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

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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

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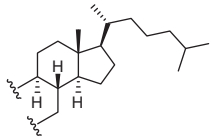
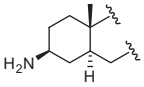
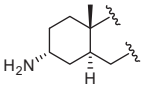
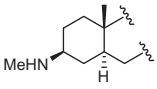
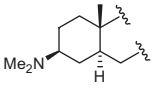
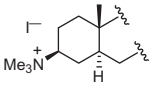
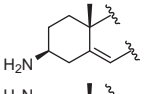
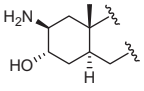
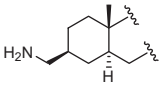
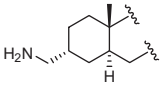
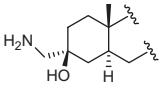
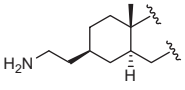
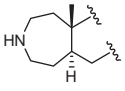
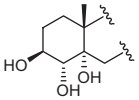
http://dx.doi.org/10.1016/j.bmcl.2013.07.015

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-