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Design and synthesis of spirotryprostatin-inspired diketopiperazine systems from prolyl spirooxoindolethiazolidine derivatives

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ABSTRACT

Based on the spirotryprostatin-A structure, we designed, synthesized, and evaluated different series of compounds belonging to the diketopiperazine structural class as potential cell cycle modulators and cytotoxic agents. Starting from the spirooxoindolthiazolidine scaffold, amide coupling with Pro derivatives and intramolecular cyclization reactions are suitable synthetic methods to generate chemically diverse diketopiperazine system, such as hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,2-*d*]pyrazine-5,10-dione (structure **I**), hexahydropyrrolo[1,2-*a*] [1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione (structure **II**) and spiroindol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione (structure **III**). Some of these compounds, especially those who belong to the series **I** and **II**, showed interesting cytotoxic activity.

1. Introduction

The progress in understanding the molecular mechanisms of the mammalian cell cycle and its involvement in cancer development has shown that cell cycle regulators have a huge prospective both as molecular probes into the process as well as potential antitumor agents.[1] Small molecule natural products have demonstrated to be invaluable tools in the discovery and characterization of critical events for the progression and the regulation of the cell cycle.[2] Therefore, the development of new and specific inhibitors of signal transduction cascade pathways will continue to be extremely important in the knowledge of the regulatory mechanism of the cell cycle.

In this context, the isolation of the spirotryprostatins A and B (Fig. 1) from the fermentation broth of *Aspergillus fumigates*[3] and the discovery of their activity as cell cycle inhibitors has challenged numerous investigators to develop concise total syntheses and analogues with superior biological activity.[4–9]

Spirotryprostatins A and B cause G2/M phase cell cycle arrest in tsFT210 cell at IC_{50}s of 197.5 and 14.0 μM, respectively According to the therapeutic potential of this class of compounds and as part of a wide program centred on the development and individuation of new modulators of cell cycle, we have focused our attention on the Spirotryprostatin A core as inspiration of biologically useful diketopiperazine analogues.[10] Thus, our initial design implicated the modification of the spirocyclic structure replacing the pyrrolidine nucleus with a thiazolidine moiety and maintaining unaltered both the oxoindole and the indolizidindione fragments (Scheme 1).

Previous work in our group on the synthesis of quinone-based cytotoxic agents, had confirmed that the incorporation of an indolizidindione moiety is an effective approach to devise new compounds with potential antitumor activity.[11]

In this paper, we report a full account of our efforts towards the synthesis of a new series of diketopiperazine and spirooxoindolethiazolidine derivatives and the preliminary results of their biological activity.

2. Results and discussion

Our approach to the synthesis of the target spiroindol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione derivatives involved the coupling of the spirooxoindolethiazolidine scaffold, obtained from isatin derivatives and cysteine, with the *N*-Boc-Pro residue, followed by *N*-Boc deprotection and intramolecular cyclization (Scheme 2).

The application of this synthetic strategy conduced to the oxoindole ring opening and to the formation of two new tricyclic systems: the hexahydropyrrolo[1,2-*a*][1,3] thiazolo[3,2-*d*] pyrazine-5,10-dione (structure **I**) and the hexahydropyrrolo[1,2-*a*][1,3]thiazol-

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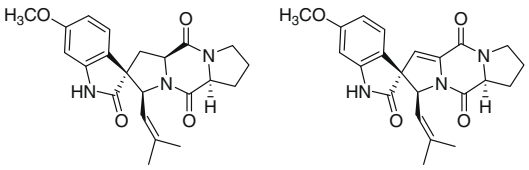
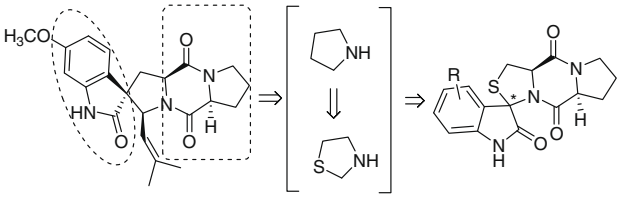


Figure 1. Structure of spirotryprostatin A and B.



Scheme 1. Design of spirooxoindolethiazolidine derivatives.

o[3,4-*d*]pyrazine-5,10-dione (structure **II**). As shown in Scheme 3, the spirooxoindolethiazolidine skeletons (**5–8**) were constructed through microwave assisted condensation between the isatin derivatives (**1–4**), and cysteine ethyl ester in MeOH under argon.[12] These derivatives were obtained with 80–90% yields, as (3*R*)/(3*S*) epimeric mixtures ranged from 60/40 to 40/60 ratios. Reaction with Boc-Pro, using DIC as coupling agent led to diasteroisomeric mixtures **9a,b–11a,b** (75–80%, **a/b**: 3/2–2/3 range) and **12a,b** (31%, **a/b**: 3/2) which were not separated in this step. Removal of the *N*-Boc protecting group of the mixture **9a,b**, or **10a,b**, or **11a,b,** using TFA in DCM, yielded the 3-ethoxycarbonyl-10a-phenyl(substituted)-hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,2-*d*]pyrazine-5,10-dione derivatives (**19–21,** 23–45% yields) as pure compounds and the 3-ethoxycarbonyl-3-phenyl(substituted)hexahydropyrrolo[1,2-*a*][1,3]thiazolo-[3,4-*d*] pyrazine-5,10-dione derivatives (**22a,b** and **24a,b** 28–33%) as diastereoisomeric mixtures (**a/b**: ~1:3 ratio), while the derivative **23a** was obtained in 41% yield as pure diastereoisomer (Table 1, entries 1, 2, and 3). Under the same conditions, the derivatives **12a**,**b** led to a complex and untreatable mixture of reaction (entry 4).

Assignments of the ^{1}H and ^{13}C NMR resonances and of the stereochemistry in final compounds were made by analysis of 2D NMR data, including COSY, HSQC, HMBC and NOESY. In the case of the regioisomers **19–21** (**I**), the stereochemistry was established on the basis of X-ray diffraction studies of compound **21** which indicated 3*R*,5a*S*,10a*S* configurations at the three stereogenic centres, as depicted in the ORTEP diagram (Fig. 2).[13]

This result showed that the stereomutation at the stereogenic centres C-3 and C-5a did not occur during the cyclization process. The stereochemical assignments for compounds **19** and **20**, were established by comparison of their ^{1}H and ^{13}C NMR spectra with those of **21** (see Section 4).

Unfortunately, we could not obtain good crystals for the analysis of any regioisomers **22–24** (**II**). The determination of the configuration at the stereogenic centres was performed by an NMR study of representative derivatives **24a** an and **24b.** Considering stereogenic centres C-5a and C-10a, we assumed that they maintain their configuration 5a*S*, 10a*R* after cyclization. NOE enhacement between H-5a and H-10a, indicating their *cis* orientation, is in accordance with this hypothesis since the inversion of both centres seems unlikely (Fig. 3). In the case of the stereogenic centre C-3, a NOE enhancement was observed between H-6′ and H-10a of **24a**, while the same enhancement was very weak in **24b**. Inspections of molecular models obtained by molecular dynamics (MD) simulations showed that the distance between H-6′ and H-10a was about 4.6 Å in the 3*S*, 5a*S*, 10a*R* isomer and about 3.5 Å in the 3*R*, 5a*S*, 10a*R* isomer. Hence, the 3*R* configuration was assigned to the **24a** isomer The stereochemical assignments for compounds **22a**, **23a**, and **22b** were made by comparison of their ^{1}H and ^{13}C NMR spectra with those of **24a** and **24b** (see Section 4).

According to these data, the formation of derivatives **I** and **II** implicates an unexpected oxoindole ring opening. To the best of our knowledge, no previous examples of this reaction performed under the above described conditions were reported.

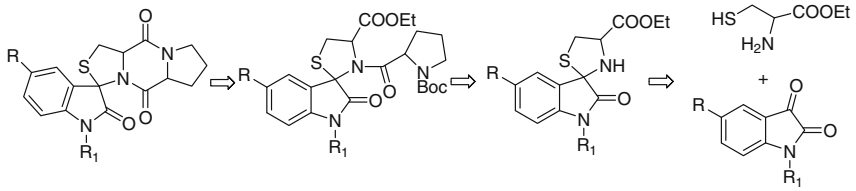
Dillman and Cardellina[14] described the isolation and characterization of an unusual sulfur-containing diketopiperazine, from extracts of the Bermudian sponge *Tedania Ignis*, analogue to our hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione (structure **II**). On the contrary, neither the isolation nor the synthesis of the hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,2-*d*]pyrazine-5,10-dione (structure **I**) were reported.

In order to define the factors that could determinate the reaction course we performed studies on the influence of solvent, time, and pH in the formation of these new derivatives and on their Concerning the solvent, the use of CH_3CN (entry 5) led to best results and the corresponding **I** and **II** structures were obtained in an overall yield higher than 85%, while in a less polar aprotic solvent as THF (entry 6) the yield was drastically lower (38%). An increase of reaction time, up to 24 h, produced a decrease in the **I** and **II** yields and a reversion to the starting spirooxoindolethiazolidine **5** (entry 7). After 120 h, this reversion was nearly complete (entry 8). The use of 1:3 2 N HCl_{aq}: MeOH solution for 24 h, led to deprotected derivatives **16**, **17** and **18** (entries 9, 11, 12).

