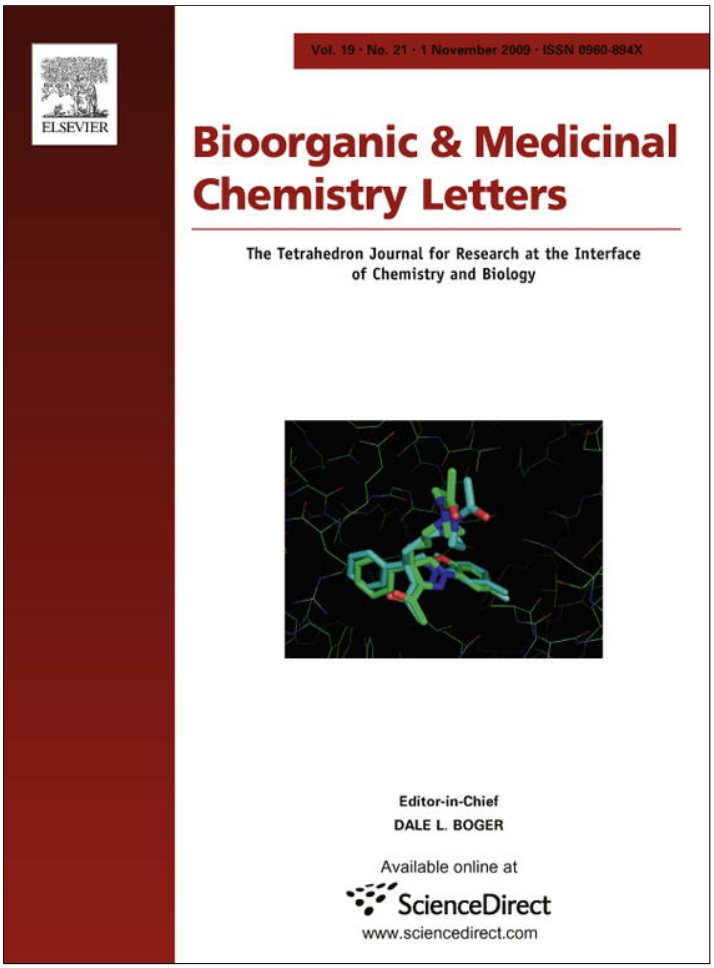

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New inhibitors of the complement system inspired in K76-COOH. A SAR study of filifolinol derivatives through modifications of the C3′ position

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ABSTRACT

A new series of tricyclic carboxylic acids with a 3*H*-spiro[benzofuran-2,10-cyclohexane] skeleton were synthesized from filifolinol, as analogs of the natural complement inhibitor K76-COOH. Their complement inhibitory activity was determined aiming to probe the importance of structural characteristics of the alicyclic part of K76-COOH. The presence and stereochemistry of *O*- and *N*-functionalities on C3′ of the filifolinol derivatives are relevant for biological activity. The IC_{50} values of the most potent compounds were comparable or surpassed the activity of K76-COOH. The results also suggest that the diol moiety of the natural product may be useful for improving compound solubility.

The complement system is a phylogenetically conserved element of the innate immune system, which plays a critical role in host-defense response against infection and in the modulation of antigen-specific immune and inflammatory responses.[1]

Controlled complement activation is fundamental for a proper response. On the contrary, the autologous activation of this system may result in significant tissue damage with devastating effects, including xenograft rejection, necrosis of infarcted heart tissue, brain damage and autoimmune tissular lesions.[2] These unwanted, often life-threatening effects, can be ameliorated by complement inhibition.[3]

Various aspects regarding therapeutic complement inhibition, the most promising complement inhibitors and their structural requirements have been repeatedly reviewed,[4] reflecting that the complement system remains a highly active area for drug discovery and lead optimization. In recent years, the growing understanding of the role of complement in the pathogenesis of various diseases has increased considerably the interest on the use of complement inhibitors. The search for these substances has lead to the discovery and development of monoclonal antibodies, recombinant complement regulatory proteins, small peptides and others.[5]

