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Original article

Design and synthesis of novel tetrahydro-2H-Pyrano[3,2-c]Pyridazin-3(6H)-one derivatives as potential anticancer agents

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ABSTRACT

Polyfunctional tetrahydro-2H-pyrano[3,2-c]pyridazin-3(6H)-one derivatives were synthesized and biologically evaluated as novel anticancer agents. These motifs were produced by a five-step reaction sequence in which the Achmatowicz oxidative cyclization, is the basic core for such synthesis. Compounds **15f**, **16c**, and **16d** showed antiproliferative activity against the SK-BR-3 breast cancer cell line. Importantly, **16c** and **16d** showed the highest efficacy, being approximately 30-fold more potent against SK-BR-3 (IC_{50} 0.21 and 0.15 μM, respectively) compared to other cancer cell lines tested. In addition, **16c** and **16d** displayed about 295 fold less toxicity against normal breast cell line MCF10A compared to SK-BR-3 breast cancer cells. These compounds form the foundation for further investigation in our continuing efforts to develop potent anticancer agents.

1. Introduction

Proliferative disorders are expected to be a major cause of death in the 21st century [1,2]. Tumor cells are a rapidly changing target leading to the selection and overgrowth of drug-resistant tumor cells, and their therapeutic uses are often hindered by the development of drug-resistance, myelosuppression and cytotoxicity towards normal cells. On the other hand, anticancer drugs tend to cause general toxicity to normally proliferating cells, which can severely limit the therapeutic value of these regimens [3].

Thus, much effort is being made to increase tissue, cell, and target selectivity for chemotherapy [4,5], and extensive synthesis and design of new structural motifs in order to overcome the aforementioned problems and improve antitumor activity are highly desirable.

To identify new chemical entities for more effective treatments of cancer, medicinal chemists can adopt many strategies, but the crucial decision is the selection of a suitable starting point from the astronomically large chemical and biological space [6,7]. The ideal starting point will be driven by novel synthetic strategies that can lead to a chemical space of skeletally diverse molecules [8–10]. The greatest challenge for synthetic chemists is therefore the improvement of overall efficiency by using atom-, step-, and economic procedures that proceed with high yield and selectivity [11,12].

In this respect, natural products can be viewed as evolved privileged structures and biologically prevalidated leads that have probably evolved evolutionarily to exert highly specialized functions [13–15]. However, due to their structural complexity, this may lead to limited commercial supplies and hinder mechanism of action studies and clinical development [16–18]. For this reason, diversity oriented synthesis to generate a plethora of novel scaffolds, is a powerful and highly productive tool for lead development and analog design [19,20].

In a recent unambiguous stereochemical synthesis of cyclopenta[b]pyranes fused to pyrazolones [21], has allowed us to gain access to an increased diversity of closely related scaffolds. This versatile synthesis has given us an opportunity to expand structure activity studies significantly and thus to test the importance of subtle skeleton alterations on anticancer activity.

In this report, the anticancer activities of a related group of simplified pyrano[b]pyridazinones were designed and their potential as anticancer agents was investigated against different panels of tumor cell lines. These efforts have been driven by our previous

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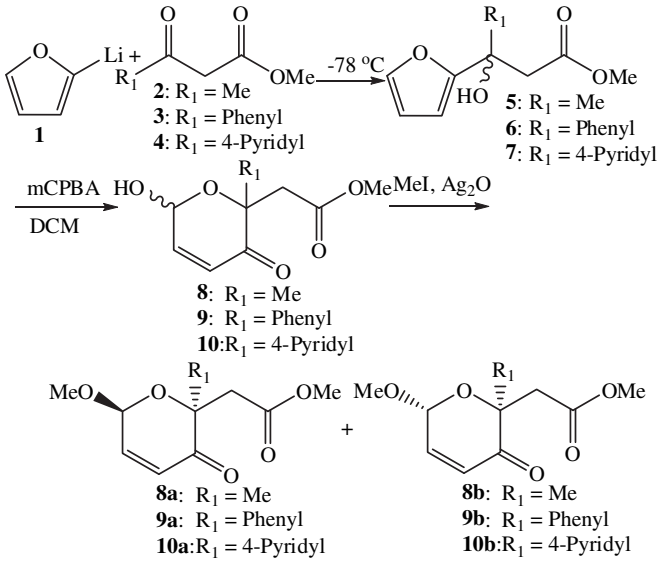
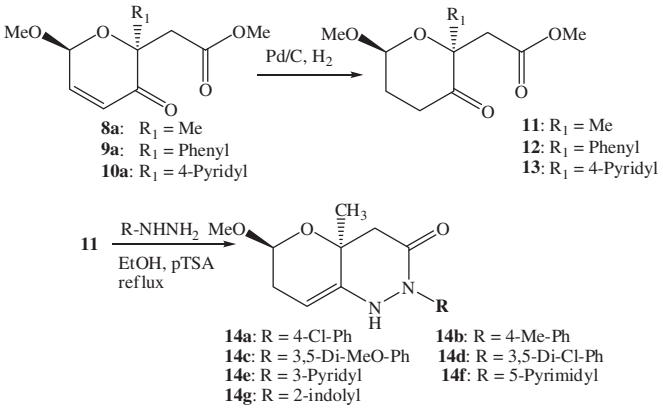


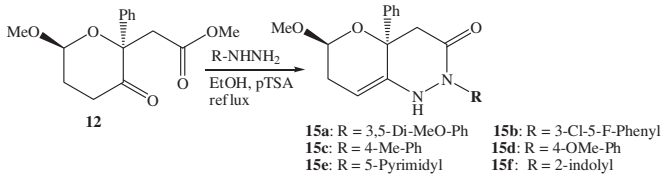
Scheme1. . Diastereoselective Synthesis of 3-oxo-3,6-dihydro-2H-pyran-2-yl)acetate derivatives.

experience in the reactivity of diketones [22] and profiting from the wide applications resulting from the Achmatowicz oxidative cyclization [23,24].

As shown in Scheme 1, this complexity-generating reaction [25] transforms a relatively simple substrate such as **5**, into a more complex product **8** with potential for diversification via functionalization of the resultant pyrenone scaffold. In connection with a program directed toward the synthesis of natural product analogs and finding leads to certain disease states [26,27], this report describes a new chemical entity, substituted tetrahydro-2H-pyrano [3,2-c]pyridazin-3(6H)-one derivatives, and their cytotoxic activity against human tumor cell line panels. Many 1,2-diaza-3-one heterocycles have been found to have potent anticonvulsant, antituberculosis, antitumor, and herbicidal activities [28–33]. It's quite interesting that the electronic substituent effects on the bioactivity changes at certain positions of such heterocycles have been observed. For instance, the bioactivities of pyridazinone and its 4,5-dihydro derivatives were found to be quite sensitive to the electronic effects of substituents at 2- or 6-positions [34]. To the best of our knowledge, however, the systems described here, represent novel scaffolds that have never been described before.



Scheme 2. Synthesis of polyfunctional tetrahydro-2H-pyrano[3,2-c]pyridazin-3(6H)-one motifs.



Scheme 3. Synthesis of polyfunctional tetrahydro-2H-pyrano[3,2-c]pyridazin-3(6H)-one motifs.

