Tandem Achmatowicz-Knoevenagel protocol: diastereoselective synthesis and anticancer evaluation of cyclopenta[b]pyrane derivatives†

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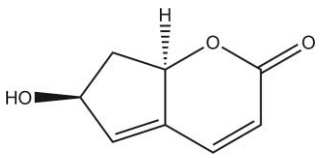
Synthesis of cyclopenta[b]pyrane derivatives *via* Achmatowicz oxidative cyclization of furanols followed by intramolecular Knoevenagel condensation of the β-ketoester arm is examined. The extent of diastereoselectivity was dependent on the nature of the chiral atom within the tethering carbon of the pyrenone ring. In some cases, this process proceeds with a high degree of stereoselectivity after protection of the anomeric hydroxyl group. Furthermore, the cytotoxic activity of pyrazolone derivatives thereof was studied against HCT116 (human colorectal cancer cells), SK–N-SH (human Caucasian bone marrow neuroblastoma) cells and the non-tumorigenic cells (MCF10A).

Introduction

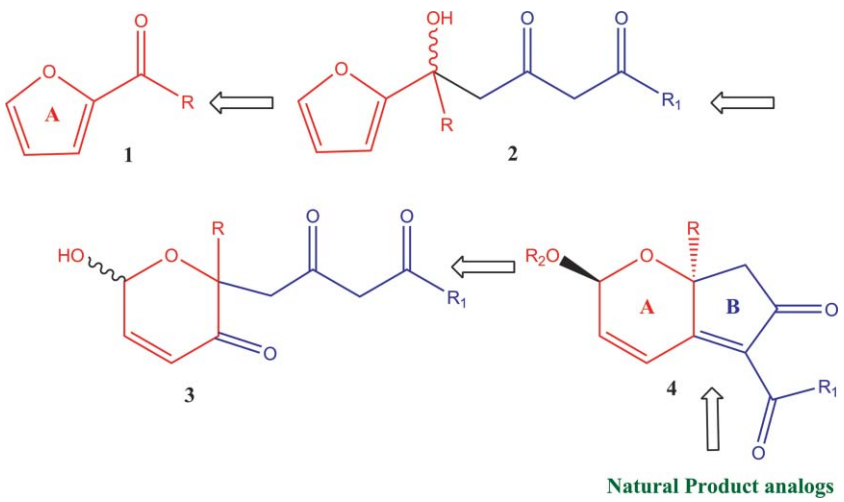
The design of new strategies for the synthesis of five-membered carbocycles continues to be of great interest for organic chemists due to the importance of this skeleton as part of biologically relevant compounds.[1] As a result, there has been an upsurge in the synthesis of cyclopenta[c]pyranes and various strategies pertinent to these scaffolds have appeared with growing frequency in concert with their theoretical and biological relevance.[1–5] In contrast, cyclopenta[b]pyrane derivatives received less attention. Only a few reports on the synthesis of such systems have been reported. A among others, the Lewis acid catalyzed Nazarov cyclization,[2] [3 + 2] cyclization of alkenyl Fischer carbene complexes,[3] [5 + 1]/[2 + 2 + 1]-cycloaddition through $Co_2(CO)_8$-mediated tandem cycloaddition of epoxyalkynes,[4] and the metal catalyzed cycloisomerization of tethered cyclic alkynyl diones.[5] Furthermore, ilexalactone (Fig. 1),[6a] a natural product containing a cyclopenta[b]pyrenone framework was synthesized using the Grubbs metathesis protocol.[6b]



Ilexalactone

Fig. 1 Ilexalactone natural product.

Our strategy however, not only describes a concise and economical route to cyclopenta[b]pyranes and pyrazolone derivatives thereof, but also possesses the potential for broad applications toward biologically significant targets. Our efforts in this regard have been driven by our previous experience in the reactivity and applications of diketones[7] and the wide applications of the Achmatowicz oxidative cyclization.[8,9] As shown in Scheme 1, this complexity-generating reaction[10] transforms a relatively simple



Scheme 1 Retrosynthetic analysis.

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substrate, such as **2**, into a more complex product **3** with the potential for diversification *via* functionalization of the resultant enone.[8,9,10]

Recently, the Ueda group described the isolation, identification, antimicrobial and anticancer activities of two cyclopenta[b]pyrane natural products.[11] Encouraged by their results, we tested the anticancer activity of our new motifs and their pyrazolone derivatives against two in-house panels of cancer cell lines.[12] This was due to our continued interests in developing new synthetic methodologies aiming at natural product synthesis,[7,13] and finding lead molecules to certain disease states.[14]

Results and Discussion

Retrosynthetic Analysis

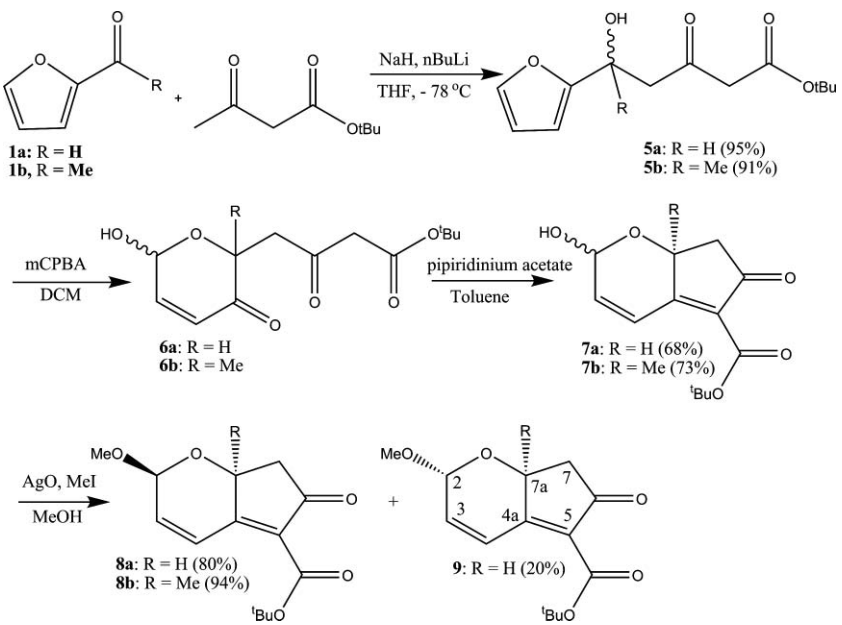
Our synthetic plan contemplated systems of type **4** in which the relative configurations of two stereogenic centers would be properly arranged (Scheme 1). For this purpose, we wished to implement a ring expansion variant of the Achmatowicz oxidative cyclization on compounds of type **2**. Analogs of this central element had previously been shown by other groups to be highly utilitarian in providing ready access to diastereoselective syntheses of furo[c]pyrane scaffolds[15,16] and other polysubstituted tetrahydropyranes.[17] Accordingly, it was envisioned that **4** might be produced from **3**, although it was not clear from the outset that **3** will undergo Knoevenagel condensation. As matters have turned out, we were able to efficiently access **4** from pyrenone **3**, which is readily accessible as a mixture of diastereoisomers from commercially available starting materials such as **1**. The latter was produced from reaction between the dianion derived from *tert*-butyl acetoacetate and furan derivative.

