Bioorganic & Medicinal Chemistry 16 (2008) 7457–7461

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Cinnamoyl- and hydroxycinnamoyl amides of glaucine and their antioxidative and antiviral activities

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ARTICLE INFO

Article history:
Received 29 January 2008
Revised 27 May 2008
Accepted 6 June 2008
Available online 13 June 2008

Keywords:
Glaucine
3-Aminoglaucine
3-Aminomethylglaucine
Cinnamic acids
Antioxidant effect
DPPH* test
Antiviral activity

ABSTRACT

The aporphine alkaloid glaucine has been converted into 3-aminomethylglaucine and its free amino group has been linked to cinnamic, ferulic, sinapic, *o*-, and *p*-coumaric acids. The antioxidative potential of the synthesized amides was studied against DPPH* test. All of the tested compounds demonstrated higher radical scavenging activity than glaucine and 3-aminomethylglaucine, and lower antioxidative effect than the free hydroxycinnamic acids. The newly synthesized compounds were tested in vitro for antiviral activity against viruses belonging to different taxonomic groups.

1. Introduction

The aporphine alkaloids possess a wide variety of pharmacological effects, including antitussive, antiparkinsonic, hypotensive, antiviral, cytotoxic, etc. activities.[1–6] A considerable antioxidative activity of these alkaloids has also been found in the recent years.[7] The non-phenolic aporphine alkaloid glaucine, isolated from the aerial parts of *Glaucium flavum* Crantz (*Papaveraceae*), is one of the important representatives of this group of compounds. Besides antitussive effect, the alkaloid possesses a promising photoprotective and antioxidative activity.[7–10] The latter biological activity of glaucine (**1**) is surprising because in its structure the phenolic hydroxyl groups are absent. According to the literature data, the antioxidative capacity of aporphine alkaloids is related to the presence of the biphenyl system rather than the phenol group. The biphenyl system can be readily oxidized to dehydroaporphine and oxoaporphine.[7] It is assumed that the benzylic C-6a-H bond may be the initial point of the free radical attack. The resulting benzylic free radicals would be stabilized by extended conjugation across the aporphine biphenyl system. The additional stabilization is provided by the nitrogen lone pair. The antioxidative property of glaucine might be of interest in regard to the cellular damage caused by the oxidative stress concomitant to various diseases. The highly potent scavenging activity of this aporphine alkaloid against the hydroxyl radicals (which are regarded as the most reactive oxygen metabolites in biological systems) could make it a potentially useful therapeutic agent. Another group of antioxidative compounds has also been the focus of our interest in the last years, namely the synthesis and activities of cinnamic acids derivatives.[11–14]

On the other hand, data exist about the antiviral effects of cinnamic acids and their esters.[15–17] An antiviral effect of some aporphine alkaloids and their derivatives as isoboldine, oxoglaucine, 3-hydroxyglaucine, and dehydroglaucine is also described.[4,5] It can be expected that coupling of cinnamoyl and glaucine moieties could result in the enhancement of the antioxidant and antiviral activities of the newly synthesized compounds.

Thus, the goal of the present study is:

- Synthesis of cinnamoyl amides of aminomethylglaucine. The new compounds will contain two structural fragments with recorded antioxidative activity.
- Investigation and comparison of the antioxidative effect of the newly synthesized compounds against DPPH*.
- Study of the antiviral activity of the new derivatives against viruses, representing several different taxonomic groups, where antiviral chemotherapy is indicated.

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doi:10.1016/j.bmc.2008.06.010

2. Results and discussion

2.1. Chemistry

The absence of an appropriate functional group in the molecule of glaucine for making the link with cinnamic acids suggested the introduction of an amino group at C-3 in its molecule (**2**) by a previously described procedure (Scheme 1).[18] The attempts to obtain cinnamoyl amides of the so modified glaucine **2** failed. The low reactivity of 3-aminoglaucine (**2**) by amidation could be explained by the decrease of the amino group nucleophility as a result of conjugation of the nitrogen lone pair electrons with the π-system of the aromatic ring. Therefore. the more reactive 3-aminomethylglaucine (**3**) was prepared by a known procedure.[19] In this case, the positive induction effect of the methylene group could increase the amino group nucleophility and the reactivity of the substrate.