However, low molecular weight complement inhibitors have been proposed as inexpensive, orally and topically active, more tissue-penetrating and patient-preferred alternatives to their labile proteic counterparts. Such advantages become more important when the drug must be administered over a long period of time, such as during the management of chronic autoimmune disorders.[6]

Several non-peptidic small molecules proved to inhibit activation of the complement system (Fig. 1); among them, FUT-175 (nafamostat, **1**),[7a,b] the failed synthetic complement inhibitor BCX 1470 (**2**)[7c] and the unique pyrazolo[3,4-*d*]pyridazine **3**.[7d]

In addition, derivatives of orlistat (**4**),[8a] epigallocatechin (**5**),[8b] as well as carboxylic acids **6**, **7**[8c] and **8**,[8d] amino acid-related carbamoylphosphinic acids (**9**),[9a] polysubstituted bisphenol A sulfates (**10**)[9b] and terpenoids, including betulinic acid sulfate (**11**),[9c] oleanolic acid (**12**)[9d–f] and disodium disuccinate astaxanthin (cardax, **13**),[9g] have exhibited anticomplementary activity.

K76-COOH (MX-1, **14b**), a partially oxidized derivative of the natural terpenoid K-76 (**14a**), which inhibits production of C5a is one of the most widely used experimental[10] low molecular weight complement inhibitors. Its simplified analogs (**15a–d**)[11] were also shown to be anticomplementary (Fig. 2).

Compounds **6**–**15** bear polar carboxylic, sulfonic or phosphinic acid features, associated to a relatively low-polarity backbone, while compounds **1–5** also display regions of different polarity, exhibiting amido, amino, amidino or phenolic groups in their polar heads.

In continuation of our previous efforts on the synthesis of K-76-COOH analogs from filifolinol (**16**)[12] and with the aim of acquiring a better understanding of the importance of the 2,3-diol motif of

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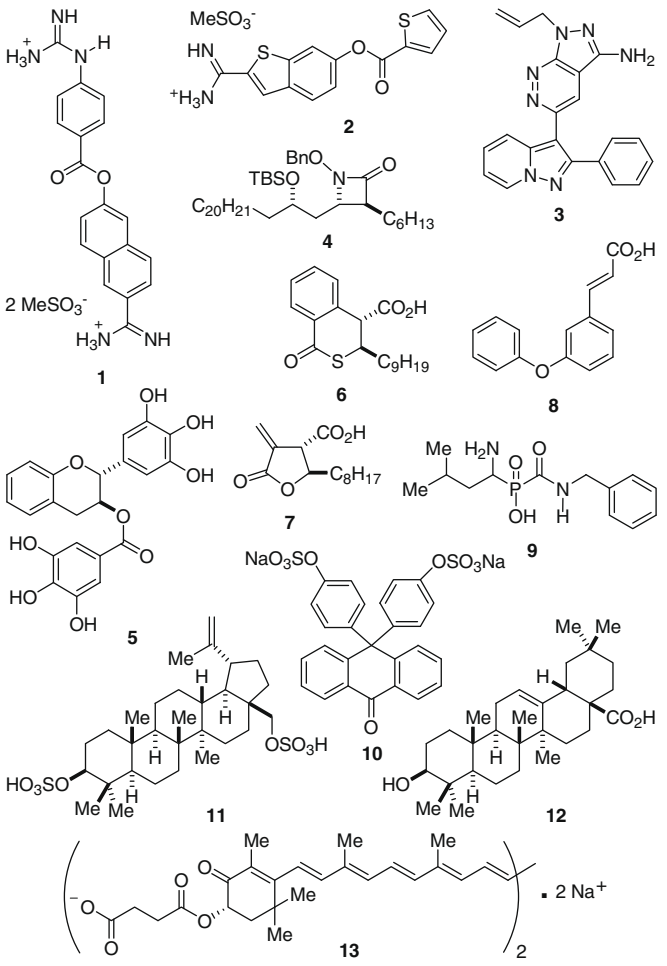


Figure 1. Chemical structures of selected natural, semi-synthetic and synthetic low molecular weight non-peptide complement inhibitors.

