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Research paper

Advances in tetrahydropyrido[1,2-*a*]isoindolone (valmerins) series: Potent glycogen synthase kinase 3 and cyclin dependent kinase 5 inhibitors

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ABSTRACT

An efficient synthetic strategy was developed to modulate the structure of the tetrahydropyridine isoindolone (Valmerin) skeleton. A library of more than 30 novel final structures was generated. Biological activities on CDK5 and GSK3 as well as cellular effects on cancer cell lines were measured for each novel compound. Additionally docking studies were performed to support medicinal chemistry efforts. A strong GSK3/CDK5 dual inhibitor (**38**, IC_{50} GSK3/CDK5 32/84 nM) was obtained. A set of highly selective GSK3 inhibitors was synthesized by fine-tuning structural modifications (**29** IC_{50} GSK3/CDK5 32/320 nM). Antiproliferative effects on cells were correlated with the *in vitro* kinase activities and the best effects were obtained with lung and colon cell lines.

1. Introduction

Cancer results mainly from cell cycle perturbations and leads to anarchic cellular proliferation. Gene mutations associated with cancer disease induce abnormality in cellular proliferation and differentiation and are often linked to resistance of several therapies s [1–5]. The four phases (G1, S, G2, M) are critical for the regulation of the cell cycle and a number of biochemical pathways have been discovered as key mechanisms for the initiation of a particular cell cycle event. Many protein kinases are activated in these biochemical pathways and mediate biological information in downstream signalling pathways through controlled phosphorylation events. Among the 518 kinases identified to date, it has been established that the ubiquitously expressed glycogen synthase kinase-3 (GSK3), mainly present in the cytoplasm, and cyclin-depend kinase 5 (CDK5), a nuclear pivot for cell cycle progression, play important roles in several biological mechanisms. GSK3 and CDK5 are reported as key mediators in neuronal migration, embryonic development, protein synthesis, cell proliferation and differentiation, microtubule dynamics, cell motility, steroidogenesis and apoptosis [2]. Deregulation of these protein kinases has been found in many human diseases and small molecules kinase inhibitor, which efficiently target GSK3 and CDK5 enzymes, are considered as potential solutions to contain the evolution of cancer. Several drugs acting on these two biological targets have entered clinical trials such as roscovitine (Seliciclib) that has progressed to phase II (Fig. 1) [6,7].

As part of our efforts in finding better therapeutic solutions, our team has been involved for several years in the synthesis of novel

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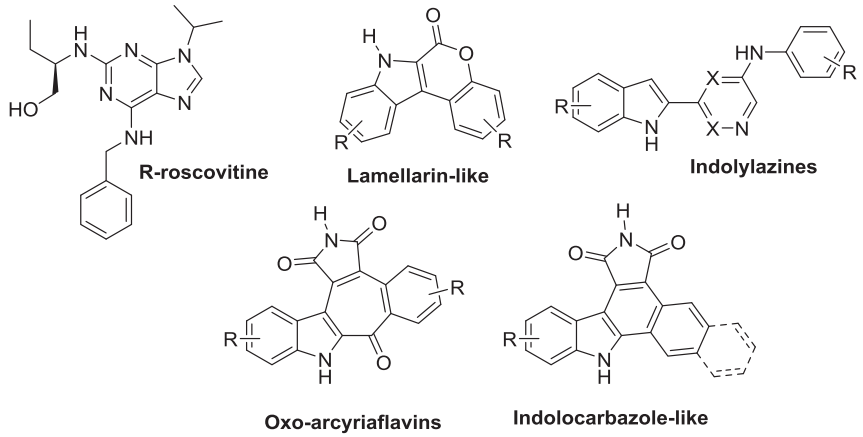


Fig. 1. Some examples of CDK5, GSK3 and DYRK1A inhibitors.

disease-relevant kinase inhibitors and in particular drugs acting on CDK5, GSK3 and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A). We have developed synthetic strategies to optimize lamellarin-like or V-shaped indolylazines derivatives as well as oxo-arcyriaflavin or indolocarbazole analogues (Fig. 1), each series providing original skeletons as well as potent ATP competitive inhibitors able to inhibit the previously mentioned protein kinases [8].

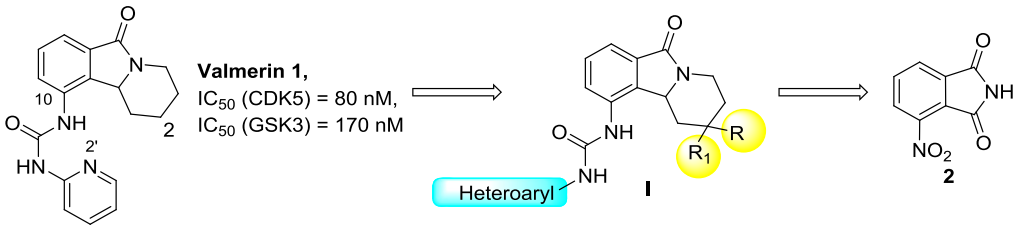
In addition, we have also performed virtual screening campaigns on CDK5, GSK3 and DYRK1A to rationalize the structure–activity relationships (SAR) and to accelerate optimization processes. During our investigations in the proposed tetrahydropyridoisoindolone series, we first identified valmerin **1** (Scheme 1) and evaluated its biological effects. This series exhibits strong kinase inhibition, induces apoptosis in cancer cells and shows *in vivo* tumour regression. We showed that the lead compound **1** acts on CDK1 or CDK5 and GSK3 in the nanomolar range without any effect on DYRK1A (up to 10 μM), the upstream regulator of CDK and GSK3.

The development of the valmerin series, acting on GSK3 and/or CDK5, required a thorough exploration of each scaffold substituent to identify the critical pharmacophore. In a previous report, we determined that the tetrahydropyridoisoindolone core linked to a (het)Ar urea can induce kinase inhibition [9] and no modification to the tetrahydropyridine ring was explored. We surmise that such modification would interfere at the ATP binding site in the recognition mechanism between the novel molecules and the enzyme and would globally enhance the binding affinities and block kinase catalytic function. Specifically, we hypothesize that the structural modulation will lead to valmerins having an activity on GSK3 and selective to CDK5 or active on both GSK3 and CDK5. Having in hand such molecules in a single chemical family will be useful to understand the implication of Valmerins in the CDK and GSK3 cell pathways. These tools will lead to a good understanding of the action mode and will offer to medicinal chemists solutions to prepare, as example, roscovitine successors. Finally, we aim to answer the following question: Is the valmerin heterocyclic scaffold a promising pharmacophoric model able to modulate kinase activity with achievable cellular effects?

To access the valmerin core, we modified the synthetic pathway and optimized novel synthetic routes. The nature and size of the urea heterocyclic group as well as the substitution of the tetrahydropyridine ring were the two main objectives of the chemistry efforts. The novel library was used to assess novel structure activity relationships. Molecular modelling studies were performed to corroborate the *in vitro* kinase activities with structural modifications. Finally, cellular effects were measured on several tumour cell lines in this study.

2. Chemistry

The synthesis of valmerins was achieved from 2-nitrophtalimide **2** and led to the interesting nitro derivatives **3** and **4** having a protected, but not necessary, ketone on the tetrahydropyridine core [9]. The transformation of dioxolane to thioacetal using ethanedithiol in the presence of $BF_3 \cdot Et_2O$ in CH_2Cl_2 was first performed from **3** [10]. Compound **5** was isolated with a yield of 86%. Ketone **4** was then subjected to a reduction using sodium borohydride in a mixture of THF/MeOH in an 86% yield. The separation of the two stereoisomers cis and trans of **4** appears as very critical. HPLC purification indicates the presence of a mixture of the two diastereoisomers in a 7:3 ratio in favour of the cis configuration [11]. The purification led to an analytical amount of pure diastereoisomer **6** in the very low yield of 14%. The next methylation step was therefore carried out on the crude cis + trans mixture which was isolated directly after reduction of the ketone.



Scheme 1. Retrosynthetic scheme for the design of novel CDK5, GSK3 and DYRK1A valmerin inhibitors.

Fortunately only the cis regioisomer **6** reacted under the special conditions involving methyl iodide in the presence of Ag_2O in THF at 50 °C in a satisfying yield (other Williamson type conditions failed) [12]. Derivative **7** was also purified satisfyingly as a single cis diastereoisomer (COSY, NOESY and HSQC NMR experiments) with a yield, calculated from **4**, of 61%. Reduction of the nitro groups of **5** and **3** were carried out using an excess of $SnCl_2$ and gave amino compounds **8** and **10** in a very good yield but, starting from **7**, the reaction failed. When the reduction of derivative **7** was carried out under a hydrogen atmosphere, compound **9** was generated in a 95% yield (Scheme 2).

Aryl amines **8**–**10** were engaged in order to generate a novel valmerin series **II**. As kinase activities are directly linked to the presence of the aryl urea function, we tried several synthetic routes. The most convenient method remained the use of amines **8**–**10** which react from *in situ* freshly prepared isocyanates. Noteworthy, the previously mentioned isocyanates were obtained starting from the chosen carboxylic acids *via* a Curtius type rearrangement. This route appears to be very advantageous since many heteroaromatic carboxylic acids are commercially available. The use of this new strategy allowed us to develop an interesting variety of ureas **11**–**31** with satisfying yields. The ureas carrying an acetal were engaged in a final deprotection step to access the corresponding ketones. This sequence is particularly interesting since the direct synthesis of final derivatives, starting from **4** or **6**, failed.

Treatment of compounds **11**, **14**, **17**, **20** and **23** with an aqueous solution of hydrochloric acid at 10% in refluxing acetone led to the desired ketones **32**–**36** with yields of up to 79%. The final compounds **37**–**41** were then directly isolated from ureas **32**–**36** by reduction with sodium borohydride in a THF/MeOH mixture. After stirring for 2 h at a temperature range of 0–5 °C, the final products **37**–**41** were straightforwardly isolated as their single cis diastereoisomers, with satisfying yields. The other stereoisomer was never observed (TLC, ^1H NMR of the crude material) (Scheme 3).

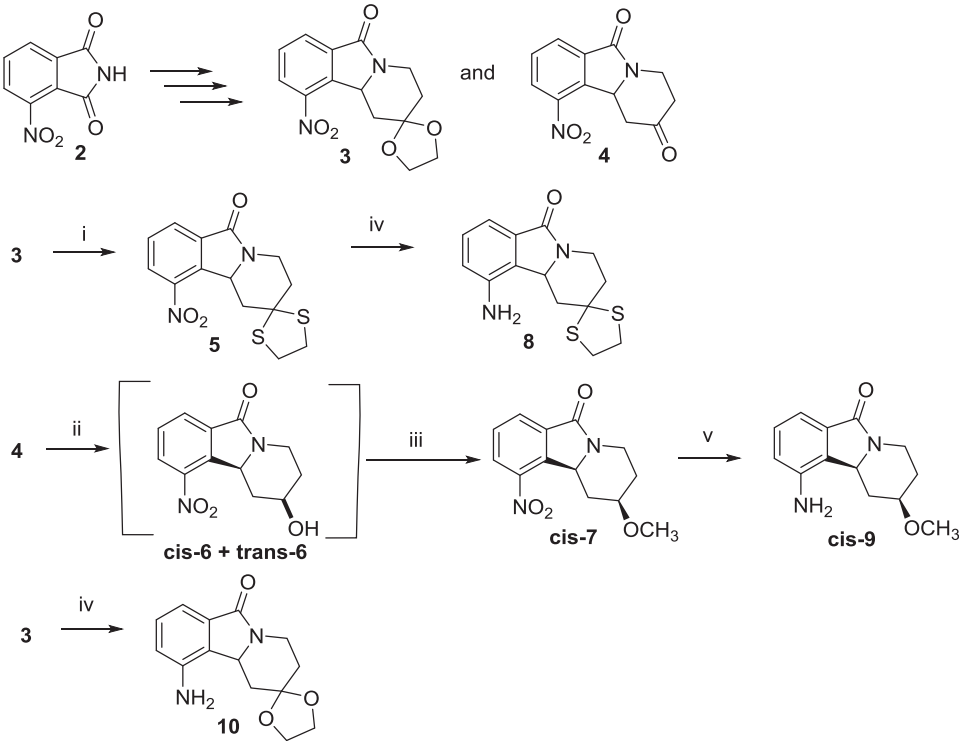
3. Kinase assays

The library of valmerins **11**–**41** was first evaluated in a primary kinase screen using the targeted enzymes CDK5 and GSK3 and the off-target kinase DYRK1A (Table 2). In a previous study, we clearly showed that the urea moiety is necessary to induce a biological effect [9]. More specifically, close to the tetrahydropyridoisoindolone scaffold, a heteroaryl urea is necessary in position *C*-10. The heterocycles contain a nitrogen atom in position *C*-2′. Valmerin **1** acts on CDK5 and GSK3 in a quite similar manner.

