Bioorganic & Medicinal Chemistry Letters xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Evaluation of steroidal amines as lipid raft modulators and potential anti-influenza agents

Sameer Agarwal [a,b,†,‡], Cornelia Schroeder [a,c,d,†], Georg Schlechtingen [a,b], Tobias Braxmeier [a,b], Gary Jennings [a], Hans-Joachim Knölker [a,b,*]

[a] JADO Technologies, Tatzberg 47-51, 01307 Dresden, Germany
[b] Department of Chemistry, Technische Universität Dresden, Bergstr. 66, 01069 Dresden, Germany
[c] Max Planck Institute for Molecular Cell Biology and Genetics, 01307 Dresden, Germany
[d] Institute of Anatomy, Technische Universität Dresden, Fiedlerstr. 42, 01307 Dresden, Germany

ARTICLE INFO

Article history:
Received 14 June 2013
Revised 10 July 2013
Accepted 11 July 2013

Keywords:
Antiviral activity
Influenza
Lipid rafts
Raft modulators
Steroidal amines

ABSTRACT

The influenza A virus (IFV) possesses a highly ordered cholesterol-rich lipid envelope. A specific composition and structure of this membrane raft envelope are essential for viral entry into cells and virus budding. Several steroidal amines were investigated for antiviral activity against IFV. Both, a positively charged amino function and the highly hydrophobic ($Clog P \geqslant 5.9$) ring system are required for IC_{50} values in the low μM range. An amino substituent is preferential to an azacyclic A-ring. We showed that these compounds either disrupt or augment membrane rafts and in some cases inactivate the free virus. Some of the compounds also interfere with virus budding. The antiviral selectivity improved in the series 3-amino, 3-aminomethyl, 3-aminoethyl, or by introducing an OH function in the A-ring. Steroidal amines show a new mode of antiviral action in directly targeting the virus envelope and its biological functions.

A large number of deadly human infectious diseases are caused by a variety of viruses, among them the prevalent pandemic or epidemic influenza, AIDS, measles, rota- and norovirus diarrheas, hepatitis A, B and C, polio and rabies, yellow fever, dengue, and newly emerging hemorrhagic fevers. Despite the availability of effective vaccines for some of these diseases, eradication has been achieved only for smallpox. Antiviral drug development is the backup strategy and for many virus infections the only approach to rescue lives and achieve a degree of epidemic control.[1] With a few exceptions, antiviral chemotherapeutics target essential viral enzymes, mostly involved in genome replication or in viral protein processing. The predominating active compounds are substrate analogues, derived from nucleoside or peptide scaffolds. Based on medical need, human immunodeficiency virus (HIV) and hepatitis C virus (HCV) have attracted strong interest of the pharmaceutical industry.[1] Drugs for these targets show several different modes of action, whereas anti-influenza drugs cover a limited mechanistic spectrum.[2] All influenza strains isolated in recent years display resistance to the 1-adamantylamines that block the influenza M2 proton channel, leaving the neuraminidase inhibitors tamiflu (oseltamivir) and relenza (zanamivir) as the only chemotherapeutic options for influenza.[3] Industry is further exploiting this verified drug target, but with increasing resistance to neuraminidase inhibitors, there is an urgency to identify new mechanisms of action.

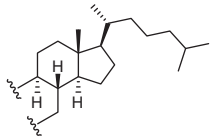
The lipid bilayer of cell membranes can be considered a two-dimensional liquid, the organization of which has been the object of intensive investigation for decades by biochemists and biophysicists.[4] The realization that epithelial cells polarize their cell surfaces into apical and basolateral plasma membranes with different protein and lipid compositions initiated a paradigm change that led to the lipid raft concept.[5] Lipid rafts are dynamic, liquid-ordered assemblies of proteins and lipids that float within the liquid-disordered bilayer of cellular membranes but can also cluster to form larger platforms, for example the envelopes of influenza virus[6] or HIV.[7] Many disease events involve membrane rafts, several of which are significant for allergy, inflammation, cancer, and viral and bacterial infections.

