them contain a cyanopiperazine core or a hemiaminal equivalent function, which is essential for effective DNA alkylation at the minor groove through the generation of intermediate iminium species.[12,34] However, biological data of renieramycin G or cribrostatin-4 show that some antiproliferative activity is maintained in members of the tetrahydroisoquinoline family possessing a C-21-amide carbonyl group and, although less active than their respective C-21 cyano- or carbinolamine-containing relatives, exhibit low micromolar cytotoxicity ($GI_{50} \approx 5$ mM for cribrostatin.4).[35–37]

Among the few studies with simpler models, Kubo and coworkers found that octahydro-1,5-imino-3-benzazocin-4,7,10-trione derivatives, in which rings *D* and *E* of the saframycin core were eliminated, showed low cytotoxic potency relative to the natural product.[38] In this context, we have shown that the pyrazino[1,2-*b*]-isoquinoline-1,4-dione system may be a synthetic precursor of

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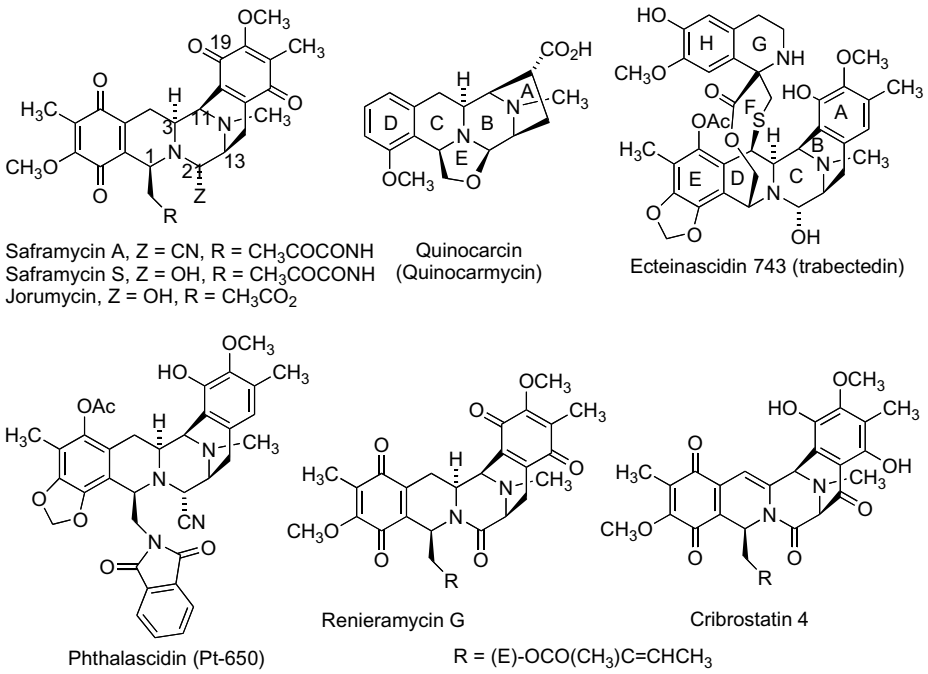


Figure 1.

the full saframycin core and of other more complex octacyclic compounds[39–41] and additionally we found that several compounds containing either rings *C–E*, *A–E*, or *A–F* have in vitro micromolar cytotoxic activities apparently uninfluenced by the ability to generate iminium species.[42]

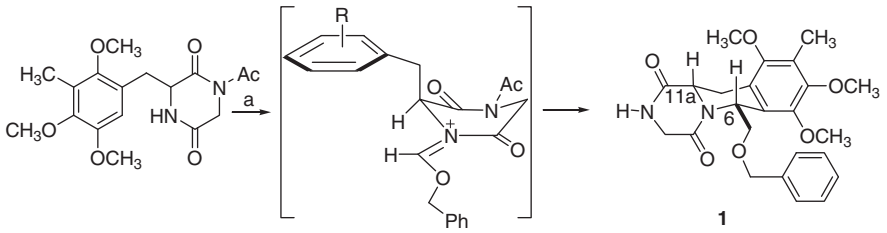
Being unaware of any investigations about the antiproliferative activity of pyrazino[1,2-*b*]-isoquinoline-4-ones, we report here their synthesis and in vitro antitumor potential, as well as the effect of representative compounds on cell cycle transitions and signaling pathways related to DNA damage and apoptosis induction. We also study the activity of the bis-cyano octacycle derivative **31** that could generate bis iminium alkylating species with cross-linking ability.

2. Results and discussion

2.1. Chemistry

Compound **1** was obtained from 2-acetyl-3-(2,4,5-trimethoxy-3-methylphenyl) piperazine-2,5-dione[43] in a one-pot procedure through in situ activation of the C(4)-lactam to a *O*-trimethylsilyl-lactim and subsequent reaction with commercial 2-benzyloxyacetaldehyde dimethyl acetal in the presence of trimethylsilyl triflate. In contrast to previous results of this reaction with other acetals, which afforded the C(6)–C(11a)-*trans* isomers,[39–41] NOE experiments with compound **1** showed that the H-6 and H-11a protons have the same *cis*-relationship as found in natural alkaloids, and that the C(6)- side chain adopts a pseudo-equatorial position. Here, the cyclized product results from the nucleophilic attack of the benzene ring to the *Re* face of the *Z*-acyliminium intermediate that, due to the greater conformational freedom of the benzyloxymethyl substituent, is more stable as compared to the previously studied compounds with different side-chains (Scheme 1).

The *N*-isopropoxycarbonyl and the *N*-Boc derivatives of **1**, compounds **2a** and **2b**, underwent a clean chemoselective *endo*-reduction of the C(1)=O carbonyl group with the exclusive formation of the *cis*-isomers **3a** and **3b** after treatment with $LiAlH(^tBuO)_3$. The relative configuration of the new stereocenter at C(1) in both compounds was assigned by ^1H NMR/NOE studies. They were later submitted to ionic reduction[44–47] expecting that the combination of trifluoroacetic acid to generate the corresponding *N*-acyliminium cation and triethyl silane as a hydride transfer reagent would be tolerant to other functions sensitive to reduction. In the case of **3a**, the desired monolactam **4a** was obtained when the reaction was performed at −10 °C while, at room temperature, this compound was obtained in a mixture with enamide **5a**. The last product results from deprotonation of the intermediate iminium species which, in contrast to other *N*-vinylamides or *N*-vinylcarbamates, are not substrates of ionic hydrogenation. Due to the lability of the *N*-Boc derivatives to trifluoroacetic acid, the ionic reduction of **3b** at −10 °C gave directly the *N*-deprotected monolactam **6** without affecting the $BnOCH_2$ chain, but deprotection of the NH group in carbamate **4a** with trifluoroacetic or hydrochloric acid



Scheme 1. Reagents and conditions: (a) TMSCl (1.1 equiv), Et_3N (1.1 equiv), DCM, rt, 1 h; (b) $BnOCH_2CH(OEt)_2$ (2 equiv), TMSOTf (3 equiv), rt, 16 h (60%).

required the use of TFA and sulfuric acid, which produced the debenzylation of the side chain to give **9**. This compound was more conveniently achieved through the previous reductive deprotection of **4a** to give the hydroxymethyl derivative **8a** followed by acid hydrolysis of the carbamate group. Compound **7** was obtained by reductive formylation of **6** (Scheme 2).

Due to the enhanced acidity of the H(11a)-proton, carbamates **2a** and **2b** are easily epimerizable and have to be handled in the absence of bases. This reactivity allowed the access to C(11a)-epimers of **4a** and **8a** in order to study the influence in biological activity of the relative configuration of C(6) and C(11a)-stereocenters. To this end, compound **2a** was equilibrated in the presence of DMAP and the nearly equimolecular mixture of this compound and its 11a-diastereoisomer thus obtained was enriched in trans-isomer by column chromatography. Partial reduction followed by ionic hydrogenation of this enriched mixture afforded pure **10a** that gave finally **11a** by reductive deprotection (Scheme 3).

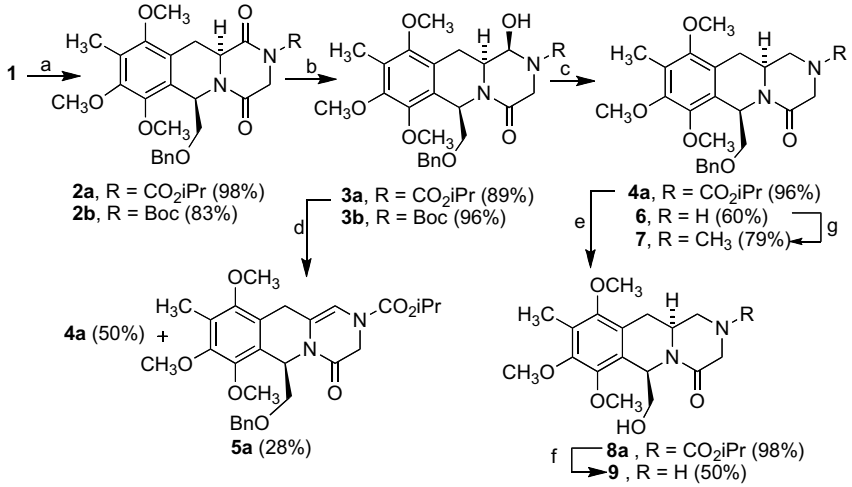
A process similar to that shown in Scheme 2 was used with the *N*-isopropoxycarbonyl derivative **14** containing the same side-chain as phthalascidin, which was obtained from the C(6)-phthalimidomethyl compound **12**.[41] Due to the fact that the C(1)-carbonyl and one of the phthalimide carbonyl groups of **14** have a similar electrophilicity, its partial reduction gave compound **15** as a mixture of diastereoisomers, from which monolactams **16** and **17** were obtained after ionic reduction at −30 °C. Enamide **18** was obtained when this reaction was performed at room temperature and, although the *cis*-relationship between H-6 and H-11a protons in **17** could not be established by conclusive ^{1}H NMR NOE experiments, it was nevertheless determined because this compound is identical to that obtained by catalytic hydrogenation of **16**, a diastereoselective reaction that is governed by the C(6)-substituent[39] (Scheme 4).

Here, hydride transfer to the intermediate bis-iminium species **II** generates compound **16**, while compound **17** comes from a second ionic reduction of the enamide portion of **16** by hydride transfer to the iminium species **III**. Isolation of both compounds requires to work at low temperatures to avoid the alternative intramolecular conjugate cyclization involving the iminium species **I** that gives compound **18** (Scheme 5).[48]

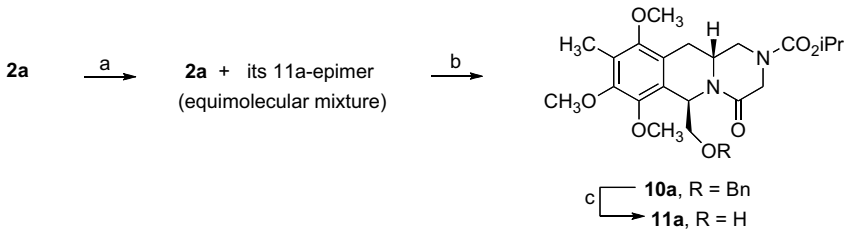
As is shown in Scheme 6, *N*-methyl derivative **20** was obtained by reductive formylation of deprotected monolactam **19**, while hydrolysis and decarboxylation of **18** gave compound **24**, most probably through the intermediacy of **23**. The structure of **24** was assigned after several NMR experiments with its C_6D_6 solution; the key features were two singlets at 4.56 and 4.01 ppm that correspond to H-11 and H-1′ protons, respectively, and correlated to two carbon atoms at 42.6 (C-11) and 63.2 (C-1′) that were previously assigned by HMQC and HMBC experiments; a double doublet at 5.03 ppm that correlated to the methylene-carbon signal at 44.6 ppm; and the ^{1}H NMR NOE enhancements observed between H-1 and H-3 protons. Compound **21** was obtained from **9** together with the *O*-formylated compound **22**.

Taking into consideration the increasing cytotoxic activity associated to *O*-acyl derivatives of simplified ecteinascidin and saframycin analogues,[25] compound **8a** was transformed under Mitsunobu conditions into the esters **26**–**29** and into the phthalimido compound **25**, the dihydro derivative of **14** (Scheme 7).