2. Results and discussion

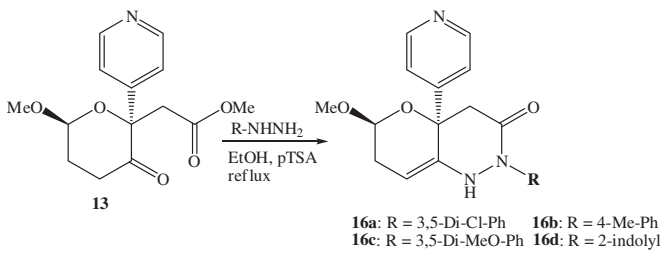
2.1. Chemistry

In a recent article from our group [21], we reported on the application of a protocol for combining the Achmatowicz oxidative cyclization and the Knoevenagel regiocontrolled condensation on the resulting cyclic enone scaffolds to deliver cyclopenta[b]pyrenones. In such a strategy, furfural derivatives were used as a nucleofuge and β-ketoester as a nucleophile. In this report however, β-ketoesters **2-4** were used as the nucleofuge and furyl lithium **1** as the nucleophile to generate systems of types **5-7** (Scheme 1)

Oxidative cyclization using *m*-chloroperbenzoic acid (*m*CPBA) or *N*-bromo succinamide (NBS) of the latter, delivered compounds **8-10**; these in turn form the building blocks for generating a library of tetrahydro-2H-pyrano[3,2-c]pyridazin-3(6H)-one derivatives. Therefore, all target compounds **14-16** were synthesized through a five-step sequence (Schemes 1-4). Various esters **2-4** were reacted at −78 °C with furyl lithium intermediate **1** to generate furanol esters **5-7** in quantitative yields (Scheme 1).Pyrenones **8-9** were smoothly produced after the application of Achmatowicz oxidative cyclization protocol using *m*CPBA in DCM. However, an attempt was made to subject compound **7** to *m*CPBA oxidative cyclization procedure; this attempt failed and tarry reaction products were formed according to TLC analysis. Alternatively, the synthesis of pyrenone **10** was achieved through the use of NBS.

The arrival at synthones **11-13** were made available through standard two step reaction sequence. For this purpose, anomeric protection of compounds **8-10** using Ag_2O/MeI, delivered compounds **8a-10a** and **8b-10b** as mixtures of diastereoisomers in fairly good combined yield. Initial efforts to purify these mixtures on silica gel using EtOAc/hexane (5% v/v) as two separate fractions, was successful. However, the fractions containing compounds **8b-10b** were always contaminated to about 30% level with difficultly separable unknown impurities. Nonetheless, since the goal is to produce pyrano[b]pyridazinone derivatives, efforts were concentrated on handling these major products for such purpose and the fractions that contain the minor products **8b-10b** were neglected.

The stereochemistry around structures **8a-10a** was confirmed through NMR nOe experiments and based on the experience gained



Scheme 4. Synthesis of polyfunctional tetrahydro-2H-pyrano[3,2-c]pyridazin-3(6H)-one motifs.

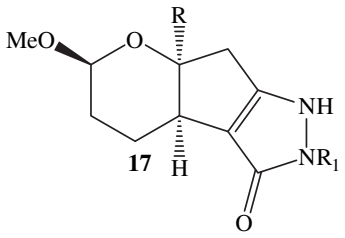


Fig. 1. Structure of octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one derivatives [21].

during the synthesis of such systems. H-2 of compound **8a** resonated at 4.95 ppm as doublets of doublets (J = 0.6 and 3.6 Hz). Irradiation of the anomeric proton produced a strong reciprocal nOe enhancement for the methyl group resident at C-6, indicating a *cis* relationship. Hydrogenation of derivatives **8a**-**10a** in the presence of Pd/C produced **11**-**13**. Having secured pure samples from compounds **11**-**13**, their stereochemistry was further deduced from NMR nOe experiments. In that, a strong reciprocal nOe interactions between the 6-Me group in compound **11** and 2-H were produced. This further confirmed the previously concluded stereochemistry.

A tandem condensation and ring-closure reaction of γ-ketoesters with hydrazines is one of the practical processes toward the construction of substituted 1,2-diaza-3-one heterocyclic ring systems [35–37]. After optimization of reaction conditions, we applied this protocol to a range of hydrazine substrates, including aryl and heteroaryl hydrazines (Schemes 2, 3 and 4). This sequential process, however, was reported to be sensitive and chemoselectively controlled by the percentage of the acidic catalyst that is added to this reaction [38,39]. High conversions of substrates were successfully realized when the condensations were carried out in refluxed EtOH under the catalysis of Bronsted acids. In particular, a good yield of the desired 4,5-dihydropyridazinone was obtained when the amount of catalyst of *p*-TSA was 10 mol % and the reaction time was 12 h. Thus, compounds **14a**-**g** were easily prepared following standard reaction protocols between compounds **11**-**13** and hydrazine derivatives in refluxing EtOH under acid catalysis (Scheme 2).

After optimization of reaction conditions, the scope of this sequential process was then successfully extended to the synthesis of other pyrano[b]pyridazinones **15a**-**16d**, under the similar conditions described above (Schemes 3 and 4).

Results from substituent effects on the ring-closure reaction described in Schemes 2, 3 and 4 indicated that the electron rich hydrazine, such as 4-methoxyphenyl hydrazine, provided higher yields of dihydropyridazinone product (compounds **14b**, **14c** and **15a** Schemes 2 and 3, respectively). On the other hand, reaction process with electron deficient hydrazine, for instance chloro substituted hydrazine **15b** and **16a** (Schemes 2 and 3), proceeded slowly with low yields.

2.2. Biological assay and SAR

Although our previous studies provided some important information about the cytotoxicity of related compounds such as **17** (Fig. 1), several questions remained unanswered: How do the skeletal complexity and substitution patterns affect the activity and selectivity? How does the tricylic framework in **17** contribute to activity, and how will the activity and selectivity change by simplification of previously described tricyclic motifs [21]. To answer these questions, chemical and biological strategies were followed. Prior article from our group, revealed that octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one derivatives (e.g., **17**, Fig. 1), in which ring-B (cyclopentanone ring) of **17** is missing in **14**, and the pyrazolone ring in **17** has been replaced by pyridazinone ring in **14**, are a new class of potent anticancer agents. These results encouraged us to further simplify the scaffold of **17** and investigate the structure activity relationships and selectivity of the newly synthesized motifs **14a**-**16d**.

