



## Original article

# Synthesis and classical pathway Complement inhibitory activity of C7-functionalized filifolinol derivatives, inspired in K-76 COOH

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## ABSTRACT

A series of carboxylic acids carrying various functionalization on C-7 of their common 3*H*-spiro [benzofuran-2,1'-cyclohexane] skeleton were synthesized from filifolinol, as analogs of the natural Complement inhibitor K-76 COOH. In order to probe the relevance of the C-7 functionalization on their bioactivity, the ability of the analogs to inhibit Complement activation through the classical pathway was determined. The observed results suggest that functionalization of C-7 can modulate the inhibitory activity of the tested compounds. The 7-trifluoromethyl derivative was the compound with the lowest IC<sub>50</sub> value among the tested analogs (IC<sub>50</sub> = 100 μM), being more potent than K-76 COOH (IC<sub>50</sub> = 570 μM).

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## 1. Introduction

Complement is part of the innate immune system. It comprises more than 30 membrane-bound elements and plasma proteins, which circulate in their inactive forms. It performs an important function in host's defense system against foreign invasive organisms, being one of the main effector mechanisms of antibody-mediated immunity [1].

Activation of the Complement system can take place through the classical, alternative or lectin pathways. Activation of either pathway is mediated by a recognition mechanism, which leads to the enzymatic cleavage of a specific Complement protein, with some of the resulting by-products usually forming the enzymatic complex able to cleave the next inactive protein of the cascade.

The three Complement pathways converge in activation of the C5 component and subsequent formation of the membrane attack complex (MAC). Complement activation also entails the production of anaphylatoxins, which promote chemotaxis and opsonins, which

facilitate phagocytosis. Both actions contribute to innate immune functions; however, they can also cause tissue damage. On the other side, the MAC is capable of producing cell lysis.

The unidirectional Complement cascade can be triggered as a defense mechanism by a host of stimuli and conditions, including autoimmune diseases, atherosclerosis, ischemia-reperfusion and infections. Although Complement activation effects are normally beneficial to the host, this response can sometimes exert adverse effects, depending on the site, extent, and duration of the event. Regardless of the activated pathway, Complement activation has been proven to participate in the pathogenesis of a number of diseases [2].

Therefore, it has been repeatedly proposed that modulation of Complement activity would clearly be beneficial in the therapy of conditions caused by excess or abnormal activation, including autoimmune and inflammatory diseases [3].

Recently, two Complement-targeting proteins have been approved for clinical use by drug regulation agencies. One of them is a recombinant C1 esterase inhibitor useful for treating the hereditary angioedema, while the other is the orphan drug eculizumab, a humanized monoclonal therapeutic antibody indicated for treatment of paroxysmal nocturnal hemoglobinuria, the potential usefulness of which is currently being extended to other conditions [4]. However, peptidic/proteic inhibitors do not possess

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many of the advantages of the small molecule compounds, including low cost, absence of immunogenicity and ease of formulation. Small molecules have also better ability to penetrate in tissues and can be administered orally, which should be very useful for treating chronic conditions. Ability to penetrate in tissues is of high importance for these compounds; lack of this quality has been a presumable cause for the failure of other potential inhibitors in clinical studies [5].

The need of low molecular weight Complement inhibitors has been recognized and various small molecule compounds have been developed as inhibitors of different stages of the Complement cascades [6]. Although none of them is commercially available nowadays, some candidates hold promise of becoming future medicines [7].

In 1978, the group of Inoue reported the isolation of K-76 (**1**) from the culture broth of *Stachybotrys complementi*, nov. sp. K-76, obtained from soil of the Ishigaki Island (Okinawa, Japan) [8], as the result of a massive effort involving the screening for Complement inhibitory activity of over 3000 strains of actinomycetes and fungi. Oxidation of the natural product yielded K-76 COOH (**2**), the sodium salt of which was less toxic and displayed better aqueous solubility [9].

In laboratory experiments, K-76 COOH has shown to play important roles in diminishing gastric mucosal damage induced by ischemia-reperfusion, increasing the survival of discordant cardiac xenografts, being potentially useful for the development of a new treatment for the ulcerative colitis [10].

K-76 COOH also demonstrated to block human lymphocyte cell-mediated cytotoxicity and antibody-dependent natural cytotoxicity [11], and protected mice from mortality in the anaphylactoid reaction induced by certain bacterial lipopolysaccharides [12]. Synthetic analogs of the natural product have been prepared and tested for their ability to inhibit activation of the classical Complement pathway [13].

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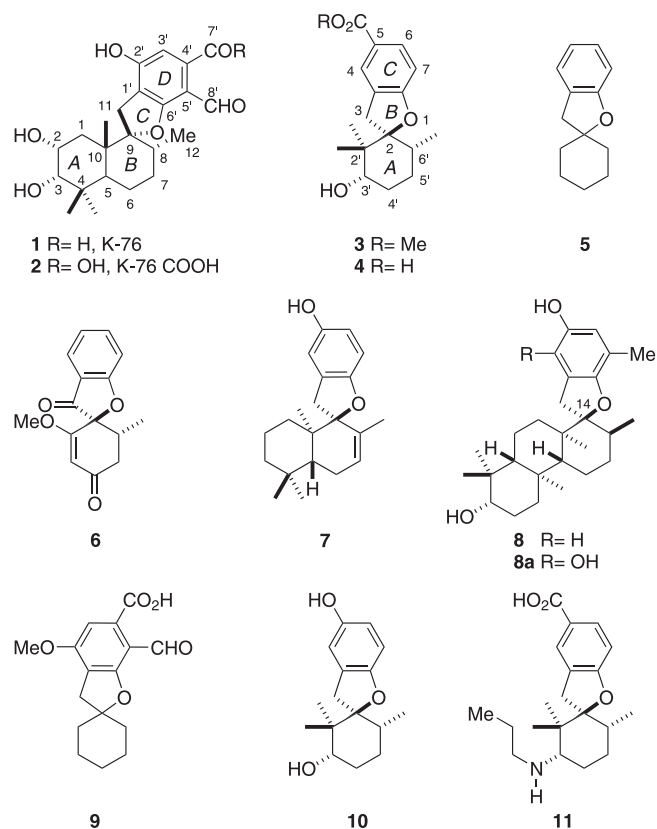
Filifolinol (**3**) is a bioactive tricyclic terpenoid with antioxidant, antibacterial, antifungal and antiviral properties, recently isolated from different *Heliotropium* species of the Atacama region of Chile [14]. The natural product **3** and the related filifoloinoic acid (**4**) carry the grisan (*3H*-spiro[benzofuran-2,1'-cyclohexane]) motif (**5**) [15], a privileged structure found in many bioactive natural products (Fig. 1), including K-76 and its derivatives, the clinically relevant antifungal agent griseofulvin (**6**) [16], the ichthiotoxic algal metabolite isochromazonarol (**7**) [17], as well as the pentacyclic stypodiol (**8**), its C-14 epimer epistypodiol and stypotriol (**8a**), which are particularly toxic and produce strong narcotic and hyperactive effects upon the reef-dwelling fish *Eupomacentrus leucostictus* [18,19].

## 2. Design

The structural analogies between filifolinol and the BCD-ring system of K-76 COOH make the former an attractive starting material for the synthesis of analogs of the latter. Simplified BCD-ring analogs of K-76 COOH such as **9** have demonstrated to be interestingly active [20].

We have previously prepared some filifolinol derivatives as Complement inhibitors, demonstrating through compound **10** that the carboxylic acid moiety is important but not essential for the biological activity [21] and that chemical modifications at the C-3' substituent level yielded inhibitors such as **11**, which were more potent than K-76 COOH itself; in addition, we found that 3'S alcohols and amines were more potent inhibitors than their 3'R counterparts [22,23].

Encouraged by these promising results and in pursuit of our interest in the development of new and more potent Complement inhibitors, we decided to use filifolinol or its known 3'-acetate (**12**)



**Fig. 1.** Chemical structures of K-76 (**1**), K-76 COOH (**2**), filifolinol (**3**), filifoloinoic acid (**4**), grisan (**5**), selected natural products carrying the *3H*-spiro[benzofuran-2,1'-cyclohexane] moiety (**6–8**) and different Complement activation inhibitors (**9–11**) inspired in K-76.

[23,24], easily available in high yield from the natural product through a conventional acetylation, as starting materials.

Therefore, the synthesis of a new series of *3H*-spiro[benzofuran-2,1'-cyclohexane] derivatives functionalized in their C-7 position was designed, as a three stage process, which included a first step for the introduction of nitro and halo moieties by means of electrophilic aromatic substitution processes.

Preparation of the former was proposed in order to reach the 7-amino derivative in the second stage, while synthesis of the latter analogs was projected as a means to install various C-7 functionalization during the second step, including trifluoromethyl, 1,2-propanediol and phenyl groups, and also different side chains carrying carboxylic acid moieties. Key transformations including hydrogenation and C–C bond forming reactions of this step were to be performed employing transition metal catalysis.

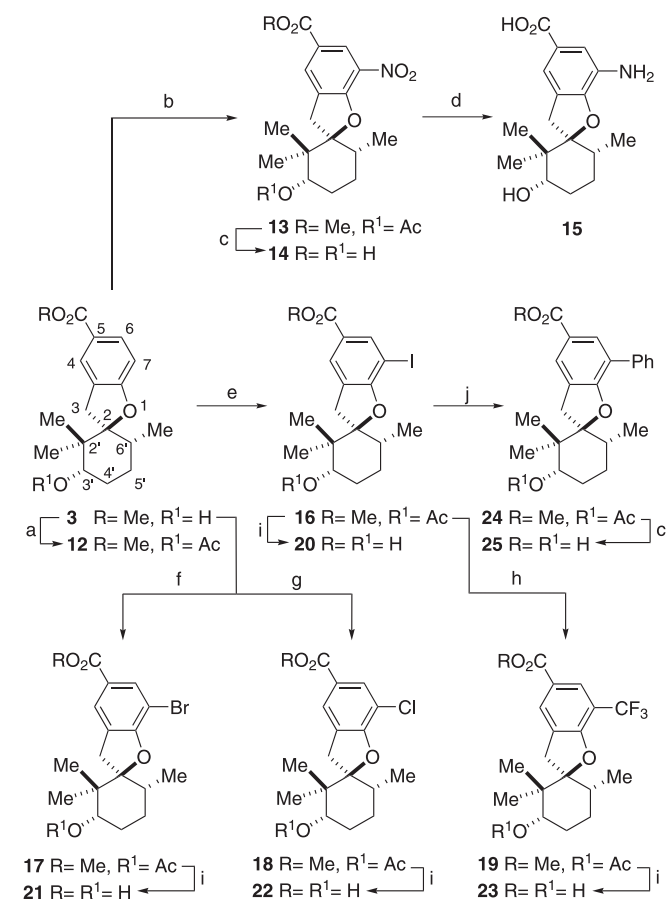
It was expected that the 3'-acetate group could withstand the conditions required for the introduction of nitro and halogen substituents of the first step as well as those needed for their further transformation into other functionalities during the second stage, while being easily removable in the third step, under basic hydrolysis, along with the methyl ester moiety found in the starting material.