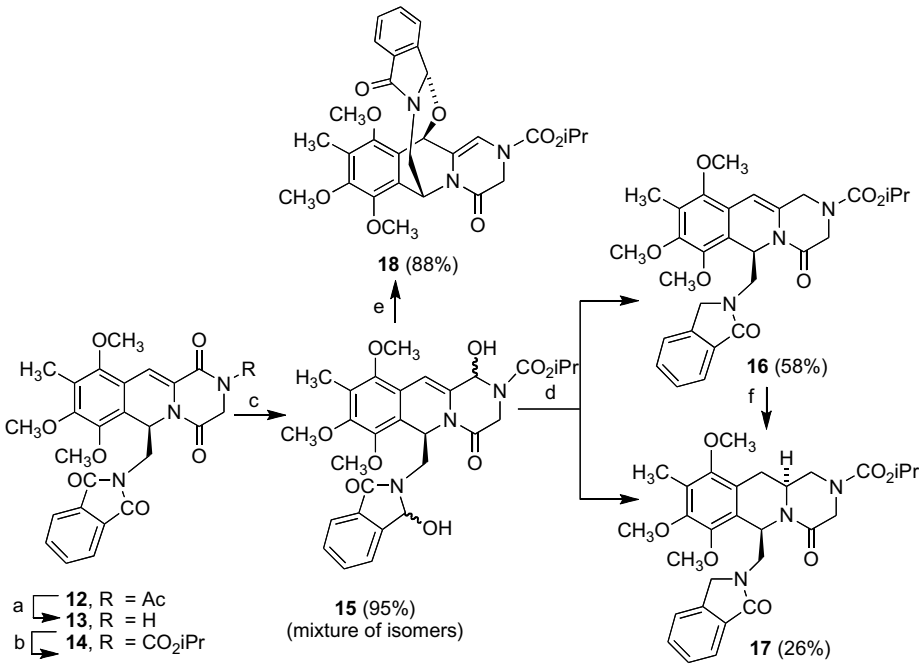
We also studied compound **31**, whose two cyano functions could generate bis iminium alkylating species with cross-linking ability. This compound was obtained in a mixture with the cyano



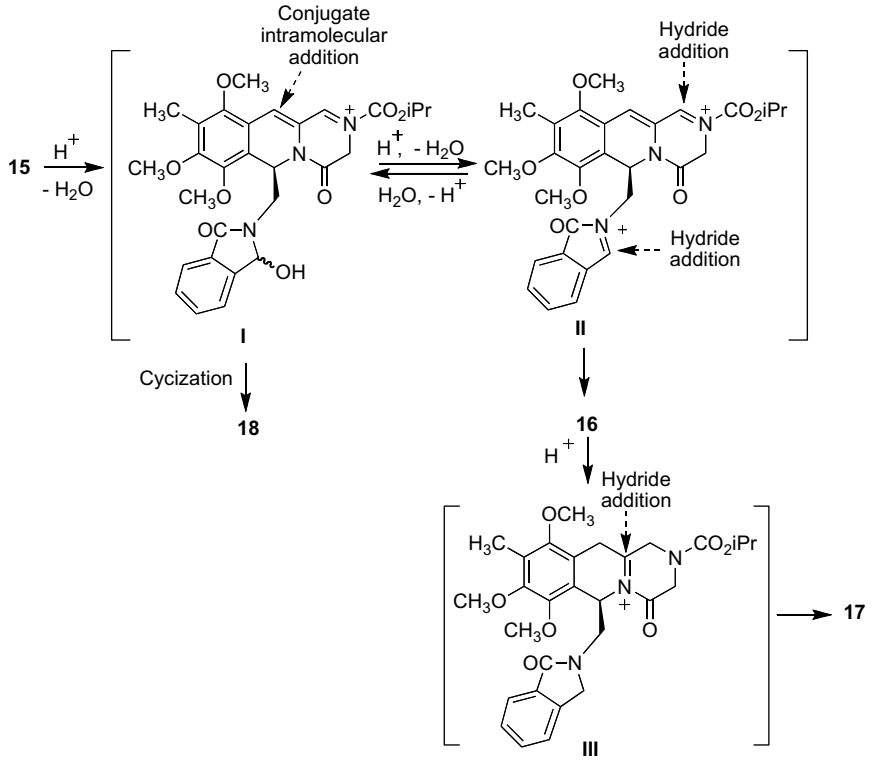
Scheme 2. Reagents and conditions: (a) DMAP (3 equiv), Et_3N (6 equiv), $ClCO_2{}^iPr$ (6 equiv), rt, 24 h or Boc_2O (1.6 equiv), DMAP (cat.), CH_3CN, rt, 12 h; (b) $LiAlH(O^tBu)_3$ (5 equiv) in anhydrous THF, rt, 16 h; (c) Et_3SiH (17 equiv), CF_3CO_2H (15.5 equiv), −10 °C, 15 h; (d) Et_3SiH (10 equiv), CF_3CO_2H (10 equiv) in anhydrous DCM, rt, 3 h; (e) H_2, 10% C/Pd, EtOH, 70 °C, 3.5 atm, 16 h; (f) TFA, H_2SO_4 (20:1), rt, 16 h; (g) HCOH, HCO_2H, MeOH, 100 °C, 1 h.



Scheme 3. Reagents and conditions: (a) DMAP (2 equiv), CH_2Cl_2, 70 °C, 1 h; (b) chromatographic enrichment, then $LiAlH(O^tBu)_3$ (5 equiv) in anhydrous THF, rt, 16 h, then Et_3SiH (17 equiv), CF_3CO_2H (15.5 equiv), −10 °C, 15 h (76%); (c) H_2, 10% C/Pd, EtOH, 70 °C, 3.5 atm, 16 h (95%).



Scheme 4. Reagents and conditions: (a) H_2SO_4, rt, 16 h; (b) DMAP (3 equiv), Et_3N (3 equiv), $ClCO_2{}^iPr$ (6 equiv), rt, 24 h; (c) $LiAlH(^tBuO)_3$ (7 equiv), THF, rt, 16 h; (d) Et_3SiH (50 equiv), TFA (50 equiv), DCM anhydrous, −30 °C, 3 h; (e) Et_3SiH (10 equiv), TFA (10 equiv), DCM anhydrous, rt, 3 h; (f) H_2/10% Pd–C, CH_3OH, 70 °C, 3.5 atm.



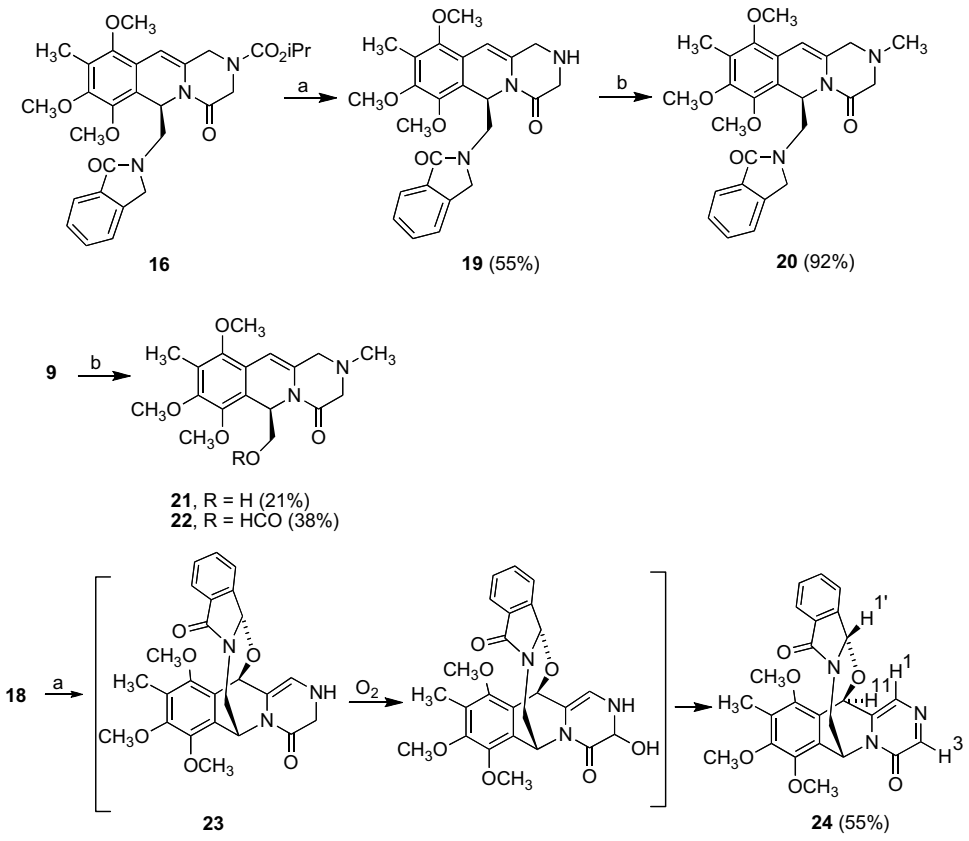
Scheme 5.

derivative at C(7) from the previously described precursor **30**[42] by a double reductive cyanation (Scheme 8).

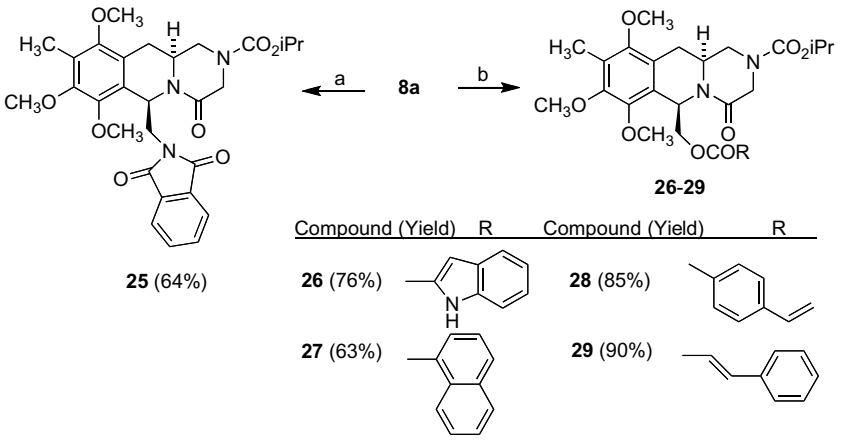
2.2. Pharmacological results

2.2.1. Antiproliferative activity

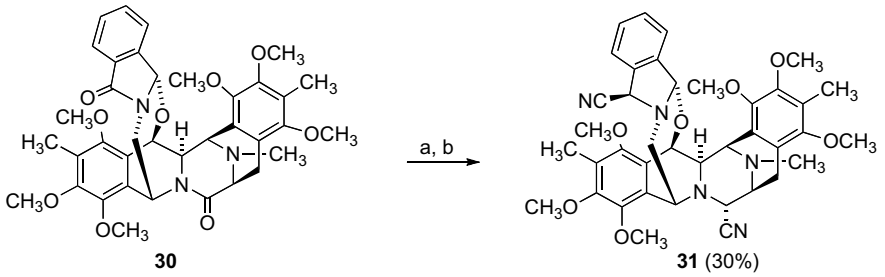
In collaboration with the biopharmaceutical company PharmaMar, the antiproliferative activity of most compounds was evaluated using a panel of three human cell lines: MDA-MB 231 (human breast carcinoma); A-549 (human lung carcinoma); HT-29 (human colon carcinoma) to determine the values GI_{50} (the drug concentration inhibiting the growth of cell lines by 50%), TGI (total growth inhibition) and LC_{50} (the half of the lethal concentration).[49] Compounds that showed mM GI_{50} values are shown in Table 1, in which data for E-743 in the same assay are included for comparison.



Scheme 6. Reagents and conditions: (a) TFA/H_2SO_4, rt, 16 h; (b) H_2CO, HCO_2H, 70 °C, 1 h.



Scheme 7. Reagents and conditions: (a) phthalimide (1 equiv), DEAD (1.1 equiv), TPP (1.2 equiv), THF, rt, 2 h; (b) R(Ar)CO_2H, EDC (2 equiv), DMAP (1.1 equiv), DCM, rt, 21 h.



Scheme 8. Reagents: (a) $LiAlH_2(OEt)_2$ (20 equiv), rt, 45 min; (b) KCN, H_2O-AcOH, rt, 15 h.