In this regard, the newly synthesized tetrahydro-2H-pyrano [3,2-c]pyridazin-3(6H)-one analogs **14a**–**14f** were evaluated for *in vitro* anticancer activity against a panel of tumor cell lines. Results from **14a**–**14g** (Table 1) showed that different substituent's around the pyrazolone ring were critical to the efficacy of these motifs. Interestingly, compounds **14c**, **14f** and **14g**, indicated approximately five-fold greater potency toward the breast cancer cell line SK-BR-3 and the human lung cancer cell line (A549) compared to other cell lines tested. Enlightened by these findings, structural modifications in the pyranopyridazinone ring system were explored with derivatives **15a**–**15f** (Scheme 3 and Table 1). Surprisingly, the introduction of an aryl ring on the ring junction and indolyl group on the pyrazolone ring; increased the potency to about twenty fold

Table 1
IC_{50} Values for Cellular Proliferation Inhibition results of Compounds **14a-16d** in Different Cancer Cell Line Panel.[a]

$IC_{50} \pm SD$ (μM)						
Compd[b]	HCT116	SK–N–SH	SK-BR-3	DU-145	A549	MCF10A
14a	35.70 ± 3.30	31.70 ± 2.00	28.70 ± 2.30	24.20 ± 2.50	25.47 ± 2.10	
14b	28.70 ± 1.20	25.30 ± 1.80	29.70 ± 1.20	23.20 ± 1.70	21.49 ± 1.40	
14c	24.30 ± 5.30	23.70 ± 4.00	5.70 ± 1.30	22.20 ± 1.90	6.15 ± 1.20	
14d	19.40 ± 1.10	20.20 ± 1.10	18.80 ± 1.00	21.29 ± 0.80	17.48 ± 1.10	
14e	17.10 ± 0.90	19.00 ± 0.80	15.20 ± 1.10	16.43 ± 0.30	14.35 ± 0.30	
14f	15.10 ± 1.40	16.60 ± 1.90	3.40 ± 1.20	17.10 ± 1.40	4.10 ± 1.80	
14g	18.30 ± 1.70	19.50 ± 1.20	4.10 ± 0.30	14.40 ± 2.30	3.92 ± 0.30	
15a	18.10 ± 1.10	19.50 ± 1.00	1.20 ± 0.11	18.30 ± 1.10	17.25 ± 1.40	
15b	16.20 ± 1.00	17.40 ± 0.90	17.60 ± 0.13	16.10 ± 1.20	14.14 ± 2.40	
15c	10.50 ± 0.80	11.10 ± 0.30	9.20 ± 0.18	11.20 ± 0.20	8.11 ± 0.50	
15d	11.30 ± 0.20	10.40 ± 0.10	8.40 ± 0.10	10.10 ± 0.09	7.10 ± 0.13	
15e	9.13 ± 0.14	8.20 ± 0.09	5.30 ± 0.12	7.33 ± 0.13	5.20 ± 0.11	
15f	8.20 ± 0.30	9.10 ± 0.70	0.53 ± 0.04	7.90 ± 0.03	8.67 ± 0.15	>38
16a	9.10 ± 0.10	8.30 ± 0.230	5.50 ± 0.08	7.60 ± 0.12	7.50 ± 0.20	
16b	7.40 ± 0.10	8.10 ± 0.13	6.20 ± 0.10	7.10 ± 0.13	5.90 ± 0.08	
16c	6.50 ± 0.10	6.40 ± 0.09	0.21 ± 0.005	5.90 ± 0.02	6.22 ± 0.10	>51
16d	4.35 ± 0.02	3.90 ± 0.04	0.15 ± 0.009	4.10 ± 0.07	4.05 ± 0.002	>55

[a] Data represent mean values (SD) for two independent determinations.
[b] Evaluated as HCl salts.

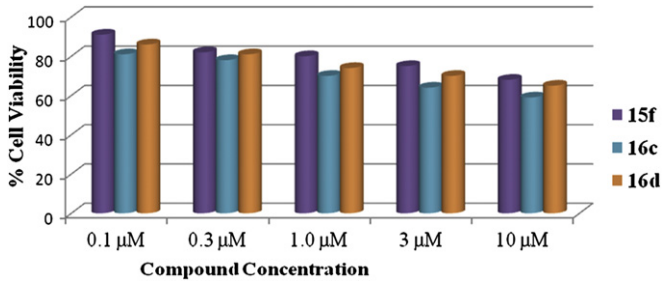


Fig. 2. *In vitro* cytotoxicity of compounds **15f**, **16c** and **16d** against MCF10A (normal breast cell line).

(compound **15f**) of these scaffolds against SK-BR-3 compared to their cytotoxicity toward A549 and other cell lines. Accordingly, compounds **16a**-**16d** (Scheme 4) were synthesized and tested. From these molecules, compounds **16c** and **16d** were found to have about thirty-fold potency toward SK-BR-3 relative to the other cell lines with IC_{50} values of 0.21 and 0.15 μM, respectively.

At this junction, selected active compounds with IC_{50} values less than 1 μM were tested against non-tumorigenic cell lines derived from breast tissue (MCF10A) vs SK-BR-3 as a positive cancer cell line control. The tested compounds showed $IC_{50} > 38$ μM against MCF10A. These results indicating that, the tested compounds are more cytotoxic against cancer cells compared to normal healthy cells.

Figs. 2 and 3 show the results of cell viability studies carried out on compound **15f**, **16c** and **16d** using the MTT assay protocol. These results further confirm not only the selectivity of these novel motifs but also indicated their lower toxicity against normal cells.

At this stage, some structure activity relationships could be construed from these results. For the compounds of type **14**, in which the ring junction carries a methyl group, there was no noticeable effect on the potency with different substituents resident on the N-2 of the pyridazinone ring. However, a phenyl ring on the ring junction as indicated in systems of type **15**, electron rich phenyl (e.g. **15a**, **15c** and **15d**) and/or heteroaryl groups (e.g. **15e** and **15f**) increased the potency of these scaffolds as anticancer agents compared to an electron poor phenyl substituents on N-2 (e.g. **15b**).

Interestingly, when the ring junction of the bicyclic framework carries a pyridyl moiety such as systems of type **16**, the anticancer activity of these motifs enhanced for both electron rich aryl and heteroaryl rings compared to electron poor aryl systems. In general, introduction of an aryl and/or heteroaryl ring at the ring junction increases the potency of these systems toward breast cancer cell line compared to other cell lines investigated.

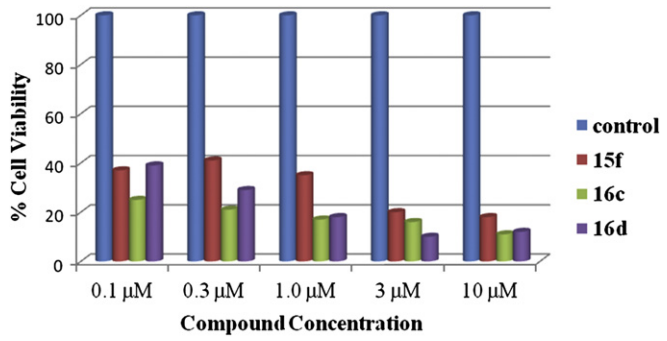


Fig. 3. *In vitro* cytotoxicity of compounds **15f**, **16c** and **16d** against SK-BR-3 (breast cancer cell line).

3. Conclusions

In conclusion, this study discovered a novel class of promising anti-breast cancer agents; substituted tetrahydro-2H-pyrano[3,2-c] pyridazin-3(6H)-one analogs. The IC_{50} values of the two most potent analogs (**16c** and **16d**) against SK-BR-3 were 0.21 and 0.15 μM, respectively. More importantly, **16c** and **16d** showed high anti-breast cancer potency, being approximately 30-fold more potent against SK-BR-3 compared to four other cancer cell lines from other tissue types. Furthermore, compounds **16c** and **16d** displayed a 290-fold increase in potency against the SK-BR-3 breast cancer cell line when compared to the non-tumorigenic breast cell line (MCF10A). These findings form the foundation for redirected efforts aimed at developing novel analogs as preclinical trials candidates for anti-breast cancer treatment.