All new compounds were evaluated for their ability to inhibit activation of the Complement cascade through the classical pathway.

## 3. Results and discussion

### 3.1. Chemistry

Preparation of the nitrogen-containing derivatives was simple and straightforward. As shown in Scheme 1, nitration of **12** with



**Scheme 1.** Reagents and conditions: a)  $\text{Ac}_2\text{O}$ , pyridine, DMAP (cat.),  $0^\circ\text{C} \rightarrow \text{RT}$ , 4 h (95%); b)  $\text{HNO}_3$ ,  $\text{Ac}_2\text{O}$  (2:1),  $0^\circ\text{C}$ , 18 h (78%); c)  $\text{NaOH}$ , dioxane– $\text{H}_2\text{O}$ ,  $60^\circ\text{C}$ , (13  $\rightarrow$  14, 17 h, 80%; 24  $\rightarrow$  25, 7 h, 99%); d)  $\text{H}_2$  (7 atm), 10% Pd/C, MeOH, RT, 15 h (95%); e)  $\text{I}_2$ ,  $\text{AgNO}_3$ , AcOH (2 drops), RT, 12 h (89%); f) 1.  $\text{Ac}_2\text{O}$ ,  $0^\circ\text{C} \rightarrow \text{RT}$ , 16 h; 2.  $\text{Br}_2/\text{AcOH}_{(\text{gl})}$ ,  $5^\circ\text{C}$ , 7 h (91%); g)  $\text{NCS}$ ,  $\text{FeCl}_3$ , MeCN, RT  $\rightarrow$   $100^\circ\text{C}$ , 22 h (92%); h)  $\text{NaCF}_3\text{CO}_2$ , CuI, NMP,  $180^\circ\text{C}$ , 1.5 h (85%); i)  $\text{LiOH}$ , THF– $\text{H}_2\text{O}$ ,  $70^\circ\text{C}$ , 7–22 h (16  $\rightarrow$  20, 88%; 17  $\rightarrow$  21, 82%; 18  $\rightarrow$  22, 95%; 19  $\rightarrow$  23, 95%); j)  $\text{PhB}(\text{OH})_2$ ,  $\text{Pd}(\text{Ph}_3\text{P})_4$ ,  $\text{Cs}_2\text{CO}_3$ ,  $\text{PhMe-EtOH}$  (2:1), microwaves, 15 min (60%).

a  $\text{HNO}_3$ – $\text{Ac}_2\text{O}$  mixture furnished 78% of 7-nitro derivative **13**, the basic hydrolysis of which provided nitro-acid **14** in 85% yield. Interestingly, the one-pot nitration of **3** under analogous conditions also furnished a similar yield of **13**. On the other hand, catalytic hydrogenation of acid **14** over 10% Pd/C gave 95% of aniline **15**.

Next, synthesis of the halogen-containing derivatives **16**–**19** was undertaken. Solvent-free iodination of **12** with the  $\text{I}_2$ – $\text{AgNO}_3$  reagent under acetic acid catalysis [25]. Conveniently provided 89% of **16**, while bromide **17** was synthesized in 85% yield by reaction of **12** with bromine in glacial acetic acid [21] and chloride **18** was obtained in 93% yield employing *N*-chlorosuccinimide as chlorinating agent, under  $\text{FeCl}_3$  catalysis [26]. On the other hand, the trifluoromethyl group was elegantly introduced following the conditions initially proposed by Matsui and coworkers, by heating iodide **16** at  $180^\circ\text{C}$  with sodium trifluoroacetate in NMP under CuI catalysis [27]; this furnished **19** in 85% isolated yield. In turn, basic hydrolysis of the esters with  $\text{LiOH}$  in a THF– $\text{H}_2\text{O}$  mixture uneventfully provided acids **20**–**23** in good yields.

The group of Bureeva [28] observed that low molecular weight compounds carrying negatively charged groups inhibit the classical pathway of Complement activation. These researchers have also shown that the presence of one negatively charged group is enough for the appearance of the activity and that the introduction of the second charged group resulted in a stronger effect.

However, the presence of three or more charged groups did not increase the activity, leading in some cases to loss of action specificity; this may be a result of inhibition of more than one stage of the Complement cascade or inhibition of more than one activation pathway.

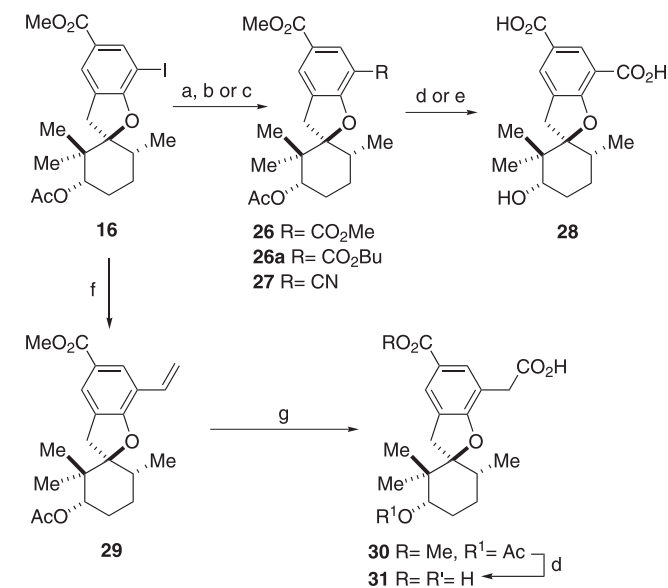
On the other hand, we have also noticed that Complement inhibition activity has been found among several carboxylic acid-type natural products [22].

Based on these observations and taking into account that introduction of hydrophilic groups will confer better solubility to the analogs, the synthesis of a set of dicarboxylic acids was designed, where the second carboxylic acid group was attached to C-7 directly or through different spacers, employing palladium-catalyzed cross-coupling reactions. The syntheses of triol **39** and the 7-phenyl derivative **25** were also included in this series.

First, the microwaves-promoted Suzuki–Miyaura coupling of aryl iodide **16** with phenylboronic acid under palladium catalysis of  $\text{Pd}(\text{Ph}_3\text{P})_4$  [29] gave 60% of biphenyl derivative **24** when  $\text{Cs}_2\text{CO}_3$  was employed as base. Basic hydrolysis of the ester moiety quantitatively afforded the expected acid **25**.

The synthesis of isophthalic acid derivative **28** was next undertaken. As depicted in Scheme 2, this was explored through the atmospheric pressure catalytic carbonylation of **16** [30]. However, this gave **26a** in only 35% yield. When the carbonylation was executed at 100 psi under  $\text{Pd}(\text{OAc})_2$  catalysis, with added MeOH to capture the intermediate species and employing  $\text{K}_2\text{CO}_3$  as the base, 50% of methyl ester **26** was realized. Basic hydrolysis of **26** furnished 85% of the desired diacid **28**.

In view of the yields of the reaction leading to **26**, an alternative route was sought. Zinc cyanide is a well-precedented source of cyanide for the cyanation of aryl halides under  $\text{Pd}(\text{Ph}_3\text{P})_4$  catalysis [31]. Therefore, aryl iodide **16** was submitted to cyanation with  $\text{Zn}(\text{CN})_2$  in DMF. The reaction was carried out under microwaves heating, to increase the reaction rate, affording an improved yield (78%) of the known benzonitrile **27**. In turn, compound **27** was hydrolyzed with the  $\text{NaOH}$ – $\text{H}_2\text{O}_2$  reagent system, to provide 90% of diacid **28**.



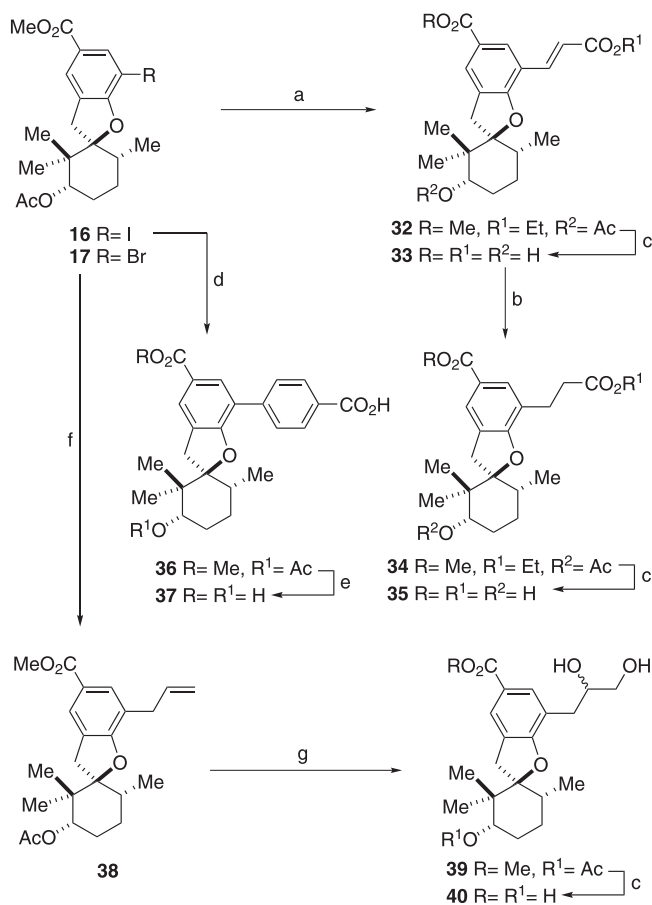
**Scheme 2.** Reagents and conditions: a) MeOH,  $\text{Pd}(\text{dppp})\text{Cl}_2$ ,  $\text{K}_2\text{CO}_3$ , MeOH, CO (100 psi), DMF,  $100^\circ\text{C}$ , 20 h (26, 50%); b)  $\text{Pd}(\text{OAc})_2$ , *n*-BuOH, *n*-Bu $_3\text{N}$ , CO (1 atm), DMF,  $100^\circ\text{C}$  (35%); c)  $\text{Zn}(\text{CN})_2$ ,  $\text{Pd}(\text{Ph}_3\text{P})_4$ , DMF, microwaves, 45 min (27, 78%); d) 10%  $\text{LiOH}$ , THF– $\text{H}_2\text{O}$  (3:1),  $80^\circ\text{C}$ , 10–18 h (26  $\rightarrow$  28, 95%; 30  $\rightarrow$  31, 99%); e) 30%  $\text{H}_2\text{O}_2$ , 3 N  $\text{NaOH}$ , dioxane,  $70^\circ\text{C}$ , 21 h (27  $\rightarrow$  28, 90%); f) *n*-Bu $_3\text{SnCH}=\text{CH}_2$ ,  $\text{Pd}(\text{AcO})_2$ , TBAF, Dabco, dioxane,  $110^\circ\text{C}$ , 2 h (98%); g) 1. 9-BBN, THF,  $-10^\circ\text{C}$ ; 2. 3 N  $\text{NaOH}$ , 30%  $\text{H}_2\text{O}_2$ ,  $0^\circ\text{C} \rightarrow 50^\circ\text{C}$ , 20 min (40%); 3.  $\text{CrO}_3/\text{H}_2\text{SO}_4$  (Jones [O]),  $\text{Me}_2\text{CO}$ ,  $0^\circ\text{C}$  (68%).