The idea that **3** would be well-suited for regioselective fusion of the cyclopentenone ring leading to **4** rests on the strong likelihood, that the Knoevenagel event required to reach **4**, would be heavily dominated by a half-chair-like transition state geometry. Consequently, the β-ketoester arm resides in quasi-axial disposition.

Diastereoselective synthesis of cyclopenta[b]pyranes

The first task in gaining access to **7a** is centered on proper functionalization of the furan derivatives such as **5a**. This in turn could be easily prepared through reaction of dianions derived from *tert*-butyl acetoacetate and furfural (**1a**). On exposure of **5a** to *m*-chloroperbenzoic acid in dichloromethane, the normal Achmatovicz reaction course was followed leading to pyranone **6a** (Scheme 2). For the arrival at **7a**, the Knoevenagel directed cyclization was selected for this purpose, although we were unaware at the time of its successful application in an example involving two possible nucleofuges contained in the pyrenone ring. Thus, the four bases EtONa, ^{t}BuOK, NaH and Cs_2CO_3, were screened as possible promoters of the desired intramolecular cyclization of **6a**. With the first two, no significant clean reaction occurred in THF at different temperatures under anhydrous conditions. When equimolar quantities of the latter two were used, substantial levels of decomposition to tarry products were observed. Parallel findings were noted with *p*-TSA in the absence of moisture. Ultimately, the use of piperidinium acetate in hot toluene was considered and found to be the ideal reagent for the Knoevenagel event. To this end, heating **6a** to 50 °C with 1.3 equiv of piperidinium acetate in toluene containing 4 Å molecular sieves, generated carbinol **7a** in 68% yield. Subsequently, anomeric protection with the help of silver oxide in MeI solution served to provide the epimeric bicylic systems **8a** and **9** in 92% combined yield as 4 : 1 separable (NMR, HPLC) mixture of diastereoisomers (Scheme 2).

The stereochemistry around **8a** and **9** was concluded from their ^{1}H NMR spectra and NOE experiments. The chemical shift of the anomeric proton in **8a** appeared at 5.0 ppm as a doublet (J = 3.3 Hz), while that in **9** resonated at 5.34 ppm as a doublet of doublets (J = 0.6, 2.0 Hz).[2,15–17] Unambiguously, the



Scheme 2 Diastereoselective synthesis of cyclopenta[b]pyran.

angular methine proton (7a-H) in **8a** showed significant reciprocal NOE interactions with the anomeric proton, indicating a *cis* relationship. However, the corresponding proton in **9**, indicated NOE enhancement of the methoxy group confirming that both reside on the same dispositions. In order to understand the delicate stereochemistry of such a process, a methyl-functionalized furanol **5b** was prepared. Subjecting this latter product to the same reaction sequence as described for **5a**, compound **7b** was delivered in 73% yield. Interestingly, subjecting **7b** to the above described anomeric protection protocol provided, the *trans* isomer **8b** as the sole reaction product. The unambiguous assignment of the stereochemistry around **8b** was concluded from its ^{1}H NMR spectrum and NOE experiments. A strong reciprocal NOE enhancement between the angular methyl group and the anomeric proton was produced upon irradiation of the set of signals at 5.10 ppm and 1.48 ppm, which were assigned to 2-H and 7a-CH_3, respectively. The coupling of the anomeric proton in **8b** is found to be 3.1 Hz, similar to that found for **8a**.[2,15–17]

Stereochemical analysis

To understand the stereochemical sensitivities to which **7b** is subject, one needs only to consider its two most accessible conformations **B** and **C** (Fig. 2). The structural elements in conformer **C** are such that steric effects should favor the equatorial methoxy epimer, for which steric factors are clearly more conducive toward the formation of such isomer. The situation is precisely reversed in **B**, where the potential for substantive 1,3-diaxial steric compression is especially obvious. Therefore, when the angular hydrogen in **8a** was replaced by a bulkier methyl group as in **8b**, the silver oxide catalyzed protection sequence delivered only the *trans* diastereoisomer **8b**. We find this easy to reconcile with the involvement of conformers **B** and **C** in the product-forming intermediate **A** (formed after treating **7b** with Ag_2O), since the ratios should be strongly affected by the added steric burden in **8b**.[17] Therefore, conformer **C** is kinetically more relevant intermediate, with adduct configuration being predominantly determined by steric control.