4. Experimental

4.1. General methods

All reagents were used as purchased from commercial suppliers without further purification. The reactions were carried out in oven dried or flamed graduated vessels. Solvents were dried and purified by conventional methods prior use. All reactions were monitored by thin layer chromatography (TLC) using Merck aluminum plates pre-coated with silica gel PF254; 20 × 20, 0.25 mm, and detected by visualization of the plate under UV lamp (l¼ 254 or 365 nm). Spots were detected by spraying with anisaldehyde–sulpharic acid in ethanol, followed by heating to 140 °C. Compounds were purified through recrystallization or using column chromatography which was performed on silica gel, packed by the slurry method. Flash column chromatography was performed with Silica gel 60, 0.040–0.063 mm (230–400 mesh). Aluminium backed plates pre-coated with silica gel 60 (UV254) were used for thin layer chromatography. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz/75 MHz spectrometer. Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Chemical shifts (δ) are given in ppm relative to the resonance of their respective residual solvent peak, $CHCl_3$ (7.27 ppm, 1H; 77.16 ppm, the middle peak, ^{13}C). Spectra were acquired in $CDCl_3$ containing 1% TMS. High resolution Mass spectra (HRMS) were recorded in positive ion mode by Electrospray Ionization (ESI) on a Bruker instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v 0.1 % formic acid) and infused using a syringe pump with a flow rate of 2 mL/min. External calibrationwas conducted using Arginine cluster in a mass range m/z 175–871. For all HRMS data, mass error: 0.00–0.50 ppm. Melting points (m.p.) were determined on an Electrothermal Melting point Apparatus and were reported uncorrected in oC.

4.2. Biology

4.2.1. Cell growth inhibition assay

All stock cultures are grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates with compounds added from DMSO diluted stock. The plates were incubated for an additional 72 h after attachment and addition of compounds, and the assay was terminated by 10% TCA. Then, 0.4% SRB dye in 1% HOAc was added to stain the cells for 10 min. Unbound dye was removed by repeated washing with 1% HOAc and the plates were air dried. Bound stain was subsequently solved with 10 mM trizma base, and the absorbance read at 515 nm. Stock solutions of agents in DMSO at 1 mg/mL were prepared fresh for each experiment and were diluted in cell culture medium to achieve desired concentrations upon addition to the target cells.

All six types of cells were incubated for 48 h at 37 °C in 96-well microtiter plates to assess inhibition of cell proliferation by measuring changes in protein content in triplicate wells. Cytotoxicity was assessed using sulforhodamine-B (SRB) by measuring the extent of inhibition of protein synthesis in treated cells compared with that in untreated cells. Following 48 h treatment, cells were fixed by the addition of cold (4 °C) 50% trichloroacetic acid (50 mL/well). After a 1-h incubation at 4 °C, plates were gently washed 5 times with deionized water and allowed to dry overnight. A 0.4% solution of SRB in 1% acetic acid (50 mL) was added to each well, and plates were incubated 10 min at room temperature. Wells were then rinsed 5 times with 1% acetic acid and allowed to dry. To solubilize the SRB dye, 150 mL/well of 10 mM Tris base (pH 10.5) was incubated for 20 min at room temperature and plates were gently vortex-mixed before absorbance at 570 nm was measured using a spectrophotometer (Spectramax-Pro; Molecular Devices). Wells containing untreated cells served as positive controls. The mean absorbance of the corresponding set of blanks was subtracted from the mean absorbance of wells incubated with each test agent. This value was then divided by the difference between the mean absorbance of the untreated cells and that of the blanks in order to calculate a percent inhibition for each concentration of agent. Growth inhibition of 50% (IC_{50}) is calculated as the drug concentration, which caused a 50% reduction in the net protein increase during the drug incubation. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average with SEM from at least three independent determinations. The following human tumor cell lines were used in the assay: A549 (non small cell lung cancer, HCT116 (human colorectal cancer cells) and SK–N–SH (human Caucasian bone marrow neuroblastoma) cells, SK-BR-3 (HER2 over-express in breast cancer), DU-145 (prostate cancer cell line). Cells propagated in RPMI-1640 supplemented with 10% FBS, penicillin (100 IU/mL), streptomycin (1 μg/mL), and amphotericin B (0.25 μg/mL), and were cultured at 37 °C in a humidified atmosphere of 95% air/5% CO_2.

4.2.2. Methodology of MTT assay

The cytotoxic activity of the cyclopenta[b]pyrane derivatives **15f**, **16c** and **16d** was determined using a standard (MTT)-based colorimetric assay. This assay quantifies viable cells by observing the reduction of tetrazolium salt, MTT, to formazan crystals by the cells. Based on the absorbance of the cell samples after the test is carried out, cell viability can be measured. Cells were plated with nutritional medium in 96 well plates (2000 and 5000 cells/well for HCT116 and SK–N–SH). After 24 h, cells were treated with a different concentration (0.1, 0.3, 1.0, 3.0 and 10 μM) of the new compounds, each concentration in 3 repetitions. The plates were incubated with the pyrazolone derivatives for 72 h. At the end of treatment, cells were washed with PBS solution. Then, 100 μL of fresh medium and 50 μL from a stock solution of MTT (3 mg/mL PBS) were added to each well. After 4 h of incubation at 37 °C, the medium was discarded and 100 μL of DMSO solution were added to each well, in order to dissolve the crystals that were formed. After a 30 min period, the absorbance of the samples was measured by an Elisa reader. The absorbance data was converted to % cell viability.

4.2.3. Synthesis

4.2.3.1. General procedure of the synthesis of compounds 5-7. A suspension of furyl lithium was prepared under argon by slow addition of *n*-BuLi (3.25 mL, 5.2 mmol of 1.6 M solution in hexane) furan (340 mg, 5 mmol) in THF (15 mL) at − 78 °C over 45 min period. To this mixture, methyl acetoacetate (580 mg, 5 mmol in 5 mL THF) was added over 5 min period. After stirring at this temperature for 45 min, the mixture was stirred warmed to 0 °C after which the mixture was quenched with a saturated solution of NH_4Cl (15 mL), extracted with EtOAc (3 × 30 mL), dried over Na_2SO_4 and concentrated under vacuum to give a yellowish crude oil. The crude was used in the next step without further purification. Compounds **5-7** were produced in quantitative yields as yellowish oils.

*4.2.3.2. General procedure for the synthesis of compounds **8-9**.* A solution of furyl alcohols **5** (920 mg, 5 mmol) or **6** (1.23 g, 5 mmol) in dicholomethane (40 mL) at 0 °C was treated with *m*-chloroperbenzoic acid (1.376 g, 8.0 mmol of 80% aqueous slurry) which was dissolved in 30 mL DCM, dried over Na_2SO_4, filtered, diluted with 50 mL toluene and dried under reduced pressure) and stirred at 0 °C for 6 h. Sodium sulfite solution (30 mL of 10%) was introduced, and the layers were separated after 1 h of rapid mixing. The aqueous phase was washed with saturated sodium bicarbonate solution (30 mL), brine and water (30 mL) prior to drying and solvent evaporation. The crude viscous yellowish oils (**8** and **9**) were used in the next step without further purification.