Table 1
In vitro cytotoxicity

Compound	Values	MDA-MB 231[a]	A-549[a]	HT-29[a]
Ecteinascidin-743	GI_{50}	5.51×10^{-8}	1.25×10^{-7}	5.17×10^{-8}
	TGI	5.78×10^{-8}	1.31×10^{-7}	6.30×10^{-8}
	LC_{50}	6.43×10^{-8}	1.71×10^{-7}	n.d.
3a	GI_{50}	9.27×10^{-6}	$5{,}68 \times 10^{-6}$	8.32×10^{-6}
	TGI	$1{,}53 \times 10^{-5}$	$1{,}17 \times 10^{-5}$	9.84×10^{-6}
	LC_{50}	n.d.	1.74×10^{-5}	n.d.
3b	GI_{50}	1.20×10^{-5}	4.79×10^{-6}	1.44×10^{-5}
	TGI	$>1.84 \times 10^{-5}$	1.75×10^{-5}	$>1.84 \times 10^{-5}$
	LC_{50}	$>1.84 \times 10^{-5}$	$>1.84 \times 10^{-5}$	$>1.84 \times 10^{-5}$
4a	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}
6	GI_{50}	n.d.	1.34×10^{-5}	n.d.
	TGI	n.d.	$>2.34 \times 10^{-5}$	n.d.
	LC_{50}	n.d.	$>2.34 \times 10^{-5}$	n.d.
7	GI_{50}	n.d.	1.27×10^{-5}	n.d.
	TGI	n.d.	$>2.27 \times 10^{-5}$	n.d.
	LC_{50}	n.d.	$>2.27 \times 10^{-5}$	n.d.
10a	GI_{50}	n.d.	8.19×10^{-6}	1.05×10^{-5}
	TGI	n.d.	1.68×10^{-5}	n.d.
	LC_{50}	n.d.	n.d.	n.d.
18	GI_{50}	n.d.	2.91×10^{-6}	1.04×10^{-5}
	TGI	n.d.	n.d.	n.d.
	LC_{50}	n.d.	n.d.	n.d.
20	GI_{50}	1.19×10^{-5}	n.d.	3.67×10^{-6}
	TGI	n.d.	n.d.	n.d.
	LC_{50}	n.d.	n.d.	n.d.
29	GI_{50}	n.d.	9.05×10^{-6}	9.23×10^{-6}
	TGI	n.d.	n.d.	n.d.
	LC_{50}	n.d.	n.d.	n.d.
31	GI_{50}	1.14×10^{-5}	1.14×10^{-5}	7.35×10^{-6}
	TGI	1.20×10^{-5}	1.20×10^{-5}	8.22×10^{-6}
	LC_{50}	1.28×10^{-5}	1.27×10^{-5}	1.02×10^{-5}

[a] Molar concentrations; n.d., activity not determined (>100 mM).

The cytotoxicity found in the 1-hydroxy derivatives **3a** and **3b** is interesting because their hemiaminal moiety could generate electrophilic alkylating species. However, the reduced products **4a** and its 11a-epimer **10a**, which are uncapable of this bioactivation, were more active than **3a** or retain the activity in the human lung carcinoma cell-line. N-unsubstituted and *N*-methyl derivatives (compounds **6** and **7**, respectively) were less active than its carbamate precursor **4a**. Substitution of the C(6)-benzyloxymethyl chain of **4a** by phthalimidomethyl (**25**) or some acyloxymethyl groups (**26**–**28**) implies the loss of activity, while the *O*-cinnamyl derivative is active (**29**) which may be rationalized if the biological activity is related to the conformational freedom of this side-chain. Among the rest of phthalimidomethyl derivatives, the *N*-methyl derivative **20** and the hexacyclic compounds **18** and **31** showed antiproliferative activity at millimolar concentrations for one or several of the three tumor cell lines. In agreement to our previously reported data,[42] this effect does not require the presence of hemiaminal or cyano functions and, apparently, the rings *A* and *B* of the model natural products are not very relevant. In general, these compounds are not potent cytotoxins as they show mid to low micromolar GI_{50}s, but LC_{50}s over 100 mM in cell lines. Two exceptions are compounds **3b** and **31** which are moderately toxic in all cell lines. Interestingly, compound **4a** is highly toxic and selective for HT-29 cells with LC_{50} values in the high nanomolar range (≈500 nM), but not in the other two cell lines.

2.2.2. Effects on cell cycle transitions

We selected compounds **3a**, **29**, and **31** to study by flow cytometric analysis their effects on cell cycle of A549 cells. Cells were treated with cisplatin as a control and with the three above-mentioned compounds (50 μM), and cell cycle was analyzed after 24 and 48 h (Figs. 2 and 3). As previously reported,[50] cisplatin was able to induce apoptosis that increases with time after 12 h of treatment reaching a maximum at 24 h (Fig. 3A), and to induce a decrease in the population of cells in G1. Compound **3a** was the only one able to induce an increase in apoptosis at 48 h and concomitantly a slight decrease in the number of cells in G1 (Fig. 3B) but, in contrast with cisplatin, an important decrease in G2/M was also observed suggesting that **3a** is affecting the G2/M checkpoint. Compound **29** also induced apoptosis, but to lesser extent than **3a** (Fig. 3C), a decrease in cell number in G2/M, and an increase in G1 population, indicating an induction of G1 arrest. The results obtained with compound **31** were similar to those observed with **29** (Fig. 3D). Taken together the above results indicate that only compound **3a** is able to efficiently induce apoptosis, that is triggered directly from the G2M phase of cell cycle, while the other two compounds are weak apoptosis inducers being inducers of G1/S arrest and therefore potentially cytostatic agents.

2.2.3. Alteration of signaling pathways related to DNA damage and apoptosis induction

The DNA damage response is activated by drugs which interact with DNA as a target and include molecules that act as sensors, transducers, and executors of the DNA damage signal. In order to get inside the mechanism of action of compounds **3a**, **29**, and **31**, we studied the alteration of some signaling pathways triggered by the DNA damage as well as the pro-apoptotic JNK/p38 signaling pathway. We first studied by Western blot, the kinetic of activation of the histone H2AX, that is phosphorylated after DNA damage,[51] but we were not able to find phosphorylation of this molecule even after 24 h of treatment, while a positive signal was found in this experiment in A549 cells treated with bleomycin (data not shown). We also studied the phosphorylation at the Ser-19 residue of CHK2, a protein kinase that may regulate cell cycle arrest and is activated in response to DNA damage induced after treatment with genotoxic agents but, again, no signals of phosphorylation induced by these compounds were detected. Equivalent results were obtained when we studied phosphorylation at the Ser-15 residue of p53. Altogether, these results indicate that it is very likely that all three compounds do not act through DNA damage, since they not activate the response at the sensor (H2AX phosphorylation), transducer (CHK2), or executors (p53) levels. Finally, since many anticancer agents induce in the treated cells activation of the mitogen activated protein kinases (MAPK) signaling pathways which are involved in apoptosis induction, such as the pro-apoptotic kinases JNK and p38, we studied the kinetic of activation for both kinases by using antibodies that detect the phosphorylated forms of both proteins and bleomycin as a positive control (data not shown). Results indicated that neither JNK nor p38 is activated by compounds **3a**, **29**, and **31**. We did not find either activation by these compounds of the AKT signaling pathway using EGF as a positive control (data not shown) (Fig. 4).

3. Conclusions

The novel pyrazino[1,2-*b*]quinazoline compounds described here are readily available from very simple starting materials through chemo- and diastereoselective reactions, and the biological results confirm that further research is needed to establish which are the targets that mediate the antiproliferative properties of the active compounds. The biological studies reported here indicate that compound **3a** is able to induce apoptosis that takes place from the G2/M phase of the cell cycle and that compounds **29** and **31** behave as cytostatic drugs since they induce a G1 arrest in the

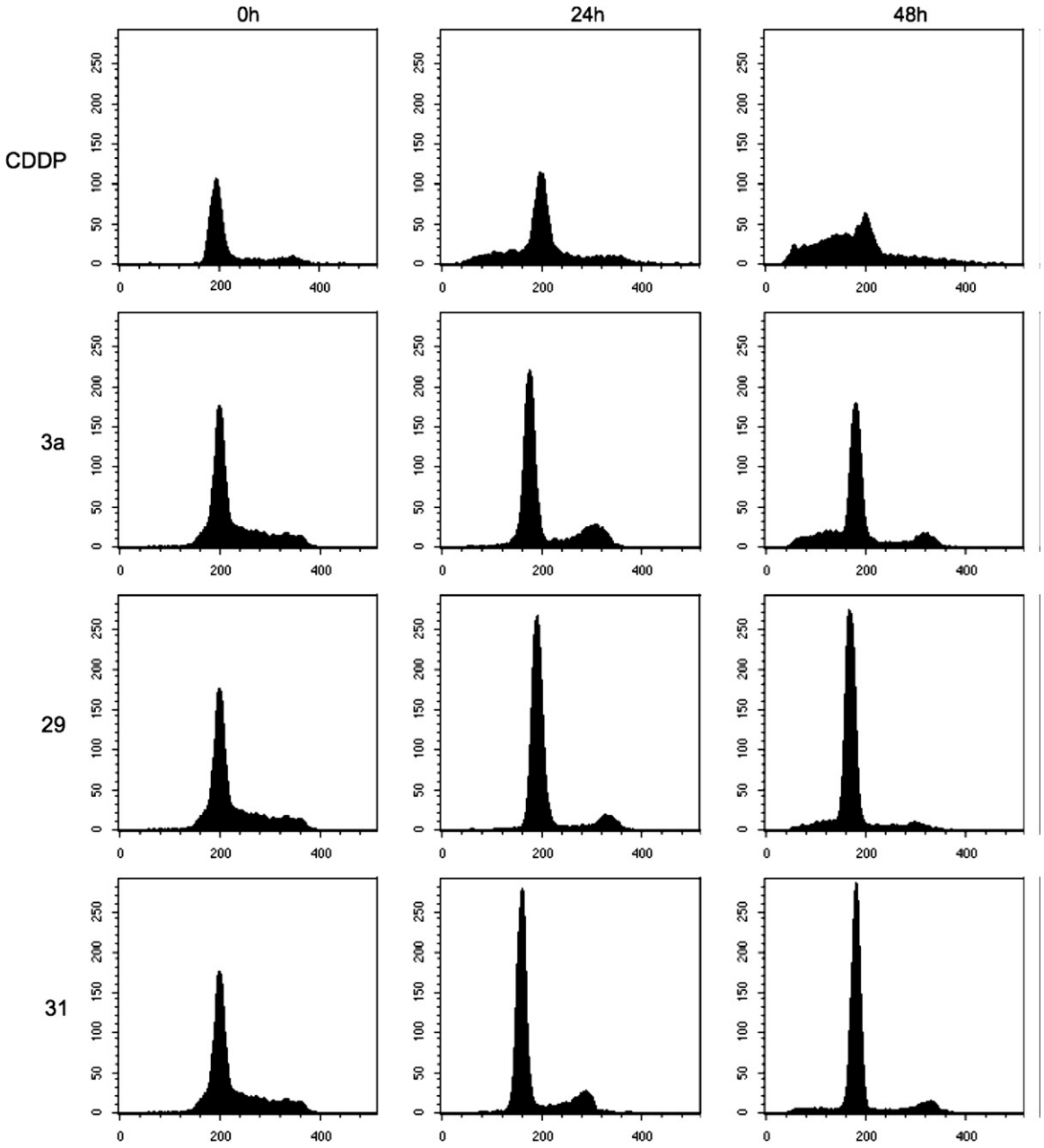


Figure 2. Histogram of the cell cycle analysis.

cell cycle. Finally, the results obtained by exploring the effect on the DNA damage response pathway, indicate that very likely none of them act through this mechanism. Up to now, we have not found evidence of other signaling pathways affected by these compounds. Other targets, such as tubulin polymerization, will be studied.

4. Experimental

4.1. Synthesis

4.1.1. 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, on aluminum 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. Infrared spectra were recorded on a FT-IR spectrophotometer, with solid compounds compressed into KBr pellets and liquid compounds examined as films on NaCl disks. 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.

4.1.2. (6*R**,11a*S**)-6-Benzyloxymethyl-7,8,10-trimethoxy-9-methyl-2,3,11,11a-tetrahydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (1)

TMSCl (2 mL, 15.7 mmol) and triethylamine (2.2 mL, 15.7 mmol) were added to a stirred solution of 1-acetyl-3-(2,4,5-trimethoxy-3-methyl)phenylmethyl-2,5-piperazinedione[43] (5.0 g, 14.3 mmol) in dry DCM (50 mL). After the reaction mixture was

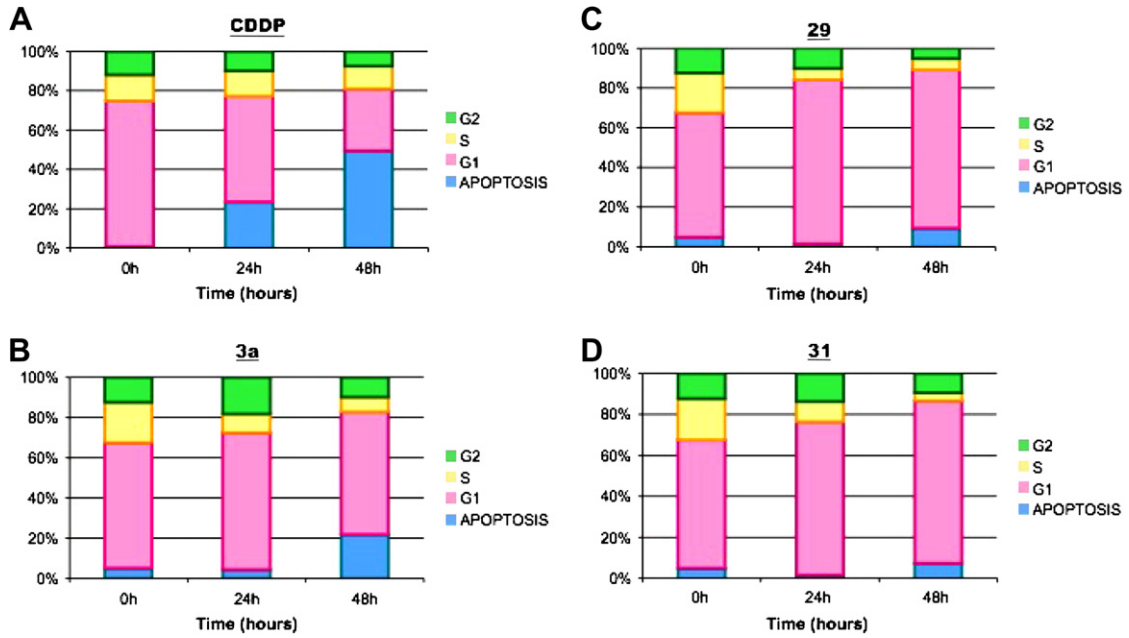


Figure 3. Flow cytometric analysis of A549 cells treated with cisplatin and compounds **3a**, **29**, and **31**. Cells were seeded, treated with 5 mg/mL of cisplatin, and 50 mg/mL of compounds **3a**, **29**, and **31** for 24 h. Cells were trypsinized and DNA stained with propidium iodide and subjected to flow cytometric analysis.