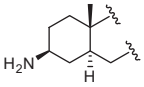
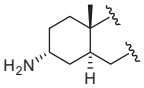
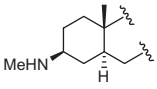
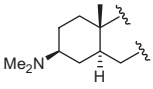
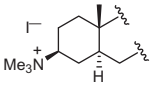
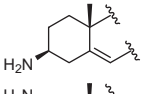
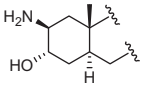
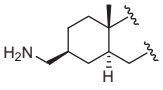
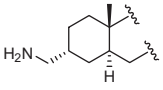
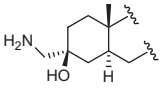
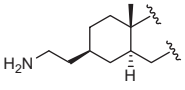
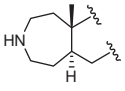
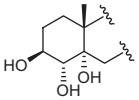
Lipid rafts are essential for membrane sorting and trafficking, cell polarization, and signal transduction processes. Several groups of pathogens, bacteria, prions, viruses, and parasites hijack lipid rafts for their purposes.[8] Cholesterol-enriched lipid rafts play critical roles at early and late stages of the influenza A virus life cycle, that is viral entry and fusion, viral protein trafficking, and assembly and budding of progeny viruses.[9] Lipids have long been known as structural elements of viral and cellular membranes, but recent

* Corresponding author. Fax: +49 351 463 37030.
E-mail address: hans-joachim.knoelker@tu-dresden.de (H.-J. Knölker).
† These authors contributed equally to this Letter.
‡ Present address: Zydus Research Centre, Cadila Healthcare Ltd, Ahmedabad 382210, India.

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.07.015

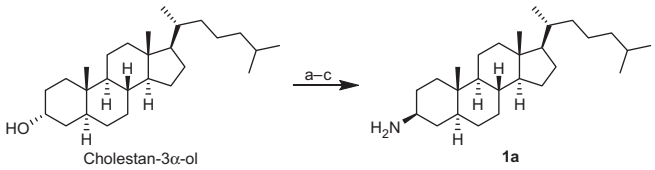
Table 1
Virus replication and functional assay results for the steroids **1–3**[a]



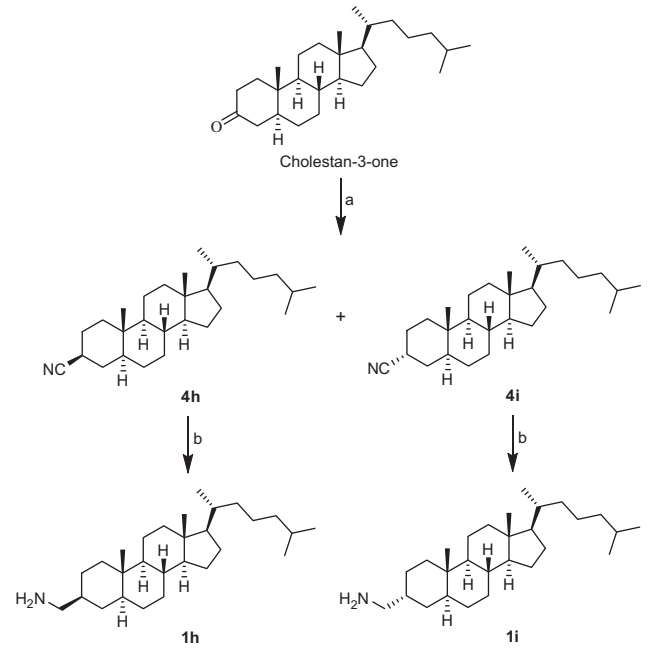
Compound	Steroid	IC_{50} (μM)	IC_{90} (μM)	MTC (μM)	TI	BA (%)	VA (%)	RM_P (%)	RM_T (%)	*C*log*P*
1a		1.7	9.0	15	8.8	51.8	47.8	−42.4	−7.1	10.1
1b		1.9	⩾MTC	15	7.9	79.9	28.5	−72.8	56.3	10.1
1c		3.3	17.5	25	7.6	33.8	0.0	−50.5	−7.0	10.3
1d		3.7	⩾MTC	15	4.1	42.8	0.0	15.5	15.6	10.8
1e		3.5	⩾MTC	12.5	3.6	94.4	16.0	90.3	83.5	5.9
1f		8.6	11.4	25	2.9	50.9	63.4	83.2	78.8	9.6
1g		4.7	62	75	16.0	53.0	75.5	26.5	32.3	8.8
1h		4.0	11.6	37.5	9.4	30.7	90.3	−66.6	−39.3	10.7
1i		3.7	7.7	37.5	10.1	12.7	50.9	−60.3	−39.6	10.7
1j		2.3	21.5	25	10.9	41.0	64.2	−41.5	15.6	9.5
1k		2.1	13.4	50	23.8	65.0	132	−38.2	31.2	11.3
2a		3.3	9.7	12.5	3.8	61.8	44.1	n.d.	n.d.	10.2
2b		5.9	—	15	2.5	20.4	0.0	2.8	36.1	9.7
3		16.1	—	15	0.9	39.4	76.7	−65.2	−36.5	8.2