*4.2.3.3. General procedure for the synthesis of compounds **8a**, **9a** and **10a**.* A solution of the anomeric mixture of **8a** (600 mg, 3 mmol) or **9a** (786 mg, 3 mmol) or **10a** (789 mg, 3 mmol) in MeI (15 mL) at rt was treated with 3.0 mmol of Ag_2O. The progress of reaction was monitored by TLC (12 h). When complete, the reaction mixture was filtered over celite. The filtrate was washed with saturated solutions of sodium sulfite, sodium bicarbonate and brine, dried and evaporated. The residue was purified on silica gel column using hexane/EtOAc (9:1) as an eluent.

*4.2.3.4. General procedure for the synthesis of compounds **11-13**.* To a stirred solution of **8a-10a** (2.0 mmol) in dry EtOAc/MeOH (1:1, 20 mL) was added Pd/C (56 mg, 15% Pd/C w/w). The mixture was placed under 1.0 atm of H_2 pressure, and the progress of reaction was monitored by TLC. After 2 h, the solid was removed by filtration through a celite pad, which was washed repeatedly with EtOAc. After concentration of the filtrate, the residue was purified on silica gel column (elution with 5% ethyl acetate in hexane) to give **11-13** as amorphous solids.

*4.2.3.5. General procedure for the synthesis of compound **10**.* Furyl alcohol **7** (494 mg, 2 mmol), 5 mL of THF, and 2 mL of H_2O were cooled to 0 °C. Solid $NaHCO_3$ (460 mg, 4.15 mmol), $NaOAc{\bullet}3H_2O$ (420 mg, 3.11 mmol), and NBS (360 mg, 2.7 mmol) were added to the solution and the mixture was stirred for 0.5 h at 0 °C. The reaction was quenched with saturated $NaHCO_3$ (5 mL), extracted (3 × 20 mL) with EtOAc, dried over Na_2SO_4, concentrated under reduced pressure and the crude product was used in the next step without further purification.

*4.2.3.6. General procedure for the synthesis of compounds **14a-16d**.* The pyranone ketoesters **11-13** derived from the reduction product of **8a-10-a** (142 mg, 0.5 mmol) and the desired hydrazine derivative (0.52 mmol) in 20 mL absolute ethanol was refluxed in the presence of 10 mol% pTSA. The progress of reaction was monitored by TLC. After 12 h, the reaction mixture was concentrated on a rotary evaporator; the residue was purified through triturating with cold EtOH containing traces of water to give **14a-16d** as light yellowish solids.

*4.2.3.7. Methyl 3-(furan-2-yl)-3-hydroxybutanoate (**5**).* Yield: quantitative, yellowish oil. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.10 (s, 1H), 6.34 (d, J = 3.0 Hz, 1H), 4.45 (s, 1H), 3.69 (s, 3H), 3.10 (d, J = 15.0 Hz, 1H), 3.0 (d, J = 15.0 Hz, 1H), 1.44 (s, 3H). ^{13}C NMR

(75 MHz, $CDCl_3$, in ppm) δ 168.6, 154.1, 140.5, 109.4, 105.3, 71.1, 54.3, 44.0, 25.9. ESIMS: *m/z* calculated for [M + Na^+]: 207.063330, found 207.063210.

*4.2.3.8. Methyl 3-(furan-2-yl)-3-hydroxy-3-phenylpropanoate (**6**).* Yield: quantitative, yellowish oil. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.53–7.22 (m, 2H), 7.10–7.15 (m, 3H), 6.81 (t, *J* = 0.2Hz, 1H), 6.37 (d, *J* = 3.0 Hz, 1H), 6.17 (dd, *J* = 0.2, 3.0 Hz, 1H), 3.69 (s, 3H), 3.44 (d, *J* = 16.0 Hz, 1H), 3.39 (d, *J* = 16.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 170.5, 154.1, 143.4, 139.9, 129.3, 128.7, 126.2, 110.3, 104.8, 81.7, 54.1, 44.1. ESIMS: *m/z* calculated for [M + Na^+]: 269.078980, found 269.078851.

*4.2.3.9. Methyl 3-(furan-2-yl)-3-hydroxy-3-(pyridin-4-yl)propanoate (**7**).* Yield: quantitative, yellowish oil. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.72 (dd, *J* = 3.0, 6.0 Hz, 2H), 7.51 (dd, *J* = 3.0, 6.0 Hz, 2H), 6.92 (t, *J* = 0.1 Hz, 1H), 6.41 (d, *J* = 3.0 Hz, 1H), 6.11 (dd, *J* = 0.2, 3.0 Hz, 1H), 3.63 (s, 3H), 3.41 (d, *J* = 15.7 Hz, 1H), 3.28 (d, *J* = 15.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 171.1, 153.9, 147.5, 139.4, 123.9, 122.4, 114.3, 105.0, 81.6, 53.2, 44.1. ESIMS: *m/z* calculated for [M + Na^+]: 270.074229, found 270.074213.

*4.2.3.10. Methyl 2-(6-hydroxy-2-methyl-3-oxo-3,6-dihydro-2H-pyran-2-yl)acetate (**8**).* Amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 6.85 (dd, *J* = 2.3, 9.2 Hz, 1H, H-5), 6.79 (dd, *J* = 3.1, 10.0 Hz, 1H, H-5), 6.10 (d, *J* = 9.6 Hz, 1H, H-4), 6.07 (dd, *J* = 1.1, 10.4 Hz, 1H, H-4), 5.65 (t, *J* = 4.0 Hz, 1H, H-6), 5.59 (t, *J* = 6.9 Hz, 1H, H-6), 3.55 (s, 3H, OMe), 3.45 (s, 3H, OMe), 3.02 (d, *J* = 15.7 Hz, 1H, H-7), 2.98 (d, *J* = 15.4 Hz, 1H, H-7), 2.53 (d, *J* = 15.7 Hz, 1H, H-7), 2.42 (d, *J* = 15.5 Hz, 1H, H-7), 1.46, 1.33 (s, 6H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 197.5, 197.4 (C-3), 170.5, 169.1 (CO_2Me), 146.5, 143.9 (C-5), 126.3 (C-4), 87.7, 87.6 (C-6), 79.6, 79.3 (C-2), 55.5, 55.3 (OMe), 45.4, 45.2 (C-7), 27.0, 23.4 (CH_3). ESIMS: *m/z* calculated for [M + Na^+]: 223.058245, found 223.058197.