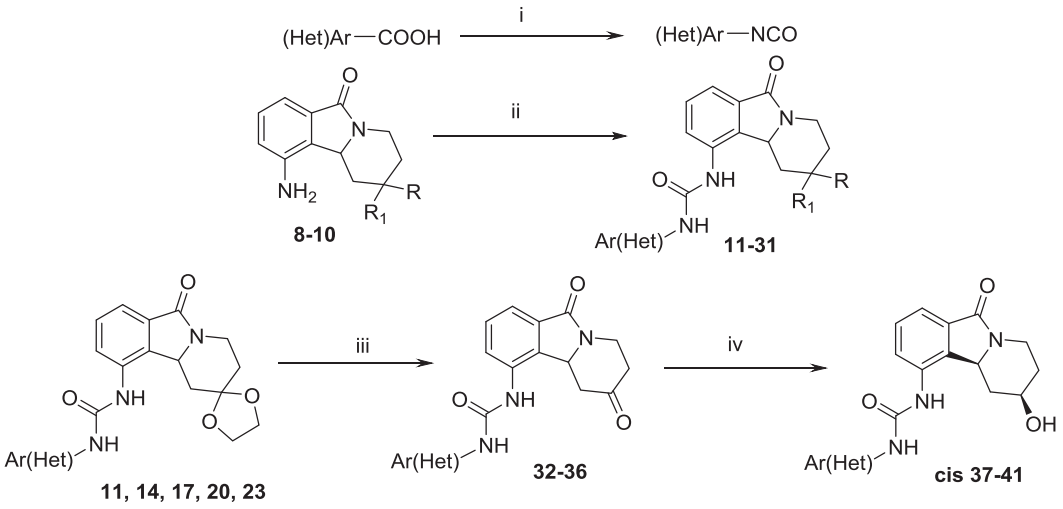
In our novel valmerins (Table 2), whatever the group used in position *C*-2, a urea in *C*-10 with a small five-membered cycle such as pyrazole (entry 6) led to the loss of kinase inhibition whereas an increase in the (het)Ar size such as a quinoline moiety (entry 4) led to some active compounds. Derivatives **40** and **22** act mainly on GSK3 (35 and 180 nM respectively; entries 4e, 4c). Valmerin **40** which possesses a *C*-2 hydroxyl group is one of the most selective derivatives so far, as it interferes with CDK5 and DYRK1A in a micromolar range. The first discrimination of CDK5 and GSK3 is achieved in this example.

We found CDK5 inhibitors with remarkable IC_{50} in the nanomolar range without effect on GSK3 but modifications of structures could modify the level of the second activity. When pyrazine was used instead of pyridine as the (het)Ar moiety, an excellent dual inhibitor was obtained. The inhibition values for compound **38** were 84 and 32 nM against CDK5 and GSK3 respectively (entry 2e). Globally each methoxylation in *C*-2 diminished activity and favoured the effect on GSK3; the previously mentioned duality was lost (entries 1c–4c, 5b–7b, 8).

Compounds bearing a ketone in *C*-2 position remained inactive (entries 1d, 2d) whatever the tested kinase. It is possible that the presence of a planar and π electron rich dipole was detrimental for kinase binding. A cyclic acetal was better tolerated by the active site despite the large size of this moiety. Valmerins which possess this



Scheme 2. *Reagents and conditions*: *i*) 1,2-ethanedithiol (5.0 eq.), $BF_3{\cdot}Et_2O$ (5.0 eq.), CH_2Cl_2, r.t., 24 h, 86%; *ii*) $NaBH_4$ (2.0 eq.), THF/MeOH (1/2), −20 °C to r.t., 5 h, 86% (mixture of **cis-6** and **trans-6**); *iii*) Ag_2O (4.0 eq.), CH_3I (10.0 eq.), THF, 50 °C, 48 h, 61% (from **4**); *iv*) $SnCl_2$ (15.0 eq.), EtOH, r.t., 12 h, **8** 80% or **10** 82%; *v*) H_2, Patm, Raney Ni, EtOH, r.t., 14 h, 95%.



Scheme 3. *Reagents and conditions*: *i*) a) Et_3N (1.3 eq.), −10 °C, THF, 5 min.; b) $ClCO_2Et$ (1.5 eq.), −10 °C, THF, 2 h; c) NaN_3 (1.7 eq.), −10 °C, H_2O, 1 h; d) toluene, reflux, 1 h; *ii*) (Het)ArNCO, dioxane, 100 °C, 24 h; *iii*) HCl 10%, acetone, reflux, 3 h; *iv*) $NaBH_4$ (2.0 eq.), THF/MeOH (1/1), 0 °C–5 °C, 2 h. For yields see Table 1.

electron rich cycle acted preferentially on GSK3 (entries 1a–5a). The best score was obtained with **14**, which bears the dioxolane ring in *C*-2 and inhibited GSK3 with an $IC_{50} = 260$ nM. The activity on CDK5 was 30 fold weaker.

Surprisingly, replacing dioxolane by dithiolane enhanced the enzymatic activities on GSK3 as well as on CDK5 (for derivatives **12**, **15** and **27**, entries 1b, 2b and 7a respectively). Adding a supplementary heavy lipophilic bromine atom on the pyridine urea increased selectivity to a very high level (entry 10). Valmerin **31** was active on GSK3 with an IC_{50} of 68 nM: the selectivity toward CDK5 increased by 100 fold.

4. Molecular modelling studies

To rationalize the structure–activity relationships, the molecules were docked into the binding site of GSK3β and CDK5. We found two main modes of binding depending on the substituent attached to the urea moiety. In one binding mode the carboxyl group of the tetrahydropyridoisoindolone scaffold points towards the catalytic lysine Lys85, forming a hydrogen bond interaction and the urea is positioned parallel to the hinge region, creating a hydrogen bond interaction between the urea and the backbone of Val135 [9]. A second mode of binding was more frequently observed in GSK3β and is shown on Fig. 2. The carboxyl group of the tetrahydropyridoisoindolone scaffold forms a hydrogen bond interaction with the NH backbone of Val35, and the urea interacts with the catalytic lysine Lys85. Interestingly, in this orientation the heterocyclic ring attached to the urea can form an additional hydrogen bond interaction with Lys85 if a nitrogen is present at the ortho position. As shown in Fig. 2, the nitrogen of the pyridine moiety from compound **29** interacts with Lys85, and a bidentate hydrogen bond is now present with Lys85. This binding mode can explain the kinase activity obtained with most of the 6-membered rings compared to 5-membered rings such as pyrazole where there is a drop in kinase activity probably because of the lack of this hydrogen bond.

The substituent attached to the saturated ring of the tetrahydropyridoisoindolone scaffold interacts with Thr138 through a hydrogen bond network or van der Waals interaction depending on the orientation of the residue side chain. Based on the binding mode analysis and sequence alignment of GSK3β, CDK5 and DYRK1A, we suggest that the low activity of the compounds for DYRK1A and CDK5 is due to the large gatekeeper residue Phe compared to Leu132 in GSK3β, creating a steric hindrance with the tetrahydropyridoisoindolone scaffold. Additionally, the lack of activity towards DYRK1A might be due to the larger residue Val306, compared to Ala143 and Cys199 in CDK5 and GSK3β respectively, located below the plane of the scaffold and at position *N*-1 in the DFG motif.

5. Cellular screening

The toxicity on synthesized molecules was assessed on six cancer cell lines; Huh7 (liver), Caco2 (colon), MDA-MB231 (breast), HCT-116 (colon), PC3 (prostate), NCI H727 (lung). The molecules were generally cytostatic, blocking cell cycle replication. These results are typical for most kinase inhibitors acting on the targeted kinases (CDK5 and GSK3β) such as roscovitine used in our test as a reference.

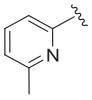
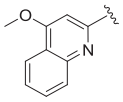
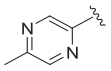
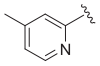
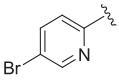
All the derivatives which were tested on the kinase assay were evaluated on the cell line panel. Surprisingly all the derivatives bearing an OH in *C*-2 position, *i.e.*, **38**, **39** and **40**, led to inactive derivatives as if the hydroxyl group was too sensitive and induced instability in the cell culture media. Among the *C*-2 OMe family which appears active against GSK3 (*i.e.*, **13** and **29**), only **29** led to interesting cellular effects. The best inhibition of growth was obtained with the colon cell line HCT-116 ($IC_{50} = 30$ nM) and the liver cell line Huh7 ($IC_{50} = 100$ nM). Other cell lines were affected with an IC_{50} between 300 and 500 nM.

Concerning the derivatives carrying *C*-2 thio acetals (**12**, **15**, **27** and **31**), cellular effects were maintained in the nanomolar range only when the urea (het)Ar moiety was a pyrazine group which could be unsubstituted or methylated (**15** and **27**). For compound **15**, the cellular activity on Huh7 was similar to the one observed with **29**. The toxicity against HCT-116 remained high ($IC_{50} = 100$ nM). A real improvement on the breast cell line was observed, with the IC_{50} reaching 150 nM (Table 3).

6. Conclusions

We have developed efficient synthetic routes able to modulate the structure of the tetrahydropyridine skeleton. A library of more than 30 novel final structures was generated and our valmerin library considerably enhanced biological activities on CDK5 and

Table 1
Synthesis of valmerins **11**–**41**.

Entry		(Het)Ar	R/R_1	Product	Yield[a]
1	a		$-OCH_2CH_2O-$	**11**	78%
	b		$-SCH_2CH_2S-$	**12**	96%
	c		cis OCH_3	**13**	81%
	d		=O	**32**	Obtained from **11**, quant.
	e		cis OH	**37**	Obtained from **32**, 76%
2	a		$-OCH_2CH_2O-$	**14**	68%
	b		$-SCH_2CH_2S-$	**15**	90%
	c		cis OCH_3	**16**	78%
	d		=O	**33**	Obtained from **14**, 95%
	e		cis OH	**38**	Obtained from **33**, 60%
3	a		$-OCH_2CH_2O-$	**17**	75%
	b		$-SCH_2CH_2S-$	**18**	78%
	c		cis OCH_3	**19**	75%
	d		=O	**34**	Obtained from **17**, 79%
	e		cis OH	**39**	Obtained from **34**, 80%
4	a		$-OCH_2CH_2O-$	**20**	72%
	b		$-SCH_2CH_2S-$	**21**	80%
	c		cis OCH_3	**22**	80%
	d		=O	**35**	Obtained from **20**, 83%
	e		cis OH	**40**	Obtained from **35**, 82%
5	a		$-OCH_2CH_2O-$	**23**	72%
	b		cis OCH_3	**24**	73%
	c		=O	**36**	Obtained from **23**, 85%
	d		H/OH	**41**	Obtained from **36**, 82%
6	a		$-SCH_2CH_2S-$	**25**	68%
	b		cis OCH_3	**26**	81%
7	a		$-SCH_2CH_2S-$	**27**	85%
	b		cis OCH_3	**28**	76%
8			cis OCH_3	**29**	76%
9			$-SCH_2CH_2S-$	**30**	57%
10			$-SCH_2CH_2S-$	**31**	82%

[a] Yields are indicated for isolated products.

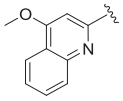
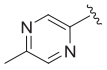
GSK3. Cellular effects on cancer cell lines were measured for each novel compound. The proposed structural modifications modulated the enzyme/drug recognition mechanism. Based on this novel scaffold, we were able to develop inhibitors exhibiting *in vitro* nanomolar activities on either both CDK5 and GSK3 with similar potency or only GSK3 with a 100 fold activity factor against CDK5. Molecular modelling provided information on the interaction of the best candidate **29** in the GSK3 binding site. Strong interactions were developed by the creation of hydrogen bonds, the carbonyl having an interaction with the hinge region (NH backbone) whereas the 2-pyridine or pyrazine urea interacts with the catalytic lysine residue. Modulations of the structure led to the three best derivatives which were evaluated in cellular assays. Antiproliferative effects were found in the nanomolar range. The strong cytostatic effect induced apoptosis in several cancer cell lines and more predominantly in lung and colon cell lines. This novel study confirms that the valmerin heterocyclic scaffold is of interest for the medicinal chemistry community and further investigations are currently in progress to envision a development of this series.

7. Experimental section

7.1. Chemistry

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 250 MHz or 400 MHz instrument using $CDCl_3$ or DMSO-d_6. The chemical shifts are reported in parts per million (δ scale) and all coupling constant (J) values are in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet doublet). Melting points are uncorrected. IR absorption spectra were obtained on a Perkin Elmer PARAGON 1000 PC and values are reported in cm^{-1}. HRMS were recorded on a Bruker maXis mass spectrometer. Monitoring of the reactions was performed using silica gel TLC plates (silica Merck 60 F254). Spots were visualized by UV light at 254 nm and 356 nm. Column chromatographies were

Table 2
Kinase inhibitions of derivatives **11**–**41**.

