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Synthesis and cytotoxic evaluation of novel paraconic acid analogs

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ABSTRACT

A novel class of 2,3-tri- and tetrasubstituted γ-butyrolactones analogous to paraconic acids has been synthesized in one step using a straightforward three-component reaction among aryl bromides, dimethyl itaconate and carbonyl compounds. The in vitro cytotoxic activity of representative compounds has been evaluated against a panel of human cancer cell lines (KB, HCT116, MCF7, HL60). While most molecules exhibit a low to moderate background activity on both KB and HL60 cancer cell lines, one compound shows increased antiproliferative activities against both cell lines with IC_{50} values in the 10^{-7}–10^{-6} mol/L range. An extended evaluation indicated that this compound also inhibits PC3, SK-OV3, MCF7R and HL60R cell growth in the same fashion.

The γ-butyrolactone scaffold is a widespread naturally-occurring motif, present in numerous compounds possessing biological activities.[1–6] In this context, paraconic acids[7–12] (bearing a carboxylic acid function at the position β to the carbonyl, see scheme 1), constitute an important group of γ-butyrolactones that both display antitumor and antibiotic activities, but also represent relevant building blocks for the synthesis of diverse pharmacologically active compounds.[13–15] Although these attractive properties have made them prominent synthetic targets in the past few years,[16–22] the straightforward synthesis of polysubstituted γ-butyrolactones still remains challenging.

In recent papers, we highlighted the use of arylmetal compounds, generated in situ from aryl bromides via a zinc dust/cobalt bromide system, in multicomponent processes.[23–27] Owing to the selective reactivity and the important functional tolerance of these reagents, it was envisaged to engage them in an extended set of diversity-oriented synthetic procedures. This would enable the further biological evaluation of newly-constituted libraries of compounds. Therefore, we report herein the preparation of novel 2,3-di and 2,2,3-trisubstituted-3-methoxycarbonyl-γ-butyrolactones analogous to paraconic acid esters by a multicomponent reaction between aryl halides, carbonyl compounds and dimethyl itaconate and their cytotoxic assessment against a panel of cancer cell lines (KB, HCT116, MCF7, HL60).

The multicomponent synthesis of the paraconic acid analogs **3** was envisaged starting from a carbonyl compound **1**, an aryl bromide **2** and dimethyl itaconate through the domino creation of three single bonds by a fourfold metallation–conjugate addition–aldol addition–intramolecular transesterification sequence (scheme 1).[28]

For the implementation of the experimental procedure, the initial metallation of the aromatic bromide was anticipated through the use of the Zn(0)/Co(II) system which had already provided reliable results in Mannich type multicomponent couplings. Preliminary experiments allowed us to determine the most convenient experimental conditions, which were defined as follows: acetonitrile is used as a solvent, zinc dust (4.6 equiv, activated by using the joined assistance of TFA and 1,2-dibromoethane) as a reductive metal, $CoBr_2$ (13 mol % vs ArBr) as a catalyst and the aryl bromide (1.5 equiv), the aldehyde (1 equiv) and dimethyl itaconate (5 equiv) are allowed to react at 60 °C for 1–3 h.[29] These conditions allowed for the preparation of a representative set of compounds, both in terms of structure and functional diversity. Results are reported in Table 1.

These results indicate that an important variety of functionalized aromatic halides and aldehydes are useable in the process. Ketones also undergo the reaction to furnish 2,2,3,3-tetrasubstituted γ-butyrolactones (Table 1, entries 11–16). It can be noted that cyclic ketones can also be employed in the process to give rise to the formation of spiranic compounds, albeit in far more limited yields (Table 1, entry 16).

Under these conditions, mixtures of diastereoisomers were generally obtained (typically 80:20 to 60:40). As the separation of the diastereoisomers proved to be generally tricky, the subsequent