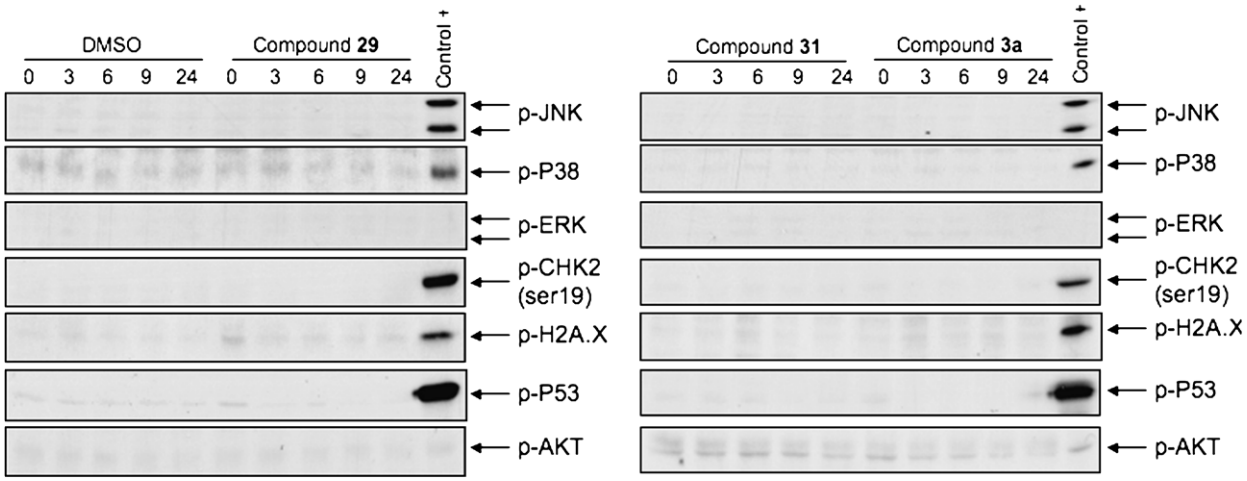


Figure 4. Analysis of phosphorylation of JNK, p38, CHK2, AKT, H2AX, and p53 in A549 cells treated with compounds **3a**, **29**, and **31** at 50 μM concentration using bleomycin as a positive control. A549 cells were treated with these compounds, lysed as previously described, and proteins were resolved in 10% SDS–PAGE. Phosphorylated forms of the corresponding proteins were detected by Western blot with specific antibodies. Arrows indicate the corresponding phosphorylated proteins.

stirred under argon atmosphere at room temperature for 1 h, benzyloxyacetaldehyde diethyl acetal (6.5 mL, 14.3 mmol) and TMSOTf (7.8 mL, 14.3 mmol) were added and stirred for an additional 12 h at room temperature. Then, the mixture was poured onto a 10% aqueous solution of $NaHCO_3$ and extracted with DCM. The combined 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 to give a residue that was purified by flash column chromatography (EtOAc) to afford 1.1 g (60% yield) of **1** as a brown solid: mp 181–182 °C; IR (film) ν, 1667 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.47 (br s, 1H), 7.30–7.00 (m, 5H), 5.99 (dd, J = 6.7 and 4.5 Hz, 1H), 4.61 (d, J = 11.8 Hz, 1H), 4.42 (dd, J = 12.4 and 4.4 Hz, 1H), 4.34 (d, J = 11.8 Hz, 1H), 3.93 (s, 2H), 3.80–3.50 (m, 2H), 3.73 (s, 3H), 3.67 (s, 3H), 3.53 (s, 3H), 3.33 (dd, J = 16.8 and 4.4 Hz, 1H), 2.66 (dd, J = 16.8 and 12.4 Hz, 1H), 2.05 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 168.5 (C), 162.4 (C), 152.7 (C), 150.6 (C), 146.4 (C), 138.3 (C), 128.9 (CH), 128.3 (CH), 128.2 (CH), 125.4 (C), 124.0 (C), 122.4 (C), 73.1 (CH_2), 70.3 (CH_2), 60.7 (CH_3), 60.4 (CH_3), 52.1 (CH), 48.7 (CH), 45.1 (CH_2), 28.5 (CH_2), 9.8 (CH_3). Anal. Calcd for $C_{24}H_{28}N_2O_6$: C, 65.44; H, 6.41; N, 6.36. Found: C, 65.12; H, 6.18; N, 6.27.

4.1.3. General procedure to obtain compounds 2a and 14

A solution of **1** or **13** (6.6 mmol), triethylamine (39.8 mmol), and 4-dimethylaminopyridine (19.2 mmol) in dry DCM (125 ml) was cooled in ice water, and isopropylchloroformiate (39.8 mmol) was added dropwise. The solution was stirred under argon atmosphere at room temperature for 24 h and then an aqueous solution of $NaHCO_3$ was added to quench the reaction. After extraction with DCM, the extracts were washed with H_2O, an aqueous solution of HCl, and a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo.

(6*R,11a*S**)-6-Benzyloxymethyl-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-2,3,11,11a-tetrahydro-6*H*-pyrazino [1,2-*b*]isoquinolin-1,4-dione (2a)**. Pure compound **2a** (3.9 g, 98%) was obtained as a white solid: mp 38–40 °C; IR (film) *v*, 1782, 1728, 1673 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.20–7.10 (m, 5H), 6.00 (dd, *J* = 7.0 and 3.5 Hz, 1H), 5.15 (sept, *J* = 6.3 Hz, 1H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.63 (dd, *J* = 10.1 and 5.2 Hz, 1H), 4.53 (d, *J* = 17.6 Hz, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.34 (d, *J* = 17.6 Hz, 1H), 3.84 (s, 3H), 3.82 (dd, *J* = 10.6 and 3.5 Hz, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.72 (dd, *J* = 10.6 and 7.0 Hz, 1H), 3.38 (dd, *J* = 16.6 and 5.2 Hz, 1H), 3.03 (dd, *J* = 16.6 and 10.1 Hz, 1H), 2.21 (s, 3H), 1.39 (d, *J* = 6.3 Hz, 6H), ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.7 (C), 163.1 (C), 152.6 (C), 151.6 (C), 150.7 (C), 146.3 (C), 138.3 (C), 128.8 (CH), 128.2 (CH), 125.5 (C), 124.3 (C), 122.1 (C), 73.2 (CH_2), 72.9 (CH), 70.6 (CH_2), 60.7 (CH_3), 60.6 (CH_3), 60.4 (CH_3), 54.5 (CH), 49.1 (CH), 48.3 (CH_2), 26.9 (CH_2), 22.2 (CH_3), 9.8 (CH_3). Anal. Calcd for $C_{28}H_{34}N_2O_8$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.72; H, 6.48; N, 5.26.

(6*R)-2-Isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-6-phthalimidomethyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (14)**. Purification of the crude product by flash column chromatography (DCM) afforded (96% yield) **14** as an orange oil; IR (film) *v* 1770, 1732, 1715, 1684, 1682 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.80–7.60 (m, 4H), 7.39 (s, 1H), 6.35 (dd, *J* = 7.5 and 4.0 Hz, 1H), 5.17 (sept, *J* = 6.2 Hz, 1H), 4.66 (d, *J* = 17.3 Hz, 1H), 4.04 (d, *J* = 17.3 Hz, 1H), 4.00 (s, 3H), 3.93 (dd, *J* = 13.9 and 7.5 Hz, 1H), 3.82 (dd, *J* = 13.9 and 4.0 Hz, 1H), 3.80 (s, 3H), 3.66 (s, 3H), 2.19 (s, 3H), 1.43 (d, *J* = 6.2 Hz, 3H), 1.41 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 167.9, 162.5, 158.5, 153.8, 152.0, 151.7, 145.9, 133.9, 131.7, 126.4, 125.6, 123.1, 121.9, 118.3, 114.0, 72.1, 62.0, 60.7, 60.0, 47.8, 47.0, 39.5, 21.7, 21.6, 9.2. Anal. Calcd for $C_{29}H_{29}N_3O_9$: C, 61.81; H, 5.19; N, 7.46. Found: C, 61.62; H, 4.96; N, 7.10.

4.1.4. (6*R*.,11a*S*.)-6-Benzyloxymethyl-2-tertbutyloxycarbonyl-7,8,10-trimethoxy-9-methyl-2,3,11,11a-tetrahydro-6*H*-pyrazino [1,2-*b*]isoquinolin-1,4-dione (2b)

A solution of **1** (300 mg, 0.6 mmol), BOC anhydride (209 mg, 0.96 mmol), and a catalytic amount of DMAP in anhydrous acetonitrile (10 mL) was stirred overnight under argon atmosphere at room temperature, and then the reaction mixture was quenched by addition of ice and extracted with DCM. The extracts were washed with H_2O, a saturated aqueous solution of HCl, and a saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on a silica gel column with hexane/EtOAc (8:2) as eluant to afford 300 mg of **2b** (yield 83%.) as a yellow oil. IR (film) *v*, 2938, 1781, 1732, 1682; ^1H NMR (250 MHz, $CDCl_3$) δ 7.33–7.22 (m, 5H), 5.99 (dd, *J* = 7.2 and 3.5 Hz, 1H), 4.68 (d, *J* = 12.1 Hz, 1H), 4.60 (dd, *J* = 10.3 and 5.3 Hz, 1H), 4.48 (d, *J* = 17.6, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.32 (d, *J* = 17.6 Hz, 1H), 3.84–3.75 (m, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 3.74 (s, 3H), 3.37 (dd, *J* = 16.7 and 5.3 Hz, 1H), 3.02 (dd, *J* = 16.7 and 10.3 Hz, 1H), 2.20 (s, 3H), 1.59 (s, 9H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.3 (C), 162.8 (C), 152.1 (C), 150.2 (C), 149.9 (C), 145.8 (C), 137.8 (C), 128.3 (CH), 127.7 (CH), 125.0 (C), 123.8 (C), 121.7 (C), 84.7 (C), 72.7 (CH_2), 70.1 (CH_2), 60.3 (CH_3), 60.1 (CH_3), 59.9 (CH_3), 53.9 (CH), 48.5 (CH), 47.8 (CH_2), 27.8 (CH_3), 26.3 (CH_2), 9.3 (CH_3). Anal. Calcd for $C_{29}H_{36}N_2O_8$: C, 64.43; H, 6.71; N, 5.18. Found: C, 64.22; H, 6.58; N, 5.06.

4.1.5. General procedure to obtain compounds 3a and 3b

To a stirred solution of lithium tritertbutoxyaluminum hydride (5.1 mmol) in dry THF (50 mL) cooled in ice water was added the corresponding **2** (1.02 mmol), and the mixture was stirred under argon atmosphere at room temperature for 16 h. The reaction mixture was quenched by addition of ice, filtered over Celite, and extracted with ethyl acetate. 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.

(1*R,6*R**,11a*S**)-6-Benzyloxymethyl-1-hydroxy-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-tetrahydro-pyrazino[1,2-*b*]isoquinolin-4-one (3a)**. Purification of the crude product by flash column chromatography (6:4 hexane/EtOAc) afforded (89% yield) **3a** as a yellow solid: mp 63–65 °C; IR (film) *v*, 3370, 1704, 1634 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5H), 6.08 (dd, *J* = 6.5 and 3.6 Hz, 1H), 5.75 (br s, 1H), 4.95 (sept, *J* = 5.8 Hz, 1H), 4.72 (d, *J* = 12.1 Hz, 1H), 4.46 (d, *J* = 12.1 Hz, 1H), 4.41 (d, *J* = 17.8 Hz, 1H), 4.14 (m, 1H), 4.05 (d, *J* = 17.8 Hz, 1H), 3.81 (s, 3H), 3.80 (m, 2H), 3.75 (s, 3H), 3.64 (s, 3H), 2.95 (dd, *J* = 16.1 and 4.1 Hz, 1H), 2.56 (dd, *J* = 16.1 and 12.2 Hz, 1H), 2.18 (s, 3H), 1.28 (d, *J* = 5.8 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 164.0 (C), 154.9 (C), 152.0 (C), 149.7 (C), 145.8 (C), 137.9 (C), 128.0 (CH), 127.5 (CH), 127.3 (CH), 124.4 (C), 124.3 (C), 122.5 (C), 74.9 (CH), 72.4 (CH_2), 69.9 (CH), 69.9 (CH_2), 60.0 (CH_3), 59.7 (CH_3), 59.6 (CH_3), 53.6 (CH), 48.4 (CH), 43.4 (CH_2), 26.9 (CH_2), 21.7 (CH_3), 9.1 (CH_3). Anal. Calcd for $C_{28}H_{36}N_2O_8$: C, 63.62; H, 6.86; N, 5.30. Found: C, 63.45; H, 6.73; N, 5.19.

