Bioorganic & Medicinal Chemistry xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

New quaternary ammonium camphor derivatives and their antiviral activity, genotoxic effects and cytotoxicity

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ARTICLE INFO

Article history:
Received 27 May 2013
Revised 31 July 2013
Accepted 5 August 2013
Available online xxxx

Keywords:
Camphor
Imine derivatives
Antivirals
Influenza
Genotoxicity
Cytotoxicity

ABSTRACT

The synthesis and biological evaluation of a novel series of dimeric camphor derivatives are described. The resulting compounds were studied for their antiviral activity, cyto- and genotoxicity. Compounds **3a** and **3d** in which the quaternary nitrogen atoms are separated by the C5H10 and C9H18 aliphatic chain, exhibited the highest efficiency as an agent inhibiting the reproduction of the influenza virus A(H1N1)pdm09. The cytotoxicity data of compounds **3** and **4** revealed their moderate activity against malignant cell lines; compound **3f** had the highest activity for the CEM-13 cells. These results show close agreement with the data of independent studies on toxicity of these compounds, in particular that the toxicity of compounds strongly depends on spacer length.

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1. Introduction

Functionalization of natural compounds exhibiting native biological activity is one of the most efficient approaches for the synthesis of biologically active substances in medicinal chemistry. Symmetric nitrogen-containing molecules bearing at least two natural fragments linked with a spacer have become widely used for medical applications. The type of natural fragments is rather diverse: alkaloids, steroids, mono- or diterpenes, etc. Thus, dimeric and trimeric compounds, which exhibited a strongly pronounced antitumor effect, have been synthesized based on artemizinine.[1] Hybrids of steroid framework have been studied as analogues of juvenile hormones.[2] The strategy of bivalent ligands has been successfully implemented for searching for acetylcholinesterase inhibitors. The alkaloids tarcine[3] and galanthamine[4] were used as starting natural compounds, and their bis-derivatives appeared promising agents for treating Alzheimer's disease. There are some examples in the literature of dimeric molecules acquiring properties that are completely different from those of the precursor molecules.[5] Indeed, symmetric derivatives of the alkaloid camphotecine enhance solubility of a compound, reduce its toxicity, and increase its specific interaction with an enzyme.[6] Meanwhile, symmetric compounds containing two quaternary nitrogen atoms separated by different size spacers are generally known to exhibit high biological activity.[7] These agents have been most widely used as myorelaxants.[8,9] Furthermore, many of them have been tested as antimalarial drugs.[10,11] However, very few examples of synthesis of dimeric compounds based on natural molecules linked to quaternary nitrogen atoms via spacers have been published. For example, ammonium derivatives of the diterpenoids steviol and isosteviol exhibited antimicrobial properties[12] and dimeric steroids can acts as a catalyst[13] Hence, synthesis of compounds containing several natural fragments linked with spacer groups, involving the insertion of two quaternized nitrogen atoms, is a promising trend in chemistry of biologically active substances.

In the present study we describe the biological activity of novel class of chemical compounds—dimeric camphor derivatives. Among other types of activity, we have investigated their ability to suppress the reproduction of influenza virus. Several examples of use of similar cage structures as anti-viral compounds are well known. Adamantane derivatives amantadine and rimantadine were the first antivirals against influenza. Isoborneol derivatives were also shown to possess antiviral activity against influenza

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0968-0896/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmc.2013.08.014

virus.[14,15] Based on the amino derivatives of isoborneol, anti-influenza drug deitiforin was licensed in the former Soviet Union for influenza treatment. Its mechanism of activity was supposed to be similar to that of rimantadine. Several attempts were undertaken to optimize the molecule of amantadine and to overcome the virus' resistance.[16–19] In particular, adamantane-based spiro-derivatives have been tested against influenza virus and demonstrated high inhibiting activity. In this regard, activity of spiro[piperidine-2,2′-adamantane] appeared comparable with that of amantadine while other 2-alkyl-2-aminoadamantanes appeared less effective. Moreover, none of them demonstrated an activity against influenza B virus thus suggesting the specific target for their activity that is absent in influenza B viruses.

Although being adamantane-based cage structures, compounds called bananins (derivatives of 1-[3-hydroxy-5-(hydroxymethyl)-2-methyl-4-pyridinyl]-2,8,9-trioxaadamantane-3,5,7-triol)[20,21] have not been tested against influenza virus. These compounds were shown to inhibit SARS coronavirus-specific helicase (SCV). In the cell-free system they were effective inhibitors of the ATPase activity of the SCV helicase with IC_{50} values in the range 0.5–3 μM.