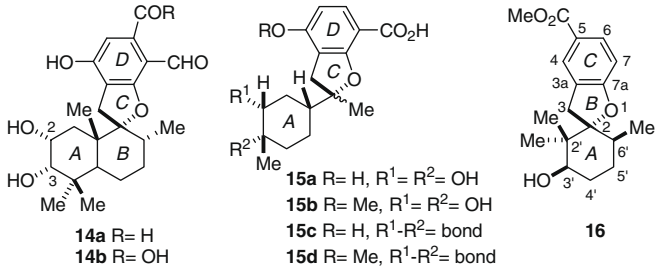


Figure 2. Chemical structures of K76 (**14a**), K76-COOH (**14b**), ACD-ring analogs of K76-COOH (**15a**–**d**) and filifolinol (**16**).

14b herein we report the synthesis of a series of filifolinol derivatives chemically modified at C3′, which can be regarded as BCD ring analogs of K76-COOH, and results of their ability to inhibit the complement. The synthetic analogs also allowed to evaluate the relevance of the nature and stereochemistry of the C3′ substituent of filifolinol derivatives on their complement inhibitory activity.

Filifolinol (**16**) is a terpenoid recently isolated from *Heliotropium filifolium* (Miers) Reiche and *Heliotropium taltalense* (Phil.) Johnst (Heliotropiaceae).[13] The natural product, which bears a 3*H*-spiro[benzofuran-2,10-cyclohexane] skeleton, has demonstrated to possess antiviral and antibacterial activity.[13b] Its structural resemblance to the BCD ring system of K76-COOH makes it attractive as starting material for the elaboration of analogs of the latter.

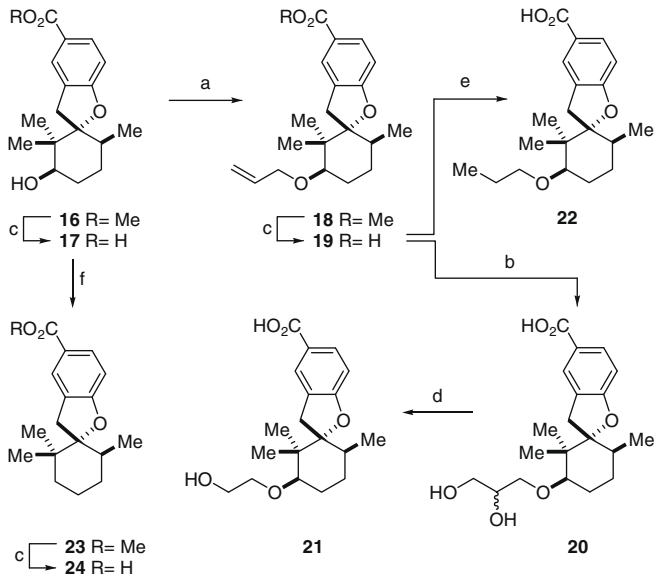
In order to prepare the proposed analogs, with different side chains attached to the C3′-hydroxyl group, filifolinol (**16**) was subjected to an O-allylation, followed by saponification (Scheme 1), and the resulting product **19** was osmylated to afford a mixture of unseparable diols **20**. The latter was further oxidatively cleavaged with potassium *m*-periodate in acetone,[14a] yielding the ethyleneglycol monoether derivative **21** after a reductive step with $NaBH_4$.

For the sake of comparison, the allyl ether **19** was catalytically hydrogenated to **22**. In addition, filifolinol was deoxygenated to **23** through its conversion into the inverted chloride with the $SOCl_2$/DMF reagent system, which underwent in situ hydrogen chloride elimination, followed by catalytic hydrogenation of the resulting cyclohexene derivative. The corresponding acids **17**, **19** and **24** were accessed by basic hydrolysis.