(1*R,6*R**,11a*S**)-6-Benzyloxymethyl-1-hydroxy-2-tertbutyloxycarbonyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-tetrahydro-pyrazino[1,2-*b*]isoquinolin-4-one (3b)**. The reaction residue was purified by flash chromatography (6:4 hexane/EtOAc) affording 1.5 g (96% yield) of **3b** as a yellow solid: mp 54–55 °C; IR (film) *v* 3344, 2926, 1704, 1644 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.35–7.30 (m, 5H), 6.10 (dd, *J* = 7.2 and 3.4 Hz, 1H), 5.71 (br s, 1H), 4.75 (d, *J* = 12.0 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.40 (d, *J* = 17.5 Hz, 1H), 4.12 (m, 1H), 4.03 (d, *J* = 17.5, 1H), 3.84 (s, 3H), 3.80-3.70 (m, 2H), 3.79 (s, 3H), 3.67 (s, 3H), 3.00 (dd, *J* = 16.5 and 4.2 Hz, 1H), 2.65 (dd, *J* = 16.5 and 11.2 Hz, 1H), 2.20 (s, 3H), 1.51 (s, 9H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 164.6 (C), 152.2 (C), 150.0 (C), 146.0 (C), 138.0 (C), 128.3 (CH), 127.7 (CH), 124.7 (C), 124.6 (C), 122.6 (CH), 122.4 (C), 81.7 (C), 77.2 (CH), 72.7 (CH_2), 70.1 (CH_2), 60.2 (CH), 59.9 (CH_3), 53.7 (CH), 48.4 (CH), 29.6 (CH_2), 28.3 (CH_3), 9.3 (CH_3). Anal. Calcd for $C_{29}H_{38}N_2O_8$: C, 64.19; H, 7.06; N, 5.16. Found: C, 64.07; H, 6.94; N, 4.99.

4.1.6. (6*R**,11a*S**)-6-Benzyloxymethyl-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino [1,2-*b*]isoquinolin-4-one (4a)

A mixture of triethylsilane (1.93 mL, 12.1 mmol) and **3a** (459 mg, 0.78 mmol) in dry DCM (20 mL) cooled to −10 °C under argon atmosphere was treated with pre-cooled TFA (0.76 mL, 13.3 mmol) in one portion and lately was stirred at −10 °C for 15 h. Then, the reaction mixture was poured onto ice-cooled saturated aqueous $NaHCO_3$, extracted with DCM, and the extracts were washed with saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified by flash column chromatography (6:4 hexane/EtOAc) to provide 391 mg of compound **4a** (96% yield) as a yellow solid: mp 51–53 °C, IR (film) *v*, 1698, 1659 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.30–7.00 (m, 5 H), 6.01 (dd, *J* = 7.6 and 3.5 Hz, 1H), 4.88 (m, 1H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.35 (d, *J* = 12.0 Hz, 1H), 4.00–3.85 (m, 2H), 3.90 (m, 1H), 3.80–3.70 (m, 3H), 3.75 (s, 3H), 3.69 (s, 3H), 3.56 (s, 3H), 3.44 (dd, *J* = 13.9 and 4.2 Hz, 1H), 2.82 (dd, *J* = 16.8 and 4.1 Hz, 1H), 2.60 (dd, *J* = 16.8 and 11.7 Hz, 1H), 2.10 (s, 3H), 1.18 (m, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 164.6 (C), 154.7 (C), 152.1 (C), 149.8 (C), 146.0 (C), 138.1 (C), 128.2 (CH), 127.7 (CH), 127.5 (CH), 124.4 (C), 124.2 (C), 122.9 (C), 72.7 (CH_2), 70.0 (CH_2), 69.4 (CH), 60.2 (CH_3), 59.9 (CH_3), 59.7 (CH_3), 48.3 (CH), 47.9 (CH_2), 47.7 (CH), 44.0 (CH_2), 27.5 (CH_2), 22.0 (CH_3), 9.2 (CH_3). Anal. Calcd for $C_{28}H_{36}N_2O_7$: C, 65.61; H, 7.08; N, 5.47. Found: C, 65.32; H, 6.97; N, 5.35.

4.1.7. 6-Benzyloxymethyl-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-2,3,6,11-tetrahydro-pyrazino[1,2-*b*]isoquinolin-4-one (5a)

To a solution of crude product **3a** (4.35, 8.24 mmol) and triethylsilane (13.2 mL, 82.4 mmol) in dry DCM (150 mL) was added TFA (4.7 mL, 82.4 mmol) and the resulting mixture was stirred under argon atmosphere at room temperature for 3 h. The reaction mixture was quenched with 10% aqueous solution of NaOH, and extracted with DCM. The organic 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 to give a mixture of products which was separated and purified by flash column chromatography. A mixture of 6:4 hexane/EtOAc as eluant gave **4a** (2.2 g, 4.3 mmol) (50% yield) and a 7:3 hexane/EtOAc mixture was used as eluant to afford **5a** (1.2 g, 2.3 mmol) (28% yield) as an orange oil: IR (film) ν 2940, 1699, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.26 (m, 5 H), 6.31 (dd, J = 8.0 and 4.0 Hz, 1 H), 6.07 (s, 1H), 4.96 (sept, J = 6.2 Hz, 1H), 4.71 (d, J = 12.0 Hz, 1H), 4.60 (m, 1 H), 4.43 (d, J = 12.0 Hz, 1H), 4.15 (d, J = 18.4 Hz, 1H), 3.85 (m, 2H), 3.76 (s, 3H), 3.72 (s, 3H), 3.61 (s, 3H), 3.50 (dd, J = 10.7 and 8.0 Hz, 1H), 3.38 (dd, J = 10.7 and 4.0 Hz, 1H), 2.20 (s, 3H), 1.27 (d, J = 6.2 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 164.0 (C), 154.0 (C), 150.8 (C), 149.5 (C), 145.5 (C), 138.1 (C), 129.3 (C), 128.2 (CH), 127.9 (CH), 127.5 (CH), 125.2 (CH), 120.8 (C), 119.8 (C), 102.3 (CH), 72.6 (CH_2), 69.8 (CH_2), 69.6 (CH), 61.3 (CH_3), 60.6 (CH_3), 60.0 (CH_3), 48.1 (CH_2), 47.1 (CH), 29.6 (CH_2), 22.0 (CH_3), 9.2 (CH_3). Anal. Calcd for $C_{28}H_{34}N_2O_7$: C, 65.87; H, 6.71; N, 5.49. Found: C, 65.64; H, 6.56; N, 5.38.

4.1.8. (6*R**,11a*R**)-6-Benzyloxymethy-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (10a)

The equimolecular mixture of **2a** and its 11a-epimer obtained after treatment of **2a** (0.17 mmol) in dry DCM (20 mL) with DMAP (0.34 mmol) at 70 °C for 1 h. and subsequent isolation of the crude product was enriched in the 11a-epimer by column chromatography (hexane/EtOAc, 8:2). This enriched mixture (1.2 g, 2.04 mmol) was added to a stirred solution of lithium tri*tert*butoxyaluminum hydride (2.60 g, 10.2 mmol) in dry THF (70 mL) cooled in ice water and the reaction was stirred under argon atmosphere at room temperature for 16 h. After quenching by addition of ice, the solid was filtered over Celite and extracted with DCM. The crude product obtained when these extracts were washed with H_2O (20 mL) and a saturated aqueous solution of NaCl (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo was treated first with triethylsilane (5.04 mL, 31.62 mmol) in dry DCM (30 mL) cooled to 0 °C under argon atmosphere, and later with pre-cooled TFA (1.98 mL, 34.68 mmol) in one portion. The reaction mixture was stirred at −10 °C for 15 h, quenched by addition of 2 mL of 10% aqueous solution of NaOH, and extracted with DCM (3× 3 mL). The combined extracts were washed with H_2O (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a mixture which was purified by flash chromatography on silica gel with (hexane/EtOAc, 7:3) as eluant to give **10a** (76% yield) as a white solid: mp 45–46 °C; IR (film) ν, 1698, 1652 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.25–7.21 (m, 3H), 7.12–7.06 (m, 2H), 773 (ws, 1H), 4.96 (sept, J = 6.2 Hz, 1H), 4.45 (d, J = 12.0 Hz, 1H), 4.36 (d, J = 12.0 Hz, 1H), 4.33 (m, 2H), 4.07 (m, 2H), 4.06 (m, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 3.68 (s, 3H), 3.60 (m, 1H), 3.50 (m, 1H), 3.07 (dd, J = 14.5 and 3.3 Hz, 1H), 2.74 (dd, J = 14.5 and 12.2 Hz, 1H), 2.20 (s, 3H), 1.27 (d, J = 6.2 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.0 (C), 154.3 (C), 150.7 (C), 150.0 (C), 146.0 (C), 138.3 (C), 128.1 (CH), 127.3(CH), 127.2 (CH), 125.7 (C), 124.5 (C), 124.0 (C), 72.8 (CH_2), 72.5 (CH_2), 69.3 (CH), 60.8 (CH_3), 60.4 (CH_3), 60.0 (CH_3), 52.7 (CH), 50.1 (CH), 47.5 (CH_2), 46.8 (CH_2), 26.0 (CH_2), 22.2 (CH_3), 9.3 (CH_3). Anal. Calcd for $C_{28}H_{36}N_2O_7$: C, 65.61; H, 7.08; N, 5.47. Found: C, 65.48; H, 6.93; N, 5.42.

4.1.9. General procedure to obtain compounds 8a and 11a

A solution of **4a** or **10a** (2.04 mmol) in ethanol (150 mL) containing 10% palladium on carbon (120 mg) was vigorously stirred under 3.5 atm of hydrogen, at 70 °C for 16 h. The reaction mixture was filtered through Celite and the solvent evaporated under reduced pressure to give a residue.

(6*R,11a*S**)-6-Hydroxymethyl-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (8a)**. This residue was purified by flash chromatography (1:9 hexane/EtOAc) to give **8a** (844 mg, 98%) as a yellow oil: IR (film) ν, 3414, 1694, 1634 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 5.89 (dd, J = 8.9 and 3.7 Hz, 1 H), 4.94 (m, 1H), 4.50 (m, 1H), 4.09 (m, 1H), 4.05 (m, 1H), 3.96 (m, 1H), 3.93 (m, 1H), 3.88 (s, 3H), 3.77 (s, 3H), 3.72 (m, 1H), 3.65 (s, 3H), 3.58 (m, 1H), 3.10 (br s, 1H), 2.93 (dd, J = 16.7 and 4.0 Hz, 1H), 2.72 (dd, J = 16.7 and 11.8 Hz, 1H), 2.16 (s, 3H), 1.25 (d, J = 7.1 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.0 (C), 154.7 (C), 152.2 (C), 150.4 (C), 146.1 (C), 124.6 (C), 123.8 (C), 122.7(C), 69.4 (CH_2), 63.4 (CH), 60.3 (CH_3), 59.9 (CH_3), 59.7 (CH_3), 51.4 (CH), 47.8 (CH_2), 47.6 (CH), 43.9 (CH_2), 27.5 (CH_2), 22.0 (CH_3), 9.2 (CH_3). Anal. Calcd for $C_{21}H_{30}N_2O_7$: C, 59.70; H, 7.16; N, 6.63. Found: C, 59.62; H, 7.06; N, 6.55.

(6*R,11a*R**)-6-Hydroxymethyl-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2- *b*]isoquinolin-4-one (11a)**. Compound **11a** (95% yield) was purified by flash chromatography on silica gel with 3:7 hexane/EtOAc as eluant. Yellow oil: IR (film) ν 3340, 1694, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 5.76 (t, J = 5.9 Hz, 1 H), 4.93 (sept, J = 6.2 Hz, 1H), 4.37 (d, J = 18.0 Hz, 1H), 4.20 (m, 1H), 4.09 (d, J = 18.0 Hz, 1H), 3.89 (s, 3H), 3.85 (m, 1H), 3.80 (s, 3H), 3.68 (s, 3H), 3.58 (dd, J = 10.9 and 5.9 Hz, 1H), 3.47 (m, 1 H), 3.15 (dd, J = 14.9 and 2.8 Hz, 1H), 2.20 (s, 3H), 1.25 (d, J = 6.2 Hz, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 167.9 (C), 154.1 (C), 150.9 (C), 150.3 (C), 146.0 (C), 125.0 (C), 124.7 (C), 122.9 (C), 69.4 (CH), 67.3 (CH_2), 60.9 (CH_3), 60.5 (CH_3), 59.9 (CH_3), 53.5 (CH), 52.4 (CH), 47.4 (CH_2), 46.5 (CH_2), 25.7 (CH_2), 22.3 (CH_3), 9.2 (CH_3). Anal. Calcd for $C_{21}H_{30}N_2O_7$: C, 59.70; H, 7.16; N, 6.63. Found: C, 59.53; H, 6.95; N, 6.32.

