Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5097–5101
Bioorganic & Medicinal Chemistry Letters

Synthesis of 3*H*-spiro[benzofuran-2,1′-cyclohexane] derivatives from naturally occurring filifolinol and their classical complement pathway inhibitory activity

Mariana Useglio,[a] Patricia M. Castellano,[b] María A. Operto,[b] René Torres[c] and Teodoro S. Kaufman[a,b,*]

[a]*Instituto de Química Orgánica de Síntesis (IQUIOS, CONICET-UNR) and Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina*
[b]*Área Análisis de Medicamentos, Departamento Farmacia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina*
[c]*Departamento de Ciencias del Ambiente, Facultad de Química y Biología, Universidad de Santiago de Chile, Avenida Bernardo O'Higgins 3363, Santiago, Chile*

Received 9 June 2006; revised 7 July 2006; accepted 11 July 2006
Available online 27 July 2006

Abstract—Six 3*H*-spiro[benzofuran-2,1′-cyclohexane] derivatives were synthesized from naturally occurring filifolinol, and their classical complement pathway inhibitory activity was determined. IC_{50} values of the most potent compounds were comparable to the activity of the natural complement inhibitor K76-COOH and some synthetic tricyclic analogs of it.

The complement system is an essential component of the innate immune system, which takes part in various functions of the adaptive immune response, provides a first defense line against foreign pathogens, and allows the clearance of immune complexes from the blood stream.[1] Interestingly, however, abnormal activation of the complement cascade such as that taking place in inflammatory diseases, including xenotransplant rejection, ischemia-reperfusion injury, and asthma, may be prejudicial and even fatal.[2] The increasing understanding of the complement system has aroused considerable interest in the development of complement inhibitors, because these have been found to prevent disease progression and ameliorate the deleterious effects caused by improper complement activation in established diseases.[2]

The role of complement in disease and the profiles of inhibitors currently being developed for potential use in therapeutics, including Alzheimer disease, cardiac disease, and transplantation, have been extensively reviewed.[3] However, well defined and characterized low molecular weight complement inhibitors are scarce. These inhibitors offer several advantages over large therapeutic proteins and other polymers, in that they are cost-effective, have better tissue penetration, and can be developed for oral use. Such considerations are of prime importance when the drug must be administered over a long period of time, such as during management of chronic autoimmune disorders.[4]

The broad-spectrum synthetic serine protease inhibitor FUT-175 (**1**, futhan, nafamostat) proved non-specific but useful (Fig. 1).[5] On the other hand, natural products and their synthetic analogs also remain as highly attractive alternatives. Oleanolic acid (**2**) has been found to be active[6a,b] and some of its synthetic analogs have been reported as inhibitors.[6c] Analogously, the natural product K76 (**3a**) and its derivative K76-COOH (**3b**) have been described as potent inhibitors of the classical complement pathway.[7a,b] They have been synthesized[7c–e] and some of their partial analogs (**4b–d** and **5a**, **b**) have demonstrated remarkable activity in vitro.[8]

Interesting potency has been found among the tricyclic analogs (**4b–d**) displaying the grisan skeleton **4a**,[9] a

Keywords: Filifolinol; Complement inhibition; 3*H*-spiro[benzofuran-2,1′-cyclohexanes]; Analogs of K76.
* Corresponding author. Tel./fax: +54 341 437 0477; e-mail: tkaufman@fbioyf.unr.edu.ar