Introduction of the acetic acid side chain was performed in three steps, through the intermediacy of polysubstituted styrene **29** which, remarkably, was cleanly prepared in almost quantitative yield by the Stille cross-coupling of **16** with *n*-Bu<sub>3</sub>SnCH=CH<sub>2</sub>.

Hydroboration–oxidation of **29** with 9-BBN and NaOH–H<sub>2</sub>O<sub>2</sub>, followed by Jones oxidation of the resulting alcohol afforded acid **30**, which was isolated in 40% overall yield. This was followed by basic hydrolysis of **30** with LiOH in a refluxing mixture of THF and H<sub>2</sub>O, which furnished the targeted phenylacetic acid derivative **31**.

As shown in Scheme 3, installation of a propionic acid side chain was accomplished by means of the Heck-coupling of **16** with ethyl acrylate, which provided polysubstituted cinnamic acid derivative **32**. The best yields were obtained in acetonitrile, when the base employed was NaHCO<sub>3</sub>, to which TEBAHSO<sub>4</sub> was added. Basic treatment of **32** furnished diacid **33** in 95% yield. On the other hand, catalytic hydrogenation of **32** with 10% Pd/C in MeOH under atmospheric pressure conditions furnished the trimer **34**, basic hydrolysis of which provided dihydrocinnamic acid derivative **35** in 90% overall yield.

Next, aryl iodide **16** was subjected to a microwave-promoted Suzuki–Miyaura cross-coupling reaction with phenylboronic acid 4-carboxylic acid, affording 60% of acid **36**, which cleanly furnished 85% of diacid **37** upon NaOH-mediated hydrolysis. Finally, the projected triol-acid **40** was prepared from **16** in 85% overall yield by



**Scheme 3.** Reagents and conditions: a) CH<sub>2</sub>=CHCO<sub>2</sub>Et, Ph<sub>3</sub>P, NaHCO<sub>3</sub>, TEBAHSO<sub>4</sub>, Pd(AcO)<sub>2</sub>, 4 Å MS, MeCN, 65 °C, 28 h (92%); b) H<sub>2</sub> (1 atm), 10% Pd/C, MeOH, RT, 3 h (95%); c) 10% LiOH, THF–H<sub>2</sub>O (3:1), 70 °C (**32** → **33**, 98%; **34** → **35**, 95%; **39** → **40**, 97%); d) 4-HO<sub>2</sub>C–C<sub>6</sub>H<sub>4</sub>–B(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, PhMe–EtOH, microwaves, 15 min (60%); e) 40% NaOH, dioxane–H<sub>2</sub>O, 60 °C, 23 h (84%); f) *n*-Bu<sub>3</sub>SnCH<sub>2</sub>CH=CH<sub>2</sub>, Pd(Ph<sub>3</sub>P)<sub>4</sub>, CsF, dioxane, 110 °C, 21 h (98%); g) K<sub>3</sub>Fe(CN)<sub>6</sub>, OsO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, *t*-BuOH–H<sub>2</sub>O 2:1, RT, 16 h (90%).

palladium-catalyzed allylation, followed by osmium tetroxide-mediated dihydroxylation of the resulting **38** to a mixture of diastereoisomeric diols **39** and basic hydrolysis of the ester moieties.

### 3.2. Biological assay

The synthetic carboxylic acids and diacids were tested for their ability to inhibit the classical Complement pathway employing the method of Weissman [32]. In this method, human Complement was activated employing an antigen–antibody complex, with antibodies raised against sheep red blood cells. The test was performed in the absence and presence of different amounts of the tested analogs and the results of the Complement-mediated hemolytic reaction were obtained as absorbance data at 410 nm. These results (*A*<sub>test</sub>) were corrected with a blank (*A*<sub>blank</sub>) and translated to percentage of hemolysis (*Y*) taking into account the lysis observed in the absence of test compounds (*A*<sub>100</sub>). A dose–response logistic function {log [*Y*/(100 – *Y*)] = *f*[log [test compound]]} was then fitted and the corresponding IC<sub>50</sub> values were obtained from the fitted parameters. The known Complement inhibitor oleanolic acid hemidiglycolate (**41**), was employed as reference [33], in order to ascertain comparability of the results.

The results of the assay (Table 1) revealed that except for phenylacetic acid derivative **31**, all the compounds inhibited activation of the classical Complement pathway. When compared to filifoloinic acid (**4**, IC<sub>50</sub> = 2000 μM), carrying an unsubstituted C-7, it was concluded that substitution of C-7 is able to modulate the ability of the analogs to inhibit activation of the classical Complement pathway. Interestingly enough, except for aniline **15** (IC<sub>50</sub> > 7000 μM) and biphenyl derivative **37** (IC<sub>50</sub> > 10,000 μM), all the analogs exhibited micro molar values of IC<sub>50</sub> lower than that of **4**, while introduction of a 7-nitro moiety proved unsuccessful to significantly improve activity of the resulting nitro-acid **14** (IC<sub>50</sub> = 1880 μM).

On the other hand, the series of dicarboxylic acids exhibited a wide degree of activity, ranging from inactive (**31**) or poorly active (**37** and **28**) compounds to moderately active tricycles (**33** and **35**). Analog **35** proved to be the most potent analog (IC<sub>50</sub> = 360 μM), outperforming K-76 COOH, followed by **33**. Surprisingly, 7-phenyl derivative **25** (IC<sub>50</sub> = 140 μM), exhibited slightly superior potency than **4**, and triol **40** ranked among the most potent members of the series (IC<sub>50</sub> = 360 μM), suggesting that a carboxylic acid moiety, when placed as part of a C-7 substituent, may not be essential to the biological activity.

Interestingly, the 7-trifluoromethyl analog **23** resulted the most potent compound among those containing halogens (IC<sub>50</sub> = 100 μM, 36 μg/mL), surpassing the performance of K-76 COOH itself (IC<sub>50</sub> = 570 μM) [13d,21]. This was followed by the 7-bromo derivative **21** (IC<sub>50</sub> = 840 μM) and the iodinated analog **20** (IC<sub>50</sub> = 1140 μM), being the 7-chloro derivative **22** the less potent of the series (IC<sub>50</sub> = 1540 μM).

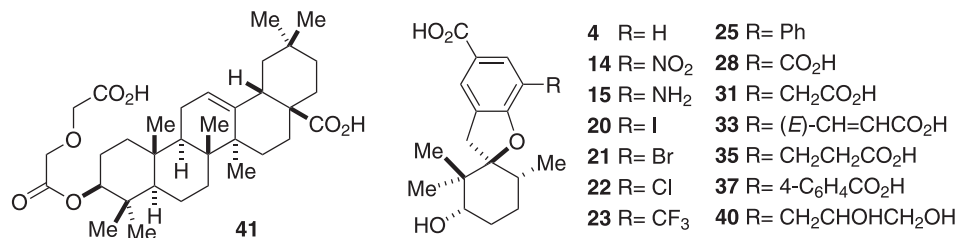
Taken together with the above observations regarding the performance of the nitro (**14**) and dicarboxylic (**28**) acids, this may be not only a result of the ability of fluorine atoms to participate in H-bonding. Clearly, this points out that there are more subtle electronic and/or H-bonding effects between these inhibitors and the active site than those revealed in this simple SAR study, which still wait to be unveiled.

### 4. Conclusions

In conclusion, with the aim of finding new and more potent Complement inhibitors carrying the BCD-ring motif found in K-76 COOH, short sequences of synthetic transformations were



**Table 1**  
Results of inhibition of the activation of the classical Complement pathway by the synthesized analogs. Comparison with data of K-76 COOH (**2**), filifoloinic acid (**4**) and oleanolic acid hemidiglycolate (**41**).



Compd. No.	Complement inhibition <sup>a</sup>		Compd. No.	Complement inhibition <sup>a</sup>	
	IC <sub>50</sub> , μM	IC <sub>50</sub> , μg/mL		IC <sub>50</sub> , μM	IC <sub>50</sub> , μg/mL
<b>2</b>	570	238 [13d,21]	<b>25</b>	140	50
<b>4</b>	2000	580	<b>28</b>	7000	2300
<b>14</b>	1880	630	<b>31</b>	Inactive	Inactive
<b>15</b>	7270	2220	<b>33</b>	970	350
<b>20</b>	1140	370	<b>35</b>	360	130
<b>21</b>	840	350	<b>37</b>	18,000	7300
<b>22</b>	1540	570	<b>40</b>	360	130
<b>23</b>	100	36	<b>41</b>	31	18

<sup>a</sup> Calculated according to the following formula: hemolysis (Y, %) = 100\*(A<sub>test</sub> - A<sub>blank</sub>)/(A<sub>100</sub> - A<sub>blank</sub>), where A<sub>test</sub>, A<sub>100</sub>, and A<sub>blank</sub> are the absorbances at 410 nm of the test sample, the 100% hemolysis control, and the blank, respectively. IC<sub>50</sub> values were recorded (Y = 50) from the linear graphs obtained by plotting log [Y/(100 - Y)] vs. log [test compound] [21,22].

performed on the natural product filifolinol, which installed different substituents on the C-7 position.

The 7-amino derivative was obtained by nitration, followed by basic hydrolysis and catalytic hydrogenation. Nuclear halogenation provided chloro, bromo and iodo derivatives, the latter two of which were employed as substrates for Cu-assisted trifluoromethylation and Pd-catalyzed cross-coupling reactions (Stille, Suzuki–Miyaura, Heck). These C–C bond forming reactions, which allowed attachment of various side chains carrying a carboxylic acid moiety, provided ready access to various dicarboxylic acids. Compounds carrying 7-phenyl and 7-propane-1',2'-diol moieties were also prepared.

The resulting filifolinol derivatives (mono- and di-carboxylic acids) were tested for their ability to inhibit the classical Complement pathway-mediated hemolysis of sheep red blood cells. Among the compounds tested, the 7-trifluoromethyl analog **23** was found to be the most potent, displaying an IC<sub>50</sub> value lower than that of K-76 COOH itself. Complementing previous observations, these new findings pave the way for the development of new analogs of K-76 COOH from filifolinol, with improved activity.