[a] IC_{50} (IC_{90}): concentration at which 50% (90%) of viral reproduction is inhibited. MTC: maximum tolerated concentration in MDCKII cells; TI: therapeutic index (MTC/IC_{50}); BA: budding assay (solvent control, DMSO: 100%); VA: virucidal assay (solvent control, DMSO: 100%); RM_P: raft modulation assay with perylene as tracer; RM_T: raft modulation assay with a tripartite structure[21] as tracer (the *N*-terminally sterol-linked peptide, Ref. 21a: example 26); lipid composition of raft liposomes [mol %]: cholesterol: 35, sphingomyelin: 10.5, ganglioside M1: 3.5, phosphatidylcholine: 25.5, phosphatidylserine: 25.5; raft modulation: disrafting [%] = $100 \times \{1 - P(\text{tracer})_{\text{steroid}}/P(\text{tracer})_{\text{DMSO}}\}$, raft augmentation [%] = $-100 \times \{1 - P(\text{tracer})_{\text{DMSO}}/P(\text{tracer})_{\text{steroid}}\}$, partition coefficients *P* are approximated by the ratio of tracer concentrations in the membrane and the aqueous phase; *C*log*P*: calculated logarithmic value for the partition coefficient, *P* = conc.(octan-1-ol)/conc.(water); n.d. = not determined.

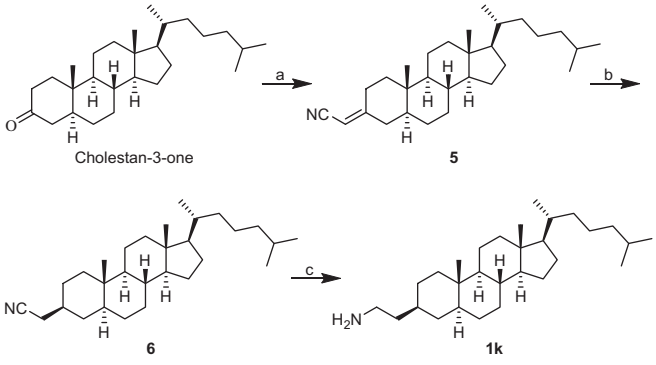
studies revealed additional roles in intricate virus-cell interactions. Understanding the manifold roles of lipids in viral replication also led to the discovery of lipid-active compounds as potential antivirals.[10] Cholesterol is a key component of cell membranes. The enrichment of cholesterol transforms liquid-disordered membranes into liquid-ordered ones (= raft membranes).[5] We proposed that molecular processes underlying infectious diseases and other disorders may be influenced by modulating raft assembly with so-



Scheme 1. Synthesis of cholestan-3β-ylamine (**1a**). Reagents and conditions: (a) MsCl, *i*-Pr_2EtN, DMAP, CH_2Cl_2, rt, 100 min (96%); (b) NaN_3, DMPU, 46 °C, 24 h (97%); (c) $LiAlH_4$, dry Et_2O, reflux, 1 h (87%).



Scheme 2. Synthesis of cholestan-3β-ylmethanamine (**1h**) and cholestan-3α-ylmethanamine (**1i**). Reagents and conditions: (a) TosMIC, KO*t*-Bu, DME–EtOH (10:1), rt, 5 h (**4h**: 50%; **4i**: 35%); (b) $LiAlH_4$, dry THF, rt to reflux, 3 h (**1h**: 68%, **1i**: 90%).



Scheme 3. Synthesis of 2-(cholestan-3β-yl)ethanamine (**1k**). Reagents and conditions: (a) Diethyl cyanomethylphosphonate, dry THF, LiOH, rt, 3 h; (b) Pd/C, H_2, MeOH, rt, 3 h; (c) $LiAlH_4$, dry THF, rt to reflux, 3 h.

called disrafters,[11,12] for example the steroidal amines described herein. Therefore, certain raft modulators may interfere with or even prevent infectious diseases.

Straightforward synthetic chemistry provided a simple access to a variety of steroids (**1**–**3**, Table 1).[12,13] The synthesis of cholestan-3β-ylamine (**1a**) started from cholestan-3α-ol, which was transformed to the corresponding mesylate followed by substitution with azide[14] and subsequent reduction to the amine **1a** by treatment with lithium aluminum hydride (Scheme 1). In a similar way, compounds **1b** and **1f** were obtained from cholestan-3β-ol and cholest-5-en-3α-ol, respectively. Compounds **1c** and **1d** were prepared in one step by reductive amination of commercial cholestan-3-one using either methylamine or dimethylamine.