The linking of 3-aminomethylglaucine to cinnamic, ferulic, sinapic, *o*-, and *p*-coumaric acids was successful. The amides were prepared by peptide chemistry methods using EDC/HOBt (Scheme 1). The amides obtained were isolated by preparative TLC and characterized by UV, ^{1}H NMR, and ESI-MS. The yields are given in Table 1.

The complete assignment of the ^{1}H NMR spectra of the compounds **4, 5, 6, 7**, and **8** measured in $CDCl_3$ is reported. In the ^{1}H NMR spectra of all the obtained amides except the signals for the 3-aminomethylglaucine, protons signals for cinnamoyl-, feruloyl-, sinapoyl -, *o*-, and *p*-coumaroyl moieties were observed.

The values of the proton–proton vicinal coupling constants (3JH/H about 15.6 Hz) measured for the olefinic protons of cinnamoyl-, feruloyl-, sinapoyl-, *o*-, and *p*-coumaroyl residues define E configuration of the double bond of all studied compounds. The chemical shift of the amide proton established the amide linkage of the 3-aminomethylglaucine with cinnamic and hydroxycinnamic acids.

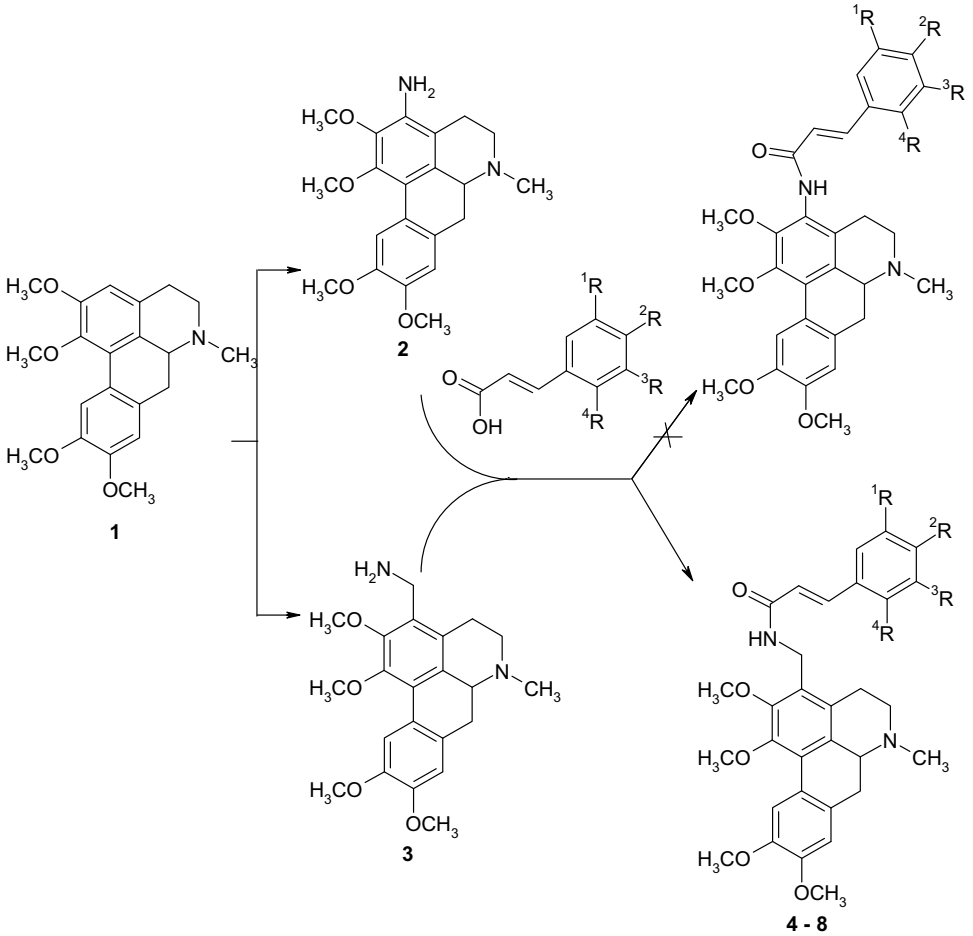
2.2. Biological evaluation

2.2.1. Antioxidant activity

The antioxidative potential of the synthesized amides was studied against DPPH* (1,1-diphenyl-2-picrylhydrazyl radical). The obtained results are summarized in Table 2. All of the tested hydroxycinnamoyl amides of 3-aminomethylglaucine demonstrated higher radical scavenging activity than glaucine and 3-aminomethylglaucine, and lower antioxidative effect than the free hydroxycinnamic acids against DPPH* test.