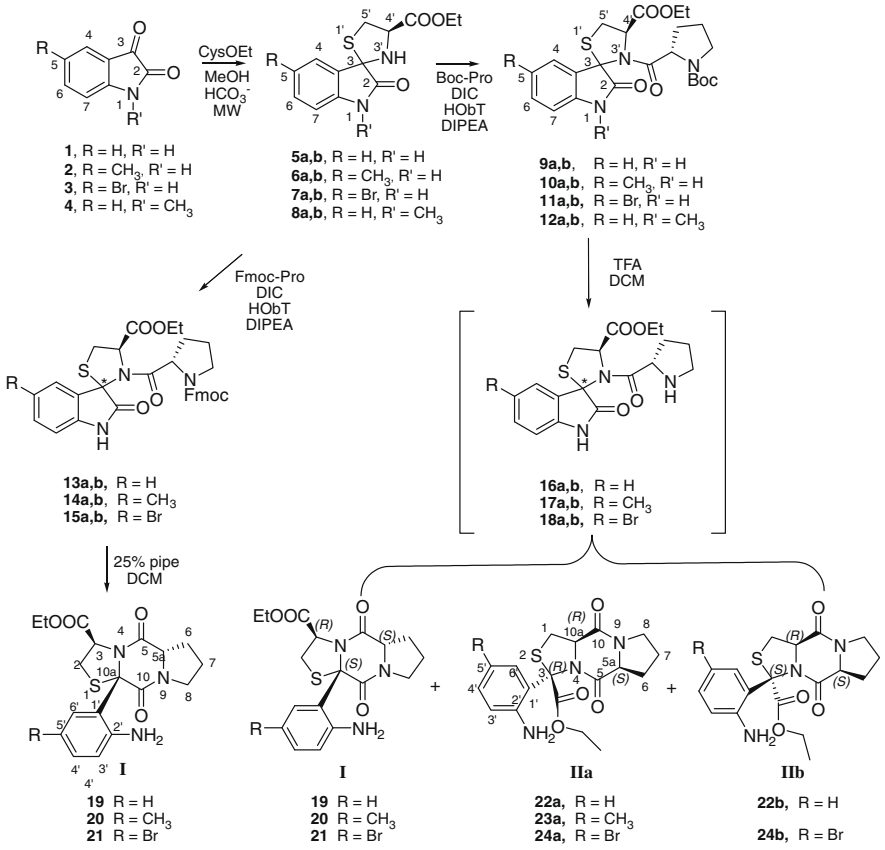
An increase of reaction time (entry 10) determined the formation of unidentifiable materials, recovering a 21% of starting isatin **1**. It is interesting to note that an augment of the proportion of 2 N HCl_{aq} up to 1:1 in MeOH solution favoured, in all cases, the reversion to starting spirooxoindolethiazolidine (entry 13). As alternative route, the Fmoc derivatives **13 a,b**, **14 a,b** and **15 a,b** (Scheme 3) were subjected to the treatment with piperidine (25%) in methylene chloride for 1 h. Under these conditions only the derivates **19**, **20** and **21** were obtained as simple regioisomers in 80%, 78%, and 81% yields, respectively (entries 14–16).

Regarding the stability, the treatment of derivatives **19–21** with 2 N HCl_{aq}/methanol solution for 24 h did not determine structural modifications and the products were recovered unchanged (Scheme 4).

The same acid treatment on the pure derivatives **22a–24a**, **22b**, and **24b**, led to oxoindole ring closing and formation of the corresponding spiro derivatives **19a–21a**, **19b**, and **21b** (**III**), that is, the

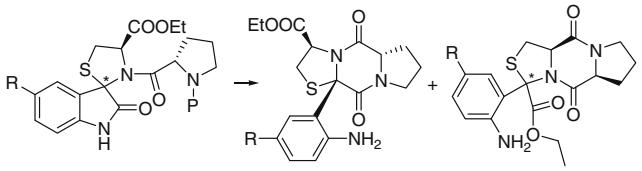


Scheme 2. Retrosynthetic route to spirooxoindolethiazolidine derivatives.



Scheme 3. Reagents: Synthesis of 3-ethoxycarbonyl-10a-phenyl(substituted)-hexahydropyrrolo[1,2-*a*][1,3] thiazolo[3,2-*d*]pyrazine-5,10-dione (structure **I**) and the 3-ethoxycarbonyl-3-phenyl(substituted)-hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione (structures **II**) derivatives.

Table 1
Results of the acid or base promoted intramolecular cyclization of 3-prolylspirooxo indolthiazolidine derivatives



N	Starting	R, R_1	Solvent	Acid/base	*T* (h)	**I** (%)	**IIa** (%)	**IIb** (%)	Other
1	**9**	H	CH_2Cl_2	TFA	2	**19** (40)	**22a** (9)	**22b** (24)	
2	**10**	CH_3	CH_2Cl_2	TFA	2	**20** (41)	**23a** (23)		
3	**11**	Br	CH_2Cl_2	TFA	2	**21** (45)	**24a** (8)	**24b** (20)	
4	**12**	H,CH_3	CH_2Cl_2	TFA	2	—		—	—
5	**9**	H	CH_3CN	TFA	2	**19** (50)	**22a** (10)	**22b** (25) [a]	
6	**9**	H	THF	TFA	12	**19** (20)	**22a** (5)	**22b** (12)[a]	
7	**9**	H	CH_3CN	TFA	24	**19** (16)	—	**22b** (22)	**5** (20)[a]
8	**9**	H	CH_3CN	TFA	120	—		—	**5** (80)[a]
9	**9**	H	MeOH	HCl[b]	24				**16** (30)[a]
10	**9**	H	MeOH	HCl[b]	120				**1** (21)
11	**10**	H	MeOH	HCl[b]	24				**17** (37)
12	**11**	H	MeOH	HCl[b]	24				**18** (31)
13	**9**	H	MeOH	HCl[c]	24				**5** (65)[a]
14	**13**	H	CH_2Cl_2	Piperidine[d]	1	**19** (80)			
15	**14**	CH_3	CH_2Cl_2	Piperidine[d]	1	**20** (78)			
16	**15**	Br	CH_2Cl_2	Piperidine[d]	1	**21** (81)			

[a] Similar results were observed from derivatives **10** and **11**.
[b] 3:1 MeOH/2 N HCl solution.
[c] 1:1 MeOH/2 N HCl solution.
[d] 25% solution.

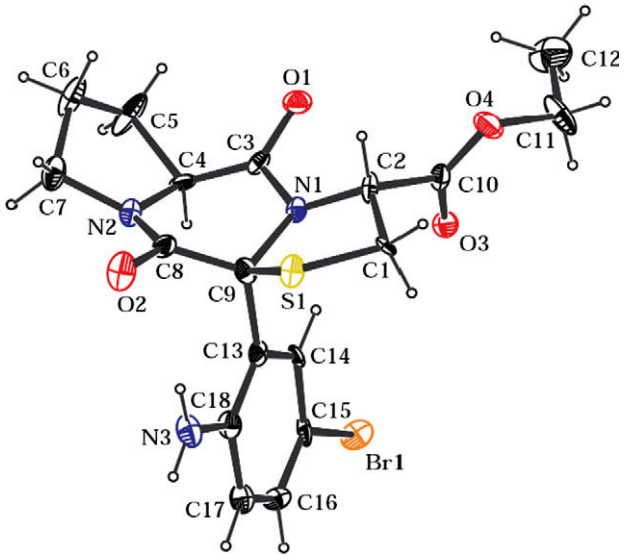


Figure 2. ORTEP representation of the structure of isomer **21**.

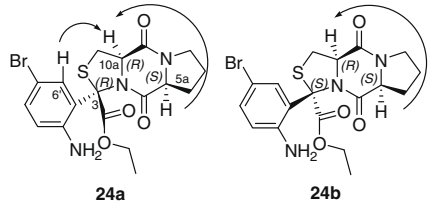
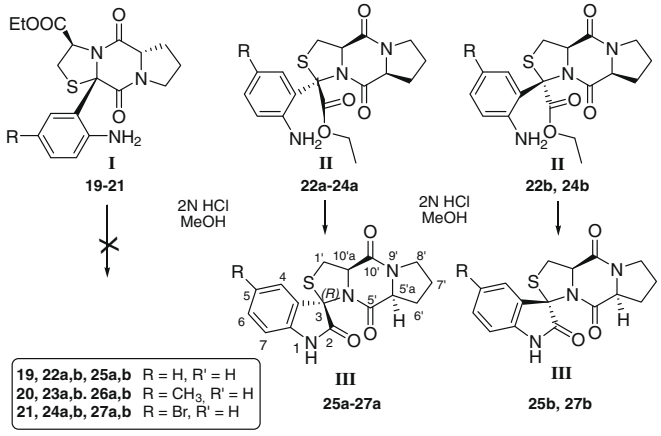


Figure 3. Significant NOEs for derivatives **24**.



Scheme 4. Reactivity of **I** and **II** derivatives in acid medium.

pentacyclic derivatives designed as analogues of spirotryprostatin A, in quantitative yields. This lactamization did not arise when the reaction was carried out in basic media.

The acid treatment on the pure derivatives **16a–18a**, **16b**, and **18b**, led to oxoindole ring closing and formation of the corresponding spiro derivatives **19a–21a**, **19b**, and **21b** (**III**), that is, the pentacyclic derivatives designed as analogues of spirotryprostatin A, in quantitative yields. This lactamization did not arise when the reaction was carried out in basic media of triethylamine/methanol at reflux. After 10 h under basic conditions, we observed a partial degradation of all derivatives.

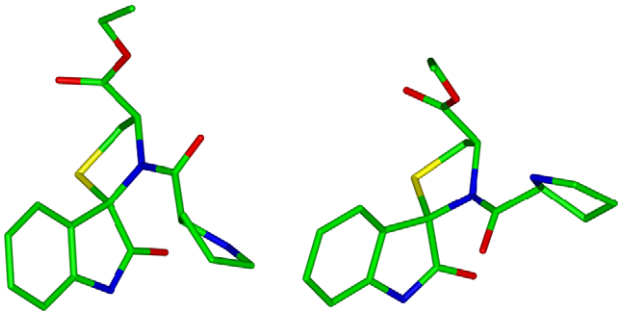


Figure 4. Lowest-energy conformers of compound **16b** containing a *trans* (left) and *cis* (right) amide bond.

