QUANTUM-CHEMICAL INVESTIGATIONS OF 5-BROMO-2′-DEOXYURIDINE DERIVATIVES WITH ANTIVIRAL ACTIVITY

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The article describes the results of semiempirical quantum-chemical calculations for a series of 3′,5′-esters of 5-bromo-2′-deoxyuridine with amino acids or peptides. These compounds were synthesized and tested for antiviral activity as potential prodrugs of the parent 5-bromonucleoside. It was not clear why only some of the compounds were active. On the basis of structure investigation and the calculated molecular descriptors, it was found that the determining factor for obtaining appropriate prodrugs of 5-bromo-2′-deoxyuridine is the lipophilicity of the esters.

Keywords: Quantum chemistry; Semiempirical calculations; Nucleosides; Antivirals; Prodrugs; Amino acids; Peptides; Lipophilicity.

The main target in the search for antiviral substances is the inhibition of DNA biosynthesis. From this point of view special attention has been paid to pyrimidine and purine analogues (as nucleosides). Many of them like 5-bromo-2′-deoxyuridine, 5-iodo-2′-deoxyuridine, 5-fluoro-2′-deoxyuridine and some thymidine analogues are bioactive[1,2]. Normally this type of compounds shows toxicity but the attachment of amino acids and short peptides at 3′- and 5′-positions in the deoxyribose ring decreases the toxicity and also prevents their rapid elimination from the organism[3,4]. These were the reasons for synthesizing amino acid and peptide esters of 5-bromo-2′-deoxyuridine in 3′- and 5′-positions (their structures are shown below) and comparing their antiviral activity with the parent 5-bromonucleoside (Scheme 1).

The compounds containing one, two and three glycine (Gly) residues (**4**, **5** and **6**) have a considerable antiviral activity, but only compound **5** has

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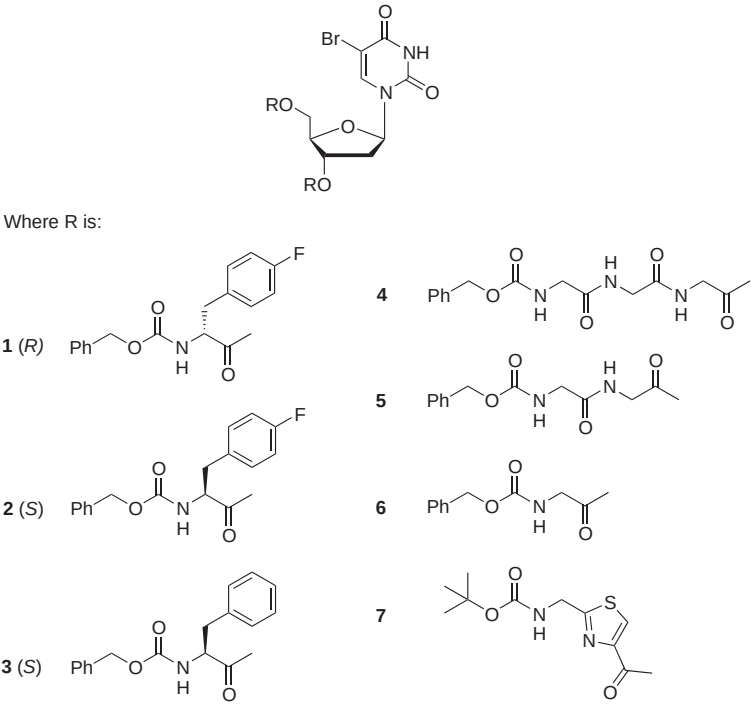
the same activity as the parent 5-bromo-2′-deoxyuridine. Furthermore, the lengthening of the peptide chain is a favorable structural change at short chain lengths but turns inappropriate when the chain becomes too long[5]. Although the compounds containing other amino acid residues have some inhibition and toxicity zone (except compound **2**), they have no antiviral activity comparable to the parent compound[5]. No structural explanation for this effect has been given so far.



SCHEME 1

On the other hand, it can be assumed that these compounds are prodrugs, hence, their hydrolysis rate determines the liberation rate of the parent active substance and its elimination rate from the organism. Chemical stability of some compounds has been evaluated at pH values and temperatures of the human gastrointestinal system (pH 7.4 and 1 and temperature 37 °C)[6]. This investigation showed higher stability of the derivatives with simple-side-chain amino acids and lower stability of those with branched side chain. These conclusions need to be particularized. Furthermore, there is no explanation why only compound **5** has the same activity as the parent 5-bromo-2′-deoxyuridine.

In the present study we try to estimate the antiviral correlation with the compound structure. This could facilitate further study of antiviral activity.

THEORY AND METHODS

The optimal geometries of the investigated compounds were obtained by structure optimization employing the restricted Hartree–Fock theory at the semiempirical AM1 level[7]. The AM1 method is chosen due to the size of the compounds under study (around 100 atoms) and limited computing resources. Furthermore, AM1 is a method of choice in the articles of Raczynska and coworkers[8–10] for calculation of microcharacteristics of heterocyclic compounds which are similar to those considered in this paper. The used software was MOPAC 2002 [11] and Hyperchem 7.0 [12].