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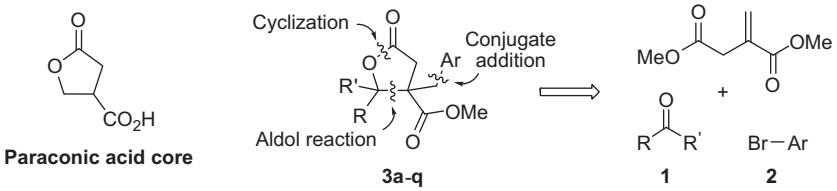
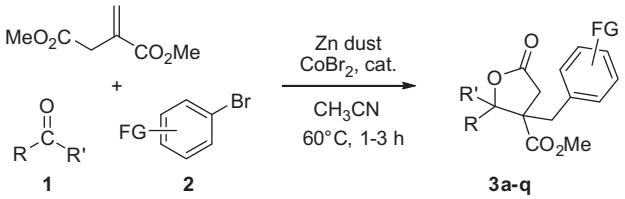
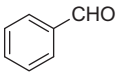
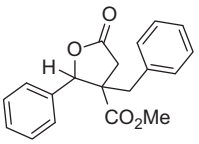
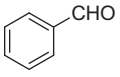
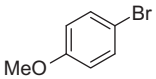
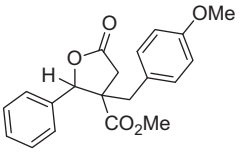
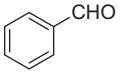
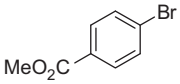
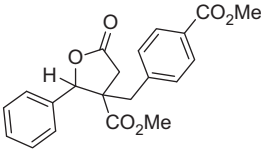
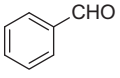
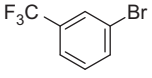
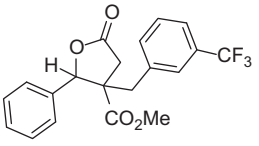
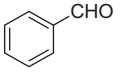
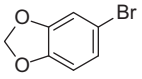
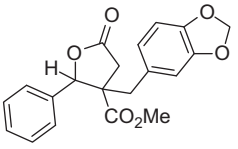
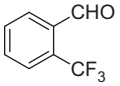
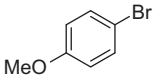
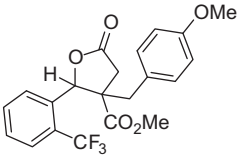
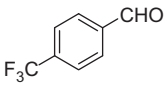
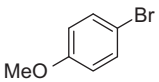
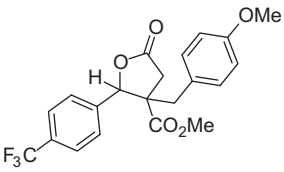


Scheme 1. Retrosynthetic approach to the paraconic acid core.

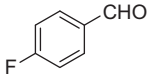
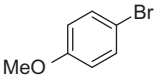
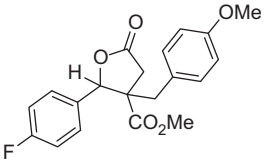
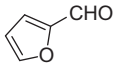
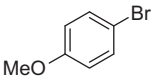
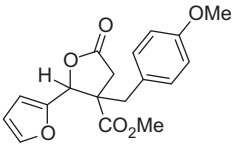
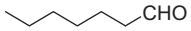
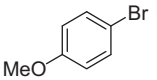
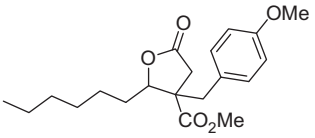
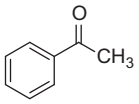
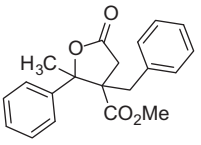
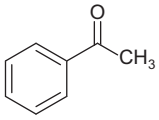
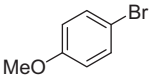
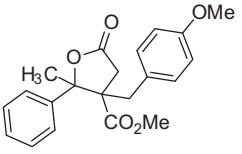
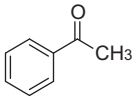
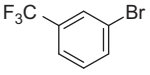
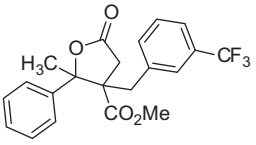
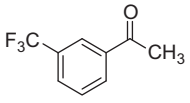
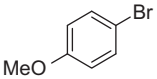
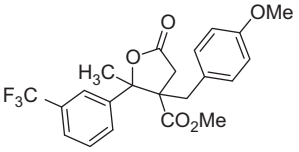
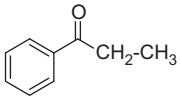
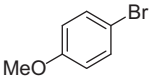
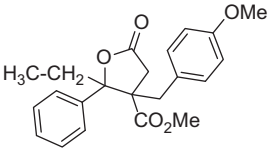
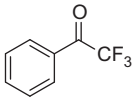
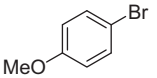
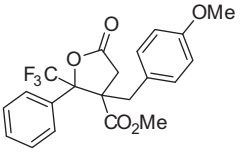
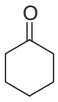
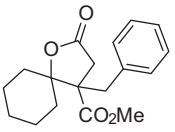
Table 1
Synthesis of γ-butyrolactones **3**[a]



Entry	Carbonyl compound **1**	Aryl bromide **2**	Product		Yield[b] (%)
1				**3a**	64
2				**3b**	95
3				**3c**	99
4				**3d**	48
5				**3e**	33
6				**3f**	56
7				**3g**	67

(*continued on next page*)

Table 1 (*continued*)