## 5. Experimental

### 5.1. Chemistry

#### 5.1.1. General conditions

Melting points were taken on an Ernst Leitz Wetzlar model 350 hot-stage microscope and are reported uncorrected. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were acquired in the specified solvent, in a Bruker Avance spectrometer (300.13 and 75.48 MHz for <sup>1</sup>H and <sup>13</sup>C, respectively), with tetramethylsilane (TMS) as internal standard. The chemical shifts (δ) are reported in ppm downfield from TMS and coupling constants (J) are expressed in hertz. DEPT 135 experiment was used to aid the interpretation of the fully decoupled <sup>13</sup>C NMR spectra. In special cases, 2D-NMR experiments (COSY, HMBC and HSQC) were also employed. Optical rotations were obtained in the specified solvent on a Jasco DIP 1000 polarimeter

(Jasco Corp., Tokyo, Japan). The high resolution mass spectra were obtained with a Bruker MicroTOF-Q II instrument (Bruker Daltonics, Billerica, MA). Detection of the ions was performed in electrospray ionization, positive ion mode. The reactions were carried out under dry Nitrogen or Argon atmospheres, employing oven-dried glassware. Microwave-assisted reactions were performed in a CEM Discover microwave oven. Fitting of the inhibition data was performed employing Origin 8.0 (OriginLab Corp., Northampton, MA).

Reagents were used as received; dry THF was prepared by distillation from Na-benzophenone ketyl; anhydrous pyridine was prepared by distillation after refluxing the reagent for 4 h over pellets of KOH; dry CH<sub>2</sub>Cl<sub>2</sub> and MeCN were prepared by a 4 h reflux over P<sub>2</sub>O<sub>5</sub> followed by distillation; anhydrous solvents were stored in dry Young ampoules. In the conventional work-up procedure, the reaction was diluted with brine (5–10 mL) and the products were extracted with EtOAc (4–5 × 20 mL); the combined organic extracts were then washed once with brine (5 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The residue was submitted to flash column chromatography with silica gel 60 H (particle size 63–200 μm). Elution was carried out with hexane–EtOAc mixtures, under positive pressure and employing gradient of solvent polarity techniques.

All new compounds gave single spots on TLC plates of Kieselgel 60 GF<sub>254</sub>, run in different hexane–EtOAc and CH<sub>2</sub>Cl<sub>2</sub>–toluene solvent systems. Chromatographic spots were detected by exposure of the plates to UV light (254 nm), followed by spraying with ethanolic ninhydrin (amines) or with ethanolic *p*-anisaldehyde/sulfuric acid reagent and careful heating for 2 min.

#### 5.1.2. General procedures for the saponification of esters

**5.1.2.1. Method A.** A solution of the ester in dioxane (2.5 mL/mmol) was treated with a 10 M NaOH solution (5 mL/mmol) and the system was warmed at 60 °C until complete consumption of the starting ester was verified by TLC (17–24 h). The mixture was cooled in an ice bath, washed with Et<sub>2</sub>O and carefully acidified with 4 N HCl. The organic products were extracted with Et<sub>2</sub>O (4 × 6 mL),

and the combined organic extracts were dried over MgSO<sub>4</sub> and concentrated under reduced pressure, leaving a residue which was chromatographed.

**5.1.2.2. Method B.** The ester was dissolved in THF (2.5 mL/mmol) and treated with a 4.3 M LiOH solution (10 mL/mmol) and warmed at 60–80 °C for 8–12 h. The reaction was cooled in an ice bath, diluted with water (5 mL), washed with Et<sub>2</sub>O (2 × 4 mL) and acidified with 4 N HCl. The organic products were extracted with Et<sub>2</sub>O (4 × 5 mL), and the combined organic phases were washed with brine (5 mL) and dried over MgSO<sub>4</sub>. Concentration of the extract under reduced pressure gave a residue which was submitted to chromatography.

**5.1.3. (1'S,3'S,6'R)-Methyl 3'-acetyloxy-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (filifolinol acetate, **12**)**

Pyridine (0.21 mL, 2.59 mmol), Ac<sub>2</sub>O (0.19 mL, 1.72 mmol) and a catalytic amount of DMAP were sequentially added to an ice cooled solution of **3** (100 mg, 0.33 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL). Then, the mixture was stirred at room temperature for 4 h, when brine (5 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine (5 mL), dried over MgSO<sub>4</sub> and concentrated under vacuum, leaving a residue which upon chromatography furnished **12** (105 mg, 95%), as a white solid, m.p. 129–131 °C (hexane–EtOAc). Physical data were identical as reported [24].

**5.1.4. (1'S,3'S,6'R)-Methyl 3'-acetyloxy-7-nitro-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**13**)**

A nitrating mixture was prepared by cautious addition of one part of freshly distilled Ac<sub>2</sub>O over 2 parts of 67% HNO<sub>3</sub>, cooled in an ice bath. This mixture was kept at 0 °C during 5–10 min and then added dropwise to a cooled solution of **12** (44 mg, 0.127 mmol) in Ac<sub>2</sub>O (0.7 mL). The reaction was stirred for 18 h at 0 °C; then, small pieces of ice were added and the products were extracted with Et<sub>2</sub>O (4 × 4 mL). The combined extracts were dried over MgSO<sub>4</sub> and NaHCO<sub>3</sub>, concentrated under reduced pressure and chromatographed to yield **13** as a yellowish oil (39 mg, 78%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> = –20.8 (c 0.97, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.79 (d, 3H, *J* = 6.6), 0.91 (s, 3H), 1.32 (s, 3H), 1.37–1.73 (m, 4H), 1.89–2.02 (m, 1H), 2.11 (s, 3H), 2.42–2.54 (m, 1H), 3.18 (d, 1H, *J* = 17.3), 3.58 (d, 1H, *J* = 17.3), 3.91 (s, 3H), 4.88 (t, 1H, *J* = 2.8), 8.03 (d, 1H, *J* = 1.4) and 8.60 (d, 1H, *J* = 1.4). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.8, 20.4, 21.3, 22.2, 25.8, 26.4, 30.2, 35.6, 42.0, 52.4, 78.8, 100.8, 122.3, 126.3, 130.4, 131.2, 134.1, 157.9, 165.3, and 169.8. HRMS (ESI) found: 392.1693 [M + H]<sup>+</sup>; C<sub>20</sub>H<sub>26</sub>NO<sub>7</sub> requires 392.1704.

**5.1.5. (1'S,3'S,6'R)-3'-Hydroxy-7-nitro-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**14**)**

Nitro-ester **13** (60 mg, 0.153 mmol) was submitted to the general procedure for the saponification of esters (Method A), yielding **14** (40 mg, 80%) as a glassy solid. [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +33.4 (c 1.0, acetone). <sup>1</sup>H NMR (MeOH-*d*<sub>4</sub>):  $\delta$  0.79 (d, 3H, *J* = 6.6), 0.99 (s, 3H), 1.15 (s, 3H), 1.45–1.69 (m, 3H), 1.91–2.03 (m, 1H), 2.24–2.36 (m, 1H), 3.03 (d, 1H, *J* = 17.3), 3.61 (brs, 1H), 3.65 (d, 1H, *J* = 17.3), 7.25 (brs, 1H) and 7.27 (brs, 1H). <sup>13</sup>C NMR (MeOH-*d*<sub>4</sub>):  $\delta$  14.2, 19.7, 21.6, 25.9, 28.2, 31.4, 36.0, 42.5, 76.4, 97.0, 116.4, 116.7, 122.6, 128.1, 129.1 and 152.1. HRMS (ESI) found: 336.1429 [M + H]<sup>+</sup>; C<sub>17</sub>H<sub>22</sub>NO<sub>6</sub> requires 336.1447.

**5.1.6. (1'S,3'S,6'R)-7-Amino-3'-hydroxy-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**15**)**

A solution of nitro-acid **13** (35 mg, 0.105 mmol) in anhydrous MeOH (3.5 mL) to which 10% Pd/C (3.5 mg) was added, was stirred under H<sub>2</sub> (7 atm) at room temperature during 15 h. The solid was filtered off through a short pad of Celite, and the filtrate was concentrated to yield **15** (30.5 mg, 95%) as a glassy solid. [ $\alpha$ ]<sub>D</sub><sup>25</sup> = –3.4 (c 0.5, MeOH). <sup>1</sup>H NMR (MeOH-*d*<sub>4</sub>):  $\delta$  0.79 (d, 3H, *J* = 6.6), 0.99 (s, 3H), 1.15

(s, 3H), 1.45–1.69 (m, 3H), 1.91–2.03 (m, 1H), 2.24–2.36 (m, 1H), 3.03 (d, 1H, *J* = 17.3), 3.61 (brs, 1H), 3.65 (d, 1H, *J* = 17.3), 7.25 (brs, 1H) and 7.27 (brs, 1H). <sup>13</sup>C NMR (MeOH-*d*<sub>4</sub>):  $\delta$  14.2, 19.7, 21.6, 25.9, 28.2, 29.3, 31.4, 36.0, 42.5, 76.4, 97.0, 116.4, 116.7, 122.6, 128.1, 129.1 and 152.1. HRMS (ESI) found: 306.1693 [M + H]<sup>+</sup>; C<sub>17</sub>H<sub>24</sub>NO<sub>4</sub> requires 306.1700.

**5.1.7. (1'S,3'S,6'R)-Methyl 3'-acetyloxy-7-iodo-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**16**)**

A mixture of **12** (674 mg, 2.65 mmol) and AgNO<sub>3</sub> (451 mg, 2.65 mmol) was gently blended in a glass mortar for 10 min, and then finely powdered **12** (288 mg, 0.825 mmol) was added. The resulting homogeneous mixture was additionally ground for 10 min, 2 drops of AcOH were added and grinding was continued for an additional 10 min. The mixture was left in the dark for 12 h at room temperature when Et<sub>2</sub>O (15 mL) was added. The resulting brown slurry was filtered through a cotton pad, and the filtrate was sequentially washed with 5% NaHSO<sub>3</sub> (2 × 5 mL), saturated NaHCO<sub>3</sub> (1 × 5 mL) and brine (5 mL). After drying the organic phase over MgSO<sub>4</sub>, the volatiles were removed in vacuum and the residue was chromatographed to afford **16** (347 mg, 89%), as an oil. [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +19.4 (c 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.79 (d, 3H, *J* = 6.6), 0.89 (s, 3H), 1.27 (s, 3H), 1.36–1.71 (m, 4H), 1.87–1.99 (m, 1H), 2.10 (s, 3H), 2.32–2.44 (m, 1H), 3.21 (d, 1H, *J* = 17.3), 3.61 (d, 1H, *J* = 17.3), 3.86 (s, 3H), 4.86 (t, 1H, *J* = 2.8), 7.76 (d, 1H, *J* = 1.4) and 8.17 (d, 1H, *J* = 1.4). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.8, 20.3, 21.3, 22.4, 25.8, 26.3, 30.5, 35.9, 41.8, 51.7, 78.8, 96.7, 116.4, 118.8, 122.0, 124.8, 129.1, 131.6, 161.3, 166.9 and 170.0. HRMS (ESI) found: 495.0631 [M]<sup>+</sup>; C<sub>20</sub>H<sub>25</sub>IO<sub>5</sub> requires 495.0639.