Compound **1g** was prepared from 2α,3α-epoxycholestane, which is available by a known procedure involving dehydration of cholestan-3β-ol to cholest-2-ene,[15] followed by epoxidation. Epoxide opening with benzylamine and debenzylation with hydrogen and palladium on charcoal provided **1g**. The epimeric methanamines **1h** and **1i** were prepared by converting cholestan-3-one into a mixture of the epimeric 3β-cyanocholestane (**4h**) and 3α-cyanocholestane (**4i**) by reaction with *p*-toluenesulfonylmethyl isocyanide (TosMIC) under van Leusen conditions (Scheme 2).[16] Subsequent chromatographic separation of the epimeric nitriles **4h** and **4i** and reduction with lithium aluminum hydride afforded cholestan-3β-ylmethanamine (**1h**) and cholestan-3α-ylmethanamine (**1i**).

Compound **1j** was prepared from cholestan-3-one by reduction of the corresponding *O*-trimethylsilyl-protected cyanohydrin derivative[17] with lithium aluminum hydride. Horner–Wadsworth–Emmons reaction[18] of cholestan-3-one with diethyl cyanomethylphosphonate provided the acrylonitrile **5** (Scheme 3). Hydrogenation of **5** followed by reduction with lithium aluminum hydride afforded 2-(cholestan-3β-yl)ethanamine (**1k**) as a single isomer.

Conversion of cholestan-3-one to the corresponding oxime followed by a Beckmann-type rearrangement as described by Doorenbos et al.[19] and reduction of the lactam with lithium aluminum hydride afforded **2a**. 4-Azacholestane (**2b**) was prepared from commercial cholest-4-en-3-one. 4,5-Dihydroxylation followed by reduction of the keto group afforded cholestane-3β,4α,5α-triol (**3**). Oxidative cleavage of **3** using lead tetraacetate in methanol, saponification of the resulting methyl ester, condensation to 4-azacholest-5-en-3-one by treatment with ammonia under pressure, hydrogenation of the 5,6-double bond, and reduction of the resulting lactam with lithium aluminum hydride provided **2b**.[20]

For the identification of raft modulators, two raft modulation assays were set up. We used a liposomal assay with raft liposomes incorporating as tracers either perylene, within the bilayer, or a tripartite structure[21] (the *N*-terminally sterol-linked peptide, example 26 in Ref. 21a), inserted in the outer leaflet with the peptide protruding into the medium. The partitioning of these tracers between liposome and buffer under the influence of test compounds was recorded via fluorescence. Partition coefficients with respect to solvent controls were transformed, so that +100% represent total disrafting and −100% maximal raft augmentation. We found that the raft modulating activities of the steroidal amines with respect to the partitioning of the tripartite structure and of perylene (RM_T and RM_P in Table 1) correlate with each other (linear regression: $R^2 = 0.566$, Fig. 1a). This is not typical of all hydrophobic compounds we have tested. Both, the disruption of rafts (positive values) and the augmentation of rafts (negative values) can be detrimental for raft-dependent steps of virus reproduction.

In order to screen the activity of raft modulators towards influenza virus, a virus reproduction and infectivity (focus reduction) assay was employed. Antiviral activity in MDCKII cells (Madin–Darby canine kidney cells) was monitored in the concentration range below the maximum tolerated concentration determined in the LDH (lactate dehydrogenase) release assay. Test compounds were evaluated against the strain influenza A/PR8/34 (H1N1). The focus reduction assay, based on virus endpoint titration, is faster

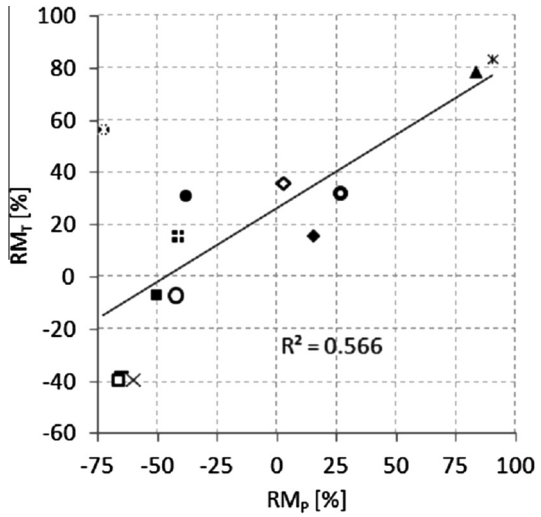


Figure 1a. Correlation of raft modulating activities of the steroids **1**–**3** detected by a tripartite structure (N-terminally sterol-linked peptide)[21a] as tracer with those detected by perylene as tracer. Quantification of raft modulation (RM): disrafting [%] = 100 × {1 − P(tracer)$_{steroid}$/P(tracer)$_{DMSO}$}; raft augmentation [%] = −100 × {1 − P(tracer)$_{DMSO}$/P(tracer)$_{steroid}$}; partition coefficients P are approximated by the ratio of tracer concentrations in the membrane and the aqueous phase.