4.1.10. (6*R**,11a*S**)-6-Benzyloxymethyl-7,8,10-trimethoxy-9-methyl-,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (6)

A mixture of triethylsilane (6.4 mL, 40.3 mmol) and **3 b** (1.5 g, 2.4 mmol) in dry DCM (100 mL) was cooled to −10 °C under argon atmosphere treated with pre-cooled TFA (3.4 mL, 44 mmol) in one portion, and lately was stirred at −10 °C for 15 h. Then, the reaction mixture was poured into ice-cooled saturated aqueous $NaHCO_3$, extracted with DCM, and the extracts were washed with saturated aqueous solution of NaCl, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude residue was purified by flash column chromatography (1:1 EtOAc/methanol) to provide 636 mg of compound **6** (60% yield) as an orange oil. IR (film) ν, 3321, 2939, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.30 (m, 5H), 6.15 (dd, J = 7.5 and 3.8 Hz, 1H), 4.74 (d, J = 11.9 Hz, 1H), 4.43 (d, J = 11.9 Hz, 1H), 3.85 (s, 3H), 3.83 (m, 2H), 3.83 (m, 1H), 3.78 (s, 3H), 3.67 (s, 3H), 3.56 (br s, 2H), 3.28 (dd, J = 13.2 and 4.5 Hz, 1H), 2.86 (d, J = 13.2 Hz, 1H), 2.83 (s, 1H), 2.80 (d, J = 2.2 Hz, 1H), 2.19 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 167.8 (C), 151.9 (C), 149.7 (C), 145.9 (C), 138.2 (C), 128.1 (CH), 127.7 (CH), 127.4 (CH), 124.4 (C), 124.1 (C), 123.0 (C), 72.4 (CH_2), 70.2 (CH_2), 60.1 (CH_3), 59.8 (CH_3), 59.6 (CH_3), 50.5 (CH_2), 47.8 (CH_2) 47.6 (CH), 47.5 (CH), 28.0 (CH_2), 9.2 (CH_3). Anal. Calcd for $C_{24}H_{30}N_2O_5$: C, 67.59; H, 7.09; N, 6.57. Found: C, 67.07; H, 7.17; N, 6.31.

4.1.11. Hydrolysis and decarboxylation, general procedure to obtain compounds 9, 19, and 24

A solution of the corresponding compound **8a**, **16**, or **18** (1.3 mmol) in dry trifluoroacetic acid (15 mL) and concd H_2SO_4 (1 mL) was stirred at room temperature for 16 h. The reaction mixture was poured into ice/water and extracted with ethyl acetate (3× 40 mL). The extracts were washed with a 10% aqueous solution of $NaHCO_3$, H_2O, and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a solid residue.

(6*R,11a*S**)-6-Hydroxymethyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a- hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (9)**. The residue was purified by flash chromatography (EtOAc) to give (50% yield) **9** as an orange solid: mp 82–83 °C; IR (film) *ν* 3307, 1681, 1633 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) *δ* 5.87 (dd, *J* = 9.2 and 4.1 Hz, 1H), 3.95 (dd, *J* = 11.6 and 4.1 Hz, 1H), 3.84 (m, 1H), 3.80 (s, 3H), 3.70 (s, 3H), 3.67 (m, 1H), 3.59 (s, 3H), 3.39 (m, 1H), 3.22 (m, 2H), 2.93 (m, 1H), 2.83 (dd, *J* = 16.8 and 4.5 Hz, 1 H), 2.67 (dd, *J* = 16.8 and 11.5 Hz, 1H), 2.10 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ* 169.5 (C), 152.5 (C), 150.4 (C), 146.6 (C), 125.0 (C), 124.1 (C), 122.8 (C), 63.9 (CH_2), 60.8 (CH_3), 60.4 (CH_3), 60.3 (CH_3), 51.3 (CH), 50.7 (CH_2), 48.4 (CH_2), 47.7 (CH), 28.7 (CH_2), 9.8 (CH_3). Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.70; H, 7.19; N, 8.33. Found: C, 60.62; H, 7.06; N, 8.15.

(6*R)-7,8,10-Trimethoxy-9-methyl-6-(1′-oxo-1′,3′-dihydro-2′-isoindolylmethyl)-1,2,3,6-tetrahydropyrazino[1,2-*b*]isoquinolin-4-one (19)**. The crude product was purified by flash chromatography (EtOAc/methanol 9:1) to give **19** (82% yield) as a yellow solid: mp 168–170 °C; IR (film) *ν*, 2932, 1689, 1657 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) *δ* 7.78 (d, *J* = 7.7 Hz, 1H), 7.55 (d, *J* = 7.0 Hz, 1H), 7.45 (d, *J* = 7.0 Hz, 1H), 7.44 (d, *J* = 7.0 Hz, 1H), 6.31 (dd, *J* = 9.5 and 2.8 Hz, 1H), 5.97 (s, 1H), 4.76 (d, *J* = 16.7 Hz, 1H), 4.24 (dd, *J* = 14.1 and 9.5 Hz, 1H), 4.19 (d, *J* = 16.7 Hz, 1H), 4.13 (d, *J* = 15.6 Hz, 1H), 3.96 (s, 3H), 3.83 (d, *J* = 15.6 Hz, 1H), 3.82 (s, 3H), 3.66 (s, 3H), 3.57 (d, *J* = 8.8 Hz, 1H), 3.45 (d, *J* = 8.8 Hz, 1H), 3.18 (dd, *J* = 14.1 and 2.8 Hz, 1H), 2.19 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ* 169.7, 165.2, 150.3, 149.3, 145.3, 141.7, 132.6, 131.9, 131.7, 127.9, 125.7, 123.4, 122.8, 120.5, 120.0, 99.4, 61.3, 60.8, 60.2, 50.8, 50.2, 46.9, 46.4, 9.3. Anal. Calcd for $C_{25}H_{27}N_3O_5$: C, 66.80; H, 6.05; N, 9.35. Found: C, 66.53; H, 5.88; N, 9.07.

(6*R,11*R**)-11,1′-Epoxy-7,8,10-trimethoxy-9-methyl-6-(3′-oxo-1′,3′-dihydro-2′-isoindolylmethyl)-6,11-dihydropyrazino[1,2-*b*]-isoquinolin-4-one (24)**. Purification by flash chromatography with EtOAc as eluant afforded **24** (55% yield) as a yellow solid: mp 190–192 °C; IR (film) *ν* 2942, 1694 cm^{-1}; ^1H NMR (500 MHz, C_6D_6) *δ* 8.31 (s, 1H); 7.69 (d, *J* = 7.5 Hz, 1H); 7.30 (s, 1H); 6.97 (d, *J* = 5.4 Hz, 1H); 6.96 (m, 2H); 6.95 (m, 1H); 5.03 (dd, *J* = 14.4 and 5.4 Hz, 1H); 4.56 (s, 1H); 4.01 (s, 1H); 3.79 (s, 3H); 3.48 (s, 1H); 3.08 (d, *J* = 14.4 Hz, 1H); 2.71 (s, 3H); 1.94 (s, 3H); ^{13}C NMR (125 MHz, C_6D_6) *δ* 167.8, 155.0, 152.6, 152.5, 148.0, 146.3, 143.3, 140.1, 132.0, 129.2, 127.5, 126.2, 124.4, 123.2, 120.1, 120.0, 63.3, 61.9, 61.1, 60.1, 48.9, 44.6, 42.6, 10.6. Anal. Calcd for $C_{25}H_{23}N_3O_6$: C, 65.07; H, 5.02; N, 9.11. Found: C, 64.92; H, 4.87; N, 9.07.

4.1.12. General procedure to obtain compounds 7, 20, 21, and 22

Formaldehyde (37%) solution in methanol (1.6 mL) was added to a stirred solution of **6**, **19**, or **9** (0.36 mmol) in formic acid (2 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.

(6*R,11a*S**)-6-Benzyloxymethyl-7,8,10-trimethoxy-2,9-dimethyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (7)**. The purification of the crude product by flash chromatography on silica gel column with EtOAc/methanol (9:1) as eluant afforded (79% yield) of **7** as an orange solid: mp 45-46 °C; IR (film) *ν*, 2938, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) *δ* 7.31 (m, 5H), 6.13 (dd, *J* = 7.5 and 3.9 Hz, 1H), 4.75 (d, *J* = 12 Hz, 1H), 4.45 (d, *J* = 12 Hz, 1H), 3.94 (m, 1H), 3.82 (s, 3H), 3.81 (m, 2H), 3.80 (s, 3H), 3.70 (s, 3H), 3.35 (d, *J* = 16.1 Hz, 1H), 3.01 (d, *J* = 16.1 Hz, 1H), 2.97 (dd, *J* = 16.6 and 11.1 Hz, 1H), 2.84 (dd, *J* = 16.6 and 4.7 Hz, 1H), 2.74 (ws, 2H), 2.40 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ*, 166.4, 152.1, 149.7, 146.0, 138.4, 128.2, 127.7, 127.4, 124.5, 124.1, 123.6, 72.6, 70.4, 60.3, 59.9, 59.8, 59.7, 56.8, 47.8, 47.6, 45.2, 28.7, 9.3. Anal. Calcd for $C_{24}H_{30}N_2O_5$: C, 68.16; H, 7.32; N, 6.36. Found: C, 67.78; H, 7.37; N, 6.18.

(6*R)-7,8,10-Trimethoxy-2,9-dimethyl-6-(1′-oxo-1′, 3′-dihydro-2′-isoindolylmethyl)-1,2,3,6-tetrahydropyrazino[1,2-*b*]isoquinolin-4-one (20)**. The purification by flash chromatography on silica gel column with EtOAc/methanol (95:5) as eluant afforded (92% yield) **20** as a white solid: mp 160–162 °C; IR (film) *ν*, 2942, 1682, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) *δ* 7.79 (d, *J* = 7.8 Hz, 1 H), 7.53 (dd, *J* = 14.3 and 7.6 Hz, 1H), 7.50–7,35 (m, 2H), 6.22 (dd, *J* = 9.5 and 2.8 Hz, 1H), 6.16 (s, 1H), 4.80 (d, *J* = 16.6 Hz, 1H), 4.25 (dd, *J* = 11.3 and 9.5 Hz, 1H), 4.22 (d, *J* = 16.6 Hz, 1H), 3.97 (s, 3H), 3.94 (d, *J* = 10.6 Hz, 1H), 3.82 (s, 3H), 3.74 (d, *J* = 10.6 Hz, 1H), 3.68 (s, 3H), 3.58 (d, *J* = 16.7 Hz, 1H), 3.26 (d, *J* = 16.7 Hz, 1H), 3.19 (dd, *J* = 11.3 and 2.8 Hz, 1H), 2.58 (s, 3H), 2.20 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ*, 171.1, 169.3, 151.0, 149.9, 145.1, 141.7, 131.9, 131.5, 127.8, 125.7, 123.3, 122.8, 120.7, 119.3, 61.4, 60.8, 60.1, 56.9, 54.2, 50.6, 46.7, 44.6, 43.1, 9.2. Anal. Calcd for $C_{26}H_{29}N_3O_5$: C, 67.37; H, 6.31; N, 9.07. Found: C, 67.09; H, 6.18; N, 8.95.

(6*R,11a*S**)-6-Hydroxymethyl-7,8,10-trimethoxy-2,9-dimethyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (21)**. The mixture obtained was purified by flash chromatography on silica gel with (EtOAc/methanol, 9:1) as eluant to afford **21** (21% yield) together with the O-formylated compound **22** (38% yield). Orange solid: mp 85–86 °C; IR (film) *ν* 3374, 2491, 1682 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) *δ* 5.86 (dd, *J* = 8.8 and 4.0 Hz, 1H), 4.09 (dd, *J* = 15.0 and 4.0 Hz, 1H), 3.91 (s, 3H), 3.89 (m, 1H), 3.81 (s, 3H), 3.76 (m, 1H), 3.60 (s, 3H), 3.34 (d, *J* = 16.3 Hz, 1H), 3.06 (d, *J* = 16.3 Hz, 1H), 2.93 (m, 2H), 2.85 (dd, *J* = 12.1 and 5.1 Hz, 1H), 2.74 (dd, *J* = 12.1 and 2.3 Hz, 1H), 2.28 (s, 3H), 2.11 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ* 167.8 (C), 151.9 (C), 150.5 (C), 146.1 (C), 124.5 (C), 123.8 (C), 123.1 (C), 64.4 (CH_2), 60.8 (CH_3), 60.4 (CH_3), 60.3 (CH_3), 60.0 (CH_2), 57.4 (CH_2), 51.0 (CH), 47.6 (CH), 45.1 (CH_3), 28.7 (CH_2), 9.3 (CH_3). Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99. Found: C, 61.62; H, 7.34; N, 7.68.