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.07.029

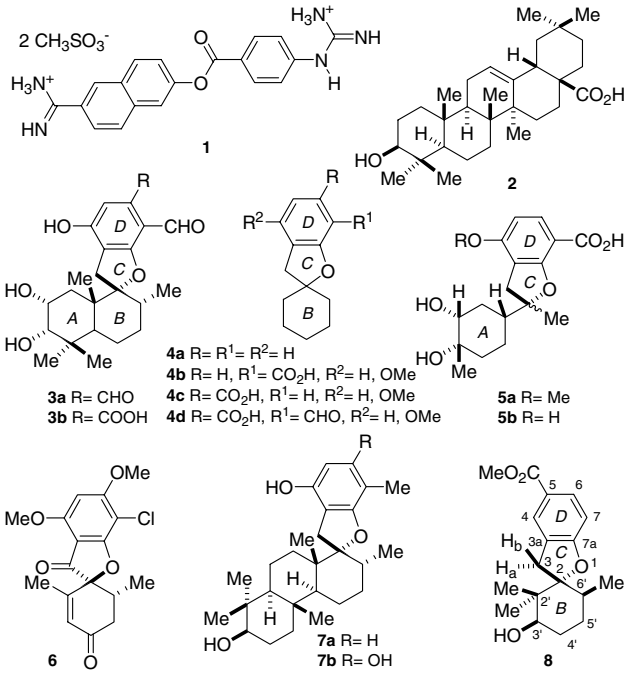


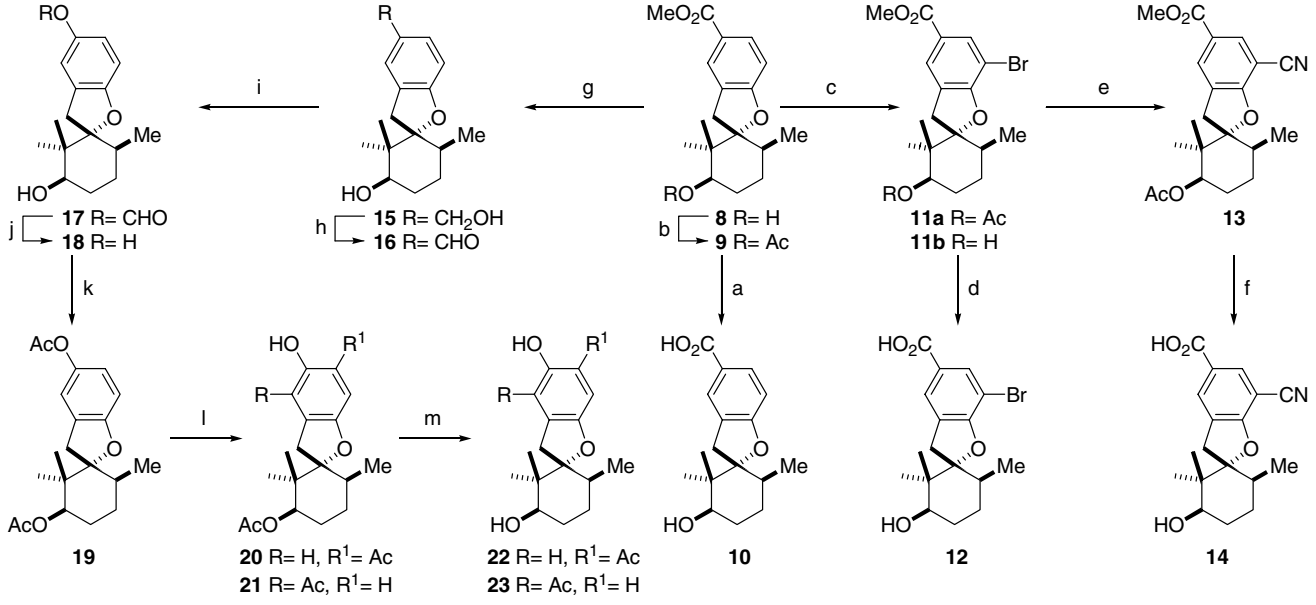
Figure 1.

structural feature shared with the antifungal drug griseofulvin (**6**) and other bioactive compounds, such as the novel myo-inositol monophosphatase inhibitor L-671,776[10a] and the ichthyotoxic terpenoids isochromazonarol,[10b] stypodiol (**7a**) and stypotriol (**7b**).[10c,d] Common features of **3b** and its most active analogs are the presence of a comparatively polar benzofuran part, carrying a carboxylic acid unit attached to the aromatic moiety and usually spiro-fused to a low polarity hydrocarbon skeleton. These cyclohexane- or decalin-based skeletons may also carry alcoholic functions.