Another group of anti-influenza compounds is represented by cage structures close to camphor scaffold–pinanamines. It was previously shown that (1*R*,2*R*,3*R*,5*S*)-3-pinanamine appeared more potent than amantadine in inhibiting amantadine-susceptible influenza virus.[22] Moreover, although at relative high concentrations, some of pinanamine derivatives were able to inhibit amantadine-resistant mutant bearing S31N mutation, the fact suggesting the principal possibility to overcome the resistance of influenza viruses to adamantane derivatives using cage scaffold-based compounds. The target of adamantane derivatives is virus-specific proton channel M2. Initially, these compounds were supposed to interact with the internal channel of M2 protein. In general, in their protonated form these compounds are considered to block the tetrameric M2 ion channel pore, formed by its transmembrane domain and hence, its proton transport function. Amino acid substitutions conferring rimantadine-resistant phenotype are localized at positions 27, 28, 31 and 34. In 2008–2009, NMR solution revealed another adamantane-binding site around the amino acid position 44.[23,24] This study suggested that rimantadine bound to the outside of the M2 protein helices facing the lipid bilayer with residue D44 participating in a hydrogen bond interaction with rimantadine. It was postulated that the high-affinity binding site corresponded to the M2 ion channel pore; whereas the secondary, low-affinity binding site could be attributed to the lipid face of the pore. These two studies proposed different sites of and different models for the interaction of adamantane drugs with M2 first, anion channel pore-binding model and, second, the lipid-facing binding model.[25] In the later case, the drug was shown to inhibit the channel from outside by an allosteric mechanism by stabilizing its closed state.

A common feature of all M2 inhibitors known so far is the presence of a primary amine group linked with a hydrophobic scaffold.[26,27] The existence of an external binding site for adamantane derivatives broadens the set of modifications of compounds for suppressing ion channel activity of M2. Narrow pore of the channel restricts the size and charge of compounds that must fit the pore in order to inhibit it effectively. On the other hand, when developing the compounds interacting with amino acids and/or lipid bilayer from outside the channel, one may use a much wider set of side substituents. In this case, other factors become important for binding, in particular, the ability of a compound to interact simultaneously with lipids of membrane bilayer and external amino acids of the transmembrane region of M2.

2. Results and discussion

2.1. Chemistry

(+)-Camphor **1**, an abundant monoterpenoid with a bicyclic framework structure, was selected to be the starting compound. Imine **2** was synthesized via the interaction between compound **1** and *N*,*N*-diethylethane-1,2-diamine under conditions of azeotropic removal of water from toluene in the presence $BF_3{\cdot}Et_2O$ with the yield of 94% (Scheme 1). In order to synthesize symmetric dimeric molecules, dihalogenides of different size and structural type were used as spacers. When interacting with compound **2** in boiling acetonitrile, they gave rise to the target compounds **3a**–**g**. The resulting salts **3a**–**g** containing two quaternary nitrogen atoms were isolated using silica gel column chromatography. In order to study the effect of the structure of compounds **3a**–**g** on their biological activity, spacer-free analogues—compounds **4a**–**b** containing a single quaternary nitrogen atom—have been synthesized.

The NOESY data and DFT quantum chemical calculation for compound **2** gave grounds to propose a structure with the *E* configuration of the imino groups for all the target compounds. Thus, the spectrum of imine **2** contains NOE cross peaks between proton signals at carbon atoms C-(2) and C-(11) (the calculated distance between the nearest protons of the specified groups is ~2.4 Å for *E* and ~4.4 Å for *Z* configurations). Cross peaks are also observed between Me-10 and H-4c, 5c, as well as between Me-9 and H-2c, which enables the unambiguous assignment of their signals (Fig. 1). It follows from the calculated data that the *E* configuration is 5 kcal/mol more energetically profitable than the *Z* configuration. Let us mention that the similar camphor imines have previously also been classified as *E* isomers.[28]

Because of insufficient solubility of compounds **3a**–**g** and **4b** in $CDCl_3$, their NMR spectra were recorded in MeOH-d_4. The substitution of a proton at the exo position 2 of the bornane framework by a deuterium atom was observed. The deuteration degree varied from sample to sample, which can presumably be attributed to different solvent exposure times and slightly varied acidity of the medium for the samples. The substitution at the vinyl position of imines in MeOH-d_4 solution is observed rather frequently and occurs due to the presence of the tautomeric equilibrium with the enamine form.[29] Broadened signals for certain carbon atoms (and the signal for carbon atom C-(1) in the imino group in particular) were observed in the ^{13}C NMR spectra of compounds **3a**–**g** and **4a**, **b**. Equimolar amounts of triethylamine were added to all the samples dissolved in MeOH-d_4 in order to 'freeze' the chemical exchange and narrow down the NMR spectral lines.

2.2. Study of biological activity

2.2.1. Antiviral activity

The obtained compounds **3a**–**g** and **4a**, **b** have been studied as potential antiviral agents. Natural compounds are considered to be the most prospective source for development of novel antivirals.[30] In USA, about half of novel drugs approved for clinical use in the past decades are natural compounds and their chemically modified derivatives.[31]

There are few antivirals against influenza; drug resistance of the virus has been reported for most of them. The best-known etiotropic drugs for influenza treatment are adamantane derivatives amantadine (Symmetrel®, 1-aminoadamantane) and rimantadine (α-methyl-1-adamantylmethylamine hydrochloride). These compounds block the viral ion channel M2, thus preventing proton flow into the virion and further cleavage of hemagglutinin and fusion of membranes of the viral envelope and lysosomal vacuole. The study of activity of compounds **3a**–**g** and **4a**, **b** against influ-

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