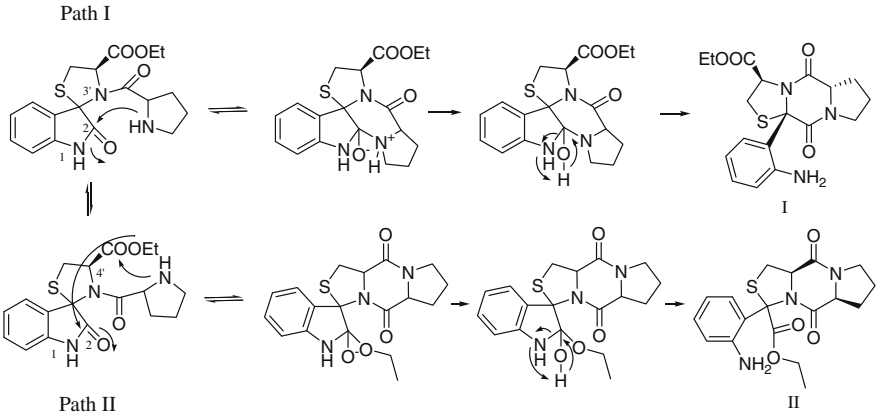
According to these findings, we hypothesized that the formation of the regioisomeric diketopiperazines (**I** and **II**) could be explained by a mechanism that involved *trans* (*E*) and *cis* (*Z*) arrangement of the amide bond of deprotected intermediates **16–18**.

A theoretical conformational analysis of **9a** (3*R*), and **9b** (3*S*) in their deprotected and deprotonated state (intermediates **16a** and **16b**) was carried out in order to examine possible low-energy structures. Amide bond was considered both in the *E* and Z configurations thus obtaining four different isomers (3*R*–*E*, 3*R*–*Z*, 3*S*–*E*, 3*S*–*Z*). Starting from randomly generated structures, minimization and a 2 ns MD simulations at a constant temperature of 500 K were run. The distances between the proline amino group and the carboxyl functions at C-2 (oxoindole carbonyl) and at C-4′ (ethoxycarbonyl) were monitored along the complete 2 ns MD trajectory. From these distances, the two possible orientations assumed by the amide bond prompted the system towards two different intramolecular cyclization pathways (Fig. 4). *E* isomers are suitable for the C-2 closure (Scheme 5, path I) while *Z* isomers are suitable for the C-4′ closure (Scheme 5, path II).

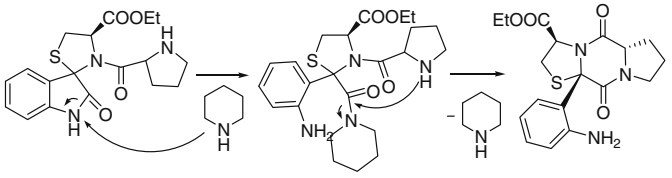
Following the hypothesized reaction mechanisms shown in Scheme 4 (path I), the amino group of proline showing an *E* disposition of the amide bond, attacks at the C-2 carbonyl of the oxoindole giving a pentacyclic intermediate which evolves to structure **I**. We calculated the molecular energies of the two intermediates with 3*R* and 3*S* configuration, and found that the 3*S* intermediate energy was about 8 kcal/mol lower than that observed for 3*R*. Evoking the Evans–Polanyi principle,[15] this means that the reaction of the 3*S* compounds through the path I is kinetically favoured compared to the 3*R* ones. Since starting compounds **9–11** quickly interconvert at the C-3 chiral centre, previous result can explain the stereospecific course of the reaction.

On the other hand, the *Z* geometry of the amide bond allows the attack of the amino group at C-4′ carbonyl ester (path II). In the reaction conditions, the oxoindole C-2 undergoes a nucleophilic attack from the generated ethoxide residue or from other nucleophilic agents present in solution (data not shown). Again, the tetrahedrical C-2 intermediate reverts to a more stable carbonyl form with ring opening and formation of the regioisomers **II**. In this case, stereogenic centre C-3 does not influence the intermediates energy.

The C-5 substituent effects are in accordance with reported mechanism. In fact, while the yield ratio of the C-5′ unsubstituted derivatives **19** with respect to **22** is 40/33, an electron-donating substituent (CH_3) which should reduce the C-2 reactivity, decreases the yield ratio of compounds **20** versus **23** to 23/41, and an electron-withdrawing substituent (Br) which should enhance the C-2 reactivity, increases the yield ratio of compounds **21** versus **24** to 45/28. In addition, the lack of reactivity observed in the *N*-methyl derivatives, **12a,b**, may be attributed to the electron-donating effect of the methyl group which prevents the nucleo-



Scheme 5. Possible reaction pathways for the formation of structures **I** and **II**.



Scheme 6. Hypothesized mechanism for the formation of the structure **I** in basic media.

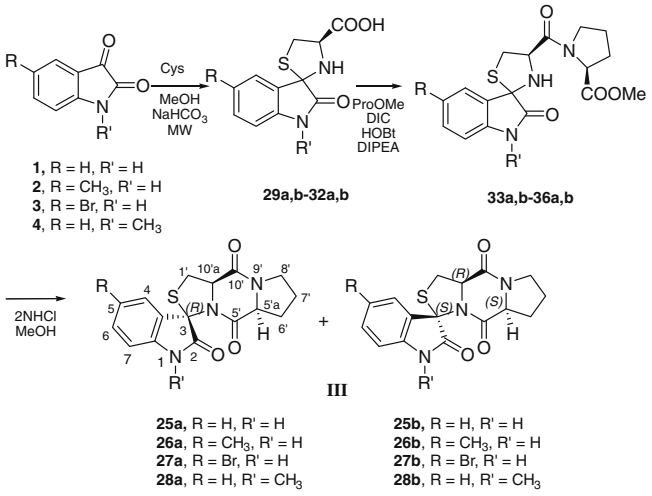
philic attack at the C-2 carbonyl group. Finally, the higher reactivity of the amino group in weak acid solution could be explained if we considered that an increase of the acidity (HCl vs TFA) should result in a shift toward the unreactive protonated form of the amine group.[16]

In basic media, the exclusive and highly efficient formation of structure **I** implies the oxoindole ring opening probably due to nucleophilic attack of piperidine at the C-2 carbonyl and successive transamidation by attack of Pro amino group (Scheme 6).

This unusual but not unexpected oxoindole ring opening in piperidine media could be considered analogous to well described aspartimide ring-opening in the peptide chemistry.[17] In that case, the nucleophilic attack of piperidine at aspartimide led to ring opening and formation of corresponding α- and β-piperidides.[18]

The route indicated in Scheme 7, was applied to the synthesis of the spiroindol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]-thiazolo[3,4-*d*]pyrazine-5,10-dione derivatives (structure **III**). The spirooxoindolethiazolidine acid derivatives **29a,b**–**32a,b**, synthesized from condensation between the corresponding isatin derivatives (**1–4**) and cysteine with 60–70% yields, were chosen as starting material. Coupling with Pro-OMe, using DIC as coupling agent, under high dilution condition, led to 1/1 diasteroisomeric mixtures of **33a**,**b–36a,b** (~70% yields), which were not separated in this step. The intramolecular lactamization of these compounds using (1/4) 2 N HCl_{aq}/MeOH solution gave directly the corresponding spiroindol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione derivatives (**25a,b**–**28a,b**) in ca 90% yields. This synthetic route did not afford any by-products due to the oxoindole ring opening

NMR analysis of the resulting crude products showed the presence of diastereoisomers **a/b** in 3/2 to 1/1 ratios. The diastereoisomer **25b** was isolated by precipitation. The diastereoisomeric mixtures **26a**,**b**, and **27a**,**b** were chromatographically separated while the mixture **28a,b** could not be separated. The physicochemical properties and purity of the final compounds were assessed by TLC, LC–MS, analytical RP-HPLC, and NMR analysis. The determination of the relative configuration at the stereogenic centres was



Scheme 7. Reagents: Synthesis of spiroindol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo-[1,2-*a*][1,3]thiazolo [3,4-*d*]pyrazine-5,10-dione derivatives (**25–28**, structure **III**).

performed by a 2D NMR study of isolate **26a**, **26b**, **27a** and **27b** derivatives. The main difference observed in the 2D NOESY spectra of derivatives **'a'** compared to **'b'** was the presence in the first of a NOE enhancement between H-4 and H-10′a (Fig. 5). Again, we hypothesized a configuration retention at C-5′a and C-10′a. Inspections of molecular models obtained by MD simulations showed that the distance between H-4 and H-10′a was about 2.9 Å in the 3*R*,5′a*S*,10′a*R* isomer and about 4.9 Å in the 3*S*, 5′a*S*,10′a*R* isomer. Hence, the 3*R* configuration was assigned to the **'a'** isomers.

All compounds **19–21 (I)**, **22a,b–24a,b (II)**, and **25a,b–28a,b (III)** were evaluated as cytotoxic agents against three human colon carcinoma (MCF-7, T47D, and A-431) cell lines. Interestingly, tricyclic derivatives **19**, **22a, 23a** and **24a** resulted active against MCF-7 cell growth with IC_{50} values of 9.1, 14.8, 1.6, and 2.2 μM, respectively. Compound **20** inhibited A431 cell growth at concentration

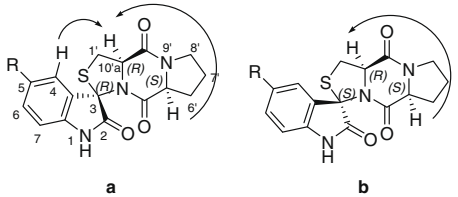


Figure 5. Significant NOEs for derivatives **a** and **b**.