Entry		(Het)Ar	R/R_1	Product	CDK5 IC_{50} (μM)	GSK3α/β IC_{50} (μM)	Selectivity CDK5/GSK3	DYRK1A IC_{50} (μM)
1	a		$-OCH_2CH_2O-$	**11**	11	0.7	1.5	80
	b		$-SCH_2CH_2S-$	**12**	0.24	0.033	7.2	>10
	c		cis OCH_3	**13**	0.55	0.081	6.8	>10
	d		=O	**32**	1.6	0.23	6.3	25
	e		cis OH	**37**	2.1	0.26	8.0	34
2	a		$-OCH_2CH_2O-$	**14**	7.1	0.26	27.3	93
	b		$-SCH_2CH_2S-$	**15**	0.15	0.044	3.4	>10
	c		cis OCH_3	**16**	0.48	0.2	2.4	>10
	d		=O	**33**	3.6	1.2	3.0	50
	e		cis OH	**38**	0.084	0.032	2.6	>10
3	a		$-OCH_2CH_2O-$	**17**	>10	0.92	>10	>10
	b		$-SCH_2CH_2S-$	**18**	>10	2	>5	>10
	c		cis OCH_3	**19**	4.3	0.32	13.4	>10
	d		=O	**34**	ND	ND	ND	ND
	e		cis OH	**39**	1.0	0.030	33.3	7.0
4	a		$-OCH_2CH_2O-$	**20**	>10	1.0	>10	>10
	b		$-SCH_2CH_2S-$	**21**	>10	4	>2.5	>10
	c		cis OCH_3	**22**	8.5	0.18	47.5	>10
	d		=O	**35**	ND	ND	ND	ND
	e		cis OH	**40**	4.9	0.035	140	>10
5	a		$-OCH_2CH_2O-$	**23**	3.1	0.61	5.0	>10
	b		cis OCH_3	**24**	1.1	0.25	4.4	>10
	c		=O	**36**	ND	ND	ND	ND
	d		cis OH	**41**	0.63	0.16		6.2
6	a		$-SCH_2CH_2S-$	**25**	≥10	1.9	≥5.2	>10
	b		cis OCH_3	**26**	2.3	1.1	2.0	>10
7	a		$-SCH_2CH_2S-$	**27**	0.22	0.055	4.0	>10
	b		cis OCH_3	**28**	0.32	0.14	10.0	>10
8			cis OCH_3	**29**	0.32	0.032	10.0	>10
9			$-SCH_2CH_2S-$	**30**	>10	0.71	>14.0	8.7
10			$-SCH_2CH_2S-$	**31**	7.3	0.068	107.3	≥10

Assays were performed in triplicate.

performed using silica gel 60 (0.063–0.200 mm, Merck).

*7.1.1. 10-Nitro-1,3,4,10b-tetrahydro-6H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dithiolan]-6-one **5***

To a stirred solution of **3** (1.0 g, 3.4 mmol, 1.0 eq.) in CH_2Cl_2 (40 mL), were added drop wise, at room temperature, ethane dithiol (1.24 mL, 17.0 mmol, 5.0 eq.) and then $BF_3{\cdot}Et_2O$ (2.15 mL, 17.0 mmol, 5.0 eq.). After 24 h of stirring at room temperature, CH_2Cl_2 (40 mL) and an aq. NaOH (1 M) solution (40 mL) were added. After extraction with CH_2Cl_2 (40 mL) the combined organic layers were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Purification by column chromatography (PE/EtOAc 40/60) led to **5** as beige solid in 86% yield. m.p. 185–187 °C. IR (ATR-Diamond, cm^{-1}) ν 1689, 1524, 1416, 1345, 1079. ^1H NMR (250 MHz, $CDCl_3$) δ 1.51 (dd, J = 11.2 Hz, J = 12.9 Hz, 1H), 1.97–2.19 (m, 1H), 2.21–2.35 (m, 1H), 2.97–3.10 (m, 1H), 3.19–3.36 (m, 1H), 3.37–3.56 (m, 4H), 4.57–4.58 (m, 1H), 5.25 (dd, J = 3.1 Hz, J = 11.2 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 8.18 (dd, J = 0.8 Hz, J = 7.8 Hz, 1H), 8.36 (dd, J = 0.8 Hz, J = 8.2 Hz, 1H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 38.7 (CH_2), 39.3 (CH_2), 39.4 (CH_2), 40.7 (CH_2), 45.5 (CH_2), 59.5 (CH), 65.5 (Cq), 127.2 (CH), 130.1 (CH), 130.3 (CH), 135.9 (Cq), 139.6 (Cq), 143.8 (Cq), 163.7 (Cq). HRMS (ESI) calcd. for $C_{14}H_{15}N_2O_3S_2$ $[M+H]^+$: 323.0524, found: 323.0509.

7.1.2. cis-2-Hydroxy-10-nitro-1,3,4,10b-tetrahydropyrido[2,1-a] isoindol-6(2H)-one cis-6

To a solution of compound **4** (500 mg, 2.1 mmol, 1.0 eq.) in a THF/MeOH (10 mL/20 mL) mixture, $NaBH_4$ (155 mg, 4.2 mmol, 2.0 eq.) was slowly added portion wise at −20 °C. The solution was stirred at this temperature for 1 h and then at room temperature for 4 h. Water (10 mL) was added, and the aqueous phase was extracted first with CH_2Cl_2 (2 × 10 mL) and then with EtOAc (10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude residue was

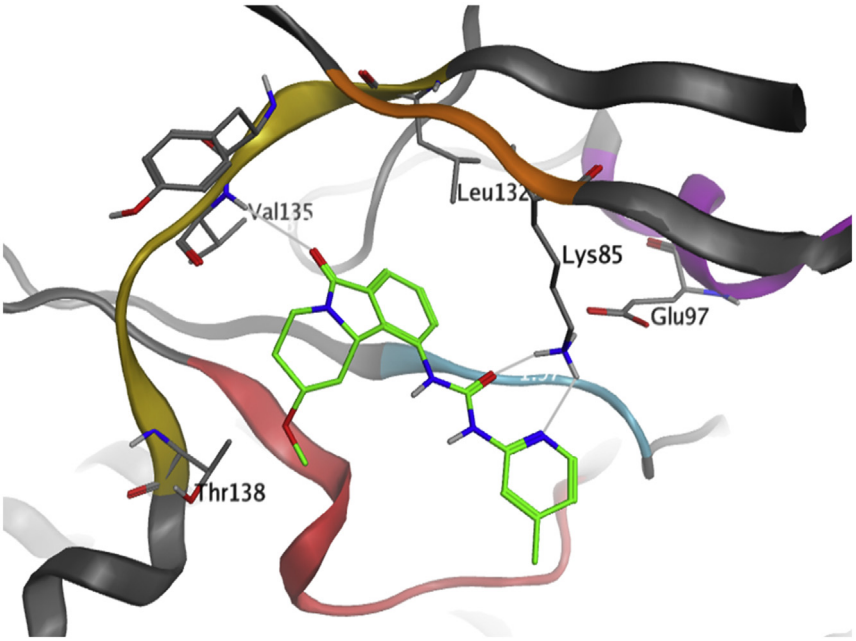
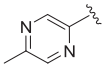


Fig. 2. Binding mode representation of compound **29** in GSK3β (PDB entry 1J1B).

Table 3
Most potent Valmerins in cell line assays.

Entry	(Het)Ar	R/R_1	Product	Human cell lines IC_{50} (μM)					
				Huh7	Caco2	MDA-MB 231	HCT 116	PC3	NCIH727
1	–	–	**Roscovitine**	5	3	3	2	2	4
2		$-SCH_2CH_2S-$	**15**	**0.1**	0.5	**0.15**	**0.1**	0.6	0.4
3		$-SCH_2CH_2S-$	**27**	0.3	0.5	0.4	0.2	0.4	0.8
4		cis OCH_3	**29**	**0.1**	0.5	0.3	**0.03**	0.4	0.4

Assays were performed in triplicate.

purified by flash chromatography on silica gel (CH_2Cl_2/MeOH 95/5) to give **cis-6** as a white solid in 14% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 2973, 1683, 1566, 1329, 1288. ^1H NMR (400 MHz, DMSO-d_6) δ 0.76 (q, J = 12.5 Hz, 1H), 1.11–1.22 (m, 1H), 1.94 (d, J = 12.5 Hz, 1H), 2.64 (d, J = 13.2 Hz, 1H), 3.08 (td, J = 5.2 Hz, J = 12.5 Hz, 1H), 3.91–3.98 (m, 1H), 4.24 (dd, J = 5.2 Hz, J = 12.5 Hz, 1H), 5.02–5.09 (m, 2H), 7.80 (t, J = 8.0 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.39 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.0 (CH_2), 36.8 (CH_2), 38.4 (CH_2), 57.9 (CH), 66.4 (CH), 127.1 (CH), 129.7 (CH), 130.3 (CH), 135.1 (Cq), 139.7 (Cq), 143.3 (Cq), 162.6 (Cq). HRMS (ESI) calcd. for $C_{12}H_{13}N_2O_4$ $[M+H]^+$: 249.0870, found: 249.0871.

7.1.3. cis-2-Methoxy-10-nitro-1,3,4,10b-tetrahydropyrido[2,1-a] isoindol-6(2H)-one **7**

To the solution of **6** (mixture cis and trans, 100 mg, 0.4 mmol, 1.0 eq.) in dry THF (5 mL), were added MeI (0.25 mL, 4.0 mmol, 10.0 eq.) and freshly prepared Ag_2O (37.0 mg, 1.6 mmol, 4.0 eq.). The mixture was stirred at 50 °C for 24 h. After cooling to room temperature, the precipitate was filtered, washed with CH_2Cl_2 (3 × 10 mL) and the combined organic layers were concentrated in vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 99/1) to afford compound **7** as a white solid in 61% yield (calcd from **4**). m.p. 132–134 °C. IR (ATR-Diamond, cm^{-1}) ν 1688, 1523, 1349, 1285, 1112, 941, 767. ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (q, J = 12.5 Hz, 1H), 1.22–1.39 (m, 1H), 2.18 (d, J = 13.2 Hz, 1H), 2.96–3.09 (m, 2H), 3.40 (s, 3H), 3.61–3.67 (m, 1H), 4.56 (dd, J = 5.0 Hz, J = 13.2 Hz, 1H), 4.96 (dd, J = 3.2 Hz, J = 11.6 Hz, 1H), 7.67 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 8.15 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 8.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.9 (CH_2), 35.0 (CH_2), 37.3 (CH_2), 56.1 (CH_3), 58.6 (CH), 76.8 (CH), 127.0 (CH), 129.9 (CH), 130.1 (CH), 135.7 (Cq), 139.6 (Cq), 143.6 (Cq), 163.5 (Cq). HRMS (ESI) calcd. for $C_{13}H_{15}N_2O_4$ $[M+H]^+$: 263.1026, found: 263.1030.

7.1.4. 10-Amino-1,3,4,10b-tetrahydro-6H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]di-thiolan]-6-one ***8***

To a stirred solution of **5** (800 mg, 2.4 mmol) in EtOH (20 mL) was added $SnCl_2$ (6.8 g, 36.0 mmol, 15.0 eq.). The mixture was stirred overnight then solvent was evaporated in vacuum keeping the temperature of the solution below 30 °C. The residue was cooled at 0 °C then neutralized successively by an aqueous NaOH (2 M) solution. After extraction with CH_2Cl_2 (40 mL), the combined organic layers were dried over $MgSO_4$, filtered and evaporated in

vacuum. The crude residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 99/1) to give compound **8** as yellow solid in 80% yield. m.p. 204–206 °C. IR (ATR-Diamond, cm^{-1}) ν 3236, 1675, 1487, 1287, 1003. ^1H NMR (250 MHz, $CDCl_3$) δ 1.63–1.77 (m, 1H), 2.02 (td, J = 5.1 Hz, J = 12.9 Hz, 1H), 2.13–2.24 (m, 1H), 2.78–2.92 (m, 1H), 3.23 (td, J = 3.2 Hz, J = 13.3 Hz, 1H), 3.31–3.50 (m, 4H), 3.81 (br s, 2H), 4.41–4.60 (m, 2H), 6.80 (dd, J = 1.3 Hz, J = 7.2 Hz, 1H), 7.17–7.37 (m, 2H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 38.4 (CH_2), 38.7 (CH_2), 39.7 (CH_2), 41.6 (CH_2), 45.7 (CH_2), 57.1 (CH), 65.7 (Cq), 114.3 (CH), 118.4 (CH), 129.0 (Cq), 129.7 (CH), 133.3 (Cq), 141.4 (Cq), 166.7 (Cq). HRMS (ESI) calcd. for $C_{14}H_{17}N_2OS_2$ $[M+H]^+$: 293.0782, found: 293.0774.