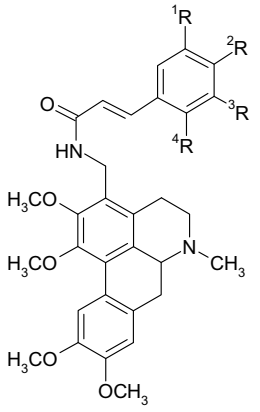
2.2.2. Antiviral activity

Compounds **5–8** were tested for antiviral activity against several viruses representing different taxonomic groups. Each virus included in the test panel was an important human pathogen. Poliovirus type 1 (LSc-2ab), coxsackievirus B1 (CV-B1), and echovirus 13 (EV-13) represented the *Enterovirus* genus of the family of *Picornaviridae*. Human rhinovirus 14 (HRV-14) represented the multitudinous genus of human rhinoviruses in the same family. Influenza virus A/Aichi/2/68 (H3N2) and the respiratory syncytial



Scheme 1.

Table 1
Synthesized *N*-cinnamoyl- and hydroxycinnamoyl-3-aminomethylglaucine amides



Compound	^{1}R	^{2}R	^{3}R	^{4}R	Yields, %
4	H	H	H	H	88.1
5	OCH_3	OH	H	H	61.6
6	OCH_3	OH	OCH_3	H	47.0
7	H	H	H	OH	43.5
8	H	OH	H	H	21.4

virus (RSV) represented *Orthomyxoviridae* and *Paramyxoviridae*, respectively. The wide spectrum of tested picornaviruses, that is, three enteroviruses and one rhinovirus was imposed by previous results of ours characterizing a derivative of glaucine, oxoglaucine, as a highly potent antipicornaviral compound.[5]

The antiviral activity was tested by the cytopathic effect (CPE) inhibition test in the viral multicycle growth setup. Disoxaril and oxoglaucine were both used as positive controls for entero- and rhinovirus inhibition. Ribavirin and rimantadine were the positive controls in the case of RSV and type A (H3N2) influenza virus inhibition, respectively. Results for the cytotoxicity, antiviral activity, and selectivity indices of the newly synthesized compounds as regards to the inhibition of the tested enteroviruses are presented in Table 3. According to the results *p*-coumaroyl amide (**8**) presented antiviral activity against the replication of one of the tested enteroviruses, namely echovirus 13 with a selectivity index 5. The highest non-toxic concentration (100 μM) of this compound revealed also some minor antiviral effect against the replication of another enterovirus in the test panel, poliovirus type 1 (LSc-2ab). Insignificant antiviral effect against poliovirus type 1 (LSc-2ab) is detected also for *o*-coumaroyl amide (**7**). None of the newly synthesized compounds showed antiviral effect against the replication of the third enterovirus in the test panel, coxsackievirus B1.

All the four newly synthesized compounds revealed some antiviral activity against the replication of the other picornavirus, representing the rhinovirus genus, HRV-14 (Table 4). Both coumaroyl amides (compounds 7 and **8**) revealed the best antirhinoviral activity. The highest non-toxic concentration of 100 μM of each of the new compounds strongly inhibited the replication of HRV-14.

An antiviral activity against the replication of influenza virus A (H3N2) was not found (data not shown).

As far as the antiviral activity against the replication of RSV is concerned, *p*-coumaroyl amide (**8**) showed detectable dose-dependent effect. The compound reached a selectivity index of 7 with an IC_{50} value of 18 μM. Interestingly, this compound showed antiviral effect against viruses representing quite different taxonomic

Table 2
Radical scavenging activity of hydroxycinnamoyl amides of 3-aminoglaucine against DPPH* test