28.2 μM. None of compounds belonging to series **III** showed significant cytotoxicity at concentrations below 10^{-4} M towards the tested cell lines. Unfortunately, none of the cytotoxic derivatives showed ability to inhibit the cell cycle of MCF-7 cell line.

3. Conclusions

The results herein described show the wide potentiality of the acid and base promoted intramolecular cyclization of 3′-prolylthiazolidinspirooxoindole derivatives to new tricyclic systems: hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,2-*d*]pyrazine-5,10-dione (structure **I**) and hexahydropyrrolo [1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione (structure **II**). Possible reaction pathways for the formation of these structures imply unusual oxoindole ring opening. In contrast, the acid promoted intramolecular cyclization of isomer 3′-prolylspirooxoindolelthiazolidine leads to new spirotryprostatin A-inspired derivatives spiroindol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-d]pyrazine-5,10-dione (structure **III**).[10,19] Application of the methodology developed in this work represents a new possibility for molecular diversification of oxoindole systems and for the development of other biologically useful members of the diketopiperazine structural class. Among the hexahydropyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione derivatives (structure **II**), the compounds **23a** and **24a** showed remarkable cytotoxic activity against MCF-7 cell lines at micromolar concentration. Moreover, they did not inhibit the cellular cycle, indicating that their mechanism of action did not include a interference with the cell cycle regulation systems. These compounds may become a promising class of cytotoxic agents and further experiments, aimed at defining the target and the mechanisms of the growth-inhibitory effect shown by some of these molecules are currently underway.

4. Experimental

4.1. General

Reagents and solvents were purchased from commercial suppliers and used as received. Analytical TLC was performed on plates coated with a 0.25 mm layer of Silica Gel 60 F254 Merck and preparative TLC on 20 × 20 cm glass plates coated with a 0.5 mm layer of silica gel PF254 Merck. Silica Gel 60 (300–400 mesh, Merck) was used for flash chromatography. Melting points are uncorrected. Optical rotations were determined in a 10 cm cell. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively. Chemical shifts are reported in δ values (ppm) relative to internal Me_4Si and *J* values are reported in hertz. Mass spectra were measured by the ES method. Elemental analyses were carried out with C, H-analyzer. DMEM, foetal bovine serum, glutamine, penicillin, streptomycin, Hepes, sodium pyruvate and PBS were from BioWhittaker (Caravaggio, BG, Italy). MTT was purchased from SIGMA (Milan, Italy).

4.2. General procedure for the synthesis of spirooxoindolethiazolidine ethyl ester derivatives (5a,b–8a,b) and spirooxoindolethiazolidine carboxylic acid (29a,b–32a,b) derivatives

The reactions were carried out in a Milestone CombiChem Microwave Synthesizer. All irradiation process, rotation of the rotor, irradiation time, temperature, and power were monitored with the 'easyWAVE' software package. Temperatures were monitored with the aid of an optical fiber inserted into one of the reaction vessels. $NaHCO_3$ (10 mmol) and the corresponding isatin derivatives (**1–4**, 12 mmol) were added to a solution of L-Cys-OEt or L-Cys-OH (10 mmol) in methanol (100 mL) and the suspension was irradiated at 300 W until 65 °C was reached. The reaction mixture was held at this temperature for 45 min and then cooled rapidly to room temperature. Then the suspensions were filtered and the filtrates were concentrated. At this step, the carboxylic acid derivatives (**29–32**) were purified by flash chromatography using EtOAc as eluent system, while the corresponding ethyl ester residues (**5–8**) were dissolved in CH_2Cl_2 and washed with water (3 × 50 mL). The combined organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. These compounds were used in the next reaction without further purification as diastereoisomeric mixtures.

4.2.1. (3*R*,4′*R*) and (3*S*,4′*R*) ethyl 2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylate (5a,b)

Oil (86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (6H, t, C*H*$_3$), 3.30–3.33 and 3.35–3.41 (2H, m, H-5'a), 3.62–3.67 and 3.91–3.95 (2H, m, H-5'b), 4.27 (4H, q, C*H*$_2$CH$_3$), 4.48–4.51 and 4.68–4.72 (2H, m, H-4′), 6.81 (2H, d, *J* = 8.0 Hz, H-4), 7.11 (2H, t, H-5), 7.34 (2H, t, H-6), 7.48 (2H, d, *J* = 8.0, H-7), 8.43 (2H, s, N*H*). ES-MS *m/z*: Calcd for $C_{13}H_{14}N_2O_3S$: 278.07. Found: 278.13.

4.2.2. (3*R*,4′*R*) and (3*S*,4′*R*) ethyl 5-methyl-2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylate (6a,b)

Oil (81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (6H, t, C*H*$_3$), 2.21 (6H, s, C*H*$_3$), 3.27–3.31 and 3.38–3.42 (2H, m, H-5'a), 3.65–3.68 and 3.89–3.91 (2H, m, H-5'b), 4.25 (4H, q, C*H*$_2$CH$_3$), 4.43–4.45 and 4.61–4.64 (2H, m, H-4′), 6.77 (2H, d, *J* = 8.0 Hz, H-7), 7.38 (2H, d, *J* = 8.0 Hz, H-6), 7.45 (2H, s, H-4), 8.70 (2H, s, N*H*). ES-MS *m/z*: Calcd for $C_{14}H_{16}N_2O_3S$: 292.09. Found: 292.13.

4.2.3. (3*R*,4′*R*) and (3*S*,4′*R*) ethyl 5-bromo-2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylate (7a,b)

Oil (89%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (6H, t, C*H*$_3$), 3.29–3.34 and 3.41–3.46 (2H, m, H-5'a), 3.70–3.75 and 3.92–3.94 (2H, m, H-5'b), 4.25 (4H, q, C*H*$_2$CH$_3$), 4.46–4.49 and 4.65–4.68 (2H, m, H-4′), 6.75 (2H, d, *J* = 8.0 Hz, H-7), 7.39 (2H, d, H-6), 7.46 (2H, s, H-4), 7.78 (2H, s, N*H*). ES-MS *m/z*: Calcd for $C_{13}H_{13}BrN_2O_3S$: 355.98. Found: 356.03.

4.2.4. (3*R*,4′*R*) and (3*S*,4′*R*) ethyl 1-methyl-2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylate (8a,b)

Oil (83%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (6H, t, CH$_2$C*H*$_3$), 3.22 (6H, s, C*H*$_3$), 3.39–3.41 and 3.45–3.49 (2H, m, H-5'a), 3.81–3.85 and 3.94–3.97 (2H, m, H-5'b), 4.24 (4H, q, C*H*$_2$CH$_3$), 4.48–4.50 and 4.69–4.72 (2H, m, H-4′), 6.82 (2H, d, *J* = 8.0 Hz, H-7), 7.11 (2H, t, H-6), 7.33 (2H, d, *J* = 8.0 Hz, H-4), 7.48 (2H, t, 5H). ES-MS *m/z*: Calcd for $C_{14}H_{16}N_2O_3S$: 292.09. Found: 292.13.

4.2.5. (3*R*,4′*R*) and (3*S*,4′*R*) 2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylic acid (29a,b)

Oil (68%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.24–3.28 and 3.37–3.42 (2H, m, H-5'a), 3.63–3.65 and 3.82–3.84 (2H, m, H-5'b), 4.26–4.28 and 4.44–4.47 (2H, m, H-4′), 6.81 (1H, d, *J* = 8.0 Hz, H-4), 7.11 (1H, t, H-5), 7.34 (1H, t, H-6), 7.48 (1H, d, *J* = 8.0 Hz, 7-H), 8.43 (1H, s, N*H*). ES-MS *m/z*: Calcd for $C_{11}H_{10}N_2O_3S$: 250.04. Found: 250.09.

4.2.6. (3*R*,4′*R*) and (3*S*,4′*R*) 5-methyl-2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylic acid (30a,b)

Oil (61%); ^{1}H NMR (400 MHz, CD_3OD) δ 2.24 (6H, s, C*H*$_3$); 3.26–3.29 and 3.34–3.38 (2H, m, H-5'a), 3.65–3.68 and 3.81–3.85 (2H, m, H-5'b), 4.28–4.30 and 4.45–4.49 (2H, m, H-4′), 6.79 (2H, d, *J* = 8.0 Hz, H-7), 7.36 (2H, d, H-6), 7.48 (2H, s, H-4), 8.83 (2H, s, N*H*). ES-MS *m/z*: Calcd for $C_{12}H_{12}N_2O_3S$: 264.06. Found: 264.13.

4.2.7. (3*R*,4′*R*) and (3*S*,4′*R*) 5-bromo-2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylic acid (31a,b)

Oil (64%); ^{1}H NMR (400 MHz, CD_3OD) δ 3.23–3.25 and 3.38–3.41 (2H, m, H-5'a), 3.64–3.67 and 3.82–3.85 (2H, m, H-5'b), 4.26–4.28

and 4.48–4.50 (2H, m, H-4′), 6.78 (2H, d, J = 8.0 Hz, H-7), 7.15 (2H, d, H-6), 7.33 (2H, s, H-4), 8.49 (2H, s, N*H*). ES-MS *m/z*: Calcd for $C_{11}H_9BrN_2O_3S$: 329.17. Found: 329.26.