Any simplifications were avoided in the generation of the starting structures. The initial geometry of the 5-bromothymidine residue was assumed planar as in all structure investigations of nucleic acids[13]. The base and the deoxyribose planes are placed in *syn* position with respect to the N-glycosidic bond. The starting conformation of the deoxyribose ring is 2′-endo-3′-exo. The initial structure of the amino acids and the peptide part was found after comprehensive conformational search including all single bonds using the AMBER 96 force field[14–17]. The spatial requirements of the side chain residues and the S-configuration at the asymmetric C_α atoms were taken into consideration where it was necessary.

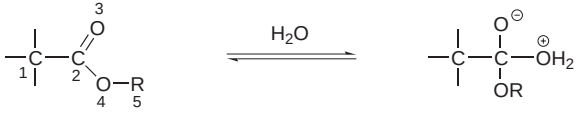
RESULTS AND DISCUSSION

Considering these compound as prodrugs, the chemical stability of some of them was investigated under biomimetic conditions (pH 1 and 7 and temperature 37 °C). However, the only conclusion was that the esters with branched side chains are not as stable as the esters with simple side chains[6].

The hydrolysis of the ester group is a typical Ac-S$_N$ reaction, mono- or bi-molecular. The proneness of the ester group to participate in this reaction can be estimated by the partial charges on the group atoms. Comparison of the charges of the ester group atoms can be used for estimation of the activity of the group participating in hydrolysis, the charges on the carbonyl atoms being the most important.

Furthermore, the obtained optimized structures indicate that there are no spatial hindrances for nucleophiles to reach the electrophilic carbon atoms of the ester groups. In Table I are collected Mulliken partial charges[18] on the atoms of the ester groups numbered according to Scheme 2. In this ap-

proach of partial charges calculation the overlap population is simply shared between the contributing atoms. Despite its known deficiencies, the Mulliken population analysis is still widely used in the literature[19].



According to the experiment, compound **7** is the most active and our results confirm unambiguously its highest activity. The partial charges at the ester carbonyl carbon atoms are respectively +0.381 and +0.384 which is much higher than the partial charges at the corresponding atoms in the other compounds. The partial charges in the other ester groups do not differ considerably.

The 3′,5′-esters of 5-bromo-2′-deoxyuridine will be appropriate prodrugs if they easily penetrate the cell, i.e., if they have high lipophilicity and sufficient stability to enter the cell unchanged and enough activity to release the parent moiety at an appropriate rate. In the mentioned paper there are no experimental results for the hydrolysis rate of the most biologically active compound **5** [6]. These data could be very useful for estimation of the capacity of all these compounds as prodrugs. According to our calculations

TABLE I
Mulliken charge distributions at the ester group atoms of 5-bromo-2′-deoxyuridine derivatives **1**–**7**

Position		**1**	**2**	**3**	**4**	**5**	**6**	**7**
5′	C1	0.037	0.049	0.044	–0.021	–0.020	–0.028	–0.119
	C2	0.325	0.270	0.269	0.280	0.276	0.265	0.379
	O3	–0.363	–0.342	–0.342	–0.337	–0.335	–0.354	–0.331
	O4	–0.244	–0.278	–0.278	–0.276	–0.275	–0.267	–0.266
	C5	–0.027	–0.028	–0.031	–0.040	–0.041	–0.032	–0.026
3′	C1	0.052	0.037	0.043	–0.020	–0.021	–0.023	–0.122
	C2	0.274	0.270	0.270	0.292	0.293	0.268	0.384
	O3	–0.325	–0.334	–0.338	–0.380	–0.378	–0.328	–0.312
	O4	–0.290	–0.287	–0.284	–0.279	–0.280	–0.290	–0.279
	C5	0.023	0.029	0.022	0.007	0.009	0.022	0.027

(Fig. 1), the activity of compound **5** must be moderate (charges of +0.278 and +0.293 at the carbonyl carbon atoms and –0.336 and –0.378 at the carbonyl oxygen atoms). There is a considerable difference in the activity of the two ester groups. From this point of view compound **7** must be eliminated as prodrug because of its fast hydrolysis.

Various molecular parameters which should correlate with the antiviral activity were also calculated with the help of Hyperchem 7.0 program package. These characteristics of the investigated compounds were compared with those of the parent 5-bromo-2′-deoxyuridine. All the molecular parameters studied are collected in Table II.

The only descriptor which correlates with antiviral activity is log *P*. A comparison of the characteristics of the prodrugs and those of the parent compound shows that both 5-bromo-2′-deoxyuridine and the most active compound 3′,5′-*O*-bis({*N*-[(benzyloxy)carbonyl]glycyl}glycyl)-5-bromo-2′-deoxyuridine have similar penetrability into the cell according to the calculated values of log *P* [20]: –1.39 for the parent compound and –2.16 for the prodrug. It is clear why 3′,5′-*O*-bis{*N*-[(benzyloxy)carbonyl]glycyl}-5-bromo-2′-deoxyuridine and 3′,5′-*O*-bis({*N*-[(benzyloxy)carbonyl]glycyl}-glycylglycyl)-5-bromo-2′-deoxyuridine are less active than 3′,5′-*O*-bis({*N*-[(benzyloxy)carbonyl]glycyl}glycyl)-5-bromo-2′deoxyuridine (Fig. 2). The former is more lipophilic and the other is less lipophilic than necessary.