Synthesis of pyrazolone derivatives

In order to exploit the structural features of **8b–c**, an attempt was made to functionalize the β-ketoester moiety of the fused cyclopentenone ring of **8b** with hydrazine derivatives, in order to assemble highly conjugated tricyclic scaffolds that might have interesting biological activities. Disappointingly, our efforts in this regard failed and produced a mixture of different products according to TLC analysis. However, subjecting **8b** to Pd/C catalyzed hydrogenation in EtOAc–methanol, compound **10** was produced in 62% yield. The production of compound **10** could be explained through the conjugated addition of hydrides on the dienone group. Having secured pure amount of **10**, the ring junction was assigned *cis* stereochemistry on the basis of its NMR NOE experiments. Irradiation of the newly introduced angular methine proton (4a-H) produced a strong NOE enhancement of the *tert*-butyl group, the angular methyl and the anomeric proton, confirming their *cis* relationship. Furthermore, irradiation of 5-H produced NOE enhancement of the anomeric methoxyl group, indicating both is in the same disposition. Further confirmation of the stereochemistry around **10** was concluded from its ^{1}H NMR spectrum. 5-H resonates at 3.29 ppm as a doublet with a coupling constant of 11.2 Hz, confirm its *trans* relationship with H-4a.[2,15–17] To further confirm these stereochemical findings, we have carried out molecular modeling calculations using force field MM2 method on compounds **10** and **10a** (Fig. 3).[18] As a result we learned that compound **10** (39.3 kcal/mol) is thermodynamically more preferred compared to compound **10a** (47.4 kcal/mol).

At this stage, a tandem ring-closure reaction of the synthesized bicyclic γ-ketoester **10** with hydrazines was followed. Such a process is considered one of the practical processes toward the construction of substituted 1,2-diaza-3-one heterocyclic ring systems.[19] Therefore, after optimization of the reaction conditions, we applied this protocol to a range of hydrazine derivatives, including aryl and heteroaryl hydrazines (Scheme 3). This sequential process, however, was reported to be sensitive and chemoselectively controlled by the percentage of the acidic catalyst added to the reaction.[20] Additionally, the regiochemistry of such a process is known to proceed first through condensation between the more basic terminal amine group of the hydrazine derivative with

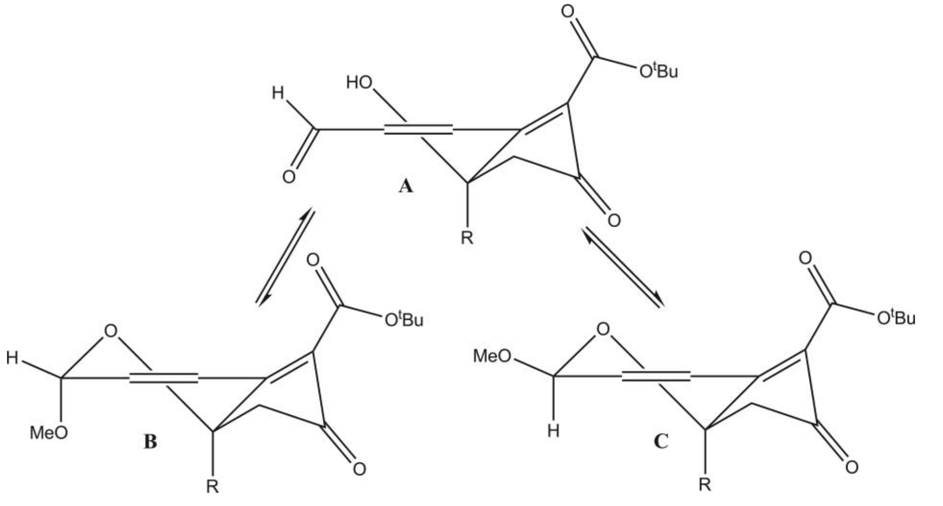


Fig. 2 Conformational analysis for the diastereoselectivity after anomeric protection.

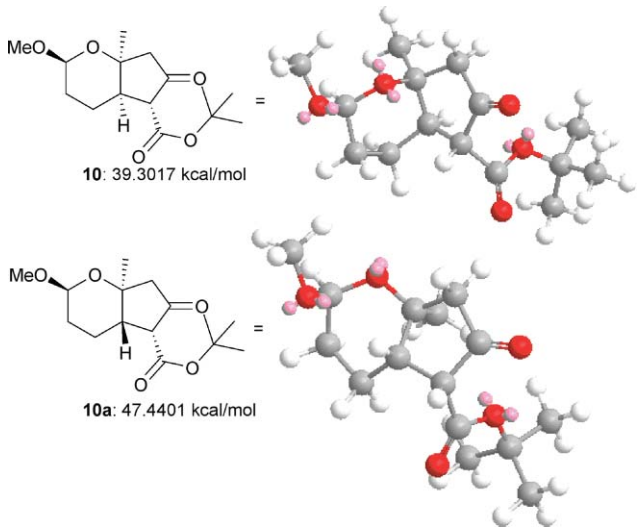
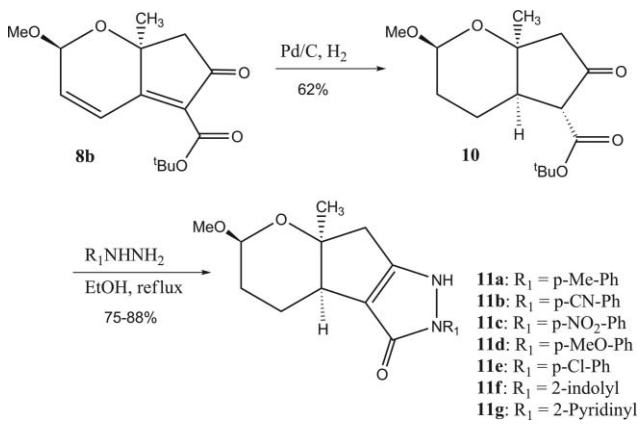


Fig. 3 Molecular modeling of compounds **10** and **10a** using ChemBio-Draw Ultra 11.[18]



Scheme 3 Synthesis of tricyclic pyrazolones.

the ketone group followed by nucleophilic acyl substitution on the ester function through the second nitrogen of the formed hydrazone, to deliver the pyrazolone scaffolds.[19,20] Thus, to arrive at the intended pyrazolone targets, compound **10** was heated with *p*-methyl phenylhydrazine in ethanol in the presence of 5 mol% of *p*-TSA, to delivered the tricyclic pyrazolone **11a** in 72% yield. Following the two-step reaction protocol with various hydrazine derivatives, a series of pyrazolones were prepared (Scheme 3).