7.1.5. *cis-10-Amino-2-methoxy-1,3,4,10b-tetrahydropyrido[2,1-a] isoindol-6(2H)-one* ***9***

To a stirred solution of compound **7** (1.0 g, 3.8 mmol) in absolute EtOH (30 mL) was added freshly prepared W Raney Nikel (ca 200 mg). The resulting suspension was hydrogenated (1 atm) at room temperature for 14 h. The mixture was filtered through a pad of celite and washed with CH_2Cl_2 (3 × 10 mL). The combined organic layers were concentrated under vacuum to give compound **9** as a yellow solid in 95% yield. m.p. 60–62 °C. IR (ATR-Diamond, cm^{-1}) ν 3236, 1683, 1566, 1288. ^1H NMR (250 MHz, $CDCl_3$) δ 1.05 (q, J = 12.2 Hz, 1H), 1.34–1.41 (m, 1H), 2.18 (d, J = 12.2 Hz, 1H), 2.80 (d, J = 12.2 Hz, 1H), 2.96 (td, J = 2.5 Hz, J = 12.0 Hz, 1H), 3.40 (s, 3H), 3.52–3.62 (m, 1H), 3.82 (br s, 2H), 4.30 (dd, J = 5.0 Hz, J = 12.0 Hz, 1H), 4.54 (dd, J = 5.0 Hz, J = 11.7 Hz, 1H), 6.80 (d, J = 8.5 Hz, 1H), 7.25 (m, 2H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 30.9 (CH_2), 35.5 (CH_2), 37.0 (CH_2), 55.9 (CH_3), 56.2 (CH), 77.1 (CH), 114.2 (CH), 118.3 (CH), 129.0 (Cq), 129.4 (CH), 133.2 (Cq), 141.2 (Cq), 166.6 (Cq). HRMS (ESI) calcd. for $C_{13}H_{17}N_2O_2$ $[M+H]^+$: 233.1285, found: 233.1289.

7.1.6. *10-Amino-1,3,4,10b-tetrahydro-6H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3] dioxolan]-6-one* ***10***

Derivative **10** was prepared from **3** as previously described for **8**. The crude residue was purified by silica gel chromatography (CH_2Cl_2/MeOH 99/1) to give compound **10** as a white solid in 82% yield. m.p. 76–78 °C. IR (ATR-Diamond, cm^{-1}) ν 1710, 1542, 1366, 1143, 1028. ^1H NMR (250 MHz, $CDCl_3$) δ 1.38 (t, J = 12.5 Hz, 1H), 1.67 (td, J = 5.7 Hz, J = 13.0 Hz, 1H), 1.81–1.83 (m, 1H), 2.44–2.46 (m, 1H), 3.23 (td, J = 3.9 Hz, J = 13.0 Hz, 1H), 3.74 (br s, 2H), 3.99–4.14 (m, 4H), 4.41–4.61 (m, 2H), 6.81 (dd, J = 1.8 Hz, J = 7.0 Hz, 1H), 7.24–7.36 (m, 2H). ^{13}C NMR (63 MHz, $CDCl_3$) δ 34.1 (CH_2), 36.6 (CH_2), 39.1 (CH_2), 55.8 (CH), 64.9 (CH_2), 65.1 (CH_2), 107.7 (Cq), 114.5 (CH), 118.4 (CH), 129.5 (Cq), 129.6 (CH), 133.5 (Cq), 141.2 (Cq), 166.8 (Cq). HRMS (ESI) calc. for $C_{14}H_{17}N_2O_3$ $[M+H]^+$: 261.1239, found: 261.1238.

7.1.7. *General procedure A for the urea synthesis*

Under Argon, a stirred solution of carboxylic acid (0.37 mmol, 1.0 eq.) and Et_3N (0.48 mmol, 1.3 eq.) in dry THF (7 mL) was cooled to −10 °C. Ethyl chloroformate (0.55 mmol, 1.5 eq.) was drop wise added and the resulting mixture was stirred for 2 h. Afterwards, a solution of sodium azide (0.63 mmol, 1.7 eq.) in water (2 mL) was added in one portion. After 1 h at −10 °C, the reaction was found to be complete (TLC) and was quenched into iced water (5 mL). The mixture was extracted with EtOAc (3 × 10 mL) and the combined organic layers were successively dried over $MgSO_4$, filtered and evaporated. The crude acyl azide was placed in dry toluene (20 mL) and heated at reflux for 1 h to give the corresponding crude isocyanate. The latter was placed in dry dioxane (7 mL) prior to adding the appropriate amine **8**, **9** or **10** (0.37 mmol, 1.0 eq.). The solution was heated at 100 °C for 24 h. The reaction mixture was cooled and the volatiles were removed to dryness in vacuum at 40 °C.

7.1.8. *1-(6-Oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dioxolan]-10-yl)-3-(pyridin-2-yl)urea* ***11***

Compound **11** was obtained following the general procedure **A** from the amine **10** and pyridin-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 78% yield. m.p. 178–180 °C. IR (ATR-Diamond, cm^{-1}) ν 3305–3224, 1651, 1529–1485–1427, 1288, 1200, 723. ^1H NMR (400 MHz, DMSO-d_6) δ 1.27 (t, J = 12.3 Hz, 1H), 1.50 (td, J = 5.7 Hz, J = 13.1, 1H), 1.85 (d, J = 13.1 Hz, 1H), 2.62 (dd, J = 2.1 Hz, J = 9.3 Hz, 1H), 3.16 (td, J = 3.4 Hz, J = 13.1 Hz, 1H), 3.85 (dd, J = 6.3 Hz, J = 14.1 Hz, 1H), 3.96 (dt, J = 6.3 Hz, J = 12.3 Hz, 1H), 4.01–4.09 (m, 2H), 4.29 (dd, J = 4.7 Hz, J = 13.1 Hz, 1H), 4.81 (dd, J = 3.4 Hz, J = 12.3 Hz, 1H), 7.09 (dd, J = 5.5 Hz, J = 6.8 Hz, 1H), 7.26 (d, J = 8.3 Hz, 1H), 7.36–7.43 (m, 1H), 7.47 (t, J = 7.7 Hz, 1H), 7.74–7.86 (m, 1H), 8.21–8.35 (m, 2H), 9.95 (s, 1H), 11.42 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 33.0 (CH_2), 36.1 (CH_2), 38.3 (CH_2), 55.5 (CH), 64.0 (CH_2), 64.3 (CH_2), 106.8 (Cq), 112.1 (CH), 117.5 (CH), 117.6 (CH), 122.5 (CH), 129.1 (CH), 132.7 (Cq), 133.9 (Cq), 134.2 (Cq), 139.1 (CH), 146.0 (CH), 152.1 (Cq), 152.9 (Cq), 164.8 (Cq). HRMS (ESI) calc. for $C_{20}H_{21}N_4O_4$ $[M+H]^+$: 381.1563, found: 381.1553.

7.1.9. *1-(6-Oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dithiolan]-10-yl)-3-(pyridin-2-yl)urea* ***12***

Compound **12** was obtained following the general procedure **A** from the amine **8** and pyridin-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in a 96% yield. m.p. 242–244 °C. IR (ATR-Diamond, cm^{-1}) ν 1703, 1677, 1553, 1510, 1483, 1418, 1310, 1153. ^1H NMR (400 MHz, DMSO-d_6) δ 1.22–1.37 (m, 1H), 1.75 (t, J = 11.3 Hz, 1H), 1.92 (d, J = 13.3 Hz, 1H), 2.72 (dd, J = 2.6 Hz, J = 12.6 Hz, 1H), 2.97–3.14 (m, 1H), 3.23–3.52 (m, 4H), 4.27 (dd, J = 4.4 Hz, J = 13.0 Hz, 1H), 4.59 (dd, J = 3.3 Hz, J = 11.5 Hz, 1H), 7.03–7.12 (m, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.39 (dd, J = 0.8 Hz, J = 7.4 Hz, 1H), 7.45 (t, J = 7.7 Hz, 1H), 7.75–7.86 (m, 1H), 8.23 (d, J = 7.9 Hz, 1H), 8.26–8.33 (m, 1H), 10.00 (s, 1H), 11.17 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.7 (CH_2), 38.2 (CH_2), 38.9 (CH_2), 40.7 (CH_2), 44.1 (CH_2), 56.9 (CH), 65.6 (Cq), 112.1 (CH), 117.4 (CH), 117.8 (CH), 123.6 (CH), 129.1 (CH), 132.7 (Cq), 133.8 (Cq), 134.1 (Cq), 139.1 (CH), 146.4 (CH), 152.2 (Cq), 152.9 (Cq), 164.8 (Cq). HRMS (ESI) calc. for $C_{20}H_{20}N_4O_2S_2Na$ $[M+Na]^+$: 435.0878, found: 435.0870.

7.1.10. *cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(pyridin-2-yl)urea* ***13***

Compound **13** was prepared following the general procedure **A** from the amine **9** and pyridin-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 81% yield. m.p. 166–168 °C. IR (ATR-Diamond, cm^{-1}) ν 1688, 1602, 1579, 1478, 1418, 1312, 1291, 751. ^1H NMR (400 MHz, $CDCl_3$) δ 1.04 (q, J = 12.5 Hz, 1H), 1.13–1.42 (m, 1H), 2.21 (d, J = 12.5 Hz, 1H), 3.01–3.09 (m, 2H), 3.37 (s, 3H), 3.59–3.65 (m, 1H), 4.60 (dd, J = 3.2 Hz, J = 12.5 Hz, 2H), 6.90 (d, J = 8.0 Hz, 1H), 7.01 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.49 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.64 (d, J = 7.5 Hz, 1H), 7.70 (td, J = 7.5 Hz, J = 8.0 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.26 (d, J = 4.0 Hz, 1H), 8.66 (s, 1H), 11.98 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.7 (CH_2), 35.6 (CH_2), 37.1 (CH_2), 55.9 (CH_3), 57.0 (CH), 77.4 (CH), 112.4 (CH), 117.7 (CH), 119.5 (CH), 124.5 (CH), 129.4 (CH), 133.2 (Cq), 133.3 (Cq), 135.0 (Cq), 139.2 (CH), 145.5 (CH), 152.7 (Cq), 153.3 (Cq), 166.0 (Cq). HRMS (ESI) calc. for $C_{19}H_{21}N_4O_3$ $[M+H]^+$: 353.1614, found: 353.1603.

7.1.11. *1-(6-Oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dioxolan]-10-yl)-3-(pyrazin-2-yl)urea* ***14***

Compound **14** was obtained following the general procedure **A** from the amine **10** and pyrazin-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a pale brown solid in 68% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 1698, 1570,

1549–1504–1478, 1298, 1244, 1073. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.23 (t, J = 12.3 Hz, 1H), 1.51 (td, J = 5.7 Hz, J = 13.1 Hz, 1H), 1.83 (d, J = 13.1 Hz, 1H), 2.55 (d, J = 11.5 Hz, 1H), 3.15 (td, J = 4.0 Hz, J = 13.1 Hz, 1H), 3.83–4.00 (m, 2H), 4.01–4.14 (m, 2H), 4.28 (dd, J = 4.0 Hz, J = 13.3 Hz, 1H), 4.78 (dd, J = 3.6 Hz, J = 12.0 Hz, 1H), 7.43 (d, J = 6.9 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 8.13 (d, J = 7.8 Hz, 1H), 8.25–8.34 (m, 2H), 8.88 (s, 1H), 10.03 (s, 1H), 10.12 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 33.1 (CH_2), 36.1 (CH_2), 38.1 (CH_2), 55.5 (CH), 64.0 (CH_2), 64.3 (CH_2), 106.8 (Cq), 118.0 (CH), 123.3 (CH), 129.1 (CH), 132.8 (Cq), 133.5 (Cq), 134.8 (Cq), 135.5 (CH), 137.8 (CH), 140.8 (CH), 149.2 (Cq), 151.7 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{19}H_{19}N_5O_4Na$ $[M+Na]^+$: 404.1335, found: 404.1327.