Filifolinol (**8**) is a naturally occurring 3*H*-spiro[benzofuran-2,1′-cyclohexane] which has recently been isolated in important quantities from *Heliotropium filifolium* Miers (Boraginaceae).[11a] The natural product, which has evidenced antiviral activity,[11b] displays many of the key structural features of the K76-COOH-based complement inhibitors **4b**–**d**. Herein, we report the synthesis of six simple derivatives of filifolinol, which can be regarded as BCD ring analogs of K76-COOH, and disclose the results of their classical complement pathway inhibitory activity.

Thus, mild hydrolysis of **8** (LiOH, THF-H_2O) furnished acid **10** in 80% yield (Scheme 1).[12] For the synthesis of acid-nitrile **14**, filifolinol was first transformed into acetate **9** (Ac_2O and Et_3N) in 92% yield, which was subjected to electrophilic bromination, yielding 91% of bromide **11a**. In turn, this was transformed into the corresponding cyanide **13** in 55% yield, with $ZnCN_2$ and catalytic amounts of $Pd(PPh_3)_4$ in dry DMF, under microwave irradiation.

Interestingly, the thermally heated version of this reaction gave very low yields of **13** and extensive decomposition of bromide **11a** took place. Final basic hydrolysis of **13** (LiOH, THF-H_2O) furnished 72% of the desired acid-nitrile **14**. On the other hand, compound **12** was easily accessed as a white, high melting point solid by basic hydrolysis of **11a**. Interestingly, however, it was found that direct bromination of **8** with bromine in



Scheme 1. Reagents and conditions: (a) LiOH, THF-H_2O, rt, 2 h (80%); (b) Ac_2O, Et_3N, CH_2Cl_2, rt, overnight (92%); (c) Br_2, AcOH, 0 °C → rt, overnight (**9** → **11a**, 91%); (d) LiOH, THF-H_2O, rt (82%); (e) $Zn(CN)_2$, $Pd(PPh_3)_4$ (5 mol%), DMF, microwaves, 400 W, 55 min (55%); (f) LiOH, THF-H_2O, rt, 2 h (72%); (g) $LiAlH_4$, THF, rt, 3 h (95%); (h) $BaMnO_4$, CH_2Cl_2, reflux, 48 h (91%); (i) *m*-CPBA, $CHCl_3$, 55 °C, 1.5 h (85%); (j) 10% $NaHCO_3$, THF-H_2O, rt, 4 h (80%); (k) Ac_2O, pyridine, DMAP, CH_2Cl_2, rt, overnight (75%); (l) hexane, hν (254 nm), rt, 2 h (63%); (m) NaOH, THF-H_2O, 2 h, rt (100%).

glacial AcOH provided **11b** albeit in very low yields (<20%).

The synthesis of phenol **18** was initiated with the $LiAlH_4$ reduction of **8** to diol **15**, which was selectively oxidized with barium manganate to the corresponding aldehyde **16**. Then, Baeyer Villiger oxidation of the latter with *m*-CPBA afforded formate **17** in 85% yield, which was finally hydrolyzed (10% $NaHCO_3$, THF-H_2O), giving 80% of **18**.

Finally, when formate **17** was treated with Ac_2O-pyridine, it was smoothly transformed into diacetate **19** in 75% yield. Irradiation of **19** in hexane at 254 nm effected its photo-Fries rearrangement, providing a 2:1 mixture of derivatives **20** and **21** in 63% combined yield. These acetophenone-acetates were quantitatively hydrolyzed (NaOH, THF-H_2O) to **22** and **23**, respectively, prior to being assayed.