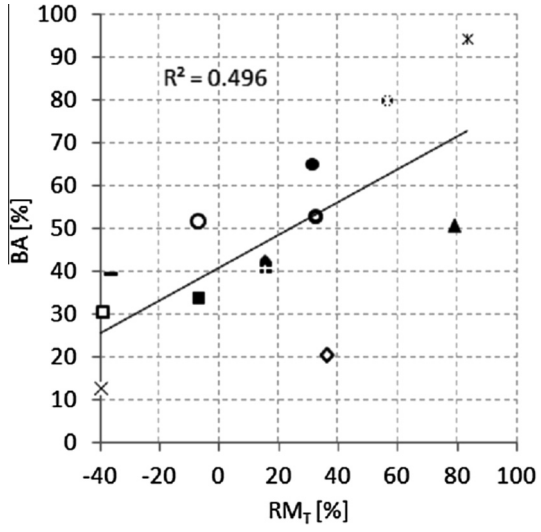


Figure 1b. Correlation of effect on virus budding with raft modulating activity for the steroids **1**–**3**. Legend for Figures 1a and 1b: ○ 1a ⛭ 1b ■ 1c ♦ 1d ⁙ 1e ▲1f ○1g □1h ×1i ✳ 1j ● 1k △ 2a ◇ 2b – 3.

than the traditional plaque reduction assay. The antiviral assay was carried out on microtiter plates and developed as a cell ELISA (enzyme-linked immunosorbent assay) using an antibody to virus nucleoprotein HB65. Cells are pre-incubated for 5 min with serial dilutions of the test compounds and then infected with the serially diluted virus. Potency in the virus reproduction and infectivity assay (characterized by IC_{50} and IC_{90} values, i.e., the concentrations at which 50% or 90% of viral reproduction is inhibited) was evaluated and compared to the maximum tolerated concentration (MTC) determined under the conditions of the focus reduction assay. The ratio of MTC and IC_{50} provides the therapeutic index (TI) as shown in Table 1. Assays were performed at least twice. Where a clear dose-effect relation was seen, we calculated the IC_{50} and IC_{90} values by regression analysis. Zero inhibition is defined as the effect of solvent (DMSO) alone. Complete or 100% inhibition is defined by the absence of virus infected cells (foci).

Our syntheses afforded several potent compounds which show strong inhibitory effects in the virus replication assay with IC_{50} and IC_{90} values in the low micromolar range (Table 1). It appears that the combination of the cholestane scaffold with an amino function either attached to the steroidal A-ring (cholesterylamines and derivatives thereof) or incorporated in the A-ring (azacholestanes or azahomocholestanes) is a structural motif triggering anti-influenza viral activity. In contrast, cholesteryl sulfate did not inhibit viral replication when tested at concentrations of up to 50 μM, the MTC for this compound. Also *O*-3β-cholesterylglycolic acid, which is negatively charged under assay conditions, and cholestan-3-one oxime exerted no inhibition. Furthermore, 4α,5α-dihydroxycholestan-3-one (IC_{50} = 16.0 μM) and cholestane-3β,4α,5α-triol (IC_{50} = 16.1 μM) (compound **3**, Table 1) provided only weak potency. Thus, strong polarity localized at the steroidal A-ring does not suffice for antiviral activity. The presence of an amino function appears to be necessary to obtain a particularly high activity of the compounds. Since *trans*-2-aminomethyl-1-cyclohexanol does not show any inhibitory effect, an amino or aminoalcohol moiety attached to a cyclic hydrocarbon motif is not the sole reason for the anti-influenza activity. Clearly, the cholestane scaffold imparts unique partitioning behavior. These compounds are obviously also distinct from the classical anti-influenza drugs, the cyclic hydrocarbons amantadine (1-adamantylamine) and rimantadine (1-(1-adamantyl)ethanamine), that target the viral M2 proton channel.[22] We conclude that the anti-influenza activity of the steroidal amines depends on their remarkable lipophilicity, combined with an amino function positively charged under physiological conditions. Their bulky cholestane scaffold is too large and hydrophobic to occupy the aqueous lumen of the proton channel like the adamantylamines. A second, intramembrane drug-binding site of the M2 protein has also been discussed.[22]

The antiviral activity of the steroidal amines is likely to be exerted by the compounds incorporated into viral or cellular membranes into which they partition nearly quantitatively. In order to examine effects of compounds partitioned into viral membranes, we set up assays for virucidal activity and the inhibition of virus budding (VA and BA, Table 1). The virucidal activity was assayed on influenza virus, incubated with the steroids **1**–**3** for 30 min and titrated by focus reduction assay.