**5.1.8. (1'S,3'S,6'R)-Methyl 3'-acetyloxy-7-bromo-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**17**)**

Filifolinol (**3**, 51.8 mg, 0.170 mmol) was treated with freshly distilled Ac<sub>2</sub>O (1 mL) at 0 °C. After stirring during 15 h, a solution of Br<sub>2</sub> in glacial AcOH (32.6 mg, 0.1 mL, 0.204 mmol) was dropwise added to the cooled solution, the mixture was allowed to warm to room temperature and additionally stirred for 7 h. The solution was poured into crushed ice, extracted with EtOAc (3 × 4 mL), washed with brine (4 mL) and dried over MgSO<sub>4</sub>. The volatiles were removed in vacuo and the residue was chromatographed giving **17** (66 mg, 91%), which physical properties were in good agreement with the literature [21].

**5.1.9. (1'S,3'S,6'R)-Methyl 3'-acetyloxy-7-chloro-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**18**)**

NCS (14 mg, 0.103 mmol) and FeCl<sub>3</sub> (17 mg, 0.103 mmol) were added to a solution of **12** (35.6 mg, 0.103 mmol) in dry MeCN (1 mL) and the mixture was stirred at reflux during 19 h. The reaction was diluted with brine (5 mL), extracted with CHCl<sub>3</sub> (3 × 3 mL) and the organic extracts were dried over MgSO<sub>4</sub> and concentrated under vacuum. Chromatography of the residue afforded **18** (36.2 mg, 92%) as an oil. [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +13.3 (c 1.03, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.79 (d, 3H, *J* = 6.6), 0.89 (s, 3H), 1.26 (s, 3H), 1.36–1.68 (m, 3H), 1.86–1.98 (m, 1H), 2.10 (s, 3H), 2.31–2.44 (m, 1H), 3.14 (d, 1H, *J* = 17.3), 3.55 (d, 1H, *J* = 17.3), 3.85 (s, 3H), 4.85 (t, 1H, *J* = 2.7), 7.70 (d, 1H, *J* = 1.3) and 7.82 (d, 1H, *J* = 1.3). <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  14.8, 20.4, 21.3, 22.2, 25.8, 26.3, 31.5, 35.7, 41.9, 52.0, 78.9, 97.7, 113.8, 123.1, 124.4, 129.9, 130.6, 159.7, 166.1 and 170.0. HRMS (ESI) found: 403.1265 [M + Na]<sup>+</sup>; C<sub>20</sub>H<sub>25</sub>ClNaO<sub>5</sub> requires 403.1283.

**5.1.10. 5.1.10 (1'S,3'S,6'R)-Methyl 3'-acetyloxy-7-trifluoromethyl-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**19**)**

CuI (71.9 mg, 0.657 mmol) and NaCF<sub>3</sub>CO<sub>2</sub> (102 mg, 0.75 mmol) were added to a solution of iodide **16** (48 mg, 0.102 mmol) in anhydrous NMP (0.6 mL). The slurry was purged with dry argon and placed in an oil bath pre-heated at 180 °C during 1.5 h. The product was chromatographed yielding **19** (45.4 mg, 85%) as an oil.

$[\alpha]_{\text{D}}^{25} = +8.7$  (c 1.1,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  0.78 (d, 3H,  $J = 6.6$ ), 0.88 (s, 3H), 1.24 (s, 3H), 1.37–1.71 (m, 3H), 1.87–1.99 (m, 1H), 2.11 (s, 3H), 2.36–2.44 (m, 1H), 3.11 (d, 1H,  $J = 17.7$ ), 3.53 (d, 1H,  $J = 17.7$ ), 3.89 (s, 3H), 4.86 (t, 1H,  $J = 2.8$ ), 7.97 (s, 1H) and 8.05 (s, 1H).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  14.8, 20.3, 21.2, 22.0, 25.9, 26.3, 28.9, 30.2, 35.6, 42.0, 52.1, 78.9, 98.8, 111.1 (q,  $J = 33.7$ ,  $\text{ArCF}_3$ ), 121.9, 127.5 (q,  $J = 4.6$ ,  $\text{ArCF}_3$ ), 129.1, 131.0, 160.9, 166.1 and 169.9. HRMS (ESI) found: 437.1532  $[\text{M} + \text{Na}]^+$ ;  $\text{C}_{21}\text{H}_{25}\text{F}_3\text{NaO}_5$  requires 437.1546.

5.1.11. (*1'S,3'S,6'R*)-3'-Hydroxy-7-iodo-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**20**)

The ester **16** (50 mg, 0.11 mmol) was submitted to the general procedure for the saponification of esters (Method B), furnishing **20** (41 mg, 88%), as a colorless oil.  $[\alpha]_{\text{D}}^{25} = +17.8$  (c 1.04,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  (acetone- $d_6$ ):  $\delta$  0.79 (d, 3H,  $J = 6.6$ ), 0.99 (s, 3H), 1.18 (s, 3H), 1.40–1.70 (m, 3H), 1.85–2.15 (m, 1H), 2.29–2.39 (m, 1H), 3.24 (d, 1H,  $J = 17.5$ ), 3.67 (t, 1H,  $J = 2.7$ ), 3.92 (d, 1H,  $J = 17.5$ ), 7.76 (d, 1H,  $J = 1.5$ ) and 8.12 (d, 1H,  $J = 1.5$ ).  $^{13}\text{C NMR}$  (acetone- $d_6$ ):  $\delta$  14.3, 20.4, 22.0, 25.9, 28.8, 32.3, 35.8, 42.6, 70.9, 75.9, 97.7, 124.1, 126.0, 129.4, 138.7, 164.3 and 165.3. HRMS (ESI) found: 417.0541  $[\text{M} + \text{H}]^+$ ;  $\text{C}_{17}\text{H}_{21}\text{IO}_4$  requires 417.0557.

5.1.12. (*1'S,3'S,6'R*)-3'-Hydroxy-7-bromo-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**21**)

Ester **17** (60 mg, 0.145 mmol) was submitted to the general procedure for the saponification of esters (Method B), giving **21** (44.2 mg, 82%) as a yellowish solid. M.p.: 238–240 °C ( $\text{CHCl}_3$ );  $[\alpha]_{\text{D}}^{25} = +3.2$  (c 0.8, acetone).  $^1\text{H NMR}$  (acetone- $d_6$ ):  $\delta$  0.80 (d, 3H,  $J = 6.5$ ), 1.02 (s, 3H), 1.18 (s, 3H), 1.46–1.72 (m, 3H), 1.90–2.15 (m, 1H), 2.23–2.46 (m, 1H), 3.22 (d, 1H,  $J = 18.0$ ), 3.60–4.60 (brs, 2H), 3.69 (d, 1H,  $J = 2.8$ ), 3.91 (d, 1H,  $J = 18.0$ ), 7.76 (d, 1H,  $J = 1.4$ ) and 7.93 (d, 1H,  $J = 1.4$ ).  $^{13}\text{C NMR}$  (acetone- $d_6$ ):  $\delta$  15.9, 21.9, 23.5, 27.5, 29.3, 33.5, 37.4, 44.2, 77.7, 100.1, 101.8, 125.4, 126.8, 132.7, 134.4, 162.7 and 167.3, in agreement with the literature [21].

5.1.13. (*1'S,3'S,6'R*)-3'-Hydroxy-7-chloro-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**22**)

Ester **18** (30 mg, 0.079 mmol) was submitted to the general procedure for the saponification of esters (Method B), giving **22** (24.2 mg, 95%) as a colorless solid. M.p.: 229–231 °C ( $\text{CHCl}_3$ );  $[\alpha]_{\text{D}}^{25} = +1.7$  (c 0.5, acetone).  $^1\text{H NMR}$  (acetone- $d_6$ ):  $\delta$  0.79 (d, 3H,  $J = 6.4$ ), 1.01 (s, 3H), 1.18 (s, 3H), 1.40–1.70 (m, 3H), 1.85–2.15 (m, 1H), 2.23–2.48 (m, 1H), 3.19 (d, 1H,  $J = 17.5$ ), 3.68 (brs, 1H), 3.88 (d, 1H,  $J = 17.5$ ), 7.74 (brs, 1H) and 7.78 (brs, 1H).  $^{13}\text{C NMR}$  (acetone- $d_6$ ): 14.3, 19.6, 20.3, 21.9, 25.9, 31.6, 35.8, 42.5, 75.9, 98.9, 112.8, 123.1, 124.6, 129.9, 131.6, 159.8 and 165.8. HRMS (ESI) found: 347.1010  $[\text{M} + \text{Na}]^+$ ;  $\text{C}_{17}\text{H}_{21}\text{ClNaO}_4$  requires 347.1021.

5.1.14. (*1'S,3'S,6'R*)-3'-Hydroxy-7-trifluoromethyl-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**23**)

The ester **19** (50 mg, 0.121 mmol) was submitted to the general procedure for the saponification of esters (Method B), providing **23** (41.2 mg, 95%) as a glassy solid.  $[\alpha]_{\text{D}}^{25} = +31.9$  (c 1.5, MeOH).  $^1\text{H NMR}$  (acetone- $d_6$ ):  $\delta$  0.68 (d, 3H,  $J = 6.7$ ), 0.85 (s, 3H), 1.04 (s, 3H), 1.33–1.58 (m, 3H), 1.74–1.91 (m, 1H), 2.18–2.32 (m, 1H), 3.06 (d, 1H,  $J = 18.1$ ), 3.48 (brs, 1H), 3.70 (d, 1H,  $J = 18.0$ ), 3.91 (d, 1H,  $J = 18.0$ ), 7.86 (s, 1H) and 7.94 (s, 1H).  $^{13}\text{C NMR}$  (acetone- $d_6$ ):  $\delta$  15.0, 21.1, 22.5, 26.2, 28.9, 30.6, 35.7, 42.9, 75.5, 100.6, 109.5 (q,  $J = 34.7$ ), 125.5, 126.6 (q,  $J = 4.4$ ), 130.1, 132.9, 160.8 and 166.6. HRMS (ESI) found: 359.1448  $[\text{M} + \text{H}]^+$ ;  $\text{C}_{18}\text{H}_{22}\text{F}_3\text{O}_4$  requires 359.1465.