Compound	Reaction time (min)					
	0.9 mM		1.8 mM		3.6 mM	
	10′	20′	10′	20′	10′	20′
	RSA (%)					
Glaucine (**1**)	0.8	1.2	1.1	1.3	1.4	1.6
3-Aminomethylglaucine (**3**)	1.6	2.5	2.2	3.0	2.9	3.8
Feruloylamide of 3-aminomethylglaucine (**5**)	8.3	10.1	16.6	19.4	31.2	36.1
Sinapoylamide of 3-aminomethylglaucine (**6**)	8.0	10.5	14.4	18.0	26.3	31.6
o-Coumaroylamide of 3-aminomethylglaucine (**7**)	1.1	0.8	2.3	3.6	3.3	4.2
p-Coumaroylamide of 3-aminomethylglaucine (**8**)	1.8	1.8	2.0	2.5	3.2	4.8
Sinapic acid	16.1	17.2	26.5	31.9	69.0	69.6
Ferulic acid	12.0	13.8	21.0	25.1	36.7	44.3
p-Coumaric acid	2.1	2.9	3.7	4.7	4.5	6.1
o-Coumaric acid	2.1	2.4	2.2	2.7	3.3	3.6

% RSA—percent radical scavenging activity; % RSA = $[Abs_{516nm\ (t=0)} - Abs_{516nm(t=t')} \times 100/Abs_{516nm(t=0)}]$, as proposed by Pekkarinen et al.[22]; glaucine-, sinapic-, ferulic-, *p*-coumaric, and *o*-coumaric acids used as standards.

Table 3
Cytotoxicity, antienteroviral activity and selectivity indices of the newly synthesized cinnamoyl- and hydroxycinnamoyl amides of glaucine

Compound	CC_{50}[a] (μM)	Poliovirus type 1 (LSc-2ab)			CV-B1			EV-13		
		IC_{50} (μM)[a]	%[b]	SI (CC_{50}/IC_{50})	IC_{50} (μM)[a]	%[b]	SI (CC_{50}/IC_{50})	IC_{50} (μM)[a]	%[b]	SI (CC_{50}/IC_{50})
5	105	—	0	—	—	0	—	—	⩽25	[c]
6	112	—	0	—	—	0	—	—	0	—
7	152	—	⩽25	[c]	—	0	—	—	0	—
8	160	—	<50	[c]	—	0	—	32	—	5
1	115	60	—	1.9	21	—	5.5	60	—	1.9
Oxoglaucine	52	1.2	—	43.3	2.5	—	20.8	0.5	—	104
Disoxaril	25	2	—	12.5	2	—	12.5	1.3	—	19.2

[a] CC_{50} and IC_{50} values represent the mean values in two different experiments with six replicates in each experiment.
[b] Percentage of viral cytopathic inhibition compared to the non-infected cells (control).
[c] Selectivity index not detected.

Table 4
Cytotoxicity, antirhinoviral activity and selectivity indices of the newly synthesized cinnamoyl- and hydroxycinnamoyl amides of glaucine

Compound	CC_{50} (μM)[a]	IC_{50} (μM)[a]	SI (CC_{50}/IC_{50})
5	113	12	9.4
6	125	28	4.5
7	155	15	10.3
8	143	13	11
1	182	22	8.3
Oxoglaucine	51	0.3	170
Disoxaril	21	1.5	14

[a] CC_{50} and IC_{50} values represent the mean values in two different experiments with six replicates in each experiment.

groups. Nevertheless, its effect is mostly expressed against the replication of human rhinovirus 14.

In conclusion, cinnamoyl-, feruloyl-, sinapoyl-, *o*-, and *p*-coumaroyl amides of 3-aminomethylglaucine have been synthesized by the methods used in the peptide chemistry. Among the tested compounds feruloyl- and sinapoyl amides demonstrated higher radical scavenging effect against DPPH* test. All of the hydroxycinnamoyl amides showed higher antioxidant activity than glaucine and 3-aminomethylglaucine. Some of the newly synthesized compounds revealed antiviral activity, especially against the replication of HRV-14.

Given the promise of the above results, the synthesis of new compounds containing structural fragments of moieties of compounds with recorded activity deserve further attention and research for optimization of their parameters.

3. Experimental

The NMR spectra of the synthesized amides were obtained on a Bruker Avance DRX-250 MHz spectrometers operating at 250.13 MHz for 1H. The measurements in CDCl3 solutions were carried out at ambient temperature (300 K) and tetramethylsilane (TMS) was used as an internal standard. The UV spectra of the compounds in CH_3OH solutions were measured with a Specord UV–vis spectrophotometer. ESI-MS spectra were obtained on Esquire 3000 plus. Optical rotations were measured with a Polarimeter 241 from Perkin Elmer. 'Agilent 8453' spectrophotometer was used for the measurement of the reduction of DPPH* absorbance at 516 nm.