Virus budding (BA, Table 1) was studied by biotinylation of infected cells 6 h after infection and, after quenching excess biotin, harvesting the virus budded during the next hour. Biotinylated virus particles were captured on streptavidin-coated microtiter plates and detected by ELISA, as described above. Generally, the effects in the functional assays do not correlate with the IC_{50} or IC_{90} values. However, there is a reasonable correlation between raft modulating activity detected with the N-terminally sterol-linked peptide as tracer and the effect in the budding assay (R^2 = 0.496, Fig. 1b). Budding was inhibited by raft augmentation, whereas disrafting had no effect, except for compound **2b**.

Despite the broad structural homogeneity of this group of compounds, several derivatives, distinct from each other by a single CH_2 or CH_3 moiety or by the configuration at C3, exhibit striking differences in one or more assay results. For example, viruses were completely inactivated (virucidal assay) by the secondary and tertiary amines **1c**, **1d**, and **2b**, whereas the quaternary ammonium salt **1e** was somewhat less active. In contrast, compound **2a**, an azacyclic steroid with a seven-membered A-ring but otherwise similar to **2b**, has a much weaker virucidal effect. Based on therapeutic indices two compounds stand out, **1g** and **1k**. Their improved TI results from the lower toxicity of these compounds. The set of functional assays employed does not cover all raft-

dependent steps of early and late virus–host interaction; for example, fusion was not investigated. Nevertheless, they illustrate that raft modulators, by integrating into viral and host membranes, can exert distinct effects on individual steps of virus entry and exit. The magnitude of these effects often depends on small structural variations of the compounds as expected for pharmacologically relevant modes of action.

In conclusion, we have identified a series of compounds as raft modulators showing significant activity towards influenza virus. It was found that steroidal amines are remarkably superior to other steroid derivatives as influenza virus inhibitors. The compounds **1a**–**1k**, **2a**, and **2b** are potential lead structures for the development of antiviral agents. They exhibit good results in the influenza virus replication assay and sufficient solubility. Based on our data, we propose that their effect is based on interference with lipid raft-dependent processes early and/or late in the infectious cycle. Both, virus entry on the one side, and virus maturation and budding on the other, involve interactions of host cell apical plasma membrane raft domains and the virus envelope which is the most ordered (i.e., raft prototypical) biological membrane investigated so far.[6]

Acknowledgments

We are grateful to Beate Michel for expert assistance in the cell and virus experiments, the Friedrich Loeffler Institute Riems for a gift of antibody HB65, and Dr. Margit Gruner for the NMR spectra. We thank Thibaut Lagny for help with the data analysis and Dr. Arndt W. Schmidt for his support in the preparation of the manuscript.