5.1.15. (*1'S,3'S,6'R*)-Ethyl 3'-acetyloxy-7-phenyl-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**24**)

A mixture of **16** (60 mg, 0.142 mmol),  $\text{Cs}_2\text{CO}_3$  (69.4 mg, 0.213 mmol) and  $\text{PhB}(\text{OH})_2$  (20.2 mg, 0.17 mmol) in  $\text{PhMe}:\text{EtOH}$  2:1

(2 mL), was purged with dry argon, treated with  $\text{Pd}(\text{Ph}_3\text{P})_4$  (6.6 mg,  $5.7 \times 10^{-3}$  mmol) and irradiated with microwaves (Temp = 150 °C, Power ~300 W) during 15 min. The resulting black suspension was diluted with EtOAc (5 mL) and filtered through Celite. The filtrate was evaporated to dryness and the residue was chromatographed yielding **24** (37.2 mg, 60%) as a colorless oil.  $[\alpha]_{\text{D}}^{25} = +6.6$  (c 0.55,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  0.79 (d, 3H,  $J = 6.6$ ), 0.96 (s, 3H), 1.24 (s, 3H), 1.38 (t, 3H,  $J = 7.1$ ), 1.49–1.69 (m, 3H), 1.94–2.06 (m, 1H), 2.13 (s, 3H), 2.26–2.38 (m, 1H), 3.14 (d, 1H,  $J = 17.2$ ), 3.56 (d, 1H,  $J = 17.2$ ), 4.36 (q, 2H,  $J = 7.1$ ), 4.88 (t, 1H,  $J = 3.0$ ), 7.33 (t, 1H,  $J = 7.3$ ), 7.43 (t, 2H,  $J = 7.5$ ), 7.74 (d, 2H,  $J = 7.3$ ), 7.81 (d, 1H,  $J = 1.6$ ) and 8.04 (d, 1H,  $J = 1.6$ ).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  14.5, 15.0, 20.6, 21.3, 22.5, 25.9, 26.3, 30.8, 35.9, 41.9, 60.7, 79.0, 96.4, 115.3, 120.6, 121.6, 124.9, 127.3, 128.2, 128.3, 129.4, 129.6, 130.4, 136.6, 160.8, 166.8 and 170.2. HRMS (ESI) found: 437.2307  $[\text{M} + \text{H}]^+$ ;  $\text{C}_{27}\text{H}_{33}\text{O}_5$  requires 437.2323.

5.1.16. (*1'S,3'S,6'R*)-3'-Hydroxy-7-phenyl-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**25**)

A solution of **24** (60 mg, 0.137 mmol) was submitted to the general procedure for the saponification of esters (Method A), furnishing **25** (50.2 mg, 99%) as a glassy solid.  $[\alpha]_{\text{D}}^{25} = -7.9$  (c 1.5, acetone).  $^1\text{H NMR}$  (acetone- $d_6$ ):  $\delta$  0.82 (d, 3H,  $J = 6.4$ ), 1.10 (s, 3H), 1.18 (s, 3H), 1.52–1.71 (m, 3H), 1.93–2.05 (m, 1H), 2.27–2.42 (m, 1H), 3.15 (d, 1H,  $J = 17.5$ ), 3.72 (d, 1H,  $J = 17.5$ ), 3.75 (brs, 1H), 7.34 (t, 1H,  $J = 7.2$ ), 7.45 (t, 2H,  $J = 7.6$ ), 7.79 (d, 2H,  $J = 7.9$ ), 7.86 (brs, 1H) and 8.14 (brs, 1H).  $^{13}\text{C NMR}$  (acetone- $d_6$ ):  $\delta$  15.2, 20.7, 22.7, 26.0, 28.8, 31.2, 36.0, 42.8, 77.2, 97.3, 121.4, 121.6, 125.7, 127.3, 128.2, 128.4, 130.3, 131.2, 136.6, 161.8, 172.1. HRMS (ESI) found: 367.1892  $[\text{M} + \text{H}]^+$ ;  $\text{C}_{23}\text{H}_{27}\text{O}_4$  requires 367.1890.

5.1.17. 5.1.17(*1'S,3'S,6'R*)-Methyl 3'-acetyloxy-7-(methoxycarbonyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**26**)

MeOH (1 mL),  $\text{K}_2\text{CO}_3$  (65 mg) and  $\text{Pd}(\text{dppp})\text{Cl}_2$  (5.3 mg, 0.09 mmol) were successively added to a solution of iodide **16** (210 mg, 0.445 mmol) in DMF (6 mL). The mixture was purged with CO and heated at 100 °C under an atmosphere of CO (100 psi) during 20 h. Then, the volatiles were removed under vacuum and the oily residue was chromatographed to yield **26** (88.8 mg, 50%) as an oil.  $[\alpha]_{\text{D}}^{25} = -10.2$  (c 1.05,  $\text{CHCl}_3$ ).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  0.44 (d, 3H,  $J = 6.6$ ), 0.99 (s, 3H), 1.20 (s, 3H), 1.50–1.65 (m, 3H), 1.93–2.05 (m, 1H), 2.32–2.44 (m, 1H), 3.05 (d, 1H,  $J = 17.7$ ), 3.64 (d, 1H,  $J = 17.7$ ), 3.72 (t, 1H,  $J = 2.7$ ), 3.87 (s, 3H), 3.89 (s, 3H), 7.89 (d, 1H,  $J = 1.4$ ) and 8.40 (d, 1H,  $J = 1.4$ ).  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  15.0, 20.5, 22.4, 26.0, 28.9, 30.5, 35.7, 42.8, 51.8, 51.9, 98.7, 111.1, 121.4, 129.5, 132.1, 132.5, 164.3, 165.2 and 166.5. HRMS (ESI) found: 363.1797  $[\text{M} + \text{H}]^+$ ;  $\text{C}_{20}\text{H}_{27}\text{O}_6$  requires 363.1802.

5.1.18. (*1'S,3'S,6'R*)-3'-Hydroxy-7-(carboxyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**28**)

*Method A:* The methyl ester **26** (37.5 mg, 0.105 mmol) was submitted to the general procedure for the saponification of esters (Method B), furnishing **28** (34.0 mg, 99%) as a glassy solid.  $[\alpha]_{\text{D}}^{25} = +0.43$  (c 1.07, acetone).  $^1\text{H NMR}$  (acetone- $d_6$ ):  $\delta$  0.79 (d, 3H,  $J = 6.6$ ), 1.00 (s, 3H), 1.19 (s, 3H), 1.44–1.54 (m, 1H), 1.57–1.76 (m, 2H), 1.92–2.01 (m, 1H), 2.32–2.44 (m, 1H), 3.13 (d, 1H,  $J = 17.9$ ), 3.69 (t, 1H,  $J = 2.7$ ), 3.81 (d, 1H,  $J = 17.9$ ), 3.87 (s, 3H), 3.89 (s, 3H), 7.95 (d, 1H,  $J = 1.4$ ) and 8.43 (d, 1H,  $J = 1.4$ ).  $^{13}\text{C NMR}$  (acetone- $d_6$ ):  $\delta$  14.4, 20.3, 21.9, 26.0, 28.8, 30.2, 35.7, 42.6, 76.0, 99.2, 111.1, 121.8, 129.5, 132.6, 132.7, 164.2, 165.1 and 166.3. HRMS (ESI) found: 335.1486  $[\text{M} + \text{H}]^+$ ;  $\text{C}_{18}\text{H}_{23}\text{O}_6$  requires 335.1489.

*Method B:*  $\text{Zn}(\text{CN})_2$  (45 mg, 0.383 mmol) and  $\text{Pd}(\text{Ph}_3\text{P})_4$  (12 mg, 0.0103 mmol) were added to a solution of **16** (158 mg, 0.371 mmol) in anhydrous DMF (3.5 mL) contained in a microwave vessel. The vessel was purged with nitrogen, sealed and irradiated (Temp.

~110 °C, Power ~100 W) until complete consumption of the starting bromide. Removal of the volatiles in vacuum was followed by dilution of the residue with EtOAc (3 mL) and filtration of the solids through a short path of Celite. Concentration of the filtrate, followed by chromatography yielded nitrile **27** (108 mg, 78%). A solution of **27** (32 mg, 0.890 mmol) in dioxane (0.4 mL) was reacted with 3 M NaOH (0.7 mL) and 30% H<sub>2</sub>O<sub>2</sub> (0.3 mL) at 70 °C until complete consumption of the starting material (21 h) was achieved. The reaction was cooled in an ice bath, diluted with H<sub>2</sub>O (3 mL), washed with Et<sub>2</sub>O (2 × 1 mL), carefully acidified with 4 N HCl (ice bath), and extracted with Et<sub>2</sub>O (4 × 2 mL). The combined organic phases were washed with brine (1 mL) and dried over MgSO<sub>4</sub>. Removal of the volatiles under reduced pressure, followed by chromatography of the residue furnished dicarboxylic acid **28** (27 mg, 90%).

5.1.19. (*1'S,3'S,6'R*)-Methyl 3'-acetyloxy-7-(2-vinyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**29**)

A solution of iodide **16** (200 mg, 0.424 mmol) in dioxane (3 mL) in a tube was treated with *n*-Bu<sub>3</sub>SnCH=CH<sub>2</sub> (136 μL, 0.466 mmol), Pd(AcO)<sub>2</sub> (2.8 mg, 0.0127 mmol), Dabco (2.9 mg, 0.0254 mmol) and TBAF (1 M in THF, 1.27 mL, 1.27 mmol). The tube was screw-capped and immersed in an oil bath pre-heated at 100 °C. After 2 h, the reaction was diluted with EtOAc (5 mL), the solids were filtered through Celite, and the filtrate was concentrated under reduced pressure. Chromatography of the oily residue afforded **29** (109.3 mg, 98%) as an oil.  $[\alpha]_D^{25} = +26.6$  (c 1.2, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.78 (d, 3H, *J* = 6.6), 0.90 (s, 3H), 1.26 (s, 3H), 1.32–1.57 (m, 2H), 1.63–1.71 (m, 1H), 2.11 (s, 3H), 1.87–1.99 (m, 1H), 2.29–2.41 (m, 1H), 3.07 (d, 1H, *J* = 17.1), 3.48 (d, 1H, *J* = 17.1), 3.87 (s, 3H), 4.87 (t, 1H, *J* = 2.8), 5.35 (dd, 1H, *J* = 1.5 and 9.9), 5.98 (dd, 1H, *J* = 1.5 and 16.3), 6.72 (dd, 1H, *J* = 9.9 and 16.3), 7.71 (d, 1H, *J* = 1.2) and 7.87 (d, 1H, *J* = 1.2). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.8, 20.3, 21.3, 22.4, 25.8, 26.3, 30.5, 35.9, 41.8, 51.7, 78.8, 96.7, 116.4, 118.8, 122.0, 124.8, 129.1, 131.6, 161.3, 166.9 and 170.0. HRMS (ESI) found: 359.1821 [M + Na]<sup>+</sup>, C<sub>22</sub>H<sub>28</sub>NaO<sub>5</sub> requires 395.1829.