3.1. General synthetic procedure for preparation of amides 4–8

The phenolic acid (cinnamic-, ferulic-, sinapic-, *o*-, and *p*-coumaric) (0.26 mM) and HOBt (0.05 g, 0.26 mM) were dissolved in CH_2Cl_2/DMF (3 mL, 1:2), the solution was stirred and cooled in an ice-water bath, while EDC (0.05 g, 0.26 mM) was added. After 5 min, to the solution, 3-aminomethylglaucine (0.100 g, 0.26 mM) and NMM (0.03 mL, 0.26 mM) were added. Stirring was continued for 1 h at 0 °C and 21 h at room temperature. The progress of the reaction was monitored by TLC ($PE/CHCl_3/CH_3COCH_3/CH_3OH$ = 4:8:1:2). After completion of reaction, the solvents were evaporated in vacuo. A mixture of CH_2Cl_2 and water was added and the organic phase was extracted with a 5% $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 filtered and evaporated to dryness under reduced pressure.

The residue was purified by TLC on Kieselgel 60 F_{254} (Merck) using solvent system ($PE/CHCl_3/CH_3COCH_3/CH_3OH$ = 4:8:1:2).

3.2. Cinnamoyl amide of 3-aminomethylglaucine 4

Yield 88.1%; $[\alpha]_D^{25}$ +20.4° (*c* 0.13, abs. CH_3OH); *UV : (CH_3OH)* λ_{max} = 219, 223, 285, 300, 302, 318 nm; ^1H *NMR:* δ 2.58 (s, 3H, NC*H*3), 2.96–3.18 (m, 6H, 3 × C*H*2), 3.72 (s, 3H, OC*H*3, (C-1)), 3.94, 3.97 (s, 9H, 3 × OC*H*3, (C-2, C-8, C-10)), 4.61–4.66 (m, 2H, N–C*H*2), 5.86 (t, 1H, N*H*) 6.36 (d, 1H, *J* = 15.6 Hz, C*H*=), 6.79 (s, 1H, Ar–H (H-8)), 7.36 (m, 3H, Ar–H), 7.48 (m, 2H, Ar–H), 7.64 (d, 1H, *J* = 15.6 Hz, C*H*=), 7.93 (s, 1H, Ar–H (H-11)); *ESI-MS*: 515 ([M+H]+), 1129 ([2M+H]+).

3.3. Feruloyl amide of 3-aminomethylglaucine 5

Yield 61.6%; $[\alpha]_D^{25}$ +28.2° (*c* 0.9, abs. CH_3OH); *UV : (CH_3OH)* λ_{max} = 222, 285, 320 nm; ^1H *NMR:* δ 2.58 (s, 3H, NC*H*3), 2.89–3.19 (m, 6H, 3 × C*H*2), 3.72 (s, 3H, OC*H*3, (C-1)), 3.80 (s, 1H, *H*-6a), 3.89, 3.94, 3.97(s, 12H, 4 × OC*H*3), 4.60–4.65 (m, 2H, NC*H*2), 5.77 (t, 1H, N*H*) 6.19 (d, 1H, *J* = 15.5 Hz, C*H*=), 6.79 (s, 1H, Ar–H (H-8)), 6.88 (d, 1H, *J* = 8.1 Hz, Ar–H(m)), 6.96 (d, 1H, *J* = 1.8 Hz, Ar–H(o)), 7.03 (dd, 1H, *J* = 8.1, 1.8 Hz, Ar–H(o)), 7.54 (d, 1H, *J* = 15.5 Hz, C*H* =), 7.94 (s, 1H, Ar–H (H-11)), *ESI-MS*: 561 ($[M+H]^+$), 1121 ($[2M+H]^+$).