4.2.8. (3*R*,4′*R*) and (3*S*,4′*R*) 1-methyl-2-oxospiro[indoline-3,2′-thiazolidine]-4′-carboxylic acid (32a,b)

Oil (66%); ^{1}H NMR (400 MHz, CD_3OD) δ 3.18 (6H, s, CH_3); 3.26–3.28 and 3.37–3.40 (2H, m, H-5'a), 3.68–3.71 and 3.84–3.86 (2H, m, H-5'b), 4.23–4.26 and 4.45–4.47 (2H, m, H-4′), 6.86 (2H, d, J = 8.0 Hz, H-7), 7.18 (2H, t, H-6), 7.41 (2H, d, J = 8.0 Hz, H-4), 7.49 (2H, t, H-5). ES-MS *m/z*: Calcd for $C_{12}H_{12}N_2O_3S$: 264.06. Found: 264.14.

4.3. General procedure for the synthesis of 3-ethyl-10a-(substituted)phenyl-5,10-dioxooctahydro-5*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,2-*d*]pyrazine-3-carboxylate (19–21) and 3-ethyl-3-(substituted)phenyl-5,10-dioxohexahydro-5*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-3-carboxylate (22a–b, 23a, 24a–b)

To a solution of the corresponding spirooxoindolthiazolidine ethyl esters (**5a,b–8a,b** 3 mmol) in dichloromethane (25 mL), *N*-Boc-Pro (1.1 equiv), DIC (1.2 equiv), HOBt (1.2 equiv), and DIPEA (2.4 equiv) were successively added. Stirring was continued at room temperature for 4 h. Afterward, the reaction mixture was diluted with dichloromethane (20 mL), and the resulting solution was washed successively with 10% citric acid (2 × 25 mL), 10% $NaHCO_3$ (2 × 25 mL), and water (2 × 25 mL), dried over Na_2SO_4, and evaporated to dryness. Flash chromatography of the residues, using different eluent systems, yielded, in each case, the correspondent 3′-(*N*-Boc)prolyl derivatives as diastereoisomeric mixture (**9a,b–12a,b),** which were not separated in this step.

A solution of derivatives **9a,b** or **10a,b** or **11a,b** or **12a,b** (1 mmol) in CH_2Cl_2 (10 mL), was treated with trifluoroacetic acid (5 mL) and stirred at room temperature. After 2 h, TEA was added until pH 7 and the resulting mixtures were washed with water (3 × 25 mL), dried over Na_2SO_4, evaporated to dryness, and purified and separated by flash column chromatography using different eluent systems to yield the title compounds.

4.3.1. (3*R*,5a*S*,10a*S*) 3-Ethyloxycarbonyl-10a-(2′-amino)-phenyl-5,10-dioxooctahydro-5*H*-pyrrolo [1,2-*a*][1,3] thiazolo[3,2-*d*]pyrazine (19)

FC in ethyl acetate/*n*-hexane 3/2. White solid (40%); mp 140–141 °C; $[\alpha]_D^{25}$ −30.1 (*c* 0.1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (3H, t, CH_2CH_3), 1.81–1.86 (1H, m, H-7$_a$), 1.96–2.04 (1H, m, H-7$_b$), 2.21–2.29 (2H, m, H-6), 2.96 (1H, t, H-2$_a$), 3.24 (1H, dd, J = 6.4 and 12.4 Hz, H-2$_b$), 3.42–3.47 (1H, m, H-8$_a$), 3.59–3.66 (1H, m, H-8$_b$), 4.12 (1H, t, H-5a), 4.27 (1H, q, CH_2CH_3), 4.31 (1H, q, CH_2CH_3), 4.83 (1H, dd, J = 6.0 and 10.4 Hz, H-3), 6.70 (1H, d, J = 7.2 Hz, H-3′), 6.75 (1H, t, H-5′), 7.16 (1H, t, H-4′), 8.02 (1H, d, J = 7.2 Hz, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$), 23.4 (C-6), 27.6 (C-7), 31.8 (C-2), 46.9 (C-8), 59.3 (C-5a), 62.2 (*C*H$_2$), 65.4 (C-3), 82.5 (C-10a), 117.1 (C-4′), 118.0 (C-6′), 127.7 (C-5′), 128.5 (C-1′), 130.4 (C-3′), 145.7 (C-2′), 164.3, 166.7, 168.8 (C=O). Anal. Calcd for $C_{18}H_{21}N_3O_4S$: C, 57.58; H, 5.64; N, 11.19; S, 8.54. Found: C, 57.41; H, 5.59; N, 11.22; S, 8.61.

4.3.2. (3*R*,5a*S*,10a*S*) 3-Ethyloxycarbonyl-10a-(2′-amino-5′-methyl)-phenyl-5,10-dioxooctahydro-5*H*-pyrrole[1,2-*a*][1,3]thiazole[3,2-*d*]-pyrazine (20)

FC in ethyl acetate/*n*-hexane 3/2. White solid (41%); mp 159–161 °C; $[\alpha]_D^{25}$ −24.9 (*c* 0.12 in $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, CH_2CH_3), 1.78–1.85 (1H, m, H-7$_a$), 1.95–2.01 (1H, m, H-7$_b$), 2.23–2.28 (2H, m, H-6), 2.27 (3H, s, CH_3-5′), 2.97 (1H, t, H-2$_a$), 3.24 (1H, dd, J = 6.4 and 12.4 Hz, H-2$_b$), 3.42–3.48 (1H, m, H-8$_a$), 3.60–3.67 (1H, m, H-8$_b$), 4.08 (1H, t, H-5a), 4.25 (1H, q, CH_2 CH_3), 4.33 (1H, q, CH_2 CH_3), 4.82 (1H, dd, J = 6.0 and 10.4 Hz, H-3), 6.63 (1H, d, J = 8.0 Hz, H-3′), 6.98 (1H, d, H-4′), 7.84 (1H, s, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$), 23.6 (C-6), 25.3(*C*H$_3$), 27.9 (C-7), 31.5 (C-2), 46.7 (C-8), 59.5 (C-5a), 62.4 (*C*H$_2$), 65.8 (C-3), 81.8 (C-10a), 120.2 (C-3′), 122.4 (C-1′), 125.6 (C-4′), 128.3 (C-5′), 131.1 (C-6′), 144.1 (C-2′), 164.2, 166.5, 168.9 (C=O). Anal. Calcd for $C_{19}H_{23}N_3O_4S$: C, 58.59; H, 5.95; N, 10.79; S, 8.23. Found C, 58.32; H, 5.51; N, 10.90; S, 8.35.

4.3.3. (3*R*,5a*S*,10a*S*) 3-Ethyloxycarbonyl-10a-(2′-amino-5′-bromo)-phenyl-5,10-dioxooctahydro-5*H*-pyrrole[1,2-*a*][1,3]thiazole[3,2-d]-pyrazine (21)

FC in dichloromethane/acetone 95/5. White solid (45%); mp 195–196 °C; $[\alpha]_D^{25}$ −27.1 (*c* 0.1 in $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ 1.34 (t, 3H, CH_2CH_3), 1.81–1.88 (1H, m, H-7$_a$), 1.98–2.04 (1H, m, H-7$_b$), 2.26–2.30 (2H, m, H-6), 2.97 (1H, t, H-2$_a$), 3.26 (1H, dd, J = 6.0 and 11.6 Hz, H-2$_b$), 3.45–3.49 (1H, m, H-8$_a$), 3.59–3.66 (1H, m, H-8$_b$), 4.14 (1H, t, H-5a), 4.22 (1H, q, CH_2 CH_3); 4.33 (1H, q, CH_2 CH_3), 4.83 (1H, dd, J = 6.0 and 10.8 Hz, H-3), 6.58 (1H, d, J = 8.4 Hz, H-3′), 7.24 (1H, d, H-4′), 8.16 (1H, s, H-6′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4 (*C*H$_3$), 23.3 (C-6), 27.6 (C-7), 31.7 (C-2), 46.9 (C-8), 59.4 (C-5a), 62.4 (*C*H$_2$), 65.6 (C-3), 81.9 (C-10a), 116.1 (C-5′), 119.5 (C-3′), 122.7 (C-1′), 129.8 (C-4′), 133.1 (C-6′), 145.1 (C-2′), 164.1, 166.6, 168.5(C=O). Anal. Calcd for $C_{18}H_{20}BrN_3O_4S$: C, 47.58; H, 4.44; Br, 17.59; N, 9.25; S, 7.06. Found: C, 47.41; H, 4.2; Br, 17.71; N, 9.12; S, 7.24.

4.3.4. (3*R*,5a*S*,10a*R*) 3-Ethyloxycarbonyl-3-(2′-amino)phenyl-5,10-dioxooctahydro-5*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine (22a)

FC in ethyl acetate/n-hexane 3/2. Oil (9%); $[\alpha]_D^{25}$ −71.2 (*c* 0.1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (3H, t, CH_2CH_3), 1.53–1.61 (1H, m, H-7$_a$), 1.85–1.93 (1H, m, H-7$_b$), 2.38–2.41 (2H, m, H-6), 3.03–3.09 (2H, m, H-1), 3.46–3.52 (1H, m, H-8$_a$), 3.62–3.68 (1H, m, H-8$_b$), 3.96–4.02 (1H, m, H-5a), 4.22–4.28 (2H, m, CH_2 CH_3), 5.13 (1H, d, J = 5.1 Hz, H-10a), 6.70 (1H, t, J = 8.0 Hz, H-5′), 6.79 (1H, d, J = 8.0 Hz, H-3′), 6.85 (1H, d, H-6′), 7.12 (1H, t, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7 (*C*H$_3$), 21.0 (C-7), 28.5 (C-6), 31.7 (C-1), 46.1 (C-8), 60.1 (C-5a), 61.2 (*C*H$_2$), 62.0 (C-10a), 63.9 (C-3), 118.0 (C-3′), 119.5 (C-5′), 122.6 (C-1′), 128.4 (C-4′), 130.9 (C-6′), 149.8 (C-2′), 164.8, 166.7, 169.6 (C=O). Anal. Calcd for $C_{18}H_{21}N_3O_4S$: C, 57.58; H, 5.64; N, 11.19; S, 8.54. Found: C, 57.42; H, 5.49; N, 11.27; S, 8.59.