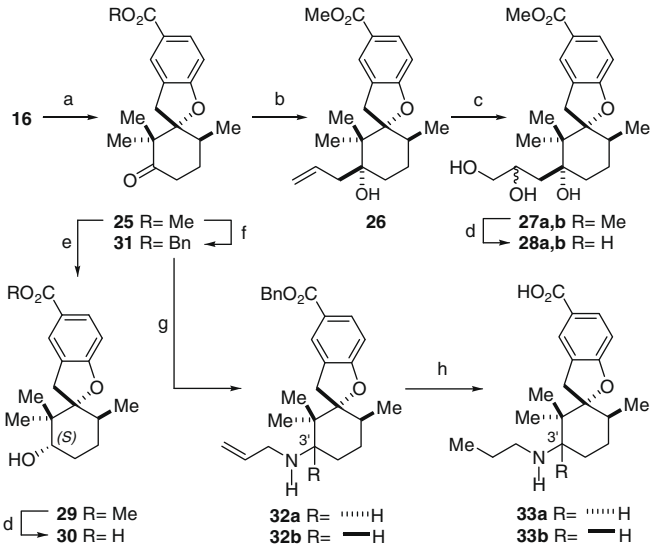
In a second sequence (Scheme 2), filifolinol was oxidized to filifolinone (**25**)[13b] under Swern conditions or with PCC/Al_2O_3 and the ketone was reacted with allylmagnesium bromide, furnishing alcohol **26** as a sole diastereomer (3′*R*). Catalytic dihydroxylation of **26** gave 87% of a 1.5:1 separable mixture of diols **27a** and **27b**. On the other hand, $NaBH_4$-mediated reduction of filifolinone provided 60% of 3′-*epi*-filifolinol (**29**), accompanied by 35% of **16**, which could be recycled. Base-catalyzed transesterification of **25** to benzyl ester **31**, followed by a reductive amination step with allylamine,[14b] furnished a separable mixture of diastereoisomeric amines **32a** (**3′***R*) and **32b** (**3′***S*).

Catalytic hydrogenation of **32a** and **32b** conveniently provided *N*-propylamino acids **33a** (**3′***R*) and **33b** (**3′***S*), respectively, while acids **28a**, **28b** and **30** were obtained by saponification of their corresponding methyl esters **27a**, **27b** and **29**.[15]

The ability of the tricyclic acids to inhibit the complement was tested, employing a modification of the method of Weisman and co-workers.[11f,12,16] with the results collected in the Table 1.[17] Addition of DMSO to a final concentration of 1.2% v/v was necessary in order to improve the solubility of the tested compounds.



Scheme 1. Reagents and conditions: (a) NaH, $BrCH_2CH{=}CH_2$, THF-DMF, rt, 25 h (89% **18**/**19**∼1); (b) OsO_4 (cat.), $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH–H_2O, rt, 21 h (78%); (c) NaOH, dioxane, 60 °C (**17**, 85%; **19**, 59%; **24**, 99%); (d) (1) KIO_4, acetone, rt, 2.5 h, (2) $NaBH_4$, *i*-PrOH, rt, 20 h (82%); (e) H_2 (1 atm.), 10% Pd/C, EtOH, rt, 5 h (98%); f) (1) $SOCl_2$, DMF (cat.), $CHCl_3$, reflux, 1.1 h (74%), (2) H_2 (1 atm.), 10% Pd/C, MeOH, rt, 2.4 h (99%).



Scheme 2. Reagents and conditions: (a) (1) Me_2SO, TFAA, CH_2Cl_2, −60 °C, 2. Et_3N, 0 °C to rt, 35 min (90%) or PCC/Al_2O_3, CH_2Cl_2, rt, 23 h (95%); (b) $BrMgCH_2CH{=}CH_2$, Et_2O, 0 °C, 2.5 h (62%); (c) OsO_4 (cat.), $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH–H_2O, rt, 23 h (87%, **27a**/**27b**∼1.5); (d) NaOH, dioxane, 60 °C, 18–22 h (**28a**, 92%; **28b**, 90%; **30**, 85%); (e) $NaBH_4$, EtOH, 0 °C, 3 h (**29**, 60%; **16**, 35%); (f) BnOH, NaH (cat.), PhMe, 70 °C, 2.5 h (65%); (g) (1) $H_2NCH_2CH{=}CH_2$, $AcOH_{(glacial)}$, $[(H_3NCH_2CH{=}CH_2)^+{\cdot}AcO^-]$, $MgSO_4$, 4 Å MS, EtOH, reflux, 19 h, (2) $NaCNBH_3$, EtOH, rt, 22 h (74%, **32a**/**32b**∼3); (h) H_2 (1 atm.), 10% Pd/C, MeOH, rt, 4 h (90%).