Next, the ability of the above-described compounds to inhibit the classical complement pathway was tested. For the estimation of the degree of inhibition, a modification of the method of Weisman and co-workers was employed,[8a,13] with the results collected in Table 1.[14]

These indicate that the novel and easily available acids **10**, **12**, and **14** as well as phenol **18** and acetophenone **23** display activity comparable to that of the monocarbonyl K76-COOH analogs, such as **4c**, being their potencies within the same order of magnitude of **3b**. On the other hand, acetophenone **22** was active but evidenced solubility problems. Interestingly, functionalization of C-7 (**12** and **14** vs. **10**) decreased IC_{50} among the acids while, surprisingly, phenol **18** displayed remarkable potency, exhibiting an IC_{50} value slightly lower than K76-COOH.

Previous findings have shown that a phenolic hydroxyl group was important for the activity of ACD-ring acid analogs of K-76 (**5a**,**b**)[9] and that methyl ethers were more active than their corresponding free phenols among the BCD-ring analogs (**4b**–**d**).[9] In the present case, however, the acids **10**, **12**, and **14** were active despite the absence of methyl ether or free phenol moieties. In addition, the compounds retained activity regardless of the fact that the carboxyl group was attached to the aromatic ring *para* to the benzofuran oxygen and not *meta* as in **3b**. Other interesting finding from this series of compounds was that, as observed in the cases of **3a**/**3b** and their lower analogs,[8b] the carboxylic acid moiety conveniently imparted better solubility to the compounds. However, results of **18** and **23** also suggest that a carboxyl group may not be essential for activity.

In conclusion, inspired by the structure of K76-COOH, we have prepared six 3*H*-spiro[benzofuran-2,1′-cyclohexane] derivatives in short synthetic sequences from filifolinol, an abundantly available natural product. We have also tested their activity as inhibitors of the classical complement pathway. Phenol **18** behaved as a good inhibitor, while the acids **10**, **12**, and **14** exhibited lower but interesting potency, despite the lack of a free phenolic hydroxyl group in their structures. Acetophenone **23** was also active at the low mM level. More functionalized inhibitors may display improved potency; work toward this goal is in progress and results will be diclosed in due time.

Table 1. Classical complement pathway inhibition by filifolinol derivatives

Entry no.	Compound	IC_{50} [a] (μM)	IC_{50}[a] (μg/mL)
1	**10**	2000	580
2	**12**	950	350
3	**14**	1320	415
4	**18**	455	260
5	**22**	—[b]	—[b]
6	**23**	1070	320
7[8b]	K76-COOH (**3b**)	570	238
8[8e]	**4b**	1300	341
9[8e]	**4c**	820	214
10[8e]	**4d**	160	44
11[8b]	**5b**	1600	515

[a] Concentration required to inhibit complement induced hemolysis by 50% compared to the control (without test compound). Values reported were obtained by interpolation in the corresponding concentration/inhibition (%) plots. Samples were run in duplicate. Results are within the ±5% error.

[b] At a concentration of 253 μg/mL, acetophenone **22** produced 17% ± 5% inhibition. Poor solubility of the compound precluded testing more concentrated solutions.

Acknowledgments

The authors thank Fundación Antorchas, SECyT-UNR, CONICET (Project N° 5439), and ANPCyT (Project N° 06-12532) for financial support.