7.1.12. 1-(6-Oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dithiolan]-10-yl)-3-(pyrazin-2-yl)urea **15**

Compound **15** was obtained following the general procedure **A** from the amine **8** and pyrazin-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 90% yield. m.p. 236–238 °C. IR (ATR-Diamond, cm^{-1}) ν 1676, 1551, 1505–1483–1421, 1298, 1138. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.63 (t, J = 12.3 Hz, 1H), 1.96 (td, J = 5.0 Hz, J = 12.3 Hz, 1H), 2.14 (d, J = 13.2 Hz, 1H), 2.89 (d, J = 11.5 Hz, 1H), 3.16 (td, J = 3.1 Hz, J = 13.3 Hz, 1H), 3.28–3.48 (m, 4H), 4.33 (dd, J = 3.1 Hz, J = 13.3 Hz, 1H), 4.79 (dd, J = 3.1 Hz, J = 11.5 Hz, 1H), 7.42–7.54 (m, 2H), 8.08 (dd, J = 0.9 Hz, J = 7.8 Hz, 1H), 8.29 (d, J = 2.7 Hz, 1H), 8.40 (dd, J = 1.5 Hz, J = 2.6 Hz, 1H), 8.83 (d, J = 1.1 Hz, 1H), 10.13 (s, 1H), 10.19 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.7 (CH_2), 38.2 (CH_2), 38.7 (CH_2), 40.5 (CH_2), 44.0 (CH_2), 56.9 (CH), 65.6 (Cq), 118.3 (CH), 124.1 (CH), 129.1 (CH), 132.8 (Cq), 133.4 (Cq), 134.7 (Cq), 135.6 (CH), 137.7 (CH), 140.8 (CH), 149.2 (Cq), 151.7 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{19}H_{19}N_5O_2S_2Na$ $[M+Na]^+$: 436.0878, found: 436.0870.

7.1.13. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(pyrazin-2-yl)urea **16**

Compound **16** was prepared following the general procedure **A** from the amine **9** and pyrazin-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 78% yield. m.p. 192–194 °C. IR (ATR-Diamond, cm^{-1}) ν 1684, 1595, 1557, 1486, 1421, 1306, 1084, 753. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (q, J = 12.5 Hz, 1H), 1.14–1.24 (m, 1H), 2.19 (d, J = 12.5 Hz, 1H), 2.97 (d, J = 13.5 Hz, 1H), 3.14 (td, J = 3.2 Hz, J = 12.5 Hz, 1H), 3.28 (s, 3H), 3.69–3.77 (m, 1H), 4.34 (dd, J = 4.0 Hz, J = 13.5 Hz, 1H), 4.73 (dd, J = 3.2 Hz, J = 13.5 Hz, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.54 (dd, J = 7.8 Hz, J = 8.0 Hz, 1H), 8.20 (d, J = 8.0 Hz, 1H), 8.35 (d, J = 3.2 Hz, 1H), 8.40 (dd, J = 1.6 Hz, J = 3.2 Hz, 1H), 9.00 (d, J = 1.6 Hz, 1H), 9.85 (s, 1H), 10.14 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.7 (CH_2), 35.3 (CH_2), 36.4 (CH_2), 55.2 (CH_3), 55.8 (CH), 76.3 (CH), 118.0 (CH), 123.1 (CH), 129.1 (CH), 132.8 (Cq), 133.5 (Cq), 134.5 (Cq), 135.5 (CH), 137.9 (CH), 141.1 (CH), 149.2 (Cq), 151.7 (Cq), 164.6 (Cq). HRMS (ESI) calc. for $C_{18}H_{19}N_5O_3Na$ $[M+Na]^+$: 376.1386, found: 376.1370.

7.1.14. 1-(6-Methylpyridin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dioxolan]-10-yl)urea **17**

Compound **17** was prepared following the general procedure **A** from the amine **10** and 6-methylpicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 75% yield. m.p. 240–242 °C. IR (ATR-Diamond, cm^{-1}) ν 3180, 1685, 1584, 1560, 1510, 1327, 1287, 751. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (t, J = 12.5 Hz, 1H), 1.69 (td, J = 5.0 Hz, J = 12.5 Hz, 1H), 1.80 (d, J = 12.5 Hz, 1H), 2.53 (d, J = 13.5 Hz, 1H), 2.60 (s, 3H), 3.27 (td, J = 2.5 Hz, J = 13.5 Hz, 1H), 3.74–4.05 (m, 4H), 4.53 (dd, J = 5.0 Hz, J = 12.5 Hz, 1H), 4.93 (dd, J = 2.5 Hz, J = 12.5 Hz, 1H), 6.73 (d, J = 7.5 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 7.47–7.59 (m, 2H), 7.67 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H), 9.16 (s, 1H), 12.14 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.8 (CH_3), 33.0 (CH_2), 35.5 (CH_2), 37.2 (CH_2), 55.5 (CH), 63.5 (CH_2), 63.6 (CH_2), 106.4 (Cq), 108.3 (CH), 115.7 (CH), 118.7 (CH), 124.9 (CH), 128.1 (CH), 131.8 (Cq), 132.3 (Cq), 135.1 (Cq), 138.2 (CH), 151.4 (Cq), 152.9 (Cq), 154.4 (Cq), 165.1 (Cq). HRMS (ESI) calc. for $C_{21}H_{23}N_4O_4$ $[M+H]^+$: 395.1719, found: 395.1733.

7.1.15. 1-(6-Methylpyridin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dithiolan]-10-yl)urea **18**

Compound **18** was prepared following the general procedure **A** from the amine **8** and 6-methylpicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 78% yield. m.p. 154–156 °C. IR (ATR-Diamond, cm^{-1}) ν 2922, 1689, 1622, 1596, 1429, 1355, 1290, 741. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.61 (t, J = 12.5 Hz, 1H), 2.03 (td, J = 5.0 Hz, J = 12.5 Hz, 1H), 2.18 (d, J = 12.5 Hz, 1H), 2.60 (s, 3H), 2.89 (d, J = 13.5 Hz, 1H), 3.14–3.29 (m, 5H), 4.53 (dd, J = 2.5 Hz, J = 13.5 Hz, 1H), 4.92 (dd, J = 2.5 Hz, J = 13.5 Hz, 1H), 6.83 (d, J = 8.0 Hz, 2H), 7.47 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.58–7.65 (m, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.0 Hz, 1H), 9.48 (s, 1H), 12.05 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.3 (CH_3), 38.3 (CH_2), 38.6 (CH_2), 38.8 (CH_2), 40.5 (CH_2), 45.1 (CH_2), 57.8 (CH), 65.5 (Cq), 109.8 (CH), 116.8 (CH), 120.4 (CH), 127.0 (CH), 129.1 (2 CH), 132.4 (Cq), 133.4 (Cq), 136.8 (2 Cq), 152.1 (Cq), 154.0 (Cq), 165.8 (Cq). HRMS (ESI) calc. for $C_{21}H_{23}N_4O_2S_2$ $[M+H]^+$: 427.1262, found: 427.1278.

7.1.16. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(6-methyl-pyridin-2-yl)urea **19**

Compound **19** was prepared from following the general procedure **A** from the amine **9** and 6-methylpicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 75% yield. m.p. 167–169 °C. IR (ATR-Diamond, cm^{-1}) ν 1689, 1589, 1556, 1436, 1283, 751. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (q, J = 12.5 Hz, 1H), 1.27–1.38 (m, 1H), 2.19 (d, J = 12.5 Hz, 1H), 2.56 (s, 3H), 2.93 (d, J = 12.5 Hz, 1H), 3.02 (td, J = 3.2 Hz, J = 13.5 Hz, 1H), 3.30 (s, 3H), 3.53–3.59 (m, 1H), 4.58 (dd, J = 4.5 Hz, J = 13.5 Hz, 1H), 4.69 (dd, J = 3.2 Hz, J = 12.5 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 6.83 (d, J = 7.5 Hz, 1H), 7.49 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.55 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 8.93 (s, 1H), 11.98 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.4 (CH_3), 30.6 (CH_2), 35.1 (CH_2), 37.1 (CH_2), 55.8 (CH_3), 57.2 (CH), 77.3 (CH), 109.4 (CH), 117.0 (CH), 120.0 (CH), 125.8 (CH), 129.2 (CH), 133.0 (Cq), 133.4 (Cq), 135.8 (Cq), 139.2 (CH), 152.3 (Cq), 153.7 (Cq), 155.0 (Cq), 166.0 (Cq). HRMS (ESI) calc. for $C_{20}H_{23}N_4O_3$ $[M+H]^+$: 367.1770, found: 367.1776.

7.1.17. 1-(4-Methoxyquinolin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dioxolan]-10-yl)urea **20**

Compound **20** was prepared following the general procedure **A** from the amine **10** and 4-methoxyquinoline-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 72% yield. m.p. 258–260 °C. IR (ATR-Diamond, cm^{-1}) ν 2967, 1689, 1621, 1585, 1414, 1332, 1289, 751. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (t, J = 12.5 Hz, 1H), 1.62–1.81 (m, 2H), 2.72 (d, J = 13.5 Hz, 1H), 3.27–3.39 (m, 2H), 3.59–3.77 (m, 3H), 4.02 (s, 3H), 4.56 (dd, J = 2.5 Hz, J = 13.5 Hz, 1H), 5.01 (dd, J = 2.5 Hz, J = 13.5 Hz, 1H), 6.47 (s, 1H), 7.38–7.49 (m, 2H), 7.67–7.73 (m, 2H), 7.95 (d, J = 7.5 Hz, 1H), 8.10 (d, J = 7.5 Hz, 1H), 8.23 (d, J = 7.5 Hz, 1H), 9.86 (s, 1H), 12.57 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.1 (CH_2), 36.6 (CH_2), 38.3 (CH_2), 55.8 (CH_3), 56.5 (CH), 64.5 (CH_2), 64.6 (CH_2), 91.5 (CH), 107.2 (Cq), 118.8 (Cq), 119.6 (CH), 121.9 (CH), 124.2 (CH), 125.3 (CH), 126.5 (CH), 128.8 (CH), 130.5 (CH), 133.0 (Cq), 133.3 (Cq), 135.7 (Cq), 145.9 (Cq), 153.4 (Cq), 154.4 (Cq), 163.9 (Cq), 166.1 (Cq). HRMS (ESI) calc. for $C_{25}H_{25}N_4O_5$ $[M+H]^+$: 461.1825, found: 461.1844.

*7.1.18. 1-(4-Methoxyquinolin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dithiolan]-10-yl)urea **21***

Compound **21** was prepared following the general procedure **A** from the amine **8** and 4-methoxyquinoline-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 80% yield. m.p. 250–252 °C. IR (ATR-Diamond, cm^{-1}) ν 2975, 1685, 1621, 1585, 1414, 1332, 1289, 751. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (t, J = 12.5 Hz, 1H), 2.06 (td, J = 5.0 Hz, J = 12.5 Hz, 1H), 2.19 (d, J = 12.5 Hz, 1H), 2.47–2.57 (m, 1H), 2.78–3.13 (m, 4H), 3.19 (td, J = 5.0 Hz, J = 13.5 Hz, 1H), 4.00 (s, 3H), 4.58 (dd, J = 2.5 Hz, J = 13.5 Hz, 1H), 5.07 (dd, J = 2.5 Hz, J = 12.5 Hz, 1H), 6.48 (s, 1H), 7.39 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.64 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.72 (d, J = 7.5 Hz, 1H), 8.00 (dd, J = 7.5 Hz, J = 8.0 Hz, 2H), 8.08 (d, J = 7.5 Hz, 1H), 10.01 (s, 1H), 12.56 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.2 (CH_2), 38.3 (CH_2), 39.0 (CH_2), 40.2 (CH_2), 45.4 (CH_2), 55.8 (CH_3), 57.7 (CH), 65.3 (Cq), 91.5 (CH), 118.7 (Cq), 120.2 (CH), 121.8 (CH), 124.2 (CH), 126.8 (CH), 127.0 (CH), 128.7 (CH), 130.4 (CH), 132.7 (Cq), 133.4 (Cq), 136.4 (Cq), 145.8 (Cq), 153.4 (Cq), 154.6 (Cq), 163.9 (Cq), 165.9 (Cq). HRMS (ESI) calc. for $C_{25}H_{25}N_4O_3S_2$ $[M+H]^+$: 493.1368, found: 493.1384.

*7.1.19. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(4-methoxyquinolin-2-yl)urea **22***

Compound **22** was prepared following the general procedure **A** from the amine **9** and 4-methoxyquinoline-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 80% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 1694, 1610, 1556, 1479, 1416, 1338, 1295, 759. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 (q, J = 12.5 Hz, 1H), 1.05–1.15 (m, 1H), 2.09 (d, J = 12.5 Hz, 1H), 2.82 (s, 3H), 2.91 (d, J = 13.5 Hz, 1H), 3.15 (td, J = 3.2 Hz, J = 13.5 Hz, 1H), 3.59 (d, J = 12.0 Hz, 1H), 4.01 (s, 3H), 4.30 (dd, J = 4.0 Hz, J = 13.5 Hz, 1H), 4.92 (dd, J = 3.2 Hz, J = 12.0 Hz, 1H), 6.84 (s, 1H), 7.41–7.51 (m, 3H), 7.77 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 10.15 (s, 1H), 11.99 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 30.8 (CH_2), 34.4 (CH_2), 36.4 (CH_2), 54.7 (CH_3), 55.9 (CH), 56.0 (CH_3), 76.4 (CH), 91.9 (CH), 117.8 (CH), 118.1 (Cq), 121.5 (CH), 123.6 (CH), 124.1 (CH), 126.3 (CH), 128.9 (CH), 130.7 (CH), 132.8 (Cq), 133.7 (Cq), 134.3 (Cq), 145.6 (Cq), 152.3 (Cq), 153.8 (Cq), 162.9 (Cq), 164.6 (Cq). HRMS (ESI) calc. for $C_{24}H_{25}N_4O_4$ $[M+H]^+$: 433.1876, found: 433.1884.