*4.2.3.11. Methyl 2-((2S,6S)-6-methoxy-2-methyl-3-oxo-3,6-dihydro-2H-pyran-2-yl)acetate (**8a**).* Yield: 82%, amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 6.87 (dd, *J* = 3.5, 10.3 Hz, 1H, H-3), 6.25 (d, *J* = 10.3 Hz, 1H, H-4), 4.95 (dd, *J* = 0.6, 3.6 Hz, 1H, H-2), 3.62 (s, 3H), 3.41 (s, 3H, OMe), 3.07 (d, *J* = 16.3 Hz, 1H, H-7), 2.54 (d, *J* = 16.3 Hz, 1H, H-7), 1.46 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 197.0 (C-3), 167.0 (CO_2Me), 140.0 (C-5), 126.3 (C-4), 87.6 (C-6), 80.2 (C-2), 45.6 (C-7), 26.7 (CH_3). ESIMS: *m/z* calculated for [M + Na^+]: 237.073895, found 237.073791.

*4.2.3.12. Methyl 2-((2R,6S)-6-methoxy-3-oxo-2-phenyl-3,6-dihydro-2H-pyran-2-yl)acetate (**9a**).* Yield: 77%, amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.44 (d, *J* = 6.6 Hz, 1H), 7.29–7.21 (m, 4 H), 6.78 (dd, *J* = 3.3, 10.1 Hz, 1H, H-3), 6.56 (dd, *J* = 0.6, 10.8 Hz, 1H, H-4), 5.29 (d, *J* = 3.3 Hz, H-2), 3.65 (s, 3H, OMe), 3.31 (s, 3H), 3.18 (d, *J* = 17.1 Hz, H-7), 2.92 (d, *J* = 17.1 Hz. H-7). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 192.7 (C-3), 169.9 (CO_2Me), 145.3 (C-5), 140.3, 129.7, 127.5, 126.1, 126.0, 101.9, 83.8 (C-6), 54.5, 51.7, 42.1 (C-7). ESIMS: *m/z* calculated for [M+Na^+299.089545 :[, found 299.089511.

*4.2.3.13. Methyl 2-((2R,6S)-6-methoxy-3-oxo-2-(pyridin-4-yl)-3,6-dihydro-2H-pyran-2-yl)acetate (**10a**).* Yield: 69%, amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.91 (d, *J* = 5.4 Hz, 2H), 7.71 (d, *J* = 6.6 Hz, 2H), 6.82 (d, *J* = 12.9 Hz, 1H), 6.33 (d, *J* = 11.8 Hz, 1H), 5.45 (d, *J* = 3.1 Hz, 1H), 3.68 (s, 3H), 3.37 (s, 3H), 3.31 (d, *J* = 16.8 Hz, 1H), 3.12 (d, *J* = 16.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 192.6, 170.1, 150.1, 144.2, 128.0, 122.3, 120.1, 101.7, 83.6, 55.2, 50.1, 41.5. ESIMS: *m/z* calculated for [M + Na^+]: 300.084794, found 300.084699.

*4.2.3.14. Methyl 2-((2S,6S)-6-methoxy-2-methyl-3-oxotetrahydro-2H-pyran-2-yl)acetate (**11**).* Yield: 91%, amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 4.52 (dd, *J* = 5.3, 9.7 Hz, 1H), 3.62 (s, 3H), 3.32 (s, 3H), 2.79-2-61 (m, 4H), 2.08–1.92 (m, 2H), 1.47 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 207.1, 171.2, 93.1, 82.7, 55.4, 50.8, 41.9, 32.6, 28.8, 21.7. ESIMS: *m/z* calculated for [M + Na^+]: 239.089545, found 239.089531.

*4.2.3.15. Methyl 2-((2R,6S)-6-methoxy-3-oxo-2-phenyltetrahydro-2H-pyran-2-yl)acetate (**12**).* Yield: 88%, amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.38–7.2 (m, 5H), 4.92 (dd, *J* = 3.1, 8.7 Hz, 1H), 3.61 (s, 3H), 3.33 (s, 3H), 3.03–2.80 (m, 4H), 2.11–2.04 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 205.1, 169.3, 140.1, 128.3, 126.4, 124.9, 95.1, 83.1, 55.1, 50.8, 41.7, 34.8, 28.1. ESIMS: *m/z* calculated for [M + Na^+]: 301.105195, found 301.105168.

*4.2.3.16. Methyl 2-((2R,6S)-6-methoxy-3-oxo-2-(pyridin-4-yl)tetrahydro-2H-pyran-2-yl)acetate (**13**).* Yield: 78%, amorphous solid. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.97 (d, *J* = 5.4 Hz, 2H), 7.75 (d, *J* = 6.6 Hz, 2H), 4.96 (dd, *J* = 3.1, 8.9 Hz, 1H), 3.59 (s, 3H), 3.11 (s, 3H), 3.09–2.80 (m, 4H), 2.13–2.09 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 206.1, 170.3, 147.2, 121.8, 118.4, 94.1, 83.7, 55.5, 50.5, 41.2, 36.0, 27.8. ESIMS: *m/z* calculated for [M + Na^+]: 302.100444, found 302.100437.

*4.2.3.17. (4 aS,6S)-2-(4-Chlorophenyl)-6-methoxy-4a-methyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14a**).* Yield: 63%, m.p. 176 °C. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.73 (d, *J* = 9.0 Hz, 2H), 7.24 (d, *J* = 9.0 Hz, 2H), 4.86 (dd, *J* = 2.6, 6.4 Hz, 1H), 4.49 (dd, *J* = 2.9 Hz, 9.1 Hz, 1H), 3.35 (s, 3H), 2.73 (d, *J* = 12.7 Hz, 1H), 2.48–2.27 (m, 3 H), 1.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 169.1, 145.4, 141.3, 133.7, 130.6, 125.9, 105.8, 105.3, 77.9, 55.7, 43.2, 29.9, 26.1. ESIMS: *m/z* calculated for [M+Na^+331.082541 :[, found 331.082532.

*4.2.3.18. (4aS,6S)-6-methoxy-4a-methyl-2-p-tolyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14b**).* Yield: 58%, m.p. 162 °C. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.71 (d, *J* = 8.7 Hz, 2H), 7.11 (d, *J* = 8.7 Hz, 2H), 4.86 (dd, *J* = 2.8, 6.3 Hz, 1H), 4.45 (dd, *J* = 6.1, 9.1 Hz, 1H), 3.36 (s, 3H), 2.69 (d, *J* = 13.1 Hz, 1H), 2.49–2.27 (m, 3 H), 2.22 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 167.1, 146.4, 141.5, 140.7, 130.8, 124.2, 106.2, 105.1, 77.9, 54.8, 41.6, 29.8, 26.4, 20.9. ESIMS: *m/z* calculated for [M + Na^+]: 311.137163, found 311.137172.

*4.2.3.19. (4aS,6S)-2-(3,5-Dimethoxyphenyl)-6-methoxy-4a-methyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14c**).* Yield: 55%, m.p. 189 °C. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 6.94 (d, *J* = 5.8 Hz, 2H), 6.55 (d, *J* = 2.7 Hz, 1H), 4.76 (dd, *J* = 3.0, 5.7 Hz, 1H), 4.51 (dd, *J* = 2.8, 5.4 Hz, 1H), 3.80 (s, 6H), 3.31 (s, 3H), 2.69 (d, *J* = 15.3 Hz, 1H), 2.46–2.25 (m, 34H), 1.42 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 167.4, 165.7, 146.1, 140.9, 110.6, 106.4, 104.9, 95.6, 77.8, 55.7, 55.6, 42.4, 29.7, 26.6. ESIMS: *m/z* calculated for [M + Na^+]: 357.142643, found 357.142633.