References and notes

1. (a) Complement, 2nd ed.; Law, S. K. A.; Reid, K. B. M., Eds.; IRL: New York, 1995; (b) Carroll, M. C. *Semin. Immunol.* **1998**, *10*, 279; (c) Dodds, A. *The Biochemist* **1998**, *20*, 18; (d) Taylor, P.; Botto, M.; Walport, M. *Curr. Biol.* **1998**, *8*, R259.
2. (a) Mollnes, T. E.; Fiane, A. E. *Allergy* **2002**, *57*, 75; (b) Saadi, S.; Platt, J. L. *Clin. Exp. Pharmacol. Physiol.* **1999**, *26*, 1016; (c) Kimura, T.; Andoh, A.; Fujiyama, Y.; Saotome, T.; Bamba, T. *Clin. Exp. Immunol.* **1998**, *111*, 484; (d) Robbins, R. A.; Russ, W. D.; Rasmussen, J. K.; Clayton, M. M. *Am. Rev. Respir. Dis.* **1987**, *135*, 651; (e) Kemp, P. A.; Spragg, J. H.; Brown, J. C.; Morgan, B. P.; Gunn, C. A.; Taylor, P. W. *J. Clin. Lab. Immunol.* **1992**, *37*, 147; (f) Rogers, J.; Griffin, W. S. T. In *Neuroinflammation: Mechanisms and Management*; Wood, P. L., Ed.; Humana Press: Totowa, 1998; p 177.
3. (a) Sahu, A.; Lambris, J. D. *Immunopharmacology* **2000**, *49*, 133; (b) Asghar, S. S.; Pasch, M. C. *Front. Biosci.* **2000**, *5*, 63; (c) Hagmann, W. K.; Sindelar, R. D. *Annu. Rep. Med. Chem.* **1992**, *27*, 199; (d) Makrides, S. C. *Pharmacol. Rev.* **1998**, *50*, 59; (e) Ward, P. A.; Czermak, B. J.; Huber-Lang, M.; Diehl, K.; Friedl, H. P. In *Therapeutic Interventions in the Complement System*; Lambris, J. D., Holers, V. M., Eds.; Humana Press: Totowa, 2000; p 237; (f) Kulkarni, A. P.; Kelleway, L. A.; Kotwall, G. J. *Ann. N. Y. Acad. Sci.* **2005**, *1056*, 413.