*7.1.20. 1-(3-Methylpyridin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dioxolan]-10-yl)urea **23***

Compound **23** was prepared following the general procedure **A** from the amine **10** and 3-methylpicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 72% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 1679, 1566, 1480–1415, 1291, 1135. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.35 (t, J = 12.5 Hz, 1H), 1.53 (td, J = 5.0 Hz, J = 12.5 Hz, 1H), 1.86 (d, J = 12.5 Hz, 1H), 2.32 (s, 3H), 2.62 (d, J = 12.5 Hz, 1H), 3.17 (td, J = 3.2 Hz, J = 13.5 Hz, 1H), 3.81–4.07 (m, 4H), 4.30 (dd, J = 3.2 Hz, J = 13.5 Hz, 1H), 4.84 (dd, J = 3.3 Hz, J = 12.5 Hz, 1H), 7.09 (dd, J = 7.0 Hz, J = 8.0 Hz, 1H), 7.39–7.50 (m, 2H), 7.69 (d, J = 7.0 Hz, 1H), 8.23 (d, J = 7.5 Hz, 1H), 8.33 (d, J = 7.5 Hz, 1H), 8.85 (s, 1H), 12.4 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.5 (CH_3) 34.4 (CH_2), 36.4 (CH_2), 37.8 (CH_2), 55.7 (CH), 64.3 (CH_2), 64.6 (CH_2), 107.4 (Cq), 110.8 (CH), 112.7 (CH), 117.5 (CH), 129.2 (CH), 133.0 (Cq), 134.6 (Cq), 137.2 (CH), 143.3 (Cq), 143.9 (Cq), 145.5 (CH), 151.6 (Cq), 152.7 (Cq), 166.2 (Cq). HRMS (ESI) calc. for $C_{21}H_{23}N_4O_4$ $[M+H]^+$: 395.1719, found: 395.1731.

*7.1.21. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(3-methylpyridin-2-yl)urea **24***

Compound **24** was prepared following the general procedure **A** from the amine **9** and 3-methylpicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 73% yield. m.p. 222–224 °C. IR (ATR-Diamond, cm^{-1}) ν 1692, 1592, 1554, 1484, 1421, 1298, 1189, 751. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (q, J = 12.5 Hz, 1H), 1.30–1.40 (m, 1H), 2.20 (d, J = 12.5 Hz, 1H), 2.31 (s, 3H), 3.03 (td, J = 3.2 Hz, J = 12.5 Hz, 2H), 3.36 (s, 3H), 3.60–3.65 (m, 1H), 4.58 (dd, J = 3.2 Hz, J = 12.0 Hz, 2H), 6.95 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.11 (s, 1H), 7.46 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H), 8.13–8.16 (m, 2H), 12.24 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.1 (CH_3), 30.9 (CH_2), 35.7 (CH_2), 37.2 (CH_2), 56.1 (CH_3), 57.1 (CH), 77.6 (CH), 117.9 (CH), 119.6 (CH), 119.7 (Cq), 124.6 (CH), 129.5 (CH), 133.2 (Cq), 133.3 (Cq), 135.0 (Cq), 139.9 (CH), 143.2 (CH), 151.2 (Cq), 152.7 (Cq), 166.2 (Cq). HRMS (ESI) calc. for $C_{20}H_{23}N_4O_3$ $[M+H]^+$: 367.1770, found: 367.1778.

*7.1.22. 1-(1-Methyl-1H-pyrazol-3-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido [2,1-a]isoindole-2,2′-[1,3]dithiolan]-10-yl)urea **25***

Compound **25** was obtained following the general procedure **A** from the amine **8** and 1-methyl-1*H*-pyrazole-3-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 68% yield. m.p. 134–136 °C. IR (ATR-Diamond, cm^{-1}) ν 3256, 2923, 1676, 1530, 1485, 1287. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.62 (t, J = 12.5 Hz, 1H), 2.01 (td, J = 5.0 Hz, J = 12.5 Hz, 1H), 2.17 (d, J = 12.5 Hz, 1H), 2.95 (d, J = 13.5 Hz, 1H), 3.16–3.33 (m, 5H), 3.87 (s, 3H), 4.52 (dd, J = 3.8 Hz, J = 13.5 Hz, 1H), 4.81 (dd, J = 3.1 Hz, J = 11.5 Hz, 1H), 5.91 (s, 1H), 7.23 (d, J = 2.2 Hz, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 8.82 (s, 1H), 10.08 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.4 (CH_2), 38.8 (CH_2), 38.9 (CH_2), 39.2 (CH_3), 41.0 (CH_2), 45.0 (CH_2), 57.8 (CH), 65.8 (Cq), 94.6 (CH), 119.8 (CH), 126.3 (CH), 129.3 (CH), 131.4 (CH), 133.2 (Cq), 133.3 (Cq), 136.0 (Cq), 148.5 (Cq), 153.5 (Cq), 166.3 (Cq). HRMS (ESI) calc. for $C_{19}H_{21}N_5O_2S_2Na$ $[M+Na]^+$: 438.1034, found: 438.1025.

*7.1.23. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(1-methyl-1H-pyrazol-3-yl)urea **26***

Compound **26** was prepared following the general procedure **A** from the amine **9** and 1-methyl-1*H*-pyrazole-3-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 81% yield. m.p. 196–198 °C. IR (ATR-Diamond, cm^{-1}) ν 1686, 1625, 1538, 1482–1421, 1316, 1284, 751. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (q, J = 12.0 Hz, 1H), 1.27–1.39 (m, 1H), 2.21 (d, J = 12.0 Hz, 1H), 2.97–3.08 (m, 2H), 3.35 (s, 3H), 3.55–3.60 (m, 1H), 3.87 (s, 3H), 4.56–4.60 (m, 2H), 5.84 (s, 1H), 7.27 (d, J = 2.2 Hz, 1H), 7.45 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.62 (d, J = 7.5 Hz, 1H), 7.98 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 10.06 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.3 (CH_2), 35.7 (CH_2), 37.0 (CH_2), 38.8 (CH_3), 55.7 (CH_3), 56.9 (CH), 77.2 (CH), 94.1 (CH), 119.5 (CH), 125.0 (CH), 129.2 (CH), 131.5 (CH), 133.2 (Cq), 133.3 (Cq), 135.2 (Cq), 148.2 (Cq), 152.8 (Cq), 166.0 (Cq). HRMS (ESI) calc. for $C_{18}H_{21}N_5O_3Na$ $[M+Na]^+$: 378.1542, found: 378.1542.

*7.1.24. 1-(5-Methylpyrazin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a] isoindole-2,2′-[1,3]dithiolan]-10-yl)urea **27***

Compound **27** was prepared following the general procedure **A** from the amine **8** and 5-methylpyrazine-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 85% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 2977, 1688, 1621, 1586, 1414, 1332, 1288, 753. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.61 (t, J = 12.2 Hz, 1H), 1.93 (td, J = 5.0 Hz, J = 12.2 Hz, 1H), 2.12 (d, J = 12.5 Hz, 1H), 2.43 (s, 3H), 2.87 (d, J = 12.5 Hz, 1H), 3.14 (t, J = 12.2 Hz, 1H), 3.29–3.35 (m, 3H), 3.39–3.44 (m, 1H), 4.32 (dd, J = 2.8 Hz, J = 12.2 Hz, 1H), 4.75 (dd, J = 2.8 Hz, J = 12.2 Hz, 1H), 7.42–7.50 (m, 2H), 8.06 (d, J = 7.5 Hz, 1H), 8.27 (s, 1H), 8.73 (s, 1H), 10.04 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.2 (CH_3), 37.8 (CH_2), 38.2 (CH_2), 38.7 (CH_2), 40.5 (CH_2), 41.0 (CH_2), 56.9 (CH), 65.6 (Cq),

118.2 (CH), 124.0 (CH), 129.1 (CH), 132.8 (Cq), 133.5 (Cq), 134.3 (CH), 134.6 (Cq), 139.7 (CH), 146.0 (Cq), 146.9 (Cq), 151.8 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{20}H_{22}N_5O_2S_2$ $[M+H]^+$: 428.1215, found: 428.1229.

7.1.25. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(5-methylpyrazin-2-yl)urea ***28***

Compound **28** was prepared following the general procedure **A** from the amine **9** and 5-methylpyrazine-2-carboxylic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 76% yield. m.p. 140–142 °C. IR (ATR-Diamond, cm^{-1}) ν 1688, 1598, 1554, 1484, 1438, 1344, 1291, 753. ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (q, *J* = 12.0 Hz, 1H), 1.30–1.40 (m, 1H), 2.22 (d, *J* = 12.5 Hz, 1H), 2.54 (s, 3H), 2.96–3.06 (m, 2H), 3.38 (s, 3H), 3.53–3.61 (m, 1H), 4.53–4.62 (m, 2H), 7.49 (dd, *J* = 7.5 Hz, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 7.5 Hz, 1H), 8.02 (s, 1H), 8.11 (d, *J* = 8.0 Hz, 1H), 8.42 (s, 1H), 9.80 (s, 1H), 11.16 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6 (CH_3), 30.5 (CH_2), 35.7 (CH_2), 37.1 (CH_2), 55.9 (CH_3), 56.9 (CH), 77.3 (CH), 120.0 (CH), 124.6 (CH), 129.5 (CH), 132.6 (Cq), 133.3 (Cq), 135.0 (Cq), 135.1 (CH), 137.7 (CH), 146.6 (Cq), 146.7 (Cq), 153.5 (Cq), 165.9 (Cq). HRMS (ESI) calc. for $C_{19}H_{22}N_5O_3$ $[M+H]^+$: 368.1723, found: 368.1722.

7.1.26. cis-1-(2-Methoxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(4-methylpyridin-2-yl)urea ***29***

Compound **29** was prepared following the general procedure **A** from the amine **9** and 4-methylpicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 76% yield. m.p. 218–220 °C. IR (ATR-Diamond, cm^{-1}) ν 1690, 1619, 1573, 1481–1439, 1310, 1293, 752. ^1H NMR (400 MHz, DMSO-d_6) δ 0.75 (q, *J* = 12.0 Hz, 1H), 1.08–1.19 (m, 1H), 2.14 (d, *J* = 12.0 Hz, 1H), 2.30 (s, 3H), 2.99 (d, *J* = 12.0 Hz, 1H), 3.10 (td, *J* = 3.2 Hz, *J* = 13.2 Hz, 1H), 3.25 (s, 3H), 3.69–3.77 (m, 1H), 4.29 (dd, *J* = 3.2 Hz, *J* = 13.2 Hz, 1H), 4.70 (dd, *J* = 3.2 Hz, *J* = 12.0 Hz, 1H), 6.92 (d, *J* = 5.2 Hz, 1H), 7.08 (s, 1H), 7.37 (d, *J* = 7.5 Hz, 1H), 7.46 (dd, *J* = 7.5 Hz, *J* = 8.0 Hz, 1H), 8.20 (d, *J* = 5.2 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 9.86 (s, 1H), 11.43 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 20.8 (CH_3), 30.7 (CH_2), 35.2 (CH_2), 36.5 (CH_2), 55.2 (CH_3), 55.8 (CH), 76.3 (CH), 112.1 (CH), 117.4 (CH), 118.8 (CH), 122.2 (CH), 129.1 (CH), 132.7 (Cq), 133.9 (Cq), 134.0 (Cq), 145.6 (CH), 150.0 (Cq), 152.2 (Cq), 152.9 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{20}H_{23}N_4O_3$ $[M+H]^+$: 367.1770, found: 367.1761.