References and notes

1. De Clercq, E. *Biochem. Pharmacol.* **2013**, *85*, 727.
2. (a) Li, Y.; Silamkoti, A.; Kolavi, G.; Mou, L.; Gulati, S.; Air, G. M.; Brouillette, W. *J. Bioorg. Med. Chem.* **2012**, *20*, 4582; (b) Venkatramani, L.; Johnson, E. S.; Kolavi, G.; Air, G. M.; Brouillette, W. J.; Mooers, B. H. *BMC Struct. Biol.* **2012**, *12*, 7; (c) Cheng, T. J.; Weinheimer, S.; Tarbet, E. B.; Jan, J. T.; Cheng, Y. S.; Shie, J. J.; Chen, C. L.; Chen, C. A.; Hsieh, W. C.; Huang, P. W.; Lin, W. H.; Wang, S. Y.; Fang, J. M.; Hu, O. Y.; Wong, C. H. *J. Med. Chem.* **2012**, *55*, 8657; (d) Shie, J. J.; Fang, J. M.; Lai, P. T.; Wen, W. H.; Wang, S. Y.; Cheng, Y. S.; Tsai, K. C.; Yang, A. S.; Wong, C. H. *J. Am. Chem. Soc.* **2011**, *133*, 17959.
3. (a) Govorkova, E. A.; Baranovich, T.; Seiler, P.; Armstrong, J.; Burnham, A.; Guan, Y.; Peiris, M.; Webby, R. J.; Webster, R. G. *Antiviral Res.* **2013**, *98*, 297; (b) Air, G. M. *Influenza Other Respir. Viruses* **2012**, *6*, 245; (c) Baranovich, T.; Webster, R. G.; Govorkova, E. A. *Curr. Opin. Virol.* **2011**, *1*, 574.
4. (a) Glaser, M. *Curr. Opin. Struct. Biol.* **1993**, *3*, 475; (b) Jain, M. K.; White, H. B. *Adv. Lipid Res.* **1977**, *15*, 1; (c) Vaz, W. L.; Almeida, P. F. *Curr. Opin. Struct. Biol.* **1993**, *3*, 482.
5. (a) Simons, K.; Van Meer, G. *Biochemistry* **1988**, *27*, 6197; (b) Simons, K.; Ikonen, E. *Nature* **1997**, *387*, 569; (c) Levental, I.; Grzybek, M.; Simons, K. *Biochemistry* **2010**, *49*, 6305.
6. (a) Gerl, M. J.; Sampaio, J. L.; Urban, S.; Kalvodova, L.; Verbavatz, J. M.; Binnington, B.; Lindemann, D.; Lingwood, C. A.; Shevchenko, A.; Schroeder, C.; Simons, K. *J. Cell Biol.* **2012**, *196*, 213; (b) Scheiffele, P.; Rietveld, A.; Wilk, T.; Simons, K. *J. Biol. Chem.* **1999**, *274*, 2038.
7. (a) Brügger, B.; Glass, B.; Haberkant, P.; Leibrecht, I.; Wieland, F. T.; Kräusslich, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 2641; (b) Chan, R.; Uchil, P. D.; Jin, J.; Shui, G.; Ott, D. E.; Mothes, W.; Wenk, M. R. *J. Virol.* **2008**, *82*, 11228.
8. Simons, K.; Ehehalt, R. *J. Clin. Invest.* **2002**, *110*, 597.
9. Barman, S.; Nayak, D. P. *J. Virol.* **2007**, *81*, 12169.
10. Ono, A.; Freed, E. O. *Adv. Virus Res.* **2005**, *64*, 311.
11. (a) Braxmeier, T.; Friedrichson, T.; Fröhner, W.; Jennings, G.; Schlechtingen, G.; Schroeder, C.; Knölker, H.-J.; Simons, K.; Zerial, M.; Kurzchalia, T. PCT Int. Appl. WO 2006/002907.; (b) Braxmeier, T.; Friedrichson, T.; Fröhner, W.; Jennings, G.; Schlechtingen, G.; Schroeder, C.; Knölker, H.-J.; Simons, K.; Zerial, M.; Kurzchalia, T. PCT Int. Appl. WO 2006/002908.; (c) Braxmeier, T.; Friedrichson, T.; Fröhner, W.; Jennings, G.; Schlechtingen, G.; Schroeder, C.; Knölker, H.-J.; Simons, K.; Zerial, M.; Kurzchalia, T. PCT Int. Appl. WO 2006/002909.; (d) Knölker, H.-J.; Agarwal, S.; Schlechtingen, G.; Braxmeier, T.; Schroeder, C.; Jennings, G. PCT Int. Appl. WO 2009/090063.; (e) Rajendran, L.; Knölker, H.-J.; Simons, K. *Nat. Rev. Drug Discov.* **2010**, *9*, 29.
12. Knölker, H.-J.; Agarwal, S.; Schlechtingen, G.; Braxmeier, T.; Schroeder, C. PCT Int. Appl. WO 2008/068037.