Table 1
Results of the complement inhibition assay for compounds **14b**, **17**, **19**, **20**, **21**, **22**, **24**, **30**, **28a**, **28b**, **33a** and **33b**. Comparison with data of **15b–d** and **16**

Compd no.	Complement inhibition[a]	
	IC_{50} (μM)	IC_{50} (μg/mL)
17	517	150
19	3075	1010
20	4720	1720
21	4396	1470
22	548	190
24		Hemolytic
15b[11f]	1600	540
15c[11c]	2150	490
30	12,140	3520
28a	480	175
28b	3287	1197
33a	211	70
33b	2746	910
14b	570	238
15d[11f]	680	210
16[12]	2000	580

[a] Calculated according to the following formulae: 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. IC_{50} values (Y = 50) were obtained from the linear graphs prepared by plotting log [Y/(100 − Y)] versus log [conc].

Under these conditions, DMSO did not affect complement activity and no hemolysis was observed in the control samples.

It was observed that blocking the C3′ carbinol moiety with a three-carbon chain (**19**, **22**) did not improve the complement inhibitory activity; moreover, introduction of alcohol/diol functionalities in the blocking chain (**20**, **21**) surprisingly lead to less active compounds. However, the activity of the parent compound was restored in **28a**, where the hydroxyl groups may be superimposed with the diol moiety of K76-COOH. On the other side, removal of the C3′ hydroxyl group furnished the hemolytic analog **24**; however, compounds with alcohol functionalities on C3′ or its associated side chain where more soluble than **24**. In the overall, this suggested a minor role of the diol present in K76-COOH (**14b**), being relevant to compound solubility.

For the sake of comparison, the complement inhibitory characteristics of tricyclic analogs **15b–d**[11c,11f] are shown in the Table 1. These also suggest that the diol moiety of K76-COOH has little influence on the inhibitory activity of these compounds.

In addition, it was observed that the stereochemistry of the C3′ functionality was relevant to activity, the acid **17** being near 24 times more active than acid **30**, its C3′ epimer with the 3′*S* configuration; the same trend was observed among the nitrogen-containing analogs **33a** and **33b**, where the 3′*R* isomer (**33a**) was approximately 13 times more potent than its 3′*S* congener **33b**. Previous work also suggested the importance of certain stereochemical features for the biological activity of K76-COOH analogs.[11f]

In conclusion, chemical modifications at the level of the C3′ substituent of the naturally-occurring filifolinol provided new simplified analogs of K76-COOH. These analogs retained complement inhibitory activity; in addition, it has been evidenced that the analog bearing a (3′*R*)-propylamino group exhibited increased bioactivity.

While additional evidence may be required in order to better define and understand the structural characteristics of acceptable complement inhibitors based on K76-COOH, the current results further reinforce the idea that there seems to be a need of a slightly polar function associated to the alicyclic moiety in order to suppress unwanted hemolytic activity and to provide better solubility.