In vitro anticancer screening

Recently, the Ueda group described the isolation, identification, antimicrobial and anticancer activity of two cyclopenta[b]pyrane natural products.[12] Triggered by these results, we screened compounds **8a–b** and **11a–f** against two in-house panels of cancer cell lines. Cytotoxicity was assessed after 72 h of exposure *via* the MTT test. Fig. 4 and 5 show that the new pyrazolone derivatives are highly cytotoxic against HCT116 (human colorectal cancer cells) and SK–N-SH (human Caucasian bone marrow neuroblastoma) cells. The results of this survival assay reveal that compound **11g** has the highest cytotoxic activity at the tested concentrations. Table 1 shows the IC_{50} values for the synthesized octahydro-3*H*-

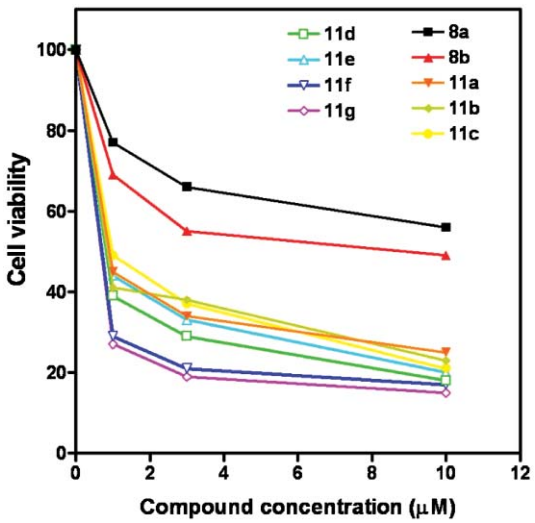


Fig. 4 Effect of pyrazolone compounds on the viability of HCT116 relative to the control.

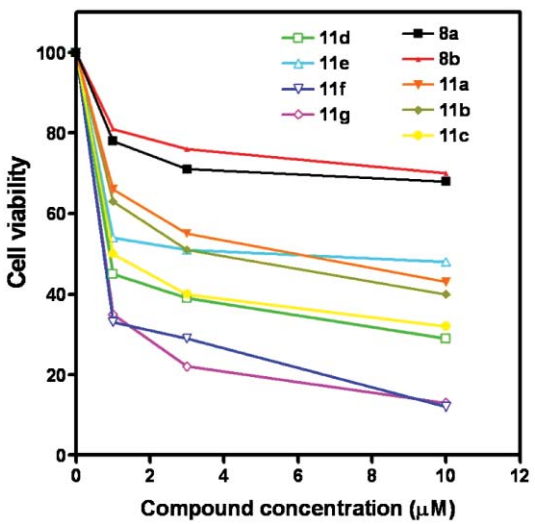


Fig. 5 Effect of pyrazolone compounds on the viability of SK–N-SH relative to the control.

pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one analogs. These results indicated that, compounds **11f** and **11g** were the most active against the breast cancer cell line SK-BR-3 and the human colorectal cancer cells (HCT116). Furthermore, both compounds indicated about 25-fold less toxicity against non-tumorigenic cells derived from breast tissues (MCF10A).

Conclusions

In summary, cyclopenta[b]pyrane systems can be assembled efficiently through the Achmatovitz oxidative ring expansion-Knoevenagel condensation strategy. This approach appears to hold considerable synthetic utility. Its particular value resides in the conciseness and efficiency with which cyclopenta[b]pyrane construction can be achieved from structurally simple and accessible furan precursors. Good stereocontrol can be relied upon during the construction process, provided that the starting furfuryl

Table 1 IC_{50} Values for cellular proliferation inhibition results of compounds **8a–11g** in different cell lines[a] IC_{50} (μM)

Compd	HCT116	SK–N-SH	MCF10A
8a	5.42	2.52	16.75
8b	4.01	2.87	18.54
11a	1.57	3.60	16.32
11b	0.63	3.34	20.11
11c	2.60	1.55	19.65
11d	1.32	0.83	21.83
11e	1.86	2.61	23.92
11f	0.28	0.71	17.58
11g	0.24	1.45	18.48

[a] Data represent mean values for three independent determinations.

unit carries a ketone rather than an aldehyde. This feature allows for incorporation of reliable stereochemical predictability into the synthetic design. Furthermore, the anticancer activity of the pyrazolone derivatives showed significant cytotoxity against HCT116 (human colorectal cancer cells) and SK–N-SH (human Caucasian bone marrow neuroblastoma) cells. Moreover, many of the synthesized compounds indicated less toxicity toward normal cell lines. These results, merits further investigations in our laboratories.

Experimental

General methods

All reagents were used as purchased from commercial suppliers without further purification. The reactions were carried out in an oven dried or flamed graduated vessels. Solvents were dried and purified by conventional methods prior use. 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, ^{13}C spectra were recorded on 250 MHz/63 MHz or 400/100.7 MHz spectrometers. 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). Elemental analysis was performed on elemental analyzer. The FD mass spectra were recorded using a mass spectrometer connected to a PDO 11/34 (DEC) computer system. All compounds were determined to be >95% pure by high-performance liquid chromatography (HPLC). Purity of compounds were determined on a Phenomenex Luna C18- (2), 3 mm column, 4.6 mm i.d.× 30 mm length, with 30–75% acetonitrile–water/0.1% trifluoroacetic acid, 1.0 mL min^{-1} elution at rt using 210, 254, or 280 nm wavelength.