4.3.5. (3*S*,5a*S*,10a*R*) 3-Ethyloxycarbonyl-3-(2′-amino)phenyl-5,10-dioxooctahydro-5*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*] pyrazine (22b)

FC in ethyl acetate/*n*-hexane 3/2. Oil (24%); $[\alpha]_D^{25}$ −152.5 (*c* 0.11 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, CH_2CH_3), 1.63–1.71 (1H, m, H-7$_a$), 1.87–1.92 (1H, m, H-6$_b$), 2.31–2.35 (2H, m, H-7), 3.02–3.11 (2H, m, H-1), 3.28–3.33 (1H, m, H-8$_a$), 3.77–3.82 (1H, m, H-8$_b$), 4.20–4.28 (3H, m, H-5a, CH_2CH_3), 4.83 (1H, d, J = 5.2 Hz, H-10a), 6.66 (1H, t, H-5′), 6.71 (1H, d, J = 8.0 Hz, H-3′); 6.78 (1H, d, J = 8.0 Hz, H-6′), 7.12 (1H, t, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1 (*C*H$_3$), 20.3 (C-7), 28.8 (C-6), 32.6 (C-1), 45.7 (C-8), 61.9 (C-5a), 62.1 (*C*H$_2$), 62.8 (C-10a), 63.7 (C-3), 118.0 (C-3′), 119.5 (C-5′), 123.4 (C-1′), 127.4 (C-4′), 131.6 (C-6′), 150.6 (C-2′), 163.9, 166.9, 168.7 (C=O). Anal. Calcd for $C_{18}H_{21}N_3O_4S$: C, 57.58; H, 5.64; N, 11.19; S, 8.54. Found: C, 57.47; H, 5.53; N, 11.29; S, 8.63.

4.3.6. (3*R*,5a*S*,10a*R*) 3-Ethyloxycarbonyl-3-(2′-amino-5′-methyl)-phenyl-5,10-dioxooctahydro-5*H*-pyrrole[1,2-*a*][1,3]thiazole[3,4-*d*]pyrazine-3-carboxylate (23a)

FC in ethyl acetate/*n*-hexane 3/2. Oil (23%); $[\alpha]_D^{25}$ −66.7 (*c* 0.12 in $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ 1.29 (3H, t, CH_2CH_3), 1.78–1.82 (1H, m, 7$_a$-H), 1.92–2.01 (1H, m, 7$_b$-H), 2.22 (3H, s, CH_3), 2.27–2.30

(2H, m, H-6), 3.12–3.18 (2H, m, H-1), 3.42–3.46 (1H, m, H-8_a), 3.69–3.74 (1H, m, H-8_b), 4.00 (1H, t, H-5a), 4.21 (2H, q, CH_2CH$_3$), 5.12 (1H, d, J = 5.2 Hz, H-10a), 6.62 (1H, d, H-3′), 6.64 (1H, s, H-6′), 6.98 (1H, d, J = 8.0 Hz, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.5 (*C*H$_3$), 20.8 (C-7), 23.9 (*C*H$_3$), 28.3 (C-6), 32.9 (C-2), 45.9 (C-8), 61.0 (C-5a), 62.5 (*C*H$_2$), 63.6 (C-10a), 64.8 (C-3), 119.8 (C-3′), 126.7 (C-4′), 129.4 (C-6′), 130.1 (C-5′), 146.3 (C-2′), 163.8, 166.4, 168.1 (C=O). Anal. Calcd for C_{19} $H_{23}N_3O_4S$: C, 58.59; H, 5.95; N, 10.79; S, 8.23. Found: C, 58.41; H, 5.57; N, 10.88; S, 8.41.

4.3.7. (3*R*,5a*S*,10a*R*) 3-Ethyloxycarbonyl-3-(2′-amino-5′-bromo)-phenyl-5,10-dioxooctahydro-5*H*-pyrrole[1,2-*a*][1,3]thiazole[3,4-d]pyrazine (24a)

FC in dichloromethane/acetone 95/5. Oil (8%); $[\alpha]_D^{25}$ −69.2 (*c* 0.2 in $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ 1. 30 (3H, t, CH_2CH_3), 1.79–1.83 (1H, m, H-7_a), 1.90–1.99 (1H, m, H-7_b), 2.29–2.34 (2H, m, H-6), 3.11–3.20 (2H, m, H-1), 3.46–3.49 (1H, m, H-8_a), 3.62–3.68 (1H, m, H-8_b), 3.99 (1H, t, H-5a), 4.23 (2H, m, CH_2CH$_3$), 5.14 (1H, d, J = 5.2 Hz, H-10a), 6.60 (1H, d, J = 8.0 Hz, H-3′), 7.01 (1H, s, H-6′), 7.21 (1H, d, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.4 (*C*H$_3$), 23.4 (C-7), 28.3 (C-6), 32.4 (C-2), 46.9 (C-8), 60.0 (C-5a), 61.0 (*C*H$_2$), 62.1 (C-10a), 63.9 (C-3), 118.2 (C-5′), 119.7 (C-3′), 127.3 (C-4′), 133.1 (C-6′), 149.1(C-2′), 163.9, 167.1, 168.1 (C=O). Anal. Calcd for $C_{18}H_{20}BrN_3O_4S$: C, 47.58; H, 4.44; Br, 17.59; N, 9.25; S, 7.06. Found: C, 47.49; H, 4.27; Br, 17.72; N, 9.09; S, 7.25.

4.3.8. (3*S*,5a*S*,10a*R*) 3-Ethyloxycarbonyl-3-(2′-amino-5′-bromo)-phenyl-5,10-dioxooctahydro-5*H*-pyrrole[1,2-*a*][1,3]thiazole[3,4-*d*]pyrazine (24b)

FC in dichloromethane/acetone 95/5. Oil (20%); $[\alpha]_D^{25}$ −176.2 (*c* 0.12 in $CHCl_3$); ^{1}HNMR (400 MHz, $CDCl_3$) δ 1.32 (3H, t, CH_2CH_3), 1.58–1.63 (1H, m, H-7_a), 1.93–1.95 (1H, m, H-7_b), 2.37–2.41 (2H, m, H-6), 3.05–3.10 (2H, m, H-1), 3.28–3.35 (1H, m, H-8_a), 3.77–3.84 (1H, m, H-8_b), 4.21–4.25 (1H, m, H-5a), 4.30–4.32 (2-H, q, CH_2CH$_3$), 4.86 (1H, d, J = 5.8 Hz, H-10a), 6.59 (1H, d, J = 8.8 Hz, H-3′), 6.92 (1H, s, H-6′), 7.20 (1H, d, H-4′); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (*C*H$_3$), 22.4 (C-7), 28.4 (C-6), 32.7 (C-2), 45.3 (C-8), 61.9 (C-5a), 62.3 (*C*H$_2$), 62.5 (C-10a), 68.3(C-3), 109.2 (C-5′), 121.1 (C-3′), 123.6 (C-1′), 128.8 (C-4′), 133.2 (C-6′), 145.2 (C-2′), 162.8, 164.9, 168.3 (C=O). Anal. Calcd for $C_{18}H_{20}BrN_3O_4S$: C, 47.58; H, 4.44; Br, 17.59; N, 9.25; S, 7.06. Found: C, 47.51; H, 4.38; Br, 17.80; N, 9.07; S, 7.19.

4.4. General procedure for the synthesis of spiro-indol-2-one[3,3′]-hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine-5,10-dione derivatives (25a,b–28a,b)

To a solution of the corresponding spirooxoindolthiazolidine carboxylic acids (**29a,b–32a,b**, 3 mmol) in 29/1 dichloromethane/DMF (300 mL), L-Pro-OMe (1.1 equiv), DIC (1.2 equiv), HOBt (1.2 equiv), and DIPEA (2.4 equiv) were successively added. Stirring was continued at room temperature for 6 h. Afterward, the reaction mixture was diluted with dichloromethane (20 mL), and the resulting solution was washed successively with 10% $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL), dried over Na_2SO_4, and evaporated to dryness. Flash chromatography of the residues, using different eluent systems, yielded, in each case, the correspondent 4′-(carbonyl-prolyl-OMe) spirooxoindol thiazolidine derivatives as diastereoisomeric mixture (**33a,b–36a,b)** which were not separated in this step.

A solution of derivatives **33a,b** or **34a,b** or **35a,b** or **36a,b** (1 mmol) was treated with 1/4: 2 N HCl_{aq}/MeOH solution (10 mL) and stirred at room temperature. After 2 h, the mixtures were concentrated and dissolved in DCM. 10% $NaHCO_3$ solution was added until pH 7 and the resulting mixtures were washed with water (3 × 25 mL), dried over Na_2SO_4, evaporated to dryness, and purified and separated by flash column chromatography using different eluent systems to yield the title compounds.

4.4.1. (3*R*,5′a*S*,10′a*R*) Spiro[[indol-2-one[3,3′]-hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine]]-5,10-dione (25a)

FC in ethyl acetate. White solid (31%); mp 175–176 °C; $[\alpha]_D^{25}$ −76.3 (*c* 0.13 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.90–2.02 (3H, m, H-6′, H-7′a), 2.17–2.25 (1H, m, H-7′b), 3.51–3.58 (3H, m, H-8′, H-1′a), 3.78–3.83 (1H, m, H-1′b), 4.22 (1H, t, H-5′a); 5.01 (1H, dd, J = 6.0 and 10.0 Hz, H-10′a), 6.79 (1H, d, J = 8.0 Hz, H-7), 7.01 (1H, t, H-5), 7.18 (1H, d, J = 8.0 Hz, H-4), 7.24 (1H, t, H-6), 8.03 (1H, s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.6 (C-6′), 27.4 (C-7′), 33.9 (C-1′), 45.8 (C-8′), 60.1 (C-5′a), 66.3 (C-10′a), 70.2 (C-3), 114.2 (C-7), 123.8 (C-4), 125.1 (C-5), 127.2 (C-3a), 131.5 (C-6), 140.7(C-7a), 163.8, 164.6, 175.2 (C=O). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76; S, 9.74. Found: C, 58.21; H, 4.48; N, 12.63; S, 9.89.