5.1.20. (*1'S,3'S,6'R*)-Methyl 3'-acetyloxy-7-(carboxymethyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**30**)

A solution of styrene **29** (109.25 mg, 0.293 mmol) in dry THF (3 mL) was cooled to –10 °C, treated dropwise with 9-BBN (0.5 M in THF, 0.67 mL, 0.337 mmol). After complete consumption of the starting olefin, 3 N NaOH (0.25 mL) and 30% H<sub>2</sub>O<sub>2</sub> (0.25 mL) were added and the system was allowed to reach room temperature, being finally heated at 50 °C during 20 min. After cooling to room temperature, solid NaHSO<sub>3</sub> was added. The products were extracted with Et<sub>2</sub>O (4 × 4 mL), dried over MgSO<sub>4</sub> and concentrated under reduced pressure. Chromatographic purification of the residue yielded **30** as a colorless oil (46 mg, 40%).  $[\alpha]_D^{25} = -9.8$  (c 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.76 (d, 3H, *J* = 6.6), 0.87 (s, 3H), 1.21 (s, 3H), 1.37–1.69 (m, 2H), 1.63–1.71 (m, 1H), 1.82–1.96 (m, 1H), 2.09 (s, 3H), 2.24–2.36 (m, 1H), 2.86 (t, 2H, *J* = 6.5), 3.08 (d, 1H, *J* = 17.3), 3.49 (d, 1H, *J* = 17.3), 3.84 (s, 3H), 3.85 (t, 2H, *J* = 6.5), 4.84 (t, 1H, *J* = 2.6), 7.69 (d, 1H, *J* = 1.5) and 7.71 (d, 1H, *J* = 1.5). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.9, 20.4, 21.3, 22.4, 25.9, 26.3, 31.0, 33.8, 35.9, 41.8, 51.8, 62.2, 78.9, 96.3, 118.9, 122.1, 124.7, 128.1, 131.6, 162.3, 167.2 and 170.1. HRMS (ESI) found: 391.2115 [M + H]<sup>+</sup>, C<sub>22</sub>H<sub>31</sub>O<sub>6</sub> requires 391.2115. A solution of carbinol **30b** (26 mg, 0.067 mmol) in dry acetone (1.5 mL) was cooled to 0 °C and treated with freshly prepared Jones reagent (~20 μL). After complete consumption of the starting material was confirmed by TLC, dry 2-propanol (1 mL) was added, the solvent was removed in vacuum and the green residue was chromatographed, yielding **31** (19 mg, 68%) as a colorless oil.  $[\alpha]_D^{25} = +5.2$  (c 1.0, acetone). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.76 (d, 3H, *J* = 6.4), 0.86 (s, 3H), 1.17 (s, 3H), 1.36–1.68 (m, 3H), 1.83–1.95 (m, 1H), 2.10 (s, 3H),

2.24–2.34 (m, 1H), 3.09 (d, 1H, *J* = 17.3), 3.50 (d, 1H, *J* = 17.3), 3.59 (d, 1H, *J* = 16.3), 3.67 (d, 1H, *J* = 16.3), 3.85 (s, 3H), 4.83 (t, 1H, *J* = 2.6), 7.71 (d, 1H, *J* = 1.6) and 7.77 (d, 1H, *J* = 1.6). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.7, 20.1, 21.3, 22.1, 25.9, 26.3, 31.0, 35.2, 35.8, 41.9, 51.8, 79.0, 96.9, 114.0, 122.1, 125.6, 128.3, 131.6, 162.4, 167.0, 170.2 and 176.4. HRMS (ESI) found: 427.1718 [M + Na]<sup>+</sup>, C<sub>22</sub>H<sub>28</sub>NaO<sub>7</sub> requires 427.1727.

5.1.21. (*1'S,3'S,6'R*)-3'-Hydroxy-7-(carboxymethyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**31**)

Compound **30** (18 mg, 0.0455 mmol) was submitted to the general procedure for the saponification of esters (Method B), affording **31** (15 mg, 95%) as a glassy solid.  $[\alpha]_D^{25} = -12.4$  (c 0.8, acetone). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 0.77 (d, 3H, *J* = 6.4), 0.98 (s, 3H), 1.12 (s, 3H), 1.23–1.47 (m, 2H), 1.55–1.73 (m, 1H), 1.86–1.99 (m, 1H), 2.25–2.34 (m, 1H), 3.08 (d, 1H, *J* = 17.3), 3.85–3.68 (m, 3H), 3.79 (d, 1H, *J* = 17.3), 7.72 (brs, 1H) and 7.74 (brs, 1H). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): δ 14.3, 20.3, 21.9, 26.0, 31.1, 34.6, 35.9, 42.6, 76.1, 97.4, 115.2, 121.7, 125.0, 128.3, 129.1, 131.6, 162.7, 167.0 and 171.3. HRMS (ESI) found: 349.1649 [M + H]<sup>+</sup>, C<sub>19</sub>H<sub>25</sub>O<sub>6</sub> requires 349.1646.

5.1.22. (*E-1'S,3'S,6'R*)-Methyl 3'-acetyloxy-7-(2-ethoxycarbonylvinyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**32**)

Ethyl acrylate (54 μL, 0.496 mmol), Ph<sub>3</sub>P (8.6 mg, 0.033 mmol), NaHCO<sub>3</sub> (35 mg, 0.412 mmol), TEBAHSO<sub>4</sub> (56 mg, 0.165 mmol), Pd(AcO)<sub>2</sub> (5.6 mg, 0.025 mmol) and activated 4 Å MS (66 mg) were successively added to a stirring solution of **16** (78 mg, 0.165 mmol) in anhydrous MeCN (2 mL) under nitrogen. The slurry was stirred at 65 °C during 28 h, when it was diluted with EtOAc (3 mL), and the solids were filtered off through a short pad of Celite. The filtrate was concentrated in vacuo and the residue was chromatographed, affording **32** (67.3 mg, 92%) as a glassy solid.  $[\alpha]_D^{25} = +33.3$  (c 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.76 (d, 3H, *J* = 6.6), 0.89 (s, 3H), 1.29 (s, 3H), 1.34 (t, 3H, *J* = 7.1), 1.36–1.70 (m, 3H), 2.11 (s, 3H), 1.88–2.00 (m, 1H), 2.34–2.46 (m, 1H), 3.10 (d, 1H, *J* = 17.3), 3.51 (d, 1H, *J* = 17.3), 3.88 (s, 3H), 4.26 (q, 2H, *J* = 7.1), 4.87 (t, 1H, *J* = 2.7), 6.69 (d, 1H, *J* = 16.1), 7.69 (d, 1H, *J* = 16.1), 7.81 (d, 1H, *J* = 1.4) and 7.95 (d, 1H, *J* = 1.4). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.2, 14.8, 20.4, 22.2, 25.9, 30.5, 36.0, 42.5, 60.3, 76.3, 99.3, 115.5, 120.3, 122.7, 127.2, 128.2, 131.2, 131.5, 140.1, 167.1, 170.1 and 172.8. HRMS (ESI) found: 483.1778 [M + K]<sup>+</sup>, C<sub>20</sub>H<sub>28</sub>KO<sub>6</sub> requires 483.1780.

5.1.23. (*1'S,3'S,6'R*)-3'-Hydroxy-7-(2-carboxyvinyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**33**)

Triester **32** (52 mg, 0.117 mmol) was submitted to the general procedure for the saponification of esters (Method B), affording **33** (42 mg, 98%).  $[\alpha]_D^{25} = +22.1$  (c 1.0, acetone). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 0.76 (d, 3H, *J* = 6.8), 1.00 (s, 3H), 1.21 (s, 3H), 1.44–1.78 (m, 3H), 1.88–2.01 (m, 1H), 2.35–2.47 (m, 1H), 3.14 (d, 1H, *J* = 18.0), 3.70 (t, 1H, *J* = 2.4), 3.82 (d, 1H, *J* = 18.0), 6.79 (d, 1H, *J* = 15.8), 7.71 (d, 1H, *J* = 15.8), 7.83 (d, 1H, *J* = 1.4) and 8.00 (d, 1H, *J* = 1.4). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): δ 14.4, 20.3, 22.2, 25.9, 30.5, 36.0, 42.7, 76.1, 99.3, 115.5, 120.3, 122.7, 127.2, 128.2, 131.2, 131.5, 140.1, 162.8, 166.6 and 167.7. HRMS (ESI) found: 383.1447 [M + Na]<sup>+</sup>, C<sub>20</sub>H<sub>24</sub>NaO<sub>6</sub> requires 383.1465.

5.1.24. (*1'S,3'S,6'R*)-Methyl 3'-acetyloxy-7-(2-ethoxycarbonyl-ethyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**34**)

10% Pd/C (5 mg) was added to a solution of ethyl cinnamate **32** (49 mg, 0.110 mmol) in absolute MeOH (6 mL). The reaction was purged with hydrogen and stirred during 3 h. The catalyst was filtered through a short pad of Celite, the filtrate was concentrated



under reduced pressure and the residue was chromatographed furnishing **34** (46 mg, 95%).  $[\alpha]_D^{25} = +4.7$  (c 0.3, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.75 (d, 3H, J = 6.6), 0.86 (s, 3H), 1.20 (t, 3H, J = 7.0), 1.22 (s, 3H), 1.36–1.68 (m, 3H), 2.09 (s, 3H), 1.84–1.96 (m, 1H), 2.24–2.36 (m, 1H), 2.62 (t, 3H, J = 7.3), 2.89 (t, 3H, J = 7.3), 3.06 (d, 1H, J = 17.1), 3.47 (d, 1H, J = 17.1), 3.83 (s, 3H), 4.09 (q, 2H, J = 7.0), 4.84 (t, 1H, J = 2.5), 7.66 (brs, 1H) and 7.69 (brs, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.2, 14.8, 20.4, 21.3, 22.3, 25.7, 25.9, 26.3, 30.8, 33.6, 35.8, 41.9, 51.7, 60.4, 78.9, 96.2, 120.5, 121.9, 124.5, 128.1, 130.8, 162.1, 167.1, 170.1 and 172.8. HRMS (ESI) found: 469.2187 [M + Na]<sup>+</sup>; C<sub>25</sub>H<sub>34</sub>NaO<sub>7</sub> requires 469.2197.

#### 5.1.25. (1'S,3'S,6'R)-3'-Hydroxy-7-(2-carboxy-ethyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylic acid (**35**)

The arylpropionic triester **34** (40 mg, 0.09 mmol) was submitted to the general procedure for the saponification of esters (Method B), affording **35** (31 mg, 95%).  $[\alpha]_D^{25} = -35.4$  (c 0.3, acetone). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 0.77 (d, 3H, J = 6.6), 0.99 (s, 3H), 1.16 (s, 3H), 1.39–1.74 (m, 3H), 1.90–2.01 (m, 1H), 2.25–2.39 (m, 1H), 2.64–2.70 (m, 2H), 2.87–2.93 (m, 2H), 3.07 (d, 1H, J = 17.5), 3.67 (t, 2H, J = 2.2), 3.77 (d, 1H, J = 17.5), 7.68 (brs, 1H) and 7.70 (brs, 1H). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): δ 14.4, 19.6, 20.4, 22.1, 25.5, 26.0, 30.9, 32.8, 35.9, 42.6, 76.1, 97.1, 120.5, 121.8, 124.5, 129.1, 130.7, 162.3, 167.3 and 173.3. HRMS (ESI) found: 385.1610 [M + Na]<sup>+</sup>; C<sub>20</sub>H<sub>26</sub>NaO<sub>6</sub> requires 385.1622.