Entry	Carbonyl compound **1**	Aryl bromide **2**	Product		Yield[b] (%)
8				**3h**	98
9				**3i**	49
10				**3j**	27
11				**3k**	82
12				**3l**	85
13				**3m**	22
14				**3n**	52
15				**3o**	41
16				**3p**	54
17				**3q**	14

[a] Experiments were conducted with 20 mL of acetonitrile, 15 mmol of the aryl bromide, 10 mmol of the carbonyl compound, 7.9 g (50 mmol) of dimethyl itaconate, 3 g (46 mmol) of zinc dust, 0.44 g (2 mmol) of $CoBr_2$. Reactions were carried out at 60 °C for 1–3 h.
[b] Isolated yield.

biological evaluation was realized on well mastered mixtures (~50:50) of the diastereoisomers to both obtain reliable results and comparable scales of biological activities.

The inhibition of cell proliferation/viability by these diversely functionalized molecules was then evaluated in vitro against a panel of cancer cell lines: KB (nasopharyngeal), HCT116 (colon), MCF7 (breast), and HL60 (leukemia). The human cell lines were obtained from ATCC, except when otherwise stated and grown in D-MEM or in RPMI medium supplemented with 10% fetal calf serum, in the presence of penicillin, streptomycin and fungizone in 75 cm^2

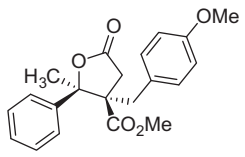
Table 2
Cellular growth inhibition percentage at 10 μM in DMSO

Entry	Compound	KB	HCT116	MCF7	HL60
1	**3a**	18 ± 6	0 ± 3	0 ± 7	22 ± 11
2	**3b**	19 ± 11	4 ± 3	0 ± 4	15 ± 7
3	**3c**	12 ± 2	0 ± 10	0 ± 13	19 ± 2
4	**3d**	39 ± 8	14 ± 2	11 ± 5	34 ± 4
5	**3e**	0 ± 17	18 ± 4	11 ± 5	18 ± 6
6	**3f**	31 ± 7	0 ± 11	0 ± 1	23 ± 7
7	**3g**	0 ± 6	0 ± 4	4 ± 7	2 ± 4
8	**3h**	33 ± 2	0 ± 5	4 ± 9	37 ± 5
9	**3i**	32 ± 4	0 ± 5	0 ± 6	29 ± 8
10	**3j**	13 ± 8	12 ± 2	28 ± 2	8 ± 4
11	**3k**	6 ± 5	9 ± 5	13 ± 9	22 ± 1
12	**3l**	95 ± 1	61 ± 1	56 ± 2	82 ± 1
13	**3m**	21 ± 7	22 ± 4	20 ± 4	21 ± 2
14	**3n**	38 ± 5	30 ± 6	46 ± 3	52 ± 1
15	**3o**	11 ± 5	28 ± 3	21 ± 7	30 ± 4
16	**3p**	56 ± 1	20 ± 3	8 ± 8	64 ± 2
17	**3q**	7 ± 13	0 ± 3	1 ± 10	4 ± 2

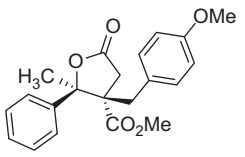
flask under 5% CO_2 at 37 °C. Cells were plated in 96-well tissue culture plates in 200 μl medium and treated 24 h later with 2 μl stock solution of compounds **3a–q** dissolved in DMSO using a Biomek 3000 (Beckman–Coulter) in sterile conditions. Controls received the same volume of DMSO (1% final volume). After 72 h exposure, MTS reagent (Promega) was added and incubated for 3 h at 37 °C: the absorbance was monitored at 490 nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control)) × 100] in triplicate experiments.[30] Results are reported in Table 2.

It can be noted that most molecules exhibit a low to moderate background activity on both KB and HL60 cancer cell lines. With benzaldehyde as the starting carbonyl compound (Table 2, entries 1–5), the presence of a CF_3 group on the starting aromatic halide seems to provide increased activities (Table 2, entry 4). Curiously, we could notice that when acetophenone is employed as the carbonyl compound, thus enabling the formation of a quaternary center at the carbon linked to the oxygen (Table 2, entries 11, 12 and 13), the CF_3 group furnishes deceiving results (Table 2, entry 13). On the contrary, a methoxy group located at the para position of the starting aryl bromide provides the best results (Table 2, entry 12). Moreover, it can be noted that compound **3l**, obtained from acetophenone, 4-bromoanisole and dimethyl itaconate, exhibits a particular increased antiproliferative activity compared to the other products, with cellular growth inhibitions higher than 50% in the four tested lines. The replacement of the CH_3 group of **3l** by a CH_2CH_3 (Table 2, entry 15) or by a CF_3 (Table 2, entry 16) results in a moderate to important decrease of the cytotoxicity. The functionalization of the phenyl moiety of acetophenone by a CF_3 group (Table 2, entry 14) also results in a slight decrease of the biological activity compared to a bare phenyl. This effect can also be observed with an aromatic aldehyde as the starting carbonyl compound, as the presence of a CF_3 group located at the para position of the phenyl ring results in a total loss of the cytotoxic activity (Table 2, entry 7). Although the cytotoxicities are far more limited than with acetophenones as starting compounds, it can be noted that aliphatic aldehydes can be used in the process to furnish products bearing a long aliphatic side chain. This latter seems to promote a particular increasing of the cytotoxicity against MCF7 line (Table 2, entry 10). On the contrary, the unusual spiranic compound, obtained from a cyclic ketone, is characterized by a general lack of activity (Table 2, entry 17).