4.4.2. (3*S*,5′a*S*,10′a*R*) Spiro[[indol-2-one[3,3′]hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine]]-5,10-dione (25b)

FC in ethyl acetate. White solid (37%); mp 190–191 °C; $[\alpha]_D^{25}$ −104.1 (*c* 0.11 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.92–2.06 (3H, m, H-6′, H-7′a), 2.23–2.30 (1H, m, H-7′b), 3.58–3.69 (3H, m, H-8′, H-1′a); 3.81 (1H, t, H-1′b); 4.27 (1H, t, H-5′a); 5.04 (1H, dd, J = 6.0 and 10.0 Hz, H-10′a), 6.81 (1H, d, J = 8.0 Hz, H-7), 7.02 (1H, t, H-5), 7.21 (1H, d, J = 8.0 Hz, H-4), 7.26 (1H, t, H-6), 7.83 (1H, s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.2 (C-6′), 28.0 (C-7′), 33.5 (C-1′), 45.6 (C-8′), 60.8 (C-5′a), 66.1 (C-10′a), 70.4 (C-3), 114.5 (C-7), 123.3 (C-4), 124.2 (C-5), 126.8 (C-3a), 130.6 (C-6), 140.9 (C-7a), 163.7, 164.5, 175.9 (C=O). Anal. Calcd for $C_{16}H_{15}N_3O_3S$: C, 58.34; H, 4.59; N, 12.76; S, 9.74. Found: C, 58.20; H, 4.50; N, 12.59; S, 9.91.

4.4.3. (3*R*,5′a*S*,10′a*R*) 5-Methyl spiro[[indol-2-one[3,3′]-hexahydro-5,10H-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*] pyrazine]]-5,10-dione (26a)

FC in ethyl acetate. White solid (29%); mp 181–183 °C; $[\alpha]_D^{25}$ −40.3 (*c* 0.12 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.93–1.97 (1H, m, H-6′a), 2.05–2.11 (2H, m, H-6′b, H-7′a), 2.22–2.26 (1H, m, H-7′b), 2.30 (3H, s, CH_3), 3.51–3.69 (3H, m, H-8′, H-1′a), 3.83 (1H, t, H-1′b), 4.28 (1H, t, H-5′a), 5.02 (1H, dd, J = 6.0 and 10.0 Hz, H-$10'_a$), 6.74 (1H, d, J = 8.0 Hz, H-7), 7.38 (1H, d, H-6), 7.41 (1H, s, H-4), 7.92 (1H, s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.2 (C-6′), 24.6 (*C*H$_3$), 27.9 (C-7′), 33.5 (C-1′), 45.6 (C-8′), 60.4 (C-5′a), 66.1 (C-10′a), 69.9 (C-3), 112.1 (C-7), 124.5 (C-3a), 127.5 (C-4), 133.0 (C-6), 134.7 (C-5), 138.2 (C-7a), 163.6, 164.9, 175.4 (C=O). Anal. Calcd for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24; S, 9.34. Found: C, 59.49; H, 5.12; N, 12.09; S, 9.41.

4.4.4. (3*S*,5′a*S*,10′a*R*) 5-Methyl spiro[[indol-2-one[3,3′]-hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*] pyrazine]]-5,10-dione (26b)

FC in ethyl acetate. White solid (35%); mp 190–191 °C; $[\alpha]_D^{25}$ −130.8 (*c* 0.2 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95–2.03 (3H, m, H-6′a, H-7′), 2.24–2.30 (1H, m, H-6′b), 2.33 (3H, s, CH_3), 3.57–3.64 (3H, m, H-8′, H-1′a); 3.82 (1H, t, H-1′b); 4.35 (1H, t, H-5′a); 5.04 (1H, dd, J = 5.6 and 10.8 Hz, H-10′a), 6.74 (1H, d, J = 8.0 Hz, H-7), 7.39 (1H, d, J = 8.0 Hz, H-6), 7.42 (1H, s, H-4), 8.59 (1H, s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.5 (C-6′), 24.8 (*C*H$_3$), 28.0 (C-7′), 32.5 (C-1′), 45.5 (C-8′), 61.0 (C-5′a), 65.8 (C-10′a), 71.1 (C-3), 112.3 (C-7), 122.7 (C-3a), 125.8 (C-4), 132.3 (C-5), 132.9 (C-6), 136.2 (C-7a), 164.2, 166.5, 174.3 (C=O). Anal. Calcd for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24; S, 9.34. Found C, 59.51; H, 5.08; N, 12.17; S, 9.52.

4.4.5. (3*R*,5′a*S*,10′a*R*) 5-Bromo Spiro[[indol-2-one[3,3′]-hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3] thiazolo[3,4-*d*] pyrazine]]-5,10-dione (27a)

FC in ethyl acetate. White solid (28%); mp 178–179 °C; $[\alpha]_D^{25}$ −68.0 (*c* 0.12 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.89–1.96 (1H, m, H-6'a), 2.04–2.09 (2H, m, H-6'b, H-7'a), 2.19–2.23 (1H, m, H-7'b), 3.49–3.65 (3H, m, H-8′, H-1'a), 3.82 (1H, t, H-1'b); 4.26 (1H, t, H-5'a), 5.00 (1H, dd, *J* = 6.0 and 10.0 Hz, H-10′a), 6.73 (1H, d, *J* = 8.0 Hz, H-7), 7.38 (1H, d, H-6), 7.41 (1H, s, H-4) 7.75 (1H, s, N*H*); ^{1}H NMR (400 MHz, CD_3OD) δ 1.93–2.03 (3H, m, H-6'a, H-7′), 2.16–2.19 (1H, m, H-6'b), 3.54–3.64 (2H, m, H-8′), 3.65–3.68 (1H, m, H-1'a), 3.65 (1H, t, *J* 10.0 Hz, H-1'b), 4.43 (1H, m, H-5'a), 5.27 (1H, dd, *J* = 6.4 and 9.6 Hz, H-10′a), 6.81 (1H, d, *J* = 8.0 Hz, H-7), 7.42 (1H, d, H-6), 7.55 (1H, s, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.6 (C-6′), 27.8 (C-7′), 33.9 (C-1′), 46.5 (C-8′), 61.9 (C-5′a), 66.0(C-10′a), 71.1 (C-3), 107.8 (C-5), 112.1 (C-7), 123.4 (C-6), 128.8 (C-3a), 130.7 (C-4), 138.9 (C-7a), 167.1, 169.2, 174.8 (C═O). Anal. Calcd for $C_{16}H_{14}BrN_3O_3S$: C, 47.07; H, 3.46; Br, 13.57; N, 10.29; S, 7.85. Found C, 46.91; H, 3.48; Br, 13.71; N, 10.40; S, 7.97.

4.4.6. (3*S*,5′a*S*,10′a*R*) 5-Bromo spiro[[indol-2-one[3,3′]-hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*] pyrazine]]-5,10-dione (27b)

FC in ethyl acetate. White solid (33%), mp 187–189 °C; $[\alpha]_D^{25}$ −128.6 (*c* 0.14 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94–2.05 (3H,m, H-6'a, H-7′), 2.25–2.30 (1H, m, H-6'b), 3.60–3.67 (3H, m, H-8′, H-1'a), 3.82 (1H, t, H-1'b), 4.32 (1H, t, H-5'a), 5.06 (1H, dd, *J* = 5.6 and 10.8 Hz, H-10′a), 6.81 (1H, d, *J* = 8.0 Hz, H-7), 7.39 (1H, d, H-6), 7.42 (1H, s, H-4), 8.33 (s, N*H*); ^{1}H NMR (400 MHz, CD_3OD) δ 1.88–2.02 (3H, m, H-6$_a$′, H-7′), 2.20–2.21 (1H, m, H-6'b), 3.54–3.64 (2H, m, H-8′), 3.65–3.68 (1H, m, H-1'a), 3.84 (1H, t, *J* = 10.4 Hz, H-1'b), 4.43 (1H, m, H-5'a); 5.14 (1H, dd, *J* = 6.8 and 9.6 Hz, H-10′a), 6.81 (1H, d, *J* = 8.0 Hz, H-7), 7.38 (1H, d, H-6); 7.42 (1H,s, H-4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.7 (C-6′), 28.2 (C-7′), 32.4 (C-1′), 45.8 (C-8′), 61.4 (C-5′a), 65.9 (C-10′a), 70.8 (C-3), 109.9 (C-5), 111.6 (C-7), 130.4 (C-6), 136.5 (C-4), 139.8 (C-7a), 164.6, 166.0, 174.6 (C═O). Anal. Calcd for $C_{16}H_{14}BrN_3O_3S$: C, 47.07; H, 3.46; Br, 13.57; N, 10.29; S, 7.85. Found C, 46.96; H, 3.39; Br, 13.68; N, 10.42; S, 7.96.