Acknowledgments

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15. *Synthesis of* ***33a*** *from filifolinol:* a stirred solution of filifolinol (**16**, 216 mg, 0.711 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with PCC/Al_2O_3 (1.90 g) at room temperature and the resulting suspension was stirred for 19 h; then, the slurry was filtered through a short column of Florisil and the adsorbent was washed with CH_2Cl_2 (5×10 mL). The filtrates were concentrated under reduced pressure and the residue was chromatographed, affording **25** (204 mg, 95%), as a solid mp 95–97 °C (hexane/AcOEt) (lit.[13b] 95–98 °C). Without further purification, NaH (3.4 mg, 0.07 mmol) was added to a solution of benzyl alcohol (0.45 mL) in anhydrous toluene (7 mL) at room temperature, under an argon atmosphere. Once gas evolution ceased, a solution of **25** (200 mg, 0.662 mmol) toluene (1.0 mL) was dropwise added and the resulting yellowish solution was heated at 70 °C for 2.5 h. Then, the reaction was cooled to room temperature and the volatiles were removed under reduced pressure, leaving an oily residue, which was chromatographed to afford benzyl ester **31** (164 mg, 65%), as a colourless oil. $[\alpha]_D^{25}$ −20.5 (*c* 1.04, $CHCl_3$); IR (film, *v*) 2942, 1705, 1610, 1490, 1333, 1231, 1165, 1089, 990, 769 and 698 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.89 (d, 3H, *J* = 6.5 Hz), 0.93 (s, 3H), 1.19–1.34 (m, 1H), 1.38 (s, H), 1.86–1.94 (m, 1H), 2.24–2.31 (m, 1H), 2.68–2.71 (m, 2H), 2.79 (d, 1H, *J* = 16.6 Hz), 5.31 (s, 2H), 6.76 (d, 1H, *J* = 8.4 Hz), 7.25–7.44 (m, 5H) 7.82 (d, 1H, *J* = 1.8 Hz) and 7.90 (dd, 1H, *J* = 1.8 and 8.4 Hz); ^{13}C NMR ($CDCl_3$, 75.48 MHz): δ 14.4, 17.4, 22.1, 27.2, 30.4, 34.3, 36.7, 42.6, 55.3, 66.4, 96.2, 108.2, 122.5, 126.6, 127.2, 128.1, 128.6, 131.5, 136.3, 163.9, 166.2 and 212.5. GC-MS (EI) *m/z* (rel. int.): 378 [M^+] (2), 292 (13) and 91 (100). Next, allylamine (0.18 mL, 2.37 mmol), AcOH (66 μL, 1.64 mmol), and allylamine acetate (506 mg, 4.32 mmol), were successively added to a mixture of ester **31** (164 mg, 0.432 mmol), $MgSO_4$ (199 mg) and activated powdered 4 Å molecular sieves (199 mg) in absolute EtOH (6.5 mL), and the reaction was refluxed 19 h. After cooling to rt, $NaCNBH_3$ (35.3 mg, 0.56 mmol) was added and the system was stirred at rt for 22 h. Then, the reaction was concentrated to dryness and diluted with H_2O (8 mL) and 1 M NaOH until pH 12. The aqueous suspension was extracted with Et_2O (5×20 mL) and the combined organic phases were dried with $MgSO_4$, concentrated under reduced pressure and the residue was chromatographed, furnishing amine **32a** (100 mg, 55%) as a colourless oil. $[\alpha]_D^{25}$ −12.6 (*c* 1.08, $CHCl_3$); IR (film, *v*) 3300, 2934, 2875, 1712, 1612, 1487, 1335, 1275, 1165, 1086, 991 and 768 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.79 (d, 3H, *J* = 6.8 Hz), 0.95 (s, 3H), 1.15 (s, 3H), 1.38–1.46 (m, 2H), 1.51–1.59 (m, 2H), 1.81–1.91 (m, 1H), 2.19–2.28 (m, 1H), 2.52 (t, 1H, *J* = 3.3 Hz), 2.96 (d, 1H, *J* = 17.2 Hz), 3.08 (dd, 1H, *J* = 6.2 and 14.1 Hz), 3.32 (dd, 1H, *J* = 