3.4. Sinapoyl amide of 3-aminomethylglaucine 6

Yield 47.0%; $[\alpha]_D^{25}$ +38.1° (*c* 0.27, abs. CH_3OH); *UV : (CH_3OH)* λ_{max} = 223, 242, 273, 285, 319 nm; ^1H *NMR:* δ 2.61 (s, 3H, NC*H*3), 2.88–3.16 (m, 6H, C*H*2), 3.71 (s, 3H, OC*H*3, (C-1)), 3.73 (s, 1H, (H-6a)), 3.81 (s, 1H, H-6a), 3.89, 3.94, 3.97 (s, 15H, OC*H*3), 4.62–4.66 (t, 2H, N–C*H*2), 5.80 (t, 1H, N*H*), 6.23 (d, 1H, *J* = 15.5 Hz, C*H*=), 6.72 (s, 2H, Ar–H(o)), 6.79 (s, 1H, Ar–H (H-8)), 7.53 (d, 1H, *J* = 15.5 Hz, C*H* =), 7.92 (s, 1H, Ar–H (H-11)); *ESI-MS*: 591 ($[M+H]^+$), 1181 ($[2M+H]^+$).

3.5. *o*-Coumaroyl amide of 3-aminomethylglaucine 7

Yield 43.5%; $[\alpha]_D^{25}$ +47.2° (*c* 0.32, abs. CH_3OH); *UV: (C_2H_5OH)* λ_{max} = 221, 282, 316 nm; ^1H *NMR:* δ 2.64 (s, 3H, NCH_3), 2.89–3.12 (m, 6H, 3 × CH_2), 3.72 (s, 3H, OCH_3, (C-1)), 3.92, 3.93, 3.94 (s, 9H, 3 × OCH_3), 4.46–4.64 (m, 2H, N–CH_2), 5.74 (br s, 1H, NH), 6.12 (d, 1H, *J* = 15.8 Hz, CH=), 6.49 (d, 1H, *J* = 8.0 Hz, Ar–H), 6.77 (s, 1H, H-8), 6.8 (d, 1H, 7.6 Hz, Ar–H), 6.95 (t, 1H, Ar–H), 7.32 (d, 1H, *J* = 6.5 Hz, Ar–H), 7.9 (d, 1H, *J* = 15.8 Hz, CH=), 7.93 (s, 1H, Ar–H (H-11)); *ESI-MS:* 531 ($[M+H]^+$), 1061 ($[2M+H]^+$).

3.6. *p*-Coumaroyl amide of 3-aminomethylglaucine 8

Yield 21.4%; $[\alpha]_D^{25}$ +24.5° (*c* 0.2, abs. CH_3OH); *UV : (C_2H_5OH)* λ_{max} = 222, 285, 309 nm; ^1H *NMR:* δ 2.65 (s, 3H, N–CH_3), 2.73–3.48 (m, 6H, 3 × CH_2), 3.71 (s, 3H, OCH_3, (C-1)), 3.82 (s, 1H, (H-6a)), 3.92, 3.93, 3.95 (s, 9H, 3 × OCH_3), 4.58 (d, 2H, *J* = 4.8 Hz, N–CH_2), 5.79 (s, 1H, NH), 5.97 (d, 1H, *J* = 15.6 Hz, CH=), 6.64 (d, 2H, *J* = 8.2 Hz, Ar–H), 6.67 (s, 1H, (H-8)), 7.18 (d, 2H, *J* = 8.2 Hz, Ar–H), 7.41 (d, *J* = 15.6 Hz, CH=), 7.93 (s, 1H, H-11); *ESI-MS*: 531 ($[M+H]^+$), 1061 ($[2M+H]^+$).

3.7. Estimation of radical scavenging activity (RSA) by the DPPH* test

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH*) was used as stabile radical. For each compound and concentration tested (0.9, 1.8, and 3.6 mM), the reduction of DPPH* radical was followed by monitoring the decrease of absorbance at 516 nm. The absorption was monitored at the start and at 10 and 20 min. The results are expressed as % RSA = [Abs_{516} nm (t = 0) − Abs_{516} nm (t = t') × 100/ Abs_{516} nm (t = 0)], as proposed by Pekkarinen et al.[22]

3.8. Estimation of the antiviral activity

The tested compounds were dissolved as 20 mM solutions in methyl alcohol and kept as stock solutions at −20 °C. Before use

in the antiviral tests, compounds were further diluted in a maintenance medium.