General procedure of the synthesis of compounds 5a and 5b

A suspension of NaH (174 mg, 4.4 mmol, 65% dispersion in mineral oil) in 10 mL of THF at 0 °C was cautiously treated with *tert*-butyl acetoacetate (0.66 mL, 4 mmol) under argon over a 15 min period. After stirring at this temperature for 30 min, a solution of n-BuLi (2.75 mL, 4.4 mmol, 1.6 M in n-hexane) was added dropwise over 10 min. The mixture was stirred at 0 °C for 30 min. The resultant milky solution was cooled to −78 °C, then 4 mmols of either furfural (384 mg) or 2-acetylfuran (440 mg) in 10 ml THF was added. The reaction mixture was then stirred at the same temperature for 1 h, after which it 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, which was purified through a short silica gel column using hexane–EtOAc (9 : 1) as eluent to afford compounds **5a** (917 mg, 95% yield) and **5b** (977 mg, 91% yield) as a yellowish oils.

tert-Butyl 5-(2-furyl)-5-hydroxy-3-oxopentanoate (5a)

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.31 (dd, J = 0.8, 1.8 Hz, 1H, H-5′), 6.30 (dd, J = 1.8, 3.2 Hz, 1H, H-4′), 6.22 (d, J = 3.2 Hz, 1H, H-3′), 5.13 (dd, J = 3.3, 8.3 Hz, 1H, H-5), 3.37 (s, 2H, H-2a, H-2b), 3.10 (dd, J = 8.3, 17.5 Hz, 1H, H-4a), 2.97 (dd, J = 3.3, 17.5 Hz, 1H, H-4b), 1.39 ($CO_2C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ 202.7 (C-2), 166.1 ($CO_2C(CH_3)_3$), 154.8 (C-2′), 110.3 (C-5′), 110.2 (C-4′), 106.3 (C-3′), 82.3 ($CO_2C(CH_3)_3$), 63.5 (C-5), 51.1 (C-2), 47.8 (C-4), 27.9 ($CO_2C(CH_3)_3$); FD-MS m/z = 255 (M + 1)$^+$. Anal. Calcd for $C_{13}H_{18}O_5$ (254.28); C, 61.40; H, 7.14. Found: C, 61.20; H, 7.31.

tert-Butyl 5-(2-furyl)-5-hydroxy-3-oxohexanoate (5b)

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.22 (dd, J = 0.9, 1.8 Hz, 1H, H-5′), 6.02 (dd, J = 1.8, 3.2 Hz, 1*H*-4′), 6.15 (dd, J = 0.9, 3.3 Hz, 1H, H-3′), 3.25 (bs, 2H, H-2a, H-2b), 3.18 (d, J = 16.5 Hz, 1H, H-4a), 2.82 (d, J = 16.5 Hz, 1H, H-4b), 1.43 (CH_3), 1.63 ($CO_2C(CH_3)_3$); ^{13}C NMR (63 MHz, CDCl3) δ 203.0 (C-3), 165.0 ($CO_2C(CH_3)_3$), 158.5 (C-2′), 110.3 (C-5′), 110.2 (C-4′), 104.7 (C = 3′), 82.0 ($CO_2C(CH_3)_3$), 70.0 (C-5), 51.7 (C-2), 51.5 (C-4), 28.2 ($CO_2C(CH_3)_3$), 27.6 (CH_3); FD-MS m/z = 269 (M + 1)$^+$. Anal. Calcd for $C_{14}H_{20}O_5$ (268.31); C, 62.67; H, 7.51. Found: C, 62.51; H, 7.42.

General procedure for the synthesis of compounds 7a and 7b

A solution of **5a** (1270 mg, 5 mmol) or **5b** (1341.5 mg, 5 mmol) in dichloromethane (40 mL) at 0 °C was treated with *m*-chloroperbenzoic acid (1376 mg, 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 with stirring 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 (**6a** and **6b**) were used in the next step without further purification.

A solution of the crude anomeric mixture of **6a** (852 mg, 3 mmol) or **6b** (810 mg, 3 mmol) in dry toluene (30 ml), freshly prepared piperidinium acetate (3.9 mmol), and 4 Å molecular sieves (250 mg) was stirred at 50 °C for 4 h. The hot mixture was filtered and evaporated under reduced pressure. The resulting brown viscous oil was dissolved in EtOAc and extracted with a saturated solution of $NaHCO_3$, brine, water and dried over Na_2SO_4. The crude material was purified on a silica gel column using hexane–EtOAc (7 : 3) as eluent to afford compounds **7a** (350 mg, 68% yield) and **7b** (425 mg, 73% yield) as amorphous solids.

tert-Butyl (7a*S*)-2-hydroxy-6-oxo-2,6,7,7a-tetrahydrocyclopenta[*b*]pyran-5-carboxylate (7a)

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.25 (bt, J = 10.1 Hz, 1H, H-3), 6.42 (dd, J = 3.3, 10.1 Hz, 1H, H-4), 5.60 (bs, 1H, H-2), 5.16 (dd, J = 4.7, 6.8 Hz, 1H, H-7a), 2.84 (dd, J = 6.8, 17.6 Hz, 1H, H-7), 2.51 (dd, J = 4.6, 17.6 Hz, 1H, H-7′), 15.3 (s, 9H, ^{t}BuO); ^{13}C NMR (63 MHz, $CDCl_3$) δ 199.1, 199.2 (C-6), 170.5, 170.7 (C-4a), 166.2 ($CO_2C(CH_3)_3$), 142, 138.2 (C-3), 125.0, 123.0 (C-4), 92.7, 89.1 (C-2), 82.0, 71.5 ($CO_2C(CH_3)_3$), 65.5 (C-7a), 42.5, 42.3 (C-7), 28.2 ($CO_2C(CH_3)_3$); FD-MS m/z = 252 (M)$^+$. Anal. Calcd for $C_{13}H_{16}O_5$ (252.10); C, 61.90; H, 6.39. Found: C, 61.85; H, 6.43.

tert-Butyl (7a*S*)-2-hydroxy-7a-methyl-6-oxo-2,6,7,7a-tetrahydrocyclopenta[*b*]pyran-5-carboxylate (7b)