7.1.27. (6-Bromopyridin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a]isoin-dole-2,2′-[1,3]dithiolan]-10-yl)urea ***30***

Compound **30** was obtained following the general procedure **A** from the amine **8** and 6-bromopicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 57% yield. m.p. 168–170 °C. IR (ATR-Diamond, cm^{-1}) ν 1654, 1565, 1530, 1486, 1430, 786. ^1H NMR (400 MHz, $CDCl_3$) δ 1.66 (t, *J* = 12.0 Hz, 1H), 2.03 (td, *J* = 5.0 Hz, *J* = 13.4, 1H), 2.18 (d, *J* = 13.5 Hz, 1H), 2.85 (dd, *J* = 1.9 Hz, *J* = 10.0 Hz, 1H), 3.09–3.38 (m, 5H), 4.55 (dd, *J* = 3.7 Hz, *J* = 13.8 Hz, 1H), 4.97 (dd, *J* = 3.3 Hz, *J* = 11.5 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 2H), 7.51 (td, *J* = 5.7 Hz, *J* = 7.8 Hz, 2H), 7.72 (dd, *J* = 7.6 Hz, *J* = 13.4 Hz, 2H), 9.91 (s, 1H), 10.88 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 38.6 (CH_2), 38.9 (CH_2), 39.1 (CH_2), 41.0 (CH_2), 45.2 (CH_2), 58.3 (CH), 65.6 (Cq), 111.2 (CH), 121.0 (CH), 121.4 (CH), 127.3 (CH), 129.4 (CH), 132.4 (Cq), 133.7 (Cq), 137.3 (Cq), 138.3 (Cq), 140.9 (CH), 153.1 (Cq), 153.6 (Cq), 166.1 (Cq). HRMS (ESI) calc. for $C_{20}H_{19}N_4O_2S_2BrNa$ $[M+Na]^+$: 513.0031, found: 513.0014.

7.1.28. (5-Bromopyridin-2-yl)-3-(6-oxo-3,4,6,10b-tetrahydro-1H-spiro[pyrido[2,1-a]isoin-dole-2,2′-[1,3]dithiolan]-10-yl)urea ***31***

Compound **31** was obtained following the general procedure **A** from the amine **8** and 5-bromopicolinic acid after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a yellow solid in 82% yield. m.p. 240–242 °C. IR (ATR-Diamond, cm^{-1}) ν 1700, 1551, 1481, 1430, 1367, 1240, 1048, 759. ^1H NMR (400 MHz, DMSO-d_6) δ 1.66 (t, *J* = 12.0 Hz, 1H), 1.95 (td, *J* = 5.0 Hz, *J* = 13.0 Hz, 1H), 2.15 (d, *J* = 13.0 Hz, 1H), 2.92 (d, *J* = 11.6 Hz, 1H), 3.17 (td, *J* = 3.2 Hz, *J* = 13.3 Hz, 1H), 3.27–3.50 (m, 4H), 4.34 (dd, *J* = 3.2 Hz, *J* = 13.5 Hz, 1H), 4.78 (dd, *J* = 3.3 Hz, *J* = 11.6 Hz, 1H), 7.33 (d, *J* = 8.9 Hz, 1H), 7.43 (d, *J* = 6.8 Hz, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 8.01 (dd, *J* = 2.5 Hz, *J* = 8.9 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.57 (d, *J* = 2.4 Hz, 1H), 10.10 (s, 1H), 10.69 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.7 (CH_2), 38.2 (CH_2), 38.8 (CH_2), 40.7 (CH_2), 44.0 (CH_2), 56.9 (CH), 65.7 (Cq), 111.4 (Cq), 114.0 (CH), 118.0 (CH), 123.6 (CH), 129.1 (CH), 132.7 (Cq), 133.6 (Cq), 134.2 (Cq), 141.3 (CH), 147.1 (CH), 151.8 (2 Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{20}H_{19}N_4O_2S_2BrNa$ $[M+Na]^+$: 513.0031, found: 513.0016.

7.1.29. General procedure B: preparation of the ketones ***32–36***

The chosen acetal (0.52 mmol) and a solution of hydrochloric acid 10% (2 mL) in acetone (4 mL) was refluxed for 3 h. After cooling, acetone was removed under reduced pressure. The resulting solid was filtered, washed with water (2 mL) and dried to give the corresponding ketones.

7.1.30. 1-(2,6-Dioxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(pyridin-2-yl) urea ***32***

Compound **32** was obtained following the general procedure **B** from compound **11** as a white solid in 98% yield. m.p. 253–255 °C. IR (ATR-Diamond, cm^{-1}) ν 1692, 1623, 1568, 1479, 1457, 1421, 1309, 1243, 751. ^1H NMR (400 MHz, DMSO-d_6) δ 2.34 (t, *J* = 12.0 Hz, 1H), 2.38–2.45 (m, 1H), 2.57–2.66 (m, 1H), 3.26 (dd, *J* = 2.8 Hz, *J* = 13.0 Hz, 1H), 3.49 (td, *J* = 4.4 Hz, *J* = 12.6 Hz, 1H), 4.47–4.50 (m, 1H), 5.08 (dd, *J* = 3.9 Hz, *J* = 11.9 Hz, 1H), 7.10 (dd, *J* = 5.4 Hz, *J* = 6.8 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 7.45 (d, *J* = 6.8 Hz, 1H), 7.51 (t, *J* = 7.7 Hz, 1H), 7.76–7.85 (m, 1H), 8.30 (d, *J* = 8.3 Hz, 1H), 8.34–8.40 (m, 1H), 9.99 (s, 1H), 11.26 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.6 (CH_2), 39.0 (CH_2), 44.0 (CH_2), 56.0 (CH), 112.2 (CH), 117.5 (CH), 117.6 (CH), 122.7 (CH), 129.4 (CH), 132.5 (Cq), 133.7 (Cq), 134.0 (Cq), 139.1 (CH), 146.2 (CH), 152.1 (Cq), 152.8 (Cq), 165.3 (Cq), 206.3 (Cq). HRMS (ESI) calc. for $C_{18}H_{17}N_4O_3$ $[M+H]^+$: 337.1301, found: 337.1294.

7.1.31. 1-(2,6-Dioxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(pyrazin-2-yl) urea ***33***

Compound **33** was obtained following the general procedure **B** from compound **14** as beige solid in 95% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 1689, 1660, 1569, 1502, 1477, 1430, 1304, 1143, 1065. ^1H NMR (400 MHz, DMSO-d_6) δ 2.31 (dd, *J* = 2.7 Hz, *J* = 13.5 Hz, 1H), 2.37–2.44 (m, 1H), 2.57–2.67 (m, 1H), 3.17 (dd, *J* = 2.7 Hz, *J* = 13.5 Hz, 1H), 3.48 (td, *J* = 4.4 Hz, *J* = 12.8 Hz, 1H), 4.44–4.49 (m, 1H), 5.05 (dd, *J* = 3.9 Hz, *J* = 11.9 Hz, 1H), 7.48 (dd, *J* = 1.2 Hz, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 8.18 (dd, *J* = 0.9 Hz, *J* = 7.5 Hz, 1H), 8.32 (d, *J* = 2.7 Hz, 1H), 8.35–8.37 (m, 1H), 8.90 (d, *J* = 1.2 Hz, 1H), 9.86 (s, 1H), 10.12 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.6 (CH_2), 39.0 (CH_2), 43.9 (CH_2), 56.0 (CH), 118.1 (CH), 123.4 (CH), 129.4 (CH), 132.6 (Cq), 133.5 (Cq), 134.4 (Cq), 135.5 (CH), 137.9 (CH), 141.0 (CH), 149.1 (Cq), 151.7 (Cq), 165.2 (Cq), 206.3 (Cq). HRMS (ESI) calc. for $C_{17}H_{15}N_5O_3Na$ $[M+Na]^+$: 360.1073, found: 360.1078.

7.1.32. 1-(2,6-Dioxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(6-methyl-pyridin-2-yl)urea ***34***

Compound **34** was obtained following the general procedure **B** from compound **17** as white solid in 79% yield. m.p. 226–228 °C. IR (ATR-Diamond, cm^{-1}) ν 1683, 1621, 1566, 1467–1435, 1323, 1287, 752. ^1H NMR (400 MHz, DMSO-d_6) δ 2.37 (t, *J* = 12.5 Hz, 1H), 2.44 (s, 3H), 2.54–2.68 (m, 1H), 3.11 (dd, *J* = 2.5 Hz, *J* = 12.8 Hz, 1H), 3.17 (d, *J* = 5.0 Hz, 1H), 3.45 (td, *J* = 5.0 Hz, *J* = 12.8 Hz, 1H), 4.48 (dd,

J = 2.8 Hz, J = 12.5 Hz, 1H), 5.08 (dd, J = 2.8 Hz, J = 12.5 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 7.17 (d, J = 8.0 Hz, 1H), 7.45–7.55 (m, 2H), 7.66 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 8.18 (d, J = 7.5 Hz, 1H), 9.84 (s, 1H), 10.70 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.6 (CH_3), 36.6 (CH_2), 39.0 (CH_2), 43.7 (CH_2), 56.1 (CH), 109.0 (CH), 116.9 (CH), 118.0 (CH), 123.9 (CH), 129.3 (CH), 132.5 (Cq), 133.8 (Cq), 134.4 (Cq), 139.2 (CH), 152.1 (Cq), 152.3 (Cq), 155.5 (Cq), 165.3 (Cq), 206.4 (Cq). HRMS (ESI) calc. for $C_{19}H_{19}N_4O_3$ $[M+H]^+$: 351.1452, found: 351.1451.

7.1.33. 1-(2,6-Dioxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(4-methoxy-quinolin-2-yl)urea ***35***

Compound **35** was obtained following the general procedure **B** from compound **20** as white solid in 83% yield. m.p. 223–225 °C. IR (ATR-Diamond, cm^{-1}) ν 2976, 1683, 1621, 1558, 1469, 1435, 1329, 1289, 751. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.29 (t, J = 12.5 Hz, 1H), 2.41 (d, J = 12.5 Hz, 1H), 2.53–2.62 (m, 1H), 3.20 (d, J = 12.8 Hz, 1H), 3.50 (td, J = 2.5 Hz, J = 12.8 Hz, 1H), 4.13 (s, 3H), 4.44 (dd, J = 2.8 Hz, J = 12.8 Hz, 1H), 5.18 (dd, J = 2.8 Hz, J = 12.5 Hz, 1H), 7.02 (s, 1H), 7.55–7.59 (m, 3H), 7.82 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 8.00–8.11 (m, 3H), 10.99 (s, 1H), 11.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.5 (CH_2), 39.0 (CH_2), 43.6 (CH_2), 56.1 (CH_3), 57.1 (CH), 92.1 (CH), 117.6 (Cq), 119.0 (CH), 122.0 (CH), 122.8 (Cq), 124.9 (CH), 125.6 (CH), 129.4 (2 × CH), 132.4 (CH), 132.7 (Cq), 132.8 (Cq), 135.8 (Cq), 152.0 (Cq), 152.3 (Cq), 165.1 (Cq), 165.3 (Cq), 205.7 (Cq). HRMS (ESI) calc. for $C_{23}H_{21}N_4O_4$ $[M+H]^+$: 417.1563, found: 417.1571.

7.1.34. 1-(2,6-Dioxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(3-methyl-pyridin-2-yl)urea ***36***

Compound **36** was obtained following the general procedure **B** from compound **23** as white solid in 85% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 1684, 1621, 1559, 1479, 1434, 1414, 1329, 1289, 751. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.22 (t, J = 12.5 Hz, 1H), 2.40 (d, J = 12.8 Hz, 1H), 2.49–2.60 (m, 4H), 3.33–3.38 (m, 1H), 3.47 (td, J = 2.5 Hz, J = 12.8 Hz, 1H), 4.40–4.46 (m, 1H), 5.20 (dd, J = 2.5 Hz, J = 12.5 Hz, 1H), 7.36 (dd, J = 5.2 Hz, J = 7.5 Hz, 1H), 7.52–7.58 (m, 2H), 8.04 (d, J = 7.5 Hz, 1H), 8.16 (d, J = 7.5 Hz, 1H), 8.28 (d, J = 5.2 Hz, 1H), 10.91 (s, 1H), 11.49 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.9 (CH_3), 36.9 (CH_2), 39.4 (CH_2), 44.1 (CH_2), 56.6 (CH), 118.8 (CH), 119.5 (CH), 124.7 (CH), 125.3 (Cq), 129.9 (CH), 132.9 (Cq), 133.3 (Cq), 136.1 (Cq), 137.1 (CH), 145.5 (CH), 148.4 (Cq), 153.2 (Cq), 165.5 (Cq), 206.3 (Cq). HRMS (ESI) calc. for $C_{19}H_{19}N_4O_3$ $[M+H]^+$: 351.1452, found: 351.1451.

7.1.35. General procedure C: synthesis of alcohols ***37–41***

At −20 °C, to a solution of ketone (0.2 mmol) in a mixture THF/MeOH 1/2 (6 mL) was added portion wise $NaBH_4$ (15 mg, 0.4 mmol, 2.0 eq.). The mixture was stirred at this temperature for 30 min and the temperature was then allowed to rise to 0–5 °C for 2 h. Water (10 mL) was added, and the aqueous phase was extracted first with CH_2Cl_2 (2 × 10 mL) and then with EtOAc (2 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure.