3.9. Cells and viruses

Poliovirus type 1 (LSc-2ab), coxsackievirus B1 (CV-B1), echovirus 13 (EV-13), human rhinovirus type 14 (HRV-14), influenza virus A/Aichi/2/68 (H3N2), and respiratory syncytial virus (RSV) were used for the antiviral tests. Poliovirus type 1 (LSc-2ab), CV-B1, and EV-13 were grown in FL cell line. HRV-14, influenza virus A (H3N2), and RSV were grown in HeLa Ohio-I cells, MDCK cell line, and HEp-2 cells, respectively. All cell lines were grown in a humidified atmosphere at 37 °C and 5% CO_2 in a growth medium of Dulbecco modified Eagle's medium (DMEM) (Gibco BRL, USA), except for HeLa Ohio cells, which were propagated in minimal essential medium (MEM) (Gibco BRL, USA), containing 5% fetal bovine serum in the case of FL and HEp-2 cells, and 10% fetal bovine serum for MDCK and HeLa cells, supplemented with antibiotics (penicillin 100 IU/mL, streptomycin 100 μg/mL and gentamycin 50 μg/mL) and 20 mM HEPES buffer (Gibco BRL, USA). Cells were routinely subcultured twice weekly. When harvesting viruses and performing antiviral assays, serum in the maintenance medium was reduced to 2% for HRV-14 and to 0.5% for the remaining viruses. Maintenance medium for influenza virus A contained also 3 μg/mL trypsin (Gibco BRL).

3.10. Cytotoxicity test

Monolayer cells were inoculated with 0.1 mL/well containing 0.5 log concentrations of the compounds diluted in a maintenance medium. After 24 h of incubation in a humidified atmosphere at 37 °C and 5% CO_2 cells were monitored for microscopic cytotoxic effects and the highest concentration, at which no visible cytotoxic effect was recorded was considered as the maximal tolerated concentration (MTC). After the microscopic evaluation, cells underwent the neutral red uptake procedure[20,21] and the 50% cytotoxic concentration (CC_{50}) was calculated. The CC_{50} value was defined as the concentration of each compound that reduced the absorbance of treated cells by 50% when compared to the untreated control. Briefly, after removal of the maintenance medium containing the tested compound, cells were washed and 0.1 mL fresh maintenance medium containing 0.005% neutral red dye (Fluka) was added to each well and cells were incubated at 37 °C for 3 h. Then cells were washed once with PBS and 0.15 mL/well dessorb solution (1% glacial acetic acid, 49% ethanol, 50% distilled water) was added. Following 10 min of mild shaking, the optical density (OD) of each well was read at 540 nm in a microplate reader (Organon Teknika reader 530). The CC_{50} values were determined by regression analysis.

3.11. Antiviral tests

The viral cytopathic effect (CPE) inhibition assay was used for evaluating the antiviral effects of the newly synthesized compounds. Monolayer cells in 96-well plates were inoculated with 0.1 mL virus suspension containing 100 CCID50 (1000 $CCID_{50}$ in the case of HRV-14). Mock-infected wells were left for toxicity and cell controls. After an hour, for virus adsorption (2 h in the case of RSV and HRV-14) excessive virus was discarded and cells were inoculated with 0.2 mL of maintenance medium containing 0.5 log non-toxic concentrations of the compounds tested. Cells were incubated in a humidified atmosphere at 37 °C (33 °C for HRV-14) and 5% CO_2. The virus cytopathic effect (CPE) was scored daily till the appearance of its maximum in the virus control wells (with no compound in the maintenance medium). Then viable cells were stained according to the neutral red uptake procedure and the percentage of CPE inhibition was calculated using the following formula: $\%\ \mathrm{CPE} = [\mathrm{OD}_{\text{test sample}} - \mathrm{OD}_{\text{virus control}}]/[\mathrm{OD}_{\text{toxicity control}} - \mathrm{OD}_{\text{virus control}}] \times 100$. The concentrations that inhibited 50% of the virus induced CPE, the 50% inhibitory concentrations (IC_{50}), were determined by regression analysis. When it was not possible to calculate the IC_{50} values, results were expressed as approximate percentages of viral inhibition. The selectivity index was calculated as the ratio between CC_{50} and IC_{50} ($SI = CC_{50}/IC_{50}$).

Acknowledgments

For the support of this work we are grateful to the National Fond for Scientific Research of Bulgaria (Contracts VUH-07/05), South-West University 'Neofit Rilski' Bulgaria, and to the CEEPUS mobility program.

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