^{1}H NMR (250 MHz, $CDCl_3$) δ 7.13 (m, 2H, H-3), 6.32 (m, 2H, H-4), 5.59, 5.51 (bs, 2H, H-2), 2.57 (m, 4H, H-7,7′), 1.42 (bs, 12H, CH_3-7a, $CO_2C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ 198.8, 198.5 (C-6), 170.5, 170.4 (C-4a), 161.1 ($CO_2C(CH_3)_3$), 141.5, 138.5 (C-3), 125.7, 125.3 (C-5), 122.5, 121.3 (C = 4), 89.6, 88.7 (C-2), 82.5 ($CO_2C(CH_3)_3$),77.5, 73.9 (C-7a) 52.2, 51.9 (C-7), 28.9, 24.2 (CH_3 = 7a), 28.1, 27.8 ($CO_2C(CH_3)_3$); FD-MS m/z = 266 (M)$^+$. Anal. Calcd for $C_{14}H_{18}O_5$ (266.12); C, 63.15; H, 6.81. Found: C, 63.09; H, 6.87.

General procedure for the synthesis of compounds 8a, 8b and 9

A solution of the anomeric mixture of **7a** (756 mg, 3 mmol) or **7b** (798 mg, 3 mmol) in MeI (15 mL) was treated at rt with 3.0 mmol of Ag_2O. The progress of the 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, brine, dried and evaporated. The residue was purified on silica gel column using hexane–EtOAc (9 : 1) as an eluent.

tert-Butyl (2*S*,7a*S*)-2-methoxy-6-oxo-2,6,7,7a-tetrahydrocyclopenta[*b*]pyran-5-carboxylate (8a)

Yield: 540 mg, 80%; Significant NMR NOEs are 2-H to 7a-H, 36%; 7a-H to 2-H, 32%. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.20 (dd, J = 0.4, 10.1 Hz, 1H, H-3), 6.30 (dd, J = 3.3, 10.1 Hz, 1H, H-4), 5.00 (dd, J = 3.3 Hz, 1H, H-2), 4.96 (dd, J = 4.8, 6.9 Hz, 1H, H-7a), 3.45 (s, 3H, OMe), 2.80 (dd, J = 7.0, 17.6 Hz, 1H, H-7), 2.48 (dd, J = 4.5, 17.6 Hz, 1H, H-7′), 1.48 (s, 9H, ^{t}BuO); ^{13}C NMR (63 MHz, $CDCl_3$) δ 197.8 (C-6), 166.1 (C-4a), 160.8 ($CO_2C(CH_3)_3$), 137.2 (C-3), 123.0 (C-4), 127.0 (C-5), 95.6 (C-2), 82.5 ($CO_2C(CH_3)_3$), 65.5 (C-7a), 56.4 (OMe), 42.3 (C-7), 28.0 ($CO_2C(CH_3)_3$); FD-MS m/z = 266 (M)$^+$. Anal. Calcd for $C_{14}H_{18}O_5$ (266.16); C 63.12, H 6.82. Found: C 63.51, H 6.55.

tert-Butyl (2*S*,7a*S*)-2-methoxy-7a-methyl-6-oxo-2,6,7,7a-tetrahydrocyclopenta[*b*]pyran-5-carboxylate (8b)

Yield: 742 mg, 94%; Significant NMR NOEs are 2-H to 7a-CH_3, 31%; 7a-CH_3 to 2-H, 33%. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.26 (dd, J = 1.7, 10.0 Hz, 1H, H-3), 6.62 (dd, J = 1.6, 10.0 Hz, 1H, H-4), 5.10 (bd, J = 3.1 Hz, 1H, H-2), 3.44 (s, 3H, OMe), 2.74 (d, J = 17.1 Hz, 1H, H-7), 2.57 (d, J = 17.1 Hz, 1H, H-7′), 1.48 (s, 3H, 7a-CH_3), 1.47 (s, 9H, ($CO_2C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ 197.7 (C-6), 169.4 (C-4a), 160.9 ($CO_2C(CH_3)_3$), 139.0 (C-2), 123.8 (C-3), 123.7 (C-5), 95.6 (C-2), 82.3 ($CO_2C(CH_3)_3$), 76.7 (C-7a), 55.1 (OMe), 52.1 (C-7), 28.2 ($CO_2C(CH_3)_3$), 24.3 (7a-CH_3); FD-MS m/z = 280 (M)$^+$. Anal. Calcd for $C_{15}H_{20}O_5$ (280.32); C 64.27, H 7.19; Found: C 63.95, H 7.32.

tert-Butyl (2*R*,7a*S*)-2-methoxy-6-oxo-2,6,7,7a-tetrahydrocyclopenta[*b*]pyran-5-carboxylate (9)

Yield: 138 mg, 20%; Significant NMR NOEs are 2-OMe to 7a-H, 37%; 7a-H to 2-OMe, 29%. ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.25 (dd, J = 2.1, 10.1 Hz, 1H, H-3), 6.32 (dd, J = 1.4, 10.1 Hz, 1H, H-4), 5.34 (dd, J = 0.6, 2.0 Hz, 1H, H-2), 4.74 (dd, J = 4.7, 6.9 Hz, 1H, H-7a), 3.47 (s, 3H, OMe), 2.83 (dd, J = 6.8, 17.7 Hz, 1H, H-7), 2.55 (dd, J = 4.6, 17.7 Hz, 1H, H-7′), 1.47 (s, 9H, ($CO_2C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.5 ($CO_2C(CH_3)_3$), 169.7 (C-4a), 141.6 (C-3), 124.8 (C-4), 98.7 (C-2), 82.0 ($CO_2C(CH_3)_3$), 71.4 (C-7a), 56.0 (OMe), 42.6 (C-7), 28.2 ($CO_2C(CH_3)_3$); FD-MS m/z = 266 (M)+. Anal. Calcd for $C_{14}H_{18}O_5$ (266.12); C 63.15, H 6.81; Found: C 62.98, H 7.11.