(6*R,11a*S**)-6-Formyloxymethyl-7,8,10-trimethoxy-2,9-dimethyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (22)**. Flash chromatography on silica gel column with (EtOAc/methanol, 9:1) as eluant afforded (38% yield) **22** as an orange oil; IR (film) *ν* 1726, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) *δ* 7.90 (s, 1H), 5.95 (dd, *J* = 9.3 and 3.4 Hz, 1H), 4.47 (dd, *J* = 11.4 and 9.3 Hz, 1H), 4.27 (dd, *J* = 11.4 and 3.4 Hz, 1H), 3.76 (s, 3H), 3.70 (m, 1 H), 3.63 (s, 3H), 3.52 (s, 3H), 3.20 (dd, *J* = 16.3 and 1.2 Hz, 1H), 2.82 (m, 1H), 2.77 (m, 1H), 2.72 (m, 1H), 2.59 (m, 2H), 2.18 (s, 3H), 2.06 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) *δ* 167.2 (C), 161.3 (C), 152.6 (C), 150.3 (C), 146.6 (C), 125.4 (C), 124.2 (C), 123.1 (C), 63.3 (CH_2), 60.8 (CH_3), 60.4 (CH_3), 60.3 (CH_3), 60.0 (CH_2), 57.0 (CH_2), 48.2 (CH), 48.1 (CH), 45.7 (CH_3), 29.0 (CH_2), 9.8 (CH_3). Anal. Calcd for $C_{19}H_{26}N_2O_6$: C, 60.30; H, 6.93; N, 7.40. Found: C, 60.22; H, 6.81; N, 7.25.

4.1.13. 7,8,10-Trimethoxy-9-methyl-6-phthalimidomethyl-2,3-dihydro-6*H*-pyrazino[1,2-*b*]isoquinoline-1,4-dione (13)

A solution of compound **12**[11] (1 g, 1.9 mmol) in dry concd trifluoroacetic acid (19 mL) and concd H_2SO_4 (1 mL) was stirred at room temperature for 16 h. The reaction mixture was poured into ice/

water and extracted with ethyl acetate (3× 200 mL). The extracts were washed with a 10% aqueous solution of $NaHCO_3$, H_2O, and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a pure compound **13** (93% yield) as a yellow oil; IR (film) ν, 3295, 1772, 1715, 1682 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.70 (m, 2H), 7.60 (m, 2H), 6.98 (s, 1H), 6.25 (dd, J = 7.2 and 3.8 Hz, 1H), 3.93 (m, 2H), 3.87 (s, 3H), 3.75 (m, 2H), 3.66 (s, 3H), 3.50 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 168.1, 162.8, 160.6, 153.1, 151.5, 145.9, 134.0, 131.8, 131.7, 126.2, 125.7, 123.5, 123.3, 123.2, 121.3, 118.3, 110.0, 62.4, 61.2, 60.5, 48.4, 45.7, 40.2, 9.8. Anal. Calcd for $C_{25}H_{23}N_3O_7$: C, 62.89; H, 4.86; N, 8.80. Found: C, 62.57; H, 4.55; N, 8.49.

4.1.14. Synthesis of compounds 16 and 17

To a stirred solution of lithium tri*tert*butoxyaluminum hydride (2.65 g, 10.4 mmol) in dry THF (40 mL) cooled in ice water was added **14** (845 mg, 1.49 mmol), and the mixture was stirred under argon atmosphere at room temperature for 16 h. The reaction mixture was quenched by addition of ice, filtered over Celite and extracted with ethyl acetate. The extracts were washed with H_2O (20 mL) and with a saturated aqueous solution of NaCl (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give nearly quantitative **15** as a mixture of diastereoisomers. A mixture of triethylsilane (12 mL, 74.5 mmol) and the crude product **15** (848 mg, 1.49 mmol) in dry DCM (30 mL) cooled to −30 °C under argon atmosphere was treated with pre-cooled TFA (5.7 mL, 74.5 mmol) in one portion and lately was stirred at −30 °C for 3 h. Then, the reaction mixture was quenched by addition of 2 mL of 10% aqueous solution of NaOH and extracted with DCM (3× 30 mL). The combined extracts were washed with H_2O (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a mixture which was purified by flash chromatography on silica gel with (hexane/EtOAc, 1:1) as eluant to give **16** (58% yield) and **17** (26% yield).

2-Isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-6-(1′-oxo-1′,3′-dihydro-2′-isoindolylmethyl)-1,2,3,6-tetrahydropyrazino[1,2-*b*]isoquinolin-4-one (16). White solid: mp 89–90 °C; IR (film) ν, 1694, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.55 (d, J = 7.3 Hz, 1H), 7.34–7.17 (m, 3H), 6.00 (dd, J = 9.8 and 3.1 Hz, 1H), 5.9o (ws, 1H), 4.72 (m, 1H), 4.67 (sept, J = 6.1 Hz, 1H), 4.64 (d, J = 16.5 Hz, 1H), 4.31 (m, 1H), 4.23 (m, 1H), 4.08 (dd, J = 14.0 and 10.0 Hz, 1H), 4.01 (d, J = 16.5 Hz, 1H), 3.73 (s, 3H), 3.56 (s, 3H), 3.48 (s, 3H), 3.44 (m, 1H), 2.92 (d, J = 14.0 Hz, 1H), 0.98 (m, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.2, 164.2, 153.8, 150.8, 149.8, 145.2, 141.7, 132.0, 131.4, 129.9, 127.7, 125.6, 123.4, 122.7, 120.8, 119.9, 102.0, 69.4, 61.3, 60.8, 60.1, 50.5, 47.9, 46.5, 44.4, 44.3, 22.1, 9.2. Anal. Calcd for $C_{29}H_{33}N_3O_7$: C, 65.03; H, 6.21; N, 7.85. Found: C, 64.92; H, 6.04; N, 7.67.

(6*R,11a*S**)-2-Isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-6-(1′-oxo-1′,3′-dihydro-2′-isoindolylmethyl)-1,2,3,6,11,11a- hexahydropyrazino[1,2-*b*]isoquinolin-4-one (17)**. White solid: mp 107-109 °C; IR (film) ν, 1694, 1652 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.80 (d, J = 7.5 Hz, 1H), 7.55 (d, J = 7.4 Hz, 1H), 7.49 (d, J = 7.4 Hz, 1H), 7.45 (dd, J = 7.5 and 7.4 Hz, 1H), 6.06 (dd, J = 11.2 and 3.5 Hz, 1H), 5.02 (d, J = 16.4 Hz, 1H), 4.94 (m, 1H), 4.38 (m, 1H), 4.37 (d, J = 16.4 Hz, 1H), 4.27 (s, 1H), 4.26 (m, 2H), 3.98 (s, 3H), 3.83 (s, 3H), 3.68 (s, 3H), 3.61 (dd, J = 14.2 and 3.7 Hz, 1H), 3.47 (m, 2H), 2.96 (d, J = 16.5 Hz, 1H), 2.74 (dd, J = 16.5 and 12.4 Hz, 1H), 2.22 (s, 3H), 1.24 (m, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 169.1, 164.9, 154.8, 152.7, 150.0, 145.9, 141.9, 132.1, 131.4, 127.8, 124.9, 124.6, 123.4, 122.9, 69.3, 60.5, 60.0, 59.8, 50.3, 48.0, 47.7, 47.6, 44.1, 43.5, 27.6, 22.1, 9.3. Anal. Calcd for $C_{29}H_{35}N_3O_7$: C, 64.79; H, 6.56; N, 7.82. Found: C, 64.45; H, 6.23; N, 7.48.

4.1.15. (6*R**,11R*)-11,1′-Epoxy-2-isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-6-(3′-oxo-1′,3′-dihydro-2′-isoindolyl methyl)-2,3,6,11-tetrahydropyrazino[1,2-*b*]isoquinolin-4-one (18)

A mixture of triethylsilane (1.18 mL, 7.4 mmol) and the crude product **15** (421 mg, 0.74 mmol) in dry DCM (20 mL) at room temperature under argon atmosphere was treated with TFA (0.57 mL, 7.4 mmol) in one portion and lately was stirred at room temperature for 3 h. Then, the reaction mixture was quenched by addition of 2 mL of 10% aqueous solution of NaOH and extracted with DCM (3× 30 mL). The combined extracts were washed with H_2O (20 mL) and brine (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give a solid residue. The crude product was purified by flash chromatography (hexane/EtOAc, 3:7) as eluant to give **18** (88% yield) as a mixture of rotamers; white solid: mp 118–120 °C; IR (film) ν, 1694 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) (5 °C) δ 7.80–7.60 (m, 3H), 7.41 (m, 1H), 6.70 (s, 0.6H), 6.54 (s, 0.4H), 6.39 (d, J = 2.0 Hz, 1H), 5.00 (m, 1H), 4.84 (m, 0.5H), 4.82 (m, 0.5H), 4.73 (m, 1H), 4.49 (s, 0.5H), 4.48 (s, 0.5H), 4.30–4.20 (m, 2H), 3.85 (s, 3H), 3.76 (s, 3H), 3.38 (t, J = 6.2 Hz, 1H), 2.80 (s, 1.5H), 2.69 (s, 1.5H), 1.96 (s, 1.5H), 1.94 (s, 1.5H), 1.28 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) (5 °C) δ 169.2, 161.9, 153.1, 152.6, 150.3, 146.2, 142.7, 131.9, 128.7, 126.0, 125.4, 123.7, 123.6, 122.7, 122.3, 120.9, 104.8, 104.4, 70.5, 66.3, 61.5, 60.9, 60.1, 47.1, 46.5, 46.0, 41.7, 41.5, 22.0, 10.2. Anal. Calcd for $C_{29}H_{31}N_3O_8$: C, 63.38; H, 5.69; N, 7.65. Found: C, 63.15; H, 5.33; N, 7.48.

4.1.16. (6*R**,11a*S**)-2-Isopropyloxycarbonyl-9-methyl-7,8,10-trimethoxy-6- phthalimidomethyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (25)

A mixture of compound **8a** (100 mg, 0.41 mmol) and phthalimide (60.3 mg, 0.41 mmol) was azeotropically dried under refluxing in dry toluene (2× 10 mL) and then was dissolved in anhydrous THF (6.2 mL). Triphenylphosphine (131 mg, 0.5 mmol) and diethyl azodicarboxylate (31 mL, 0.45 mmol) were added and this mixture was stirred at 23 °C for 2 h. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (1:2 hexane/EtOAc) to provide 98 mg of compound **25** (64% yield) as a white solid: mp 188–190 °C; IR (film) ν 1772, 1715, 1652, 1650 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.78 (m, 2H), 7.67 (m, 2H), 5.99 (dd, J = 10.9 and 3.4 Hz, 1H), 4.85 (m, 1H), 4.32 (dd, J = 13.8 and 3.4 Hz, 1H), 4.30 (m, 1H), 4.22 (m, 1H), 4.00 (m, 1H), 3.96 (s, 3H), 3.87 (m, 1H), 3.77 (s, 3H), 3.59 (s, 3H), 3.50 (m, 1H), 3.42 (d, J = 18.1 Hz, 1H), 2.89 (dd, J = 16.7 and 2.9 Hz, 1H), 2.65 (dd, J = 16.7 and 12.2 Hz, 1H), 2.12 (s, 3H), 1.18 (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 168.5 (C), 165.6 (C), 154.4 (C), 152.4 (C), 150.3 (C), 146.6 (C), 133.9(CH), 132.0 (C), 124.1 (C), 123.2 (CH), 123.2 (C), 69.4 (CH), 60.5 (CH_3), 60.1 (CH_3), 59.9 (CH_3), 48.9 (CH), 47.8 (CH), 47.6 (CH_2), 44.0 (CH_2), 38.7 (CH_2), 27.6 (CH_2), 22.5 (CH_3), 9.4 (CH_3). Anal. Calcd for $C_{29}H_{33}N_3O_8$: C, 63.15; H, 6.03; N, 7.62. Found: C, 63.12; H, 5.96; N, 7.47.

4.1.17. General procedure to obtain *O*-acylderivatives 26–29

A 0.1 M solution of **8a** (0.4 mmol) in dry DCM, EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.81 mmol), DIMAP (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 dissolved 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.