4.4.7. (3*R* and 3*S*,5′a*S*,10′a*R*) 1-Methyl spiro[[indol-2-one[3,3′]-hexahydro-5,10*H*-pyrrolo[1,2-*a*][1,3]thiazolo[3,4-*d*]pyrazine]]-5,10-dione (28a,b)

FC in ethyl acetate. White solid (72%) ^{1}HNMR (400 MHz, $CDCl_3$) δ 1.83–2.04 (3H, m, H-6′, H-7'a), 2.11–2.23 (1H, m, H-7'b), 3.21 (3H, s, C*H*$_3$); 3.42–3.61 (3H, m, H-8′, H-1'a), 3.78 (1H, t, H-1'b), 4.19 (1H, m, H-5'a), 4.24 (1H, m, H-5'a); 5.00 (1H, m, H-10′a), 5.03 (1H, m, H-10′a), 6.81 (1H, d, *J*= 8.0 Hz, H-7), 7.02 (1H, t, H-6), 7.22 (1H, d, *J* = 8.0 Hz, H-4), 7.33 (1H, t, H-5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.6, 24.9 (C-6′), 26.5(C-7′), 29.1 and 29.8 (*C*H$_3$), 39.0 and 42.1 (C-1′), 46.9 and 52.9 (C-8′), 59.1 (C-5′a), 62.9 and 66.7 (C-10′a), 73.2 (C-3), 108.7, 109.0 (C-7), 123.5 (C-4), 124.3 (C-5), 129.2 and 130.7 (C-6), 130.1 (C-5), 142.1 (C-7a), 168.1, 169.2, 172.4, 176.7 (C═O). Anal. Calcd for $C_{17}H_{17}N_3O_3S$: C, 59.46; H, 4.99; N, 12.24; S, 9.34. Found: C, 59.63; H, 4.89; N, 12.59; S, 9.29.

4.5. NMR spectroscopy

NMR experiments were performed on a Varian Mercury 400 MHz spectrometer at 298 K. NMR samples were prepared by dissolving each compound (about 5 mM) in 0.6 ml of $CDCl_3$. 2D NOESY spectra of compounds **24a**, **24b**, **26a**, **26b** and **27a**, **27b** were recorded in the phase-sensitive mode, data block sizes were 2048 addresses in *t*2 and 512 equidistant *t*1 values. Before Fourier transformation, the time domain data matrixes were multiplied by shifted sin^2 functions in both dimensions. A mixing time of 400 ms was used.

4.6. Computational data

Molecular modeling and graphics manipulations were performed using the InsightII software package (Accelrys, San Diego, CA). The 3D structures of the compounds were constructed using the module Builder of InsightII program and then optimized using minimization steps (conjugate gradient method), in vacuo, using the CVFF force field[20] in InsightII/Discover software packages. MD simulations were performed at 500 K period (time step = 1 fs). MD results were analyzed with the Analysis module of InsightII.

4.7. Crystallographic analysis

A suitable crystal of **21** (0.04 × 0.6 × 0.04 mm) was selected and mounted on a glass fibre. The diffraction data were collected at 100 K with graphite monochromatized Mo Kα radiation (λ = 0.71069 Å, $2\theta_{max} \leqslant 54.52°$, φ and ω scan mode) on a Rigaku AFC7S diffractometer equipped with a Mercury CCD detector and corrected for Lorentz, polarization and absorption effects. The data collection was performed with a detector to crystal distance *D* = 414 mm using an oscillation angle of $\Delta\varphi = 0.5°$ and consisted of 892 images. It covered a resolution range of *d* = 26.76–0.80 Å. Intensity data were corrected for absorption.[21] Lattice constants and crystal orientation were obtained using 1D FFT with DPS algorithm,[22] the number of reflections used was 970 with a refinement of 30 best directions and lengths. The structure was solved by direct methods using SIR92[23] and the refinement, realized by SHELXL97,[24] was based on F^2 considering all non-hydrogen atoms anisotropically and hydrogens included on calculated positions, riding on their parent atoms and then refined isotropically. A total of 514 parameters were refined. Maximum and minimum residual density were 0.85 and −0.45 e/Å^3. Final disagreement indices: R_1 = 0.0578 for 3556 reflections with $F_o > 4\sigma(F_o)$ and wR_2 = 0.1711 for all 8860 data. ORTEP drawings were prepared by means of the program ORTEP32.[25]

4.8. Cytotoxic activity assay

The human breast adenocarcinoma MCF-7, human ductal breast carcinoma T47D, and human epidermoid carcinoma A431 cells were cultured at 37 °C in humidified 5%CO_2/95% air in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% foetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 mM Hepes and 5 mM sodium pyruvate. The cells were plated in 24 culture wells at a density of 2.5 × 10^5 cells/ml per well or 10 cm diameter culture dishes at a density of 3 × 10^6 cells/ml per dish and allowed to adhere for 2 h. Thereafter the medium was replaced with fresh medium and cells were incubated with isatins (10^{-7}, 10^{-6}, 10^{-5} M) added to the cells for 48 h. Stocks of antitumoral compounds were dissolved in saline or saline and 1% DMSO, sterilized by 30 min of UV irradiation and stored at −20 °C. The cell viability was determined by using 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2*H*-tetrazolium dimethylthiazol-2yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) conversion assay as previously described.[26] Briefly, 100 ml MTT (5 mg/ml in complete DMEM) was added and the cells were incubated for an additional 3 h. After this time point the cells were lysed and the dark blue crystals solubilized with 500 ml of a solution containing 50% (v:v) *N*,*N*-dimethylformamide, 20% (w:v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer equipped with a 620 nm filter. The cell viability in response to treatment with test compounds was calculated as % dead cells = 100 − (OD treated/OD control) × 100.

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Supplementary data

Supplementary data (spectroscopic data for 3-(prolyl) and 4′-(carbonylproly) siprooxoindolethiazolidine intermediates (**9a,b**–**12a,b**, **13a,b**–**15a,b**, and **33a,b**–**36a,b**), copies of ^{1}H and ^{13}C NMR spectra for compounds **19**–**121**, **22a**–**24a**, **22b**, **24b**, **25a**–**27a**, **19b**–**27b**, and **28a,b** and NOESY spectra for compounds **24a**, **24b**, **26a, 26b, 27a,** and **27b)** associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.079.

References and notes

1. (a) Sherr, C. J. *Science* **1996**, *274*, 1672; (b) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57; (c) Sandal, T. *Oncologist* **2002**, *7*, 73.
2. (a) Hung, D. T.; Jamison, T. F.; Schreiber, S. L. *Chem. Biol.* **1996**, *3*, 623; (b) Crews, C. M.; Shotwell, J. B. *Prog. Cell Cycle Res.* **2003**, *5*, 125.
3. (a) Osada, H.; Cui, C. B.; Onose, R.; Hanaoka, F. *Bioorg. Med. Chem.* **1997**, *5*, 193; (b) Osada, H. *J. Antibiot.* **1998**, *51*, 973.
4. Von Nussbaum, F.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2175.
5. Wang, H.; Ganesan, A. *J. Org. Chem.* **2000**, *65*, 4685.
6. (a) Edmondson, S.; Danishefsky, S. J.; Sepp-Lorenzino, L.; Rosen, N. *J. Am. Chem. Soc.* **1999**, *121*, 2147; (b) Onishi, T.; Sebahar, P. R.; Williams, R. M. *Org. Lett.* **2003**, *5*, 3135.
7. (a) Sebahar, P. R.; Williams, R. M. *J. Am. Chem. Soc.* **2000**, *122*, 5666; (b) Sebahar, P. R.; Osada, H.; Usui, T.; Williams, R. M. *Tetrahedron* **2002**, *58*, 6311.
8. Miyake, F. Y.; Yakushijin, K.; Horne, D. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5357.
9. Bagul, T. D.; Lakshmaiah, G.; Kawabata, T.; Fuji, K. *Org. Lett.* **2002**, *4*, 249.
10. Wilson, R. M.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8329.
11. Gomez-Monterrey, I.; Campiglia, P.; Carotenuto, A.; Califano, D.; Pisano, C.; Vesci, L.; Lama, T.; Bertamino, A.; Sala, M.; Mazzella di Bosco, A.; Grieco, P.; Novellino, E. *J. Med. Chem.* **2007**, *50*, 1787.
12. Kutschy, P.; Suchý, M.; Dzurilla, M.; Pazdera, P.; Takasugi, M.; Kováčik, V. C. C. *Chem. Commun.* **2000**, *65*, 425.
13. Crystallographic data for **21**: formula $2(C_{18}H_{20}BrN_3O_4S)?CH_2Cl_2$, $M_w = 454.34$, orthorhombic system, space group $P2_12_12_1$, $Z = 8$, $a = 9.2803(11)$ Å, $b = 15.114(2)$ Å, $c = 29.275(4)$ Å, $V = 4106.0(9)$ Å^3, $D_x = 1.607$ g cm^{-3}, $\mu_{calcd} = 4.53$ mm^{-1}.
14. Dillman, R. L.; Cardellina, J. H., II *J. Nat. Prod.* **1991**, *54*, 1159.
15. Grunwald, E. *Prog. Phys. Org. Chem.* **1990**, *17*, 55.
16. Capasso, S.; Mazzarella, L. *J. Chem. Soc., Perkin Trans. 2* **1999**, 329.
17. Fields, G. B. *Methods in Molecular Biology. Peptide Synthesis Protocols. Methods for Removing the Fmoc Group*; Pennington, M. W., Dunn, B. M., Eds., doi: 10.1385/0-89603-273-6:17.
18. Isidro-Llobet, A.; Guasch-Camell, J.; Álvarez, M.; Albericio, F. *Eur. J. Org. Chem.* **2005**, 3031. and references cited therein.
19. Galliford, C. V.; Scheidt, K. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 8748.
20. Maple, J.; Dinur, U.; Hagler, A. T. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5350.
21. Blessing, R. H. *Acta Crystallogr., Sect. A* **1995**, *51*, 33.
22. Steller, I.; Bolotovsky, R.; Rossman, J. *Appl. Crystallogr.* **1997**, *30*, 1036.
23. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Cavalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435.
24. Sheldrick, G. M. SHELX97, A program for refining crystal structure, University of Göttingen, Germany, 1997.
25. Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.
26. Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.