tert-Butyl (2*S*,4a*S*,5*R*,7a*S*)-2-methoxy-7a-methyl-6-oxooctahydrocyclopenta[*b*]pyran-5-carboxylate (10)

To a stirred solution of **8b** (560 mg, 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 the 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 a silica gel column (elution with 5% ethyl acetate in hexane) to give **10** as amorphous solid (430 mg, 87%). Significant NMR NOEs are 4a-H to _tert_-butyl, 30%; 7a-CH_3 to 2-H, 31%; 7a-CH_3 to 2-H, 35%; 4a-H to 2-H, 21%. 7a-CH_3 to _tert_-butyl, 25%. ^{1}H NMR (250 Mz, $CDCl_3$) δ 4.73 (dd, J = 2.4, 9.8 Hz, 1H, H-2), 3.29 (d, J = 11.2 Hz; 1H, H-5), 3.24 (s, 3H, OMe), 2.79 (d, J = 18.4 Hz,; H-7), 2.45 (ddd, J = 2.3, 4.6, 11.2 Hz, 1H, H-4a), 2.31 (d, J = 18.4 Hz, 1H, H-7′), 1.97 (m, 2H), 1.74 (dm, J = 17.0 Hz, 1H) 1.65 (dm, J = 13.0 Hz, 1H), 1.49 (s, 3H, CH_3-7a), 1.41 (s, 9H, $CO_2C(CH_3)_3$); ^{13}C NMR (63 MHz, $CDCl_3$) δ 210.3 (C-6), 171.0 ($CO_2C(CH_3)_3$), 95.7 (C-2), 82.2 ($CO_2C(CH_3)_3$), 81.7 (C-7a), 60.2 (C-5), 56.8 (OMe), 55.0 (C-7), 51.5 (C-4a), 34.0 (C-3), 28.4($CO_2C(CH_3)_3$), 25.4 (C-4), 24.7 (CH_3-7a); FD-MS m/z = 284 (M)$^+$. Anal. Calcd for $C_{15}H_{24}O_5$ (284.35); C 63.40, H 8.51; Found: C 63.52, H 8.46.

General procedure for the synthesis of compounds 11a–f

The bicyclic β-ketoester **10** derived from the reduction product of **8b** (142 mg, 0.5 mmol) and the desired hydrazine derivative (0.52 mmol) in 20 ml absolute ethanol were refluxed, and the progress of the reaction was monitored by TLC. After 6 h, the reaction mixture was concentrated on a rotary evaporator, purified on silica gel column (elution with 10% ethyl acetate in hexane) to give **11a–f** as amorphous solids.

(3b*S*,6*S*,7a*S*)-6-Methoxy-7a-methyl-2-(4-methylphenyl)-1,2,3b,4,5,6,7a,8-octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one (11a)

Yield: 122 mg, 88%; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.70 (d, J = 7.3 Hz, 2H, Ar–H), 7.17 (d, J = 7.3 Hz, 2H, Ar–H), 4.64 (dd, J = 2.7, 7.9 Hz, 1H, H-2), 3.04 (bd, J = 6.2 Hz; 1H), 3.30 (s, 3H, OMe), 2.25 (dd, J = 4.6, 11.3 Hz, 1H), 2.20 (m, 3H), 2.17 (s, 3H, Ar–CH_3), 1.90 (m,, 3H), 1.45 (s, 3H, CH_3-7a); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 159.6, 143.0, 141.7, 137.4, 133.4, 132.6, 125.9, 115.6, 94.2, 84.1, 60.2, 56.8, 55.0, 34.1, 25.4, 24.7, 20.9; FD-MS m/z = 314 $(M)^+$. Anal. Calcd for $C_{18}H_{22}N_2O_3$ (314.38); C, 68.77; H, 7.05; N, 8.91. Found: C, 68.56; H, 7.24; N, 9.07.

4-[(3b*S*,6*S*,7a*S*)-6-Methoxy-7a-methyl-3-oxo-1,3,3b,4,5,6,7a,8-octahydro-2*H* pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-2-yl]benzonitrile (11b)

Yield: 91.5 mg, 75% ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.81 (dd, J = 1.2, 7.4 Hz, 2H, Ar–H), 7.73 (dd, J = 1.1, 7.4 Hz, 2H, Ar–H), 4.90 (dd, J = 3.1, 8.2 Hz, 1H, H-2), 3.01 (bd, J = 6.4 Hz; 1H), 3.41 (s, 3H, OMe), 2.62 (m, 2H), 2.46 (dd, J = 4.3, 10.1 Hz, 1H), 2.23 (m, 2H), 1.92 (m,, 2H), 1.49 (s, 3H, CH_3-7a); FD-MS m/z = 325 $(M)^+$. Anal. Calcd for $C_{18}H_{19}N_3O_3$ (325.14); C, 66.45; H, 5.89; N, 12.91. Found: C, 66.27; H, 5.95; N, 12.70.

(3b*S*,6*S*,7a*S*)-6-Methoxy-7a-methyl-2-(4-nitrophenyl)-1,2,3b,4,5,6,7a,8-octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one (11c)

Yield: 104 mg, 78%; ^{1}H NMR (400 Mz, $CDCl_3$) δ 8.34 (dd, J = 0.9, 6.9 Hz, 2H, Ar–H), 8.18 (dd, 1.0, J = 6.9 Hz, 2H, Ar–H), 4.85 (dd, J = 2.9, 7.0 Hz, 1H, H-2), 3.01 (m, 1H), 3.53 (s, 3H, OMe), 3.01 (dd, J = 2.1, 4.5 Hz, 1H), 2.71–2.79 (m, 2H), 2.45 (dt, J = 3.3, 10.1 Hz, 1H), 2.20 (m, 2H), 1.89 (m, 2H), 1.47 (s, 3H, CH_3-7a); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 161.6, 144.3, 142.1, 133.0, 122.7, 119.5, 115.0, 94.9, 84.7, 55.7, 49.2, 44.1, 30.8, 22.4, 22.0; FD-MS m/z = 345 (M)+. Anal. Calcd for $C_{17}H_{19}N_3O_5$ (345.13); C, 59.12; H, 5.55; N, 12.1. Found: C, 59.22; H, 5.67; N, 12.31.