(6*R,11a*S**)-2-Isopropyloxycarbonyl-6((1*H*-indole-2-carbonyloxyl)methyl)-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (26)**. The residue was purified by flash column chromatography on silica gel with hexane/ethyl acetate (3:7) as eluant yielding **26** (76% yield) as yellow

solid: mp 150–152 °C; IR (film) ν 3304, 1704, 1651 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 9.20 (br s, 1H), 7.60 (d, J = 6.7 Hz, 1H), 7.36 (d, J = 6.9 Hz, 1H), 7.25 (m, 1H), 7.19 (s, 1H), 7.06 (m, 1H), 6.13 (dd, J = 8.4 and 3.3 Hz, 1H), 4.84 (m, 1H), 4.71 (dd, J = 9.4 and 8.4 Hz, 1H), 4.52 (dd, J = 9.4 and 3.3 Hz, 1H), 4.35 (d, J = 15.0 Hz, 1H), 4.05 (m, 1H), 3.86 (s, 3H), 3.72 (s, 3H), 3.63 (d, J = 15.0 Hz, 1H), 3.58 (s, 3H), 3.40 (m, 2H), 2.90 (dd, J = 13.9 and 3.2 Hz, 1H), 2.65 (dd, J = 13.9 and 10.0 Hz, 1H), 2.12 (s, 3H), 1.18 (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 165.6 (C), 161.3 (C), 154.7 (C), 152.4 (C), 150.2 (C), 146.3 (C), 137.1(C), 127.4 (C), 126.8 (C), 125.4 (CH), 125.3 (C), 123.0 (C), 122.5 (CH), 122.4 (C), 120.7 (CH), 112.1 (CH), 109.5 (CH), 69.6 (CH), 63.1 (CH_2), 60.5 (CH_3), 60.0 (CH_3), 59.8 (CH_3), 49.0 (CH), 48.0 (CH_2), 47.8 (CH), 44.0 (CH_2), 27.7 (CH_2), 22.5 (CH_3), 9.3 (CH_3). Anal. Calcd for $C_{30}H_{35}N_3O_8$: C, 63.70; H, 6.24; N, 7.43. Found: C, 63.62; H, 6.16; N, 7.35.

(6*R,11a*S**)-2-Isopropyloxycarbonyl-6-(1-naphthyloxymethyl)-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (27)**. Compound **27** was purified by flash chromatography on silica gel with (1:1 hexane/EtOAc) as eluant to give a white solid (63% yield): mp 110–112 °C; IR (film) ν 1700, 1659, 1510 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 8.58 (d, J = 8.2 Hz, 1 H), 7.98 (dd, J = 7.3 and 1.2 Hz, 1H), 7.82 (d, J = 4.9 Hz, 1H), 7.68 (dd, J = 9.4 and 1.7 Hz, 1H), 7.45–7.25 (m, 3H), 6.08 (dd, J = 9.8 and 3.6 Hz, 1H), 4.81 (dd, J = 11.5 and 9.8 Hz, 1H), 4.70 (m, 1H), 4.39 (dd, J = 11.5 and 3.6 Hz, 1H), 4.21 (m, 1H), 3.95 (m, 1H), 3.87 (m, 1H), 3.76 (s, 3H), 3.60 (s, 3H), 3.52 (m, 1H), 3.45 (s, 3H), 3.23 (m, 1H), 2.81 (m, 1H), 2.54 (dd, J = 16.6 and 12.0 Hz, 1H), 2.54 (s, 3H), 1.05 (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 767.3 (C), 165.0 (C), 154.7 (C), 152.4 (C), 150.1 (C), 146.3 (C), 133.7 (CH), 133.4 (C), 131.2 (CH), 130.6 (C), 128.5 (CH), 127.7 (CH), 126.6 (C), 126.1 (CH), 125.5 (CH), 125.2 (C), 124.9 (CH), 123.3 (C), 122.9 (C), 69.4 (CH), 63.1 (CH_2), 60.6 (CH_3), 60.0 (CH_3), 59.8 (CH_3), 48.8 (CH), 47.9 (CH), 47.8 (CH_2), 43.9 (CH_2), 27.6 (CH_2), 22.0 (CH_3), 9.3 (CH_3). Anal. Calcd for $C_{32}H_{36}N_2O_8$: C, 66.65; H, 6.29; N, 4.86. Found: C, 66.52; H, 6.26; N, 4.75.

(6*R,11a*S**)-2-Isopropyloxycarbonyl-7,8,10-trimethoxy-9-methyl-6-(4-vinyl-benzyloxymethyl)-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (28)**. Compound **28** was purified by flash chromatography on silica gel with (1:1 hexane/EtOAc) as eluant to give a white solid (85% yield): mp 122–124 °C; IR (film) ν 2250, 1715, 1652, 1608 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.98 (d, J = 8.2 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 6.76 (dd, J = 17.6 and 10.9 Hz, 1H), 6.23 (dd, J = 9.7 and 3.7 Hz, 1H), 5.87 (d, J = 17.6 Hz, 1H), 5.39 (d, J = 10.9 Hz, 1H), 5.00 (s, 1 H), 4.82 (dd, J = 11.3 and 9.7 Hz, 1 H), 4.56 (dd, J = 11.3 and 3.7 Hz, 1H), 4.42 (m, 1H), 4.12 (m, 1H), 4.02 (m, 1H), 3.95 (s, 3H), 3.81 (s, 3H), 3.71 (m, 1H), 3.67 (s, 3H), 3.45 (dd, J = 13.2 and 2.9 Hz, 1H), 2.96 (d, J = 16.6 Hz, 1H), 2.72 (dd, J = 16.6 and 12.0 Hz, 1H), 2.20 (s, 3H), 1.23 (s, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.3 (C), 164.9 (C), 154.6 (C), 152.4 (C), 150.1 (C), 146.3 (C), 142.1 (C), 135.9 (CH), 130.0 (CH), 128.9 (C), 126.2 (CH), 125.1 (C), 123.1 (C), 122.8 (C), 116.5 (CH_2), 69.4 (CH), 63.1 (CH_2), 60.4 (CH_3), 60.0 (CH_3), 59.8 (CH_3), 48.7 (CH), 47.7 (CH), 47.7 (CH_2), 44.4 (CH_2), 27.6 (CH_2), 22.0 (CH_3), 9.3 (CH_3). Anal. Calcd for $C_{30}H_{36}N_2O_8$: C, 65.20; H, 6.57; N, 5.07. Found: C, 65.12; H, 6.46; N, 5.05.

(6*R,11a*S**)-2-Isopropyloxycarbonyl-6-*trans*-cinnamoylmethyl-7,8,10-trimethoxy-9-methyl-1,2,3,6,11,11a-hexahydro-pyrazino[1,2-*b*]isoquinolin-4-one (29)**. Compound **29** was purified by flash chromatography on silica gel with (1:1 hexane/EtOAc) as eluant to give a yellow solid (90% yield): mp 62–64 °C; IR (film) ν 704, 1660 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.72 (d, J = 16.0 Hz, 1 H), 7.60–7.50 (m, 2H), 7.48–7.40 (m, 3H), 6.48 (d, J = 16.0 Hz, 1H), 6.16 (dd, J = 10.0 and 3.7 Hz, 1H), 4.97 (m, 1H), 4.79 (dd, J = 11.5 and 10.0 Hz, 1H), 4.53 (m, 1H), 4.45 (dd, J = 11.5 and 3.7 Hz, 1H), 4.22 (m, 1H), 4.04 (m, 1H), 3.96 (s, 3H), 3.83 (s, 3H), 3.77 (m, 1H), 3.68 (s, 3H), 3.50 (m, 1H), 2.98 (dd, J = 16.6 and 3.2 Hz, 1H), 2.74 (dd, J = 16.6 and 12.0 Hz, 1H), 2.23 (s, 3H), 1.27 (m, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 166.9 (C), 165.1 (C), 154.9 (C), 152.4 (C), 150.1 (C), 146.3 (C), 145.5 (CH), 134.2 (C), 130.4 (CH), 128.9 (CH), 128.2 (CH), 125.1 (C), 123.3 (C), 122.9 (C), 117.5 (CH), 65.5 (CH), 62.7 (CH_2), 60.5 (CH_3), 60.0 (CH_3), 59.8 (CH_3), 48.9 (CH), 47.8 (CH), 47.7 (CH_2), 43.7 (CH_2), 27.6 (CH_2), 22.1 (CH_3), 9.4 (CH_3). Anal. Calcd for $C_{30}H_{36}N_2O_8$: C, 65.20; H, 6.57; N, 5.07. Found: C, 65.12; H, 6.46; N, 5.05.

4.1.18. (6*S**,7*R**,9*R**,14*R**,15*R**)-14.1′-Epoxi-3,12,16-trimethyl-1,2,4,10,11,13- hexamethoxy-9(3′-cyano-1′,3′-dihydro-2′-isoin-dolylmethyl)-6,7,9,14,14a,15-hexahydro-5*H*-6,15-iminoisoquino[3,2-*b*]-3-benzazocin-7-carbonitrile (31)

A solution of **30**[42] (0.03 mmol) in dry THF (1 mL) was added dropwise to a solution of $LiAlH_2(OEt)_2$ (0.6 mmol), and the mixture was stirred for 45 min. at room temperature. Then, AcOH (37 mL, 0.6 mmol) and a 4.8 M solution of KCN in H_2O (0.6 mL, 2.9 mmol) were added. After stirring at room temperature for 15 h, the reaction mixture was quenched with aqueous 10% $NaHCO_3$ (12 mL) and extracted with ethyl acetate (3× 10 mL). The combined organic layers were washed with H_2O and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel column with hexane/ethyl acetate (1:9) as eluant to give **31** (30% yield) as a white solid: mp 155 °C; IR (film) ν 1935, 1465, 1408, 1068 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 7.18 (dd, J = 7.1 and 7.0 Hz, 1H), 7.00 (dd, J = 7.5 and 7.2 Hz, 1H), 6.90 (d, J = 7.2 Hz, 1H), 4.26 (d, J = 3.8 Hz, 1H), 4.18 (d, J = 1.9 Hz, 1H), 4.06 (d, J = 0.8 Hz, 1H), 4.01 (d, J = 2.1 Hz, 1H), 3.92 (s, 3H), 3.91 (s, 3H), 3.78 (s, 3H), 3.72 (s, 3H), 3.49 (d, J = 12.9 Hz, 1H), 3.35 (d, J = 18.2 Hz, 1H), 3.27 (m, 2H), 3.23 (s, 3H), 3.08 (s, 3H), 3.01 (dd, J = 11.2 and 3.8 Hz, 1H), 3.01(s, 3H), 2.67 (d, J = 11.2 Hz, 1H), 2.52 (d, J = 18.2 Hz, 1H), 2.22 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 151.7 (C), 150.2 (C), 149.5 (C), 149.2 (C), 147.6 (C), 145.0 (C), 144.3 (C), 140.1 (C), 129.9 (C), 126.1 (C), 125.9 (CH), 125.8 (CH), 125.0 (C), 124.0 (C), 123.7 (C), 123.5 (CH), 123.3 (CH), 121.4 (C), 118.7 (C), 118.0 (C), 63.8 (CH), 62.4 (CH), 62.3 (CH), 61.3 (CH_3), 60.9 (CH_3), 60.3 (CH_3), 60.1 (CH_3), 60.0 (CH_3), 59.9 (CH_3), 58.1 (CH), 57.5 (CH), 57.4 (CH), 56.3 (CH_2), 55.6 (CH), 41.7 (CH), 41.5 (CH_3), 22.4 (CH_2), 9.8 (CH_3), 9.3 (CH_3). Anal. Calcd for $C_{39}H_{43}N_5O_7$: C, 67.52; H, 6.25; N, 10.09. Found: C, 67.45; H, 6.16; N, 9.97.

4.2. Biological materials and methods

4.2.1. Cytotoxicity determinations on MDA-MB 231, A-549, and HT-29 cell lines

Cells were placed in 96-well microtiter plates at a density of 5×10^3/well and incubated for 24 h. After that, they 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. 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 is measured spectrophotometrically. Tumor 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.

4.2.2. Western blotting and antibodies

A549 cells were lysed as previously described,[50] and 20 μg of protein lysate was resolved in 10% SDS–PAGE. Antibodies used were anti p-P53 Ser15 (Cell Signaling, Charlottesville, USA), Phospho-Histone H2A.XSer139 (Cell Signaling, Charlottesville, USA), Phosho-Chk1/2 Antibody Sampler Kit (Cell Signaling, Charlottesville, USA), p-JNK, pP38, pAKT, and pERK (Cell Signaling, Charlottesville, USA).

4.2.3. Flow cytometry

A549 cells were treated with CDDP (2.5 μg/mL) and compounds **3a**, **29**, and **31** (50 μg/ml) for 24 h. Adherent and non-adherent cells were harvested and fixed in 70% ethanol in phosphate-buffered saline (PBS) overnight. For DNA content analysis, the cells were pelleted and re-suspended in PBS containing 1 μg/mL RNase (Qiagen Ltd, Crawley, UK) and 25 μg/mL propidium iodide, incubated at room temperature for 30 min. and then analyzed using a Beckton Dickinson Flow Cytometer (Cowley, UK). Data were plotted by using CellQuest software; 10,000 events were analyzed for each sample. All experiments were repeated at least three times.

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