*4.2.3.20. (4aS,6S)-2-(3,5-Dichlorophenyl)-6-methoxy-4a-methyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14d**).* Yield: 62%, m.p. 194 °C. ^1H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.78 (d, *J* = 1.8 Hz, 2H), 7.42 (s, 1H), 4.84 (dd, *J* = 5.7, 8.4 Hz, 1H), 4.53 (dd, *J* = 3.1, 6.2 Hz, 1H), 3.34 (s, 3H), 2.71–2.29 (m, 4H), 1.41 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 167.2, 145.5, 139.1, 136.4, 134.2, 131.9, 124.6, 122.8, 106.7, 105.3, 79.1, 55.6, 42.3, 29.4, 26.5. ESIMS: *m/z* calculated for [M + Na^+]: 365.043569, found 365.043544.

*4.2.3.21. (4aS,6S)-6-methoxy-4a-methyl-2-(pyridin-3-yl)-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14e**).* Yield: 68%,

m.p. 181 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 9.25 (d, J = 2.9 Hz, 1H), 8.62 (dd, J = 2.8, 5.8 Hz, 1H), 8.12 (dd, J = 2.7, 5.9 Hz, 1H), 7.42 (dd, J = 2.8, 5.7 Hz, 1H), 4.78 (dd, J = 2.6, 6.2 Hz, 1H), 4.39 (dd, J = 1.2, 3.2 Hz, 1H), 3.34 (s, 3H), 2.69 (d, J = 15.7 Hz, 1H), 2.46–2.28 (m, 3H), 1.42 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 167.2, 146.4, 146.1, 143.4, 136.7, 127.9, 126.8, 106.5, 105.2, 78.3, 55.9, 42.1, 29.7, 26.7. ESIMS: m/z calculated for [M + Na^+]: 298.116762, found 298.116759.

*4.2.3.22. (4aS,6S)-6-methoxy-4a-methyl-2-(pyrimidin-5-yl)-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14f**).* Yield: 59%, m.p. 159 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 9.33 (s, 2H), 8.91 (s, 1H), 4.85 (dd, J = 2.6, 5.2 Hz, 1H), 4.39 (dd, J = 1.1, 8.7 Hz, 1H), 3.36 (s, 3H), 2.76 (d, J = 14.3 Hz, 1H), 2.47–2.29 (m, 3H), 1.41 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 167.7, 151.2, 147.9, 146.3, 131.5, 106.8, 105.4, 79.2, 55.6, 43.0, 29.3, 25.9. ESIMS: m/z calculated for [M + Na^+]: 299.112011, found 299.112023.

*4.2.3.23. (4aS,6S)-2-(1H-indol-2-yl)-6-methoxy-4a-methyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**14g**).* Yield: 48%, m.p. 191 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.78 (d, J = 8.9 Hz, 1H), 7.43 (d, J = 8.7 Hz, 1H), 7.21 (s, 1H), 7.12 (dd, J = 5.4, 8.6 Hz, 1H), 6.67 (t, J = 8.8 Hz, 1H), 4.85 (dd, J = 2.7, 6.1 Hz, 1H), 4.37 (dd, J = 1.0, 8.5 Hz, 1H), 3.30 (s, 3H), 2.71 (d, J = 13.1 Hz, 1H), 2.48–2.28 (m, 3H), 1.39 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.7, 148.0, 141.5, 133.1, 129.3, 123.9, 121.7, 121.0, 109.9, 106.6, 105.1, 97.1, 78.7, 55.8, 42.3, 29.1, 25.9. ESIMS: m/z calculated for [M + Na^+]: 336.132412, found 336.132443.

*4.2.3.24. (4aR,6S)-2-(3,5-Dimethoxyphenyl)-6-methoxy-4a-phenyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**15a**).* Yield: 57%, m.p. 186 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.31–7.09 (m, 5H), 6.79 (d, J = 2.8 Hz, 2H), 6.30 (s, 1H), 5.11 (dd, J = 2. 4, 5.4 Hz, 1H), 4.66 (bs, 1H), 3.83 (s, 6H), 3.35 (s, 3H), 3.12 (d, J = 15.1 Hz, 1H), 2.80 (d, J = 15.1 Hz, 1H), 2.50 (dt, J = 5.7, 10.4, 11.6 Hz, 1H), 2.34 (dt, J = 5.3, 10.1, 11.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 166.2, 165.0, 145.1, 142.3, 139.7, 126.5, 123.9, 122.1, 110.0, 107.9, 107.3, 95.8, 82.1, 55.7, 55.2, 42.5, 29.7. ESIMS: m/z calculated for [M + Na^+]: 419.158293, found 419.158281.

*4.2.3.25. (4aR,6S)-2-(3,5-Dichlorophenyl)-6-methoxy-4a-phenyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**15b**).* Yield: 51%, m.p. 166 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.92 (s, 2H), 7.36–7.09 (m, 6H), 5.18 (t, J = 2.7 Hz, 1H), 4.31 (bs, 1H), 3.31 (s, 3H), 3.15 (d, J = 14.5 Hz, 1H), 2.78 (d, J = 14.4 Hz, 1H), 2.54 (dd, J = 4.2, 12.6 Hz, 1H), 2.42 (dd, J = 4.5, 8.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.8, 145.0, 142.3, 138.1, 136.2, 126.4, 124.8, 123.9, 123.3, 122.8, 107.9, 107.5, 82.1, 55.7, 43.1, 29.8. ESIMS: m/z calculated for [M + Na^+]: 427.059219, found 427.059207.

*4.2.3.26. (4aR,6S)-6-methoxy-4a-phenyl-2-p-tolyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**15c**).* Yield: 44%, m.p. 198 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.62 (d, J = 12.2 Hz, 2H), 7.31–7.09 (m, 7H), 5.22 (t, J = 5.4 Hz, 1H), 4.59 (dd, J = 2.6, 5.3 Hz, 1H), 3.34 (s, 3H), 3.13 (d, J = 14.6 Hz, 1H), 2.81 (d, J = 14.6 Hz, 1H), 2.53 (dt, J = 5.6, 17.2 Hz, 1H), 2.39 (dt, J = 2.8, 17.2 Hz, 1H), 2.31 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.9, 145.1, 143.0, 140.6, 131.2, 126.9, 124.4, 123.5, 122.9, 108.0, 107.5, 82.3, 55.7, 42.4, 29.8, 20.9. ESIMS: m/z calculated for [M+Na^+373.152813 :[, found 373.152827.

*4.2.3.27. (4aR,6S)-6-methoxy-2-(4-methoxyphenyl)-4a-phenyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**15d**).* Yield: 53%, m.p. 177 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 7.51 (dd, J = 2.2, 5.1 Hz, 2H), 7.32–7.07 (m, 5H), 6.92 (dd, J = 2.1, 5.3 Hz, 2H), 5.08 (dd, J = 2.2, 5.6 Hz, 1H), 4.71 (bd, J = 8.6 Hz, 1H), 3.73 (s, 3H), 3.30 (s, 3H), 3.09 (d, J = 13.7 Hz, 1H), 2.76 (d, J = 13.7 Hz, 1H), 2.53 (dt, J = 5.4, 14.3 Hz, 1H), 2.38 (dt, J = 5.1, 14.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 166.1, 161.6, 145.1, 142.3, 136.7, 126.3, 126.0, 124.9, 123.0, 115.1, 107.9, 107.2, 82.7, 55.8, 55.1, 43.0, 29.3. ESIMS: m/z calculated for [M + Na^+]: 389.147728, found 389.147719.