13. Compounds **1a–1f**: (a) Windaus, A.; Adamla, J. *Ber. Dtsch. Chem. Ges.* **1911**, *44*, 3051; (b) Dodgson, D. P.; Haworth, R. D. *J. Chem. Soc.* **1952**, 67; (c) Haworth, R. D.; McKenna, J.; Powell, R. G. *J. Chem. Soc.* **1953**, 1110; (d) Pierce, J. H.; Shoppee, C. W.; Summers, G. H. R. *J. Chem. Soc.* **1955**, 690; (e) Sauers, R. R. *J. Am. Chem. Soc.* **1958**, *80*, 4721; (f) Bird, C. W.; Cookson, R. C. *J. Chem. Soc.* **1960**, 2343; (g) Boutigue, M.-H.; Jacquesy, R. *Bull. Soc. Chim. Fr.* **1973**, 750; (h) Boonyarattanakalin, S.; Martin, S. E.; Dykstra, S. A.; Peterson, B. R. *J. Am. Chem. Soc.* **2004**, *126*, 16379; Compound **1g**: (i) Svoboda, M.; Tichý, M.; Fajkoš, J.; Sicher, J. *Tetrahedron Lett.* **1962**, *3*, 717; (j) Hassner, A.; Heathcock, C. *J. Org. Chem.* **1965**, *30*, 1748; Compound **1i**: (k) Smith, P. B.; McKenna, J. *Tetrahedron* **1933**, *1964*, 20; Compound **2a**: (l) Shoppee, C. W.; Sly, J. C. P. *J. Chem. Soc.* **1958**, 3458; (m) Shoppee, C. W.; Lack, R. E.; Roy, S. K. *J. Chem. Soc.* **1963**, 3767; (n) Takahashi, T. T.; Satoh, J. Y. *J. Chem. Soc., Perkin Trans. 1* **1916**, 1980; (o) Kim, J. C.; Choi, S.-K.; Moon, S.-H. *Arch. Pharm. Res.* **1986**, *9*, 215; Compound **2b**: (p) McKenna, J.; Tulley, A. *J. Chem. Soc.* **1960**, 945; (q) Edward, J. T.; Morand, P. F. *Can. J. Chem.* **1960**, *38*, 1316; (r) Shoppee, C. W.; Killick, R. W.; Krüger, G. *J. Chem. Soc.* **1962**, 2275; (s) Prelog, V.; Tagmann, E. *Helv. Chim. Acta* **1867**, *1944*, 27; (t) Bowers, A.; Denot, E.; Sánchez, M. B.; Sánchez-Hidalgo, L. M.; Ringold, H. J. *J. Am. Chem. Soc.* **1959**, *81*, 5233; (u) Zhao, K.; Wang, Y.; Han, L. *Steroids* **2007**, *72*, 95.
14. Davis, A. P.; Dresen, S.; Lawless, L. J. *Tetrahedron Lett.* **1997**, *38*, 4305.
15. Cruz Silva, M. M.; Riva, S.; Melo, M. L. S. *Tetrahedron* **2005**, *61*, 3065.
16. (a) Oldenziel, O. H.; van Leusen, A. M. *Tetrahedron Lett.* **1973**, *14*, 1357; (b) Oldenziel, O. H.; van Leusen, D.; van Leusen, A. M. *J. Org. Chem.* **1977**, *42*, 3114.
17. Evans, D. A.; Carroll, G. L.; Truesdale, L. K. *J. Org. Chem.* **1974**, *39*, 914.
18. Lattanzi, A.; Orelli, L. R.; Barone, P.; Massa, A.; Iannece, P.; Scettri, A. *Tetrahedron Lett.* **2003**, *44*, 1333.
19. Doorenbos, N. J.; Wu, M. T. *J. Org. Chem.* **1961**, *26*, 2548.
20. (a) Turner, R. B. *J. Am. Chem. Soc.* **1950**, *72*, 579; (b) Doorenbos, N. J.; Huang, C. L.; Tamorria, C. R.; Wu, M. T. *J. Org. Chem.* **1961**, *26*, 2546; (c) Bończa-Tomaszewski, Z. *Tetrahedron Lett.* **1986**, *27*, 3767.
21. (a) Braxmeier, T.; Friedrichson, T.; Fröhner, W.; Jennings, G.; Munick, M.; Schlechtingen, G.; Schroeder, C.; Knölker, H.-J.; Simons, K.; Zerial, M.; Kurzchalia, T. PCT Int. Appl. WO 2005/097199.; (b) Rajendran, L.; Schneider, A.; Schlechtingen, G.; Weidlich, S.; Ries, J.; Braxmeier, T.; Schwille, P.; Schulz, J. B.; Schroeder, C.; Simons, M.; Jennings, G.; Knölker, H.-J.; Simons, K. *Science* **2008**, *320*, 520; (c) Schieb, H.; Weidlich, S.; Schlechtingen, G.; Linning, P.; Jennings, G.; Gruner, M.; Wiltfang, J.; Klafki, H.-W.; Knölker, H.-J. *Chem. Eur. J.* **2010**, *16*, 14412.
22. (a) Hay, A. J.; Zambon, M. C.; Wolstenholme, A. J.; Skehel, J. J.; Smith, M. H. *J. Antimicrob. Chemother.* **1986**, *18*, 19; (b) Pielak, R. M.; Chou, J. J. *Protein Cell* **2010**, *1*, 246.