#### 5.1.26. (1'S,3'S,6'R)-Ethyl 3'-hydroxy-7-(4-carboxyphenyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**36**)

A mixture of **16** (80 mg, 0.188 mmol), Cs<sub>2</sub>CO<sub>3</sub> (92 mg, 0.282 mmol) and 4-HO(O)CC<sub>6</sub>H<sub>4</sub>B(OH)<sub>2</sub> (37 mg, 0.226 mmol) in toluene–EtOH 2:1 (2 mL) was purged with dry argon during 5 min and Pd(Ph<sub>3</sub>P)<sub>4</sub> (8.7 mg, 7.5 × 10<sup>-3</sup> mmol) was added. The vessel was sealed and irradiated in a microwave oven (Temp. = 150 °C, Power ~175 W) during 15 min. The black suspension was diluted with EtOAc (5 mL), and the solids were filtered through a short pad of Celite. The filtrate was evaporated to dryness and the remaining solid was chromatographed, yielding **36** (50 mg, 60%).  $[\alpha]_D^{25} = -3.5$  (c 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.81 (d, 3H, J = 6.7), 1.07 (s, 3H), 1.17 (s, 3H), 1.39 (t, 3H, J = 7.0), 1.49–1.69 (m, 3H), 1.94–2.06 (m, 1H), 2.26–2.38 (m, 1H), 3.12 (d, 1H, J = 17.4), 3.71 (d, 1H, J = 17.4), 3.74 (brs, 1H), 4.37 (q, 2H, J = 7.0), 7.82 (d, 1H, J = 1.4), 7.87 (d, 1H, J = 8.6), 8.08 (d, 1H, J = 1.4) and 8.17 (d, 1H, J = 8.6). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.5, 15.1, 20.7, 22.7, 26.0, 29.0, 31.2, 36.0, 42.7, 60.7, 76.1, 97.5, 120.0, 122.9, 125.9, 127.6, 128.2, 130.1, 130.3, 130.4, 142.3, 161.1, 166.5 and 170.9. HRMS (ESI) found: 461.1925 [M + Na]<sup>+</sup>; C<sub>26</sub>H<sub>30</sub>NaO<sub>6</sub> requires 461.1935.

#### 5.1.27. (1'S,3'S,6'R)-3'-Hydroxy-7-(4-carboxyphenyl)-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**37**)

Compound **36** (56 mg, 0.128 mmol) was submitted to the general procedure for the saponification of esters (Method A), furnishing **37** (44 mg, 84%) as a glassy solid.  $[\alpha]_D^{25} = +3.6$  (c 1.0, Me<sub>2</sub>CO). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 0.79 (d, 3H, J = 6.6), 1.07 (s, 3H), 1.17 (s, 3H), 1.42–1.74 (m, 3H), 1.92–2.01 (m, 1H), 2.26–2.39 (m, 1H), 3.17 (d, 1H, J = 17.9), 3.69 (t, 1H, J = 2.7), 3.86 (d, 1H, J = 17.9), 7.84 (d, 1H, J = 1.2), 7.94 (d, 1H, J = 8.3), 8.07 (d, 1H, J = 1.2) and 8.13 (d, 1H, J = 8.3). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): δ 14.5, 20.5, 22.2, 25.9, 28.8, 30.8, 36.0, 42.6, 76.1, 97.9, 119.9, 122.9, 125.9, 128.0, 129.3, 129.8, 129.9, 131.1, 141.3, 161.2, 166.6 and 166.3. HRMS (ESI) found: 433.1605 [M + Na]<sup>+</sup>; C<sub>24</sub>H<sub>26</sub>NaO<sub>6</sub> requires 433.1622.

#### 5.1.28. (1'S,3'S,6'R)-Methyl 3'-acetyloxy-7-allyl-2',2',6'-trimethyl-3H-spiro[1-benzofuran-2,1'-cyclohexane]-5-carboxylate (**38**)

A mixture of bromide **17** (100 mg, 0.235 mmol), *n*-Bu<sub>3</sub>SnCH<sub>2</sub>CH=CH<sub>2</sub> (160 μL, 0.493 mmol) and CsF (150 mg, 0.987 mmol) in anhydrous dioxane (3.0 mL) was purged with

anhydrous argon and Pd(Ph<sub>3</sub>P)<sub>4</sub> (21.9 mg, 0.018 mmol) was added. The vessel was tightly closed with a screw cap and immersed in an oil bath pre-heated at 110 °C during 21 h. The reaction was diluted with EtOAc (5 mL) and the solids were filtered off through Celite. The filtrate was concentrated in vacuum and the oily residue was chromatographed furnishing **38** (91 mg, 98%), as an oil.  $[\alpha]_D^{25} = +15.2$  (c 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.77 (d, 3H, J = 6.6), 0.87 (s, 3H), 1.22 (s, 3H), 1.38–1.70 (m, 3H), 1.86–1.98 (m, 1H), 2.11 (s, 3H), 2.25–2.37 (m, 1H), 3.08 (d, 1H, J = 17.1), 3.31–3.35 (m, 2H), 3.49 (d, 1H, J = 17.1), 3.85 (s, 3H), 4.85 (t, 1H, J = 2.8), 5.03–5.09 (m, 2H), 5.89–6.02 (m, 1H), 7.66 (brs, 1H) and 7.70 (brs, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.9, 20.4, 21.3, 22.3, 25.9, 26.3, 31.0, 34.2, 35.9, 41.9, 51.7, 78.9, 96.0, 115.8, 120.3, 121.9, 124.3, 128.0, 130.8, 135.9, 162.1, 167.3 and 170.1. HRMS (ESI) found: 387.2157 [M + H]<sup>+</sup>; C<sub>23</sub>H<sub>31</sub>O<sub>5</sub> requires 387.2166.

#### 5.1.29. Synthesis of diols (**39**)

K<sub>3</sub>Fe(CN)<sub>6</sub> (136.2 mg, 0.414 mmol), K<sub>2</sub>CO<sub>3</sub> (57.2 mg, 0.414 mmol) and OsO<sub>4</sub> (0.36 mg, 1.410<sup>-3</sup> mmol) were added to a solution of allyl derivative **37** (53 mg, 0.138 mmol) in a 2:1 mixture of *t*-BuOH–H<sub>2</sub>O (3 mL). The slurry was stirred at room temperature until the reaction was deemed complete (16 h). Then, 10% NaHSO<sub>3</sub> (2 mL) was added, and the organic products were recovered by extraction with EtOAc (5 × 3 mL). The extract was washed with brine (3 mL), dried over MgSO<sub>4</sub>, concentrated in vacuo and chromatographed to afford **39** (54 mg, 90%) as an inseparable and roughly equimolar mixture of epimers.  $[\alpha]_D^{25} = +5.7$  (c 1.1, CHCl<sub>3</sub>). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.78 (d, 3H, J = 6.3), 0.87 (s, 3H), 1.22 (s, 3H), 1.38–1.71 (m, 3H), 1.85–1.97 (m, 1H), 2.11 (s, 3H), 2.25–2.36 (m, 1H), 2.80–2.83 (m, 2H), 3.10 (d, 1H, J = 17.3), 3.31–3.35 (m, 2H), 3.47–3.56 (m, 2H), 3.63–3.68 (m, 1H), 3.86 (s, 3H), 3.96–4.05 (m, 1H), 4.86 (brs, 1H), 7.70 (brs, 1H) and 7.73 (brs, 1H). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 14.9, 15.0, 20.4, 21.3, 22.4, 26.0, 26.2, 26.3, 31.0, 34.1, 34.2, 35.8, 35.9, 41.8, 51.8, 65.9, 66.0, 71.8, 71.9, 78.8, 96.6, 96.7, 118.0, 118.2, 122.2, 122.3, 124.8, 128.2, 132.0, 162.1, 167.1 and 170.1. HRMS (ESI) found: 433.2032 [M + Na]<sup>+</sup>; C<sub>23</sub>H<sub>32</sub>NaO<sub>7</sub> requires 433.2040.

#### 5.1.30. Hydrolysis of the mixture of diols **39**

The epimeric mixture of diols **39** (48 mg, 0.114 mmol) was submitted to the general procedure for the saponification of esters (Method B), yielding **40** (40.3 mg, 97%) as an inseparable and roughly equimolar mixture of epimeric diols.  $[\alpha]_D^{25} = -7.8$  (c 1.0, acetone). <sup>1</sup>H NMR (acetone-*d*<sub>6</sub>): δ 0.76 (d, 3H, J = 6.4), 0.77 (d, 3H, J = 6.1), 0.98 (s, 3H), 0.99 (s, 3H), 1.14 (s, 6H), 1.17–1.31 (m, 3H), 1.36–1.51 (m, 2H), 1.53–1.76 (m, 4H), 2.01–2.07 (m, 4H), 2.22–2.37 (m, 4H), 2.61–2.91 (m, 4H), 3.07 (d, 2H, J = 17.8), 3.42–3.60 (m, 3H), 3.62–3.88 (m, 3H), 3.91–4.7 (m, 2H), 7.66 (brs, 2H) and 7.72 (brs, 2H). <sup>13</sup>C NMR (acetone-*d*<sub>6</sub>): δ 14.5, 14.8, 17.8, 19.7, 20.5, 22.1, 26.0, 28.9, 31.0, 33.9, 34.1, 35.9, 42.6, 63.2, 68.8, 75.1, 75.2, 76.1, 97.0, 103.6, 117.7, 117.9, 121.9, 124.3, 124.6, 124.7, 128.9, 129.1, 131.7, 162.5 and 167.2. HRMS (ESI) found: 387.1769 [M + Na]<sup>+</sup>; C<sub>20</sub>H<sub>28</sub>NaO<sub>6</sub> requires 387.1778.

#### 5.2. Determination of Complement inhibition [21]

Fresh human sera was pooled, diluted 1/50 and the complement was titrated against a 0.85% suspension of sensitized sheep red blood cells (SRBCs). The serum was further diluted with diluted Mayer buffer (DMB) according to its titer. Compounds were tested as their more soluble sodium salts. In the typical procedure, accurately weighed 20 mg of each test compound was dissolved in 120 μL DMSO to which 1.0 equivalents of 0.2 N NaOH was added, and the resulting solution was diluted to 10 mL with DMB to give final concentrations of 2.0 mg/mL. Then, 0.1–0.5 mL of the test compound solution were placed (in duplicate) into a series of 5-mL

test-tubes, and completed to 1.0 mL with DMB containing 1.2% DMSO; diluted human complement (0.5 mL) was added and, after 20 min at 37 °C, the mixtures were treated with the SRBCs suspension (0.5 mL). After incubating at 37 °C for 30 min, the tubes were centrifuged for 5 min at 2500 rpm to pellet the intact SRBCs and the absorbances of the supernatants were read at 410 nm against a blank processed in the same form, but devoid of complement activity. Appropriate 100% hemolysis and vehicle controls (blank, 1.2% DMSO in DMB) were run concomitantly.

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