7.1.36. cis-1-(2-Hydroxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(pyridin-2-yl)urea ***37***

Compound **37** was obtained following the general procedure **C** from compound **32** after purification by flash chromatography (CH_2Cl_2/MeOH 93/7) as a white solid in 76% yield. m.p. 218–220 °C. IR (ATR-Diamond, cm^{-1}) ν 3217, 1695, 1562, 1512–1478–1430, 1309. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.77 (q, J = 12.0 Hz, 1H), 1.10–1.28 (m, 1H), 1.95 (d, J = 11.7 Hz, 1H), 2.89 (d, J = 11.7 Hz, 1H), 3.08 (td, J = 4.1 Hz, J = 12.0 Hz, 1H), 3.60 (s, 1H), 3.96 (t, J = 10.7 Hz, 1H), 4.24 (dd, J = 4.1 Hz, J = 13.2 Hz, 1H), 4.69 (dd, J = 2.8 Hz, J = 11.8 Hz, 1H), 7.04–7.13 (m, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 7.3 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.81 (t, J = 7.3 Hz, 1H), 8.21 (d, J = 7.7 Hz, 1H), 8.32 (d, J = 4.5 Hz, 1H), 9.96 (s, 1H), 11.21 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.5 (CH_2), 36.6 (CH_2), 38.7 (CH_2), 56.0 (CH), 66.7 (CH), 112.1 (CH), 117.5 (CH), 117.6 (CH), 122.6 (CH), 128.9 (CH), 132.7 (Cq), 133.8 (Cq), 134.4 (Cq), 139.1 (CH), 146.2 (CH), 152.1 (Cq), 152.8 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{18}H_{19}N_4O_3$ $[M+H]^+$: 339.1457, found: 339.1462.

7.1.37. cis-1-(2-Hydroxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(pyrazin-2-yl)urea ***38***

Compound **38** was obtained following the general procedure **C** from compound **33** after purification by flash chromatography (CH_2Cl_2/MeOH 96/4) as a white solid in 60% yield. m.p. 250–252 °C. IR (ATR-Diamond, cm^{-1}) ν 1694, 1671, 1622, 1568, 1545, 1500, 1485, 1427, 1296, 1056. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75 (q, J = 11.8 Hz, 1H), 1.14–1.23 (m, 1H), 1.94 (d, J = 12.1 Hz, 1H), 2.78 (d, J = 11.8 Hz, 1H), 3.07 (td, J = 3.1 Hz, J = 13.2 Hz, 1H), 3.88–3.95 (m, 1H), 4.25 (dd, J = 3.9 Hz, J = 13.2 Hz, 1H), 4.65 (dd, J = 3.1 Hz, J = 11.8 Hz, 1H), 5.01 (d, J = 4.9 Hz, 1H), 7.42 (dd, J = 0.8 Hz, J = 7.4 Hz, 1H), 7.48 (t, J = 7.7 Hz, 1H), 8.10 (dd, J = 0.6 Hz, J = 7.7 Hz, 1H), 8.31 (d, J = 2.7 Hz, 1H), 8.32–8.38 (m, 1H), 8.94 (d, J = 1.2 Hz, 1H), 9.85 (s, 1H), 10.08 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.4 (CH_2), 36.6 (CH_2), 38.7 (CH_2), 56.0 (CH), 66.7 (CH), 118.1 (CH), 123.3 (CH), 129.0 (CH), 132.8 (Cq), 133.4 (Cq), 135.0 (Cq), 135.4 (CH), 137.9 (CH), 141.1 (CH), 149.2 (Cq), 151.7 (Cq), 164.6 (Cq). HRMS (ESI) calc. for $C_{17}H_{17}N_5O_3Na$ $[M+Na]^+$: 362.1229, found: 362.1223.

7.1.38. cis-1-(2-Hydroxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(6-methylpyridin-2-yl)urea ***39***

Compound **39** was obtained following the general procedure **C** from compound **34** after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 80% yield. m.p. 240–242 °C. IR (ATR-Diamond, cm^{-1}) ν 3143, 1685, 1589, 1508, 1484, 1424, 1270. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.76 (q, J = 12.5 Hz, 1H), 1.11–1.25 (m, 1H), 1.94 (d, J = 12.5 Hz, 1H), 2.51 (s, 3H), 2.76 (d, J = 12.5 Hz, 1H), 3.07 (td, J = 2.5 Hz, J = 12.8 Hz, 1H), 3.82–3.93 (m, 1H), 4.26 (dd, J = 2.8 Hz, J = 12.2 Hz, 1H), 4.71 (dd, J = 2.8 Hz, J = 12.2 Hz, 1H), 5.01 (d, J = 5.0 Hz, 1H), 6.93 (d, J = 7.5 Hz, 1H), 7.16 (d, J = 8.2 Hz, 1H), 7.39–7.51 (m, 2H), 7.67 (dd, J = 7.5 Hz, J = 8.2 Hz, 1H), 8.17 (d, J = 7.5 Hz, 1H), 9.84 (s, 1H), 10.75 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.8 (CH_3), 34.4 (CH_2), 36.6 (CH_2), 38.4 (CH_2), 56.1 (CH), 66.8 (CH), 108.9 (CH), 116.8 (CH), 117.7 (CH), 123.6 (CH), 128.8 (CH), 132.7 (Cq), 133.7 (Cq), 134.7 (Cq), 139.1 (CH), 152.2 (Cq), 152.3 (Cq), 155.4 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{19}H_{21}N_4O_3$ $[M+H]^+$: 353.1614, found: 353.1619.

7.1.39. cis-1-(2-Hydroxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(4-methoxyquinolin-2-yl)urea ***40***

Compound **40** was obtained following the general procedure **C** from compound **35** after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 82% yield. m.p. >260 °C. IR (ATR-Diamond, cm^{-1}) ν 2977, 1686, 1585, 1512, 1479, 1414, 1289. ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.76 (q, J = 12.5 Hz, 1H), 1.04–1.21 (m, 1H), 1.93 (d, J = 12.5 Hz, 1H), 2.79 (d, J = 12.5 Hz, 1H), 3.14 (td, J = 2.8 Hz, J = 12.5 Hz, 1H), 3.77–3.90 (m, 1H), 4.02 (s, 3H), 4.26 (dd, J = 2.8 Hz, J = 12.5 Hz, 1H), 4.82–4.91 (m, 2H), 6.87 (s, 1H), 7.42–7.53 (m, 3H), 7.73 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 8.21 (d, J = 7.5 Hz, 1H), 10.16 (s, 1H), 11.95 (s, 1H). ^{13}C NMR (63 MHz, DMSO-d_6) δ 34.5 (CH_2), 36.6 (CH_2), 38.1 (CH_2), 56.1 (CH_3), 56.2 (CH), 66.7 (CH), 91.9 (CH), 117.9 (CH), 118.1 (Cq), 121.4 (CH), 123.8 (CH), 124.0 (CH), 126.1 (CH), 128.9 (CH), 130.8 (CH), 132.8 (Cq), 133.5 (Cq), 135.0 (Cq), 145.7 (Cq), 152.3 (Cq), 153.7 (Cq), 163.0 (Cq), 164.7 (Cq). HRMS (ESI) calc. for $C_{23}H_{23}N_4O_4$ $[M+H]^+$: 419.1719, found: 419.1728.

7.1.40. cis-1-(2-Hydroxy-6-oxo-1,2,3,4,6,10b-hexahydropyrido[2,1-a]isoindol-10-yl)-3-(3-methylpyridin-2-yl)urea ***41***

Compound **41** was obtained following the general procedure **C** from compound **36** after purification by flash chromatography (CH_2Cl_2/MeOH 99/1) as a white solid in 82% yield. m.p. 204–206 °C. IR (ATR-Diamond, cm^{-1}) ν 2960, 1684, 1584, 1503, 1486–1420, 1262. ^1H NMR (400 MHz, DMSO-d_6) δ 0.80 (q, J = 12.5 Hz, 1H), 1.12–1.23 (m, 1H), 1.97 (d, J = 12.5 Hz, 1H), 2.32 (s, 3H), 2.90 (d, J = 12.5 Hz, 1H), 3.08 (td, J = 2.5 Hz, J = 12.8 Hz, 1H), 3.95–4.02 (m, 1H), 4.25 (dd, J = 2.8 Hz, J = 12.2 Hz, 1H), 4.70 (dd, J = 2.8 Hz, J = 12.2 Hz, 1H), 5.01 (d, J = 5.0 Hz, 1H), 7.06 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.37 (d, J = 7.5 Hz, 1H), 7.47 (dd, J = 7.5 Hz, J = 8.0 Hz, 1H), 7.67 (d, J = 7.5 Hz, 1H), 8.22 (d, J = 7.5 Hz, 1H), 8.27 (d, J = 8.0 Hz, 1H), 8.87 (s, 1H), 12.32 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 17.4 (CH_3), 31.1 (CH_2), 35.0 (CH_2), 37.1 (CH_2), 56.5 (CH), 67.2 (CH), 117.9 (CH), 118.3 (CH), 121.8 (Cq), 123.0 (CH), 129.5 (CH), 133.2 (Cq), 134.3 (Cq), 134.8 (Cq), 140.6 (CH), 143.5 (CH), 151.6 (Cq), 152.8 (Cq), 165.2 (Cq). HRMS (ESI) calc. for $C_{19}H_{21}N_4O_3$ $[M+H]^+$: 353.1614, found: 353.1616.

7.2. Kinase assay

Kinase activities were assayed in Buffer A or C, at 30 °C, at a final ATP concentration of 15 μM. Blank values were subtracted and activities expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO. The kinase peptide substrates were obtained from Proteogenix (Oberhausbergen, France).

DYRK1A (human, recombinant, expressed in *E. coli* as a GST fusion protein) was purified by affinity chromatography on glutathione-agarose and assayed in buffer A (+0.5 mg BSA/mL) using Woodtide (KKISGRLSPIMTEQ) (1.5 μg/assay) as a substrate, in the presence of 15 μM [γ-^{33}P] ATP (3000 Ci/mmol; 10 mCi/mL) in a final volume of 30 μl. After 30 min incubation at 30 °C, the reaction was stopped by harvesting onto P81 phosphocellulose papers (Whatman) using a FilterMate harvester (Packard) and filters were washed in 1% phosphoric acid. Scintillation fluid was added and the radioactivity measured in a Packard counter. CDK5/p25 (human, recombinant) was prepared as previously described [13]. Its kinase activity was assayed in buffer B, with 1 mg histone H1/mL. GSK-3α/β (porcine brain, native) was assayed in Buffer A using a GSK3 specific substrate (GS-1: RRAAVPPSPSLSRHSSPH QSpEDEEE) (pS stands for phosphorylated serine) [14].

7.3. Cell culture and survival assay

HuH7, CaCo-2, MDA-MB-231, HCT116, PC3, HaCaT and NCI-H727 cell lines were obtained from the ECACC collection. Skin diploid fibroblastic cells were provided by BIOPREDIC International Company (Rennes, France). Cells were grown according to ECACC recommendations. The toxicity test of the compounds on these cells was as follows: 2.10^3 cells/well for HCT116 cell line or 4.10^3 cells/well for the other cell lines were seeded in 96 well plates. 24 h after cell seeding, cells were exposed to increasing concentrations of the compounds (0.1 μM–0.3 μM–0.9 μM–2.7 μM–8.3 μM–25 μM). After 48 h of treatment, the cells were washed in PBS and fixed in cooled 90% ethanol/5% acetic acid for 20 min. Then, the nuclei were stained with Hoechst 3342 (Sigma). Image acquisition and analysis was performed using a Cellomics ArrayScan VTI/HCS Reader (Thermo Scientific). The Ic50 were determined using Xlfit software.

7.4. Molecular modelling

Hardware and software: molecular modelling studies were performed with the Schrodinger Molecular Modelling Suite 2014 update 3 [15] with Maestro, the interface piloting the diverse modules. Glide was used to dock ligands. Analysis and visualization tasks were performed with MOE software [16].

Structure preparation: crystal structures were retrieved from the protein data bank: GSK3b with the PDB code 1J1B [17], CDK5 with the PDB code 4AU8 [18] and DYRK1A with the PDB code 4MQ1 [19]. Subunit A was conserved regarding the three structures which were next prepared using the Protein Preparation Wizard workflow of the Schrodinger Molecular Modelling Suite. Proteins were pre-processed (hydrogen atoms added, incomplete residues filled), bond orders and connections of ligands were manually corrected. An exhaustive sampling was conducted regarding hydrogen bond assignment and the complex was finally refined by a minimization stage with a constraint to converge to a structure with an RMSD of 0.3 _A (OPLS2005 force field), essentially in order to remove steric clashes. Ligands, other than the one cocrystallized, were built within Marvin Sketch 5.8.0 [20] and were submitted to Corina [21], a 3D structure generator. Next 3D structures were submitted to the LigPrep module of the Schrodinger Molecular Modelling Suite in order to take into account tautomerization and ionization *via* the Epik module. The resulting structures became the starting point for docking simulations. Docking parameters: docking calculations were performed with extra precision. Ligand flexibility was taken into account and the option of sampling of ring conformation was activated.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.06.046.

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