Table 3
In vitro cytotoxicity profile of diastereoisomeric mixtures of **3l**



3l$_A$



3l$_B$

3l$_A$/**3l$_B$** ratio	Percentage of cell inhibition at 10 μM in DMSO			
	KB	HCT116	MCF7	HL60
75/25	91 ± 1	64 ± 2	55 ± 5	64 ± 2
35/65	90 ± 1	63 ± 2	44 ± 9	64 ± 2
0/100	98 ± 1	56 ± 2	59 ± 3	68 ± 1

Table 4
IC_{50} values for compound **3l**

	KB	HCT116	MCF7	HL60
IC50 min/max (10^{-6} M)	0.59/1.52	1.35/2.37	2.76/6.44	0.87/1.99

Table 5
Cellular growth inhibition percentage of compound **3l** at 10 μM in DMSO: global results

KB	HCT116	MCF7	HL60	PC3	SK-OV3	MCF7R	HL60R
95 ± 1	61 ± 1	56 ± 2	82 ± 1	68 ± 1	76 ± 1	82 ± 1	92 ± 2

As biological activities generally depend on stereochemical features, we envisaged to further assess independently the biological activities of diastereoisomers of the lead compound **3l**. Therefore, a meticulous chromatographic separation of a reaction mixture of **3l** was undergone. Unfortunately, we only succeeded in the isolation of the *trans* diastereoisomer **3l$_B$** as no Table amounts of this latter were remaining in **3l$_A$**-containing fractions. However, three representative fractions were submitted to a cytotoxic evaluation on KB, HCT116, MCF7 and HL60 cells. Results are indicated in Table 3.

This evaluation revealed quite surprising results, similar activities being observed independently from the sample composition. These results account for a negligible role of **3l** stereochemistry over the cytotoxic activities. Consequently, we did not try to further assess the other compounds of the **3** series as pure or enriched mixtures of stereoisomers. In addition, we envisaged implementing a deeper investigation of **3l** with the more reliable 50:50 diastereoisomeric mixture of **3l$_A$** and **3l$_B$**. Thus, compound **3l** was subjected to a deeper biological assessment which first revealed that IC_{50} values were in the 10^{-7}–10^{-6} mol/L range in KB, HCT116, MCF7 and HL60 cancer cell lines, as indicated in Table 4.

These results prompted us to undertake a more accurate evaluation of the biological activities of compound **3l**. Therefore, this latter compound was subjected to a MTS assay against an additional panel of cancer cell lines including, among others, multidrug resistant MCF7R and HL60R cell lines. Global results are indicated in Table 5.

These latter results confirm the general important in vitro activities of compound **3l** against various cancer cell lines. Indeed, the inhibition of the proliferation of the additional PC3 (prostate) and SK-OV3 (ovary) cancer cell lines is already significant at 10^{-5} M. In addition, these results might indicate that compound **3l** is not substrate of P-gp, since multidrug resistant cell lines MCF7R and HL60R are affected in the same fashion as the others.

In conclusion, we have developed a rapid and efficient method for the synthesis of 2,3-substituted 3-methoxy-γ-butyrolactones bearing, in particular, a functionalized benzyl side chain at the position β to the carbonyl. This domino process provides a reliable access to a wide variety of paraconic acids analogs, with an important

range of functions compatible with the process. The biological activities of the final compounds have been evaluated against a representative set of cancer cell lines (KB, HCT116, MCF7 and HL60). One of the molecules proves to exhibit a promising antitumor activity with IC_{50}s in the 10^{-7}–10^{-6} M range. Additional assays on an extended panel of cancer cell lines (PC3, SK-OV3 and MCF7R and HL60R) indicate very similar cytotoxicities. Current works are devoted to a deeper investigation of the structure–activity relationship and the mechanism of action in order to either obtain a global increase of the cytotoxic activities or provide an optimized specific cellular growth inhibition against targeted cancer cell lines.

Acknowledgments

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