6.2 and 14.1 Hz), 3.63 (d, 1H, *J* = 17.2 Hz), 5.08 (ddd, 1H, *J* = 1.6, 3.3 and 10.4 Hz, CH=CH_{2cis}), 5.17 (ddd, 1H, *J* = 1.6, 4.9 and 17.1 Hz, CH=CH_{2trans}), 5.31 (s, 2H), 5.89 (dddd, 1H, *J* = 3.3, 4.9, 10.4 and 17.1, CH=CH_2), 6.69 (d, 1H, *J* = 8.2 Hz), 7.32–7.44 (m, 5H) 7.82 (d, 1H, *J* = 1.8 Hz) and 7.86 (dd, 1H, *J* = 1.8 and 8.2 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 15.3, 20.4, 23.4, 24.1, 26.4, 31.6, 36.4, 42.5, 51.8, 63.5, 66.2, 97.7, 107.9, 116.2, 121.7, 126.4, 128.1, 128.5, 128.7, 131.0, 136.5, 136.9, 164.0 and 166.5. GC–MS (EI) *m/z* (rel. int.) 419 [M^+] (6), 362 (13), 280 (29), 96 (100), 91 (93) and 83 (52). Increasing solvent polarity afforded amine **32b** (34 mg, 19%), as an oil. $[\alpha]_D^{25}$ −5.12 (*c* 1.03, $CHCl_3$); IR (film, *v*) 3350, 2927, 2875, 1708, 1611, 1489, 1333, 1275, 1166, 1088, 968 and 768 cm^{-1}; ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.72 (d, 3H, *J* = 6.5 Hz), 1.01 (s, 3H), 1.11 (dt, 1H, *J* = 4.6, 12.5 Hz), 1.21 (s, 3H), 1.59–1.74 (m, 2H), 1.82–1.90 (m, 1H), 2.01 (br s, $w_{1/2}$ = 9.2 Hz, 1H), 2.19–2.29 (m, 1H), 2.52 (dd, 1H, *J* = 4.6 and 11.4 Hz), 2.94 (d, 1H, *J* = 16.8 Hz), 3.00 (d, 1H, *J* = 16.8 Hz), 3.21 (dd, 1H, *J* = 8.1 and 14.2 Hz), 3.66 (dd, 1H, *J* = 5.1 and 14.2 Hz), 5.26–5.24 (m, 2H), 5.31 (s, 2H), 5.89–6.02 (m, 1H), 6.74 (d, 1H, *J* = 8.5 Hz), 7.31–7.44 (m, 5H), 7.83 (d, 1H, *J* = 1.5 Hz) and 7.89 (dd, 1H, *J* = 1.5 and 8.5 Hz); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 14.6, 16.0, 20.5, 25.4, 28.0, 30.1, 34.7, 43.4, 49.8, 60.7, 66.3, 96.7, 108.1, 120.1, 122.1, 126.5, 127.4, 128.1, 128.6, 131.4, 133.0, 136.4, 164.2 and 166.3. Without further purification, 10% Pd/C (3.2 mg) was added to a solution of allylamine **32a** (33 mg, 0.078 mmol) in anhydrous MeOH (2.5 mL) and the reaction was hydrogenated at 1 atm. The solids were filtered through a short pad of Celite, and the pad was washed with small portions of MeOH. Removal of the solvent gave **33a** (24 mg, 90%). $[\alpha]_D^{25}$ −4.0 (*c* 1.05, MeOH); IR (film, *v*) 3415, 2964, 2945, 1712, 1693, 1643, 1601, 1566, 1444, 1371, 1269, 1114, 1045, 922 and 731 cm^{-1}; ^1H NMR (acetone-d_6, 300.13 MHz): δ 0.91 (d, 3H, *J* = 7.1 Hz), 0.93 (t, 3H, *J* = 7.2 Hz), 1.13 (s, 3H), 1.19 (s, 3H), 1.63–1.73 (m, 4H), 1.81–1.86 (m, 2H), 1.91–2.02 (m, 2H), 2.12–2.18 (m, 1H), 2.68–2.78 (m, 1H), 2.89–2.97 (m, 2H), 3.12 (d, 1H, *J* = 16.8 Hz), 2.95–3.38 (bm, 1H), 3.59 (d, 1H, *J* = 16.8 Hz), 6.71 (d, 1H, *J* = 8.4 Hz), 7.80 (d, 1H, *J* = 8.4 Hz) and 7.82 (br s, $w_{1/2}$ ~3.9 Hz, 1H); ^{13}C NMR (acetone-d_6, 75.48 MHz): δ 11.0, 14.9, 19.0, 22.1, 22.3, 26.0, 32.3, 36.5, 42.3, 43.9, 49.8, 63.9, 97.4, 107.6, 123.8, 126.5, 128.2, 130.8, 163.0 and 172.2. LC–MS (ESI^+) *m/z* (rel. int) 332.2 $[M+H]^+$ (18), 203.0 (7), 189.1 (100) and 176.7 (3).
16. 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.
17. 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 equiv 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, 0.2, 0.3, 0.4, and 0.5 mL of the test compound solution were placed into 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.