*4.2.3.28. (4aR,6S)-6-methoxy-4a-phenyl-2-(pyrimidin-5-yl)-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**15e**).* Yield: 50%, m.p. 183 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 9.33 (s, 2H), 8.92 (s, 1H), 7.27–7.09 (m, 5H), 5.03 (dd, J = 2.2, 5.1 Hz, 1H), 4.62 (dd, J = 2.2, 5.3 Hz, 1H), 3.35 (s, 3H), 3.08 (d, J = 14.2 Hz, 1H), 2.75 (d, J = 14.2 Hz, 1H), 2.52 (dt, J = 5.3, 14.1 Hz, 1H), 2.41 (dt, J = 2.3, 14.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.7, 151.9, 148.2, 145.3, 142.2, 132.6, 126.8, 124.5, 122.8, 107.7, 107.5, 82.4, 55.3, 43.1, 29.4. ESIMS: m/z calculated for [M + Na^+]: 361.127661, found 361.127672.

*4.2.3.29. (4aR,6S)-2-(1H-indol-2-yl)-6-methoxy-4a-phenyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**15f**).* Yield: 49%, m.p. 156 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 10.6 (s, 1H), 8.4 (s, 1H), 7.8 (s, 1H), 7.41–7.09 (m, 6H), 6.57 (t, J = 8.6 Hz, 2H), 5.05 (t, J = 4.2 Hz, 1H), 4.61 (t, J = 6.7 Hz, 1H), 3.33 (s, 3H), 3.11 (d, J = 12.7 Hz, 1H), 2.79 (d, J = 12.7 Hz, 1H), 2.51 (bd, J = 5.7 Hz, 1H), 2.41 (bd, J = 4.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 164.9, 147.8, 144.1, 140.6, 133.7, 128.3, 126.5, 125.1, 124.8, 123.2, 121.9, 121.4, 110.3, 108.1, 107.8, 98.2, 83.1, 55.7, 43.2, 30.1. ESIMS: m/z calculated for [M + Na^+]: 398.148062, found 398.148058.

*4.2.3.30. (4aR,6S)-2-(3,5-Dichlorophenyl)-6-methoxy-4a-(pyridin-4-yl)-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**16a**).* Yield: 60%, m.p. 171 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.65 (d, J = 5.3 Hz, 2H), 7.87 (d, J = 2.1 Hz, 2H), 7.41 (t, J = 2.1 Hz, 1H), 7.32 (d, J = 5.4 Hz, 2H), 5.12 (dd, J = 2.0, 5.6 Hz, 1H), 4.60 (dd, J = 2.1, 5.4 Hz, 1H), 3.36 (s, 3H), 3.11 (d, J = 14.6 Hz, 1H), 2.72 (d, J = 14.6 Hz, 1H), 2.54 (dt, J = 5.3, 8.9 Hz, 1H), 2.41 (dt, J = 2.3, 12.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.9, 146.1, 145.8, 144.2, 137.9, 136.1, 124.3, 123.1, 120.7, 108.2, 107.8, 82.9, 55.6, 43.2, 29.7. ESIMS: m/z calculated for [M + Na^+]: 428.054468, found 428.054454.

*4.2.3.31. (4aR,6S)-6-methoxy-4a-(pyridin-4-yl)-2-p-tolyl-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**16b**).* Yield: 46%, m.p. 145 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.67 (d, J = 5.7 Hz, 2H), 7.61 (d, J = 8.9 Hz, 2H), 7.33 (d, J = 5.6 Hz, 2H), 7.22 (d, J = 10.5 Hz, 2H), 5.09 (dd, J = 2.2, 5.6 Hz, 1H), 4.61 (t, J = 5.7 Hz, 1H), 3.35 (s, 3H), 3.12 (d, J = 14.3 Hz, 1H), 2.82 (d, J = 14.3 Hz, 1H), 2.53 (dt, J = 5.2, 14.5 Hz, 1H), 2.35 (dt, J = 2.6, 13.7 Hz, 1H), 2.23 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.8, 146.1, 145.3, 144.9, 142.6, 141.0, 130.7, 123.6, 121.1, 107.9, 107.2, 83.2, 56.1, 42.3, 31.1, 21.2. ESIMS: m/z calculated for [M + Na^+]: 374.148062, found 374.148071.

*4.2.3.32. (4aR,6S)-2-(3,5-Dimethoxyphenyl)-6-methoxy-4a-(pyridin-4-yl)-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**16c**).* Yield: 47%, m.p. 162 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.65 (d, J = 5.7 Hz, 2H), 7.29 (d, J = 5.5 Hz, 2H), 6.78 (d, J = 2.4 Hz, 2H), 6.35 (bs, 1H), 5.04 (dd, J = 2.7, 5.6 Hz, 1H), 4.65 (bs, 1H), 3.87 (s, 6H), 3.34 (s, 3H), 3.12 (d, J = 14.3 Hz, 1H), 2.88 (d, J = 14.3 Hz, 1H), 2.55 (dt, J = 5.4, 12.2 Hz, 1H), 2.34 (dt, J = 5.5, 12.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 165.9, 165.3, 146.1, 145.5, 144.8, 141.1, 120.8, 109.9, 107.8, 107.1, 95.7, 83.2, 55.8, 55.3, 43.1, 29.0. ESIMS: m/z calculated for [M + Na^+]: 420.153542, found 420.153550.

*4.2.3.33. (4aR,6S)-2-(1H-indol-2-yl)-6-methoxy-4a-(pyridin-4-yl)-4,4a,6,7-tetrahydro-1H-pyrano[3,2-c]pyridazin-3(2H)-one (**16d**).* Yield:

41%, m.p. 178 °C. ^{1}H NMR (300 MHz, $CDCl_3$, in ppm) δ 8.61 (d, J = 5.6 Hz, 2H), 7.75 (d, J = 5.4 Hz, 1H), 7.41–7.21 (m, 4H), 6.66 (d, J = 5.7 Hz, 1H), 5.07 (t, J = 4.2 Hz, 1H), 4.61 (t, J = 6.9 Hz, 1H), 3.11 (s, 3H), 3.09 (d, J = 12.1 Hz, 1H), 2.81 (d, J = 12.1 Hz, 1H), 2.55 (bd, J = 4.2 Hz, 1H), 2.42 (bd, J = 4.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$, in ppm) δ 164.9, 148.1, 146.7, 146.0, 140.3, 133.4, 129.2, 125.0, 121.8, 121.6, 120.4, 110.1, 108.0, 107.2, 97.2, 81.4, 55.7, 43.1, 28.7. ESIMS: m/z calculated for [M + Na^+]: 399.143311, found 399.143322.

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