4. Taylor, S. M.; Fairlie, D. P. *Expert Opin. Ther. Patents* **2000**, *10*, 1.
5. (a) Inagi, R.; Miyata, T.; Madea, K.; Sugiyama, S.; Miyama, A.; Nakashima, I. *Immunol. Lett.* **1991**, *27*, 49; (b) Blum, M. G.; Collins, B. J.; Chang, A. C.; Zhang, J. P.; Knaus, S. A.; Pierson, R. N. *Xenotransplantation* **1998**, *5*, 35.
6. (a) Kapil, A.; Sharma, S. *J. Pharm. Pharmacol.* **1994**, *46*, 922; (b) Kapil, A.; Sharma, S. *J. Pharm. Pharmacol.* **1995**, *47*, 585; (c) Assefa, H.; Nimrod, A.; Walker, L.; Sindelar, R. D. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1619.
7. (a) Hong, K.; Kinoshita, T.; Miyazaki, W.; Izawa, T.; Inoue, K. *J. Immunol.* **1979**, *122*, 2418; (b) Miyazaki, W.; Tamoka, H.; Shinohara, M.; Kaise, H.; Izawa, T.; Nakano, Y.; Kinoshita, T.; Hong, K.; Inoue, K. A. *Microbiol. Immunol.* **1980**, *24*, 1091; (c) Corey, E. J.; Das, J. *J. Am. Chem. Soc.* **1982**, *104*, 5551; (d) McMurry, J. E.; Erion, M. D. *J. Am. Chem. Soc.* **1985**, *107*, 2712; (e) Mori, K.; Komatsu, M. *Liebigs Ann. Chem.* **1988**, 107.
8. (a) Kaufman, T. S.; Srivastava, R. P.; Sindelar, R. D.; Scesney, S. M.; Marsh, H. C. *J. Med. Chem.* **1995**, *38*, 1437; (b) Kaufman, T. S.; Srivastava, R. P.; Sindelar, R. D.; Scesney, S. M.; Marsh, H. C. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 501; (c) Srivastava, R. P.; Zhu, X.; Walker, L. A.; Sindelar, R. D. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1751; (d) Bradbury, B. J.; Sindelar, R. D. *J. Heterocycl. Chem.* **1989**, *26*, 1827; (e) Bradbury, B. J.; Bartyzel, P.; Kaufman, T. S.; Nieto, M. J.; Sindelar, R. D.; Scesney, S. M.; Gaumond, B. R.; Marsh, H. C. *J. Med. Chem.* **2003**, *46*, 2697.
9. Kaufman, T. S.; Sindelar, R. D. *J. Heterocycl. Chem.* **1989**, *26*, 879, and references cited therein.
10. (a) Falck, J. R.; Reddy, K. K.; Chandrasekar, S. *Tetrahedron Lett.* **1997**, *38*, 5245; (b) Dave, M.-N.; Kusumi, T.; Ishitsuka, M.; Iwashita, T.; Kakisawa, H. *Heterocycles* **1984**, *22*, 2301; (c) González, A. G.; Alvarez, M. A.; Martín, J. D.; Norte, M.; Pérez, C.; Rovirosa, J. *Tetrahedron* **1982**, *38*, 719; (d) Gerwick, W. H.; Fenical, W. *J. Org. Chem.* **1981**, *46*, 22.
11. (a) Torres, R.; Virrarroel, L.; Urzúa, A.; Delle Monache, F.; Delle Monache, G.; Gacs-Baitz, E. *Phytochemistry* **1994**, *36*, 249; (b) Torres, R.; Modak, B.; Urzúa, A.; Delle Monache, F.; Damonte, E.; Pujol, C. A. *Bol. Soc. Chil. Quím.* **2002**, *47*, 259.
12. Data for selected compounds: Compound **10**—mp: 162–164 °C; $[\alpha]_D^{25} = -16.3$ (*c* 1.76, $CHCl_3$); IR (KBr) *v* 3550–2450, 3416, 1680, 1406, 1382, 1202, 1175, 1028, 955, 835, 741 and 652 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.79 (s, 3H, *J* = 6.7, Me-6′), 1.01 (s, 3H, Me-2′$_{eq}$), 1.14 (s, 3H, Me-2′$_{ax}$), 1.45–1.70 (m, 3H, H-4′ and H-5′$_{eq}$), 1.82–2.05 (m, 1H, H-5′$_{ax}$), 2.15–2.40 (m, 1H, H-6′), 3.05 (d, 1H, *J* = 17.8, H-3$_b$), 3.64 (d, 1H, *J* = 17.8, H-3$_a$), 3.69 (br s, 1H, H-3′), 4.85–5.70 (br s, 2H, OH and CO_2H), 6.72 (d, 1H, *J* = 8.2, H-7), 7.86 (d, 1H, *J* = 2.4, H-4) and 7.87 (dd, 1H, *J* = 2.4 and 8.2, H-6). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.88 (Me-6′), 20.42 (Me-2′$_{eq}$), 22.33 (Me-2′$_{ax}$), 25.89 (C-5′), 28.81 (C-4′), 30.97 (C-3), 35.87 (C-6′), 42.59 (C-2′), 77.17 (C-3′), 97.11 (C-2), 107.70 (C-7), 120.82 (C-5), 126.73 (C-4), 129.16 (C-3a), 131.53 (C-6), 164.66 (C-7a) and 171.67 (CO_2H-5). HRMS (CI)—found: 291.15980 (M^++1); $C_{17}H_{23}O_4$ requires 291.15964. Compound **12**—mp: 238–240 °C; $[\alpha]_D^{25} = -44.1$ (*c* 0.8, acetone); IR (KBr) *v* 3600–3000, 3446, 2924, 1680, 1602, 1473, 1259, 989, 916 and 748 cm^{-1}. ^1H NMR (acetone-d_6, 200 MHz): δ 0.80 (d, 3H, *J*= 6.4, Me-6′), 1.01 (s, 3H, Me-2′$_{eq}$), 1.18 (s, 3H, Me-2′$_{ax}$), 1.40–1.70 (m, 3H, H-4′ and H-5′$_{eq}$), 1.85–2.15 (m, 1H, H-5′$_{ax}$), 2.23–2.48 (m, 1H, H-6′), 3.22 (d, 1H, *J* = 18.0, H-3$_b$), 3.60–4.60 (br s, 2H, OH and CO_2H), 3.69 (d, 1H, *J* = 2.8, H-3′), 3.91 (d, 1H, *J* = 18.0, H-3$_a$), 7.76 (s, 1H, H-4) and 7.93 (s, 1H, H-6). ^{13}C NMR (acetone-d_6, 50 MHz): δ 15.90 (Me-6′), 21.93 (Me-2′$_{eq}$), 23.48 (Me-2′$_{ax}$), 27.50 (C-5′), 30.38 (C-4′), 33.46 (C-3), 37.37 (C-6′), 44.17 (C-2′), 77.65 (C-3′), 100.11 (C-2), 101.73 (C-7), 125.41 (C-5), 126.76 (C-4), 132.67 (C-3a), 134.44 (C-6), 162.71 (C-7a) and 167.23 (CO_2H-5). HRMS (CI)- found: 369.06996 (M^++1); $C_{17}H_{22}BrO_4$ requires 369–07015. Compound **14**—mp: 80–82 °C; $[\alpha]_D^{25} = -2.2$ (*c* 1.39, $CHCl_3$); IR (KBr) *v* 3500–2600, 2924, 2854, 1703, 1695, 1613, 1464, 1386, 1215, 1194, 1098 and 990 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.79 (d, 3H, *J* = 6.5, Me-6′), 1.02 (s, 3H, Me-2′$_{eq}$), 1.20 (s, 3H, Me-2′$_{ax}$), 1.50–165 (m, 3H, H-4′ and H-5′$_{eq}$), 1.85–2.05 (m, 1H, H-5′$_{ax}$), 2.10–3.30 (br s, 2H, OH and CO_2H), 2.23–2.48 (m, 1H, H-6′), 3.11 (d, 1H, *J* = 18.0, H-3$_b$), 3.74 (d, 1H, *J* = 18.0, H-3$_a$), 3.73 (br t, 1H, *J* = 2.8, H-3′), 7.97 (d, 1H, *J* = 1.3, H-6) and 8.11 (d, 1H, *J* = 1.3, H-4). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.71 (Me-6′), 20.42 (Me-2′$_{eq}$), 22.16 (Me-2′$_{ax}$), 25.82 (C-5′), 28.66 (C-4′), 31.66 (C-3), 35.51 (C-6′), 42.61 (C-2′), 76.90 (C-3′), 92.30 (C-2),100.95 (C-7), 115.00 (C-5), 122.03 (CN-7), 130.20 (C-4), 131.45 (C-3a), 134.24 (C-6), 165.80 (C-7a) and 169.57 (CO_2H-5). HRMS (CI)—found: 316.15466 (M^++1); $C_{18}H_{22}NO_4$ requires 315.15488. Compound **18**— $[\alpha]_D^{25} = -23.5$ (*c* 0.99, $CHCl_3$); IR (film) *v* 3394, 3273, 2928, 2850, 1496, 1244, 1190, 993 and 806 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.81 (s, 3H, *J* = 6.5, Me-6′), 1.02 (s, 3H, Me-2′$_{eq}$), 1.12 (s, 3H, Me-2′$_{ax}$), 1.40–1.70 (m, 3H, H-4′ and H-5′$_{eq}$), 1.70–2.40 (br s, 2H, 2× OH), 1.85–2.07 (m, 1H, H-5′$_{ax}$), 2.10–2.35 (m, 1H, H-6′), 2.98 (d, 1H, *J* = 17.4, H-3$_b$), 3.54 (d, 1H, *J* = 17.4, H-3$_a$), 3.69 (br t, 1H, *J* = 2.8, H-3′), 6.52 (s, 2H, H-3 and H-4) and 6.61 (s, 1H, H-7). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.99 (Me-6′), 20.38 (Me-2′$_{eq}$), 22.40 (Me-2′$_{ax}$), 25.94 (C-5′), 28.74 (C-4′), 32.06 (C-3), 35.98 (C-6′), 42.57 (C-2′), 77.44(C-3′), 94.65 (C-2), 107.62 (C-7), 111.67 (C-4), 113.67 (C-6), 129.34 (C-3a), 148.91 (C-5) and 154.05 (C-7a). HRMS (CI)—found: 263.16496 (M^++1); $C_{16}H_{23}O_3$ requires 263.16472. Compound **23**— $[\alpha]_D^{25} = -19.8$ (*c* 0.54, $CHCl_3$); IR (film) *v* 3450, 2954, 2930, 2875, 2863, 1641, 1592, 1463, 1391, 1204, 1184, 1032, 943, 864, 776 and 604 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ 0.86 (d, 3H, *J* = 6.6, Me-6′), 0.97 (s, 3H, Me$_{eq}$-2′), 1.25 (s, 3H, Me$_{ax}$-2′), 1.41–1.75 (m, 3H, H-4′ and H$_{eq}$-5′), 1.88–2.05 (m, 2H, OH-3′ and H − 5′$_{ax}$), 2.23–2.41 (m, 1H, H-6′), 2.68 (s, 3H, *Me*CO-4), 3.29 (d, 1H, *J* = 17.8, H-3$_b$), 3.70 (t, 1H, *J* = 2.9, H-3′), 3.82 (d, 1H, *J* = 17.8, H-3$_a$), 6.77 (d, 1H, *J* = 8.8, H-7), 6.94 (d, 1H, *J* = 8.8, H-6) and 12.09 (s, 1H, OH-5). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.86 (Me-6′), 20.34 (Me$_e$-2′$_{eq}$), 21.07 (MeCO-4), 21.94 (Me-2′$_{ax}$), 25.65 (C-5′), 26.11 (C-4′), 35.24 (C-3), 35.64 (C-6′), 41.66 (C-2′), 79.08 (C-3′), 93.38 (C-2), 116.78 (C-7), 117.01 (C-6), 117.42 (C-4), 126.38 (C-3a), 152.90 (C-7a), 156.89 (C-5) and 203.49 (MeCO-4). HRMS (CI)—found: 305.17552 (M^++1); $C_{18}H_{25}O_4$ requires 305.17529.
13. Weisman, H. F.; Batow, T.; Leppo, M. K.; Marsh, H. C.; Carson, G. R.; Concino, M. F.; Boyle, M. P.; Roux, K. H.; Weisfeldt, M. L.; Fearon, D. T. *Science* **1990**, *249*, 146.
14. A pool of fresh human sera was diluted 1/50 and the complement was titrated against a 0.85% suspension of sensitized sheep erythrocytes (SRBCs). The serum was further diluted with diluted Mayer buffer (DMB) according to its titer. Compounds were tested as their sodium salts. Accurately weighed 20 mg of each test compound was dissolved in DMSO (120 μL) to which 1.0 equivalent of 2 N NaOH was added, and the resulting solution was diluted to 10 mL with DMB giving a final concentration of 2.0 mg/mL. Then, appropriate volumes of the test compound solution (0.1, 0.2, 0.3, 0.4, and 0.5 mL) were added into 5-mL test-tubes, followed by DMB containing 1.2% DMSO to a final volume of 1.0 mL. This was followed by addition of 0.5 mL of diluted human complement and, after 20 min at 37 °C, 0.5 mL of the SRBCs suspension. The tubes were incubated at 37 °C for additional 30 min. After incubation,

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% lysis and vehicle controls (blank) were run concomitantly. Response data for complement inhibition were calculated according to the following formula: Hemolysis (Y, %) = $100*(A_{test} - A_{blank})/(A_{100} - A_{blank})$, where A_{test}, A_{100}, and A_{blank} are the absorbances of the test sample, the 100% hemolysis control, and the blank, respectively. DMSO did not affect complement activity at the final assay concentrations of 1.2%. Percent of hemolysis was plotted (Origin 6.0 software) against concentration and IC_{50} values (concentration of test compound inhibiting 50% of the hemolysis) were obtained by interpolation. Graphs were linearized by plotting log [Y/(100 − Y)] vs. log [conc].