(3b*S*,6*S*,7a*S*)-6-Methoxy-2-(4-methoxyphenyl)-7a-methyl-1,2,3b,4,5,6,7a,8-octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one (11d)

Yield: 110 mg, 82%; ^{1}H NMR (400 Mz, $CDCl_3$) δ 7.61 (dd, J = 0.9, 6.5 Hz, 2H, Ar–H), 6.95 (dd, 1.0, J = 6.5 Hz, 2H, Ar–H), 4.93 (dd, J = 2.2, 6.2 Hz, 1H, H-2), 3.77 (s, 3H, Ar–OMe), 3.00 (m, 1H), 3.40 (s, 3H, OMe), 2.75 (dd, J = 2.3, 4.4 Hz, 1H), 1.88 (m, 1H), 2.42(dt, J = 3.3, 9.5 Hz, 1H), 2.20 (m, 1H), 1.50 (s, 3H, CH_3-7a); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 161.75, 132.5, 132.4, 115.1, 112.6, 94.2, 84.6, 55.7, 55.6, 46.1, 39.8, 32.8, 25.7, 25.2; FD-MS m/z = 330 $(M)^+$. Anal. Calcd for $C_{18}H_{22}N_2O_4$ (330.16); C, 65.44; H, 6.71; N, 8.48, 12.1. Found: C, 65.53; H, 6.65; N, 8.52.

(3b*S*,6*S*,7a*S*)-2-(4-Chlorophenyl)-6-methoxy-7a-methyl-1,2,3b,4,5,6,7a,8-octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one (11e)

Yield: 120.7 mg, 85%; ^{1}H-NMR (400 Mz, $CDCl_3$) δ 7.85 (dd, J = 0.8, 6.1 Hz, 2H, Ar–H), 7.30 (dd, 0.9, J = 6.1 Hz, 2H, Ar–H), 5.00 (dd, J = 2.3, 5.7 Hz, 1H, H-2), 3.03 (m, 1H), 3.99 (s, 3H, OMe), 2.76 (dd, J = 2.2, 4.1 Hz, 1H), 2.22–2.46 (m, 3H), 1.89 (m, 3H), 1.47 (s, 3H, CH_3-7a); ^{13}C NMR (100.7 MHz, $CDCl_3$) δ 160.9, 151.8, 151.3, 139.0, 132.4, 116.8, 115.1, 94.7, 84.6, 55.7, 49.3, 46.1, 30.2, 21.8, 21.4; FD-MS m/z = 334 $(M)^+$. Anal. Calcd for $C_{17}H_{19}ClN_2O_3$ (334.11); C, 60.99; H, 5.72; Cl, 10.59; N, 8.37. Found: C, 60.85; H, 5.79; Cl, 10.61; N, 8.42.

(3b*S*,6*S*,7a*S*)-2-(1*H*-Indol-2-yl)-6-methoxy-7a-methyl-1,2,3b,4,5,6,7a,8-octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one (11f)

Yield: 98 mg, 76%; ^{1}H NMR (400 Mz, $CDCl_3$) δ 10.62 (bs, 1H), 10.26 (s, 1H), 7.73 (d, J = 1.8 Hz, 1H), 7.52 (s, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.16 (t, J = 1.8, 3.2 Hz, 1H), 6.50 (t, J = 1.8, 3.3 Hz, 1H), 4.84 (dd, J = 2.1, 5.2 Hz, 1H, H-2), 3.00 (m, 1H), 3.49 (s, 3H, OMe), 2.77 (m, 1H), 2.21–2.46 (m, 2H), 1.88 (m, 2H), 1.47 (s, 3H, CH_3-7a); FD-MS m/z = 334 $(M)^+$. Anal. Calcd for $C_{19}H_{21}N_3O_3$ (339.16); C, 67.24; H, 6.24; N, 12.38. Found: C, 67.33; H, 6.13 N, 12.45.

(3b*S*,6*S*,7a*S*)-6-Methoxy-7a-methyl-2-pyridin-2-yl-1,2,3b,4,5,6,7a,8-octahydro-3*H*-pyrano[3′,2′:3,4]cyclopenta[1,2-*c*]pyrazol-3-one (11g)

Yield: 119 mg, 79%; ^{1}H NMR (400 Mz, $CDCl_3$) δ 8.41 (d, J = 1.1 Hz, 1H), 7.92 (d, J = 0.9 Hz, 1H), 7.63 (t, J = 1.3 Hz, 1H), 6.98 (t, J = 1.2 Hz, 1H), 5.19 (t, J = 4.4 Hz, 1H, H-2), 3.08 (m, 1H), 3.77 (s, 3H, OMe), 2.65 (m, 2H), 2.21–2.38 (m, 3H), 1.85 (m, 2H), 1.42 (s, 3H, CH_3-7a); FD-MS m/z = 301 $(M)^+$. Anal. Calcd for $C_{16}H_{19}N_3O_3$ (301.14); C, 63.77; H, 6.36; N, 13.94. Found: C, 63.65; H, 6.42; N, 13.81.

Molecular Modeling

ChemBio3D Ultra 11 was used to calculate the thermodynamically more preferred conformations of compounds **10** and **10a** using force field MM2 method.[18]

In vitro cytotoxicity assay

The cytotoxic activity of the cyclopenta[b]pyrane derivatives **11a–11g** was determined using a standard (MTT)-based colorimetric assay.[12] 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, SK–N-SH and the non-tumorigenic cell line derived from breast tissue (MCF10A)). After 24 h, cells were treated with different concentrations (0.1, 0.5, 1, 3 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^{-1} 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 were converted to % cell viability. The IC_{50} were calculated using Graphpad Prism software.

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