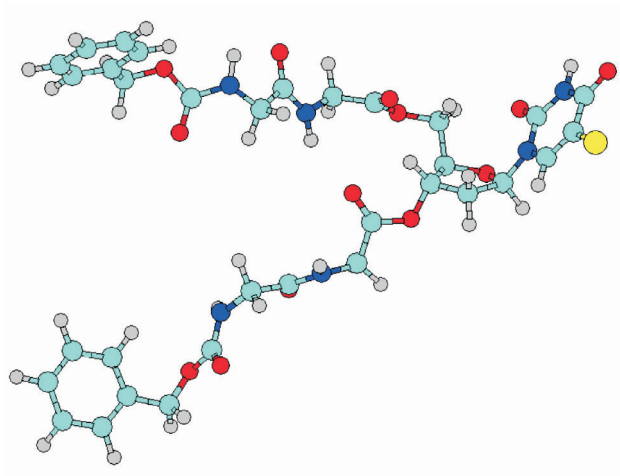


FIG. 1
Optimized structure of compound **5**. Legend: green, C; gray, H; red, O; blue, N; yellow, Br

Moreover, the barely active and the inactive compounds have positive log *P* and much higher lipophilicity than the parent compound. In the case of compound **7** the lack of activity is probably due to its fast hydrolysis before entering in the cells.

TABLE II
Molecular parameters of 5-bromo-2′-deoxyuridine derivatives **1**–**7** and data demonstrate their antivirus activity according to ref.[5]

Compd	Heat of formation kcal/mol	Energy kcal/mol	Dipole moment D	Surface Å^2	Volume Å^3	Hydration energy kcal/mol	log *P*[a]	DI[b] mm
P.c.[c]	–84715.16	–170.337	5.120	359.31	678.88	–12.21	–1.39	63.2
1	–270204.94	–374.51	4.717	1051.43	2203.78	–15.75	1.71	12.2
2	–270204.94	–374.51	4.717	1087.40	2202.57	–15.94	1.71	0.0
3	–248470.66	–292.42	5.152	1060.24	2184.88	–16.51	2.91	13.2
4	–279439.90	–484.34	1.667	1175.87	2264.48	–25.61	–4.32	29.0
5	–241392.51	–414.24	5.687	1029.51	1983.35	–21.59	–2.16	51.0
6	–203343.52	–342.54	5.471	869.02	1699.13	–16.23	–0.01	35.7
7	–238464.55	–338.53	5.783	1131.52	1987.27	–20.16	–0.36	0.0

[a] Octanol–water partition coefficient log *P* is a measure of molecular hydrophobicity. Hydrophobicity affects drug absorption, bioavailability, hydrophobic drug–receptor interactions, metabolism of molecules, as well as their toxicity. [b] DI, diameter of inhibition zone. [c] Parent compound: 5-bromo-2′-deoxyuridine.

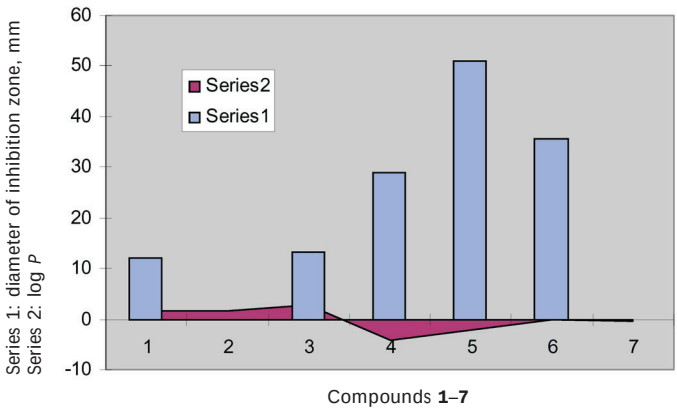


FIG. 2
Correlation between antiviral activity and log *P*

CONCLUSION

Based on semiempirical SCF quantum-chemical calculations on a series 3′,5′-amino acid and peptide esters of 5-bromo-2′-deoxyuridine a descriptor is determined which explains why some of them are active and other are not. Comparison of the log *P* values of the parent compound and the prodrug candidates shows that the cell membrane permeability is a decisive feature for the selection of appropriate prodrugs in this case. This finding could be a useful indication in the search of bioactive molecules similar to the ones reported here.

REFERENCES

1. Herrmann E. E.: *Proc. Soc. Exp. Biol. Med.* **1961**, *107*, 142.
2. Kaufmann H. E.: *Proc. Soc. Exp. Biol. Med.* **1962**, *109*, 251.
3. Aggarwal S., Gogu S., Rangan S. R. S., Agrawal C.: *J. Med. Chem.* **1990**, *33*, 1505.
4. Nguyen C., Kasinathan G., Leal-Cortijo I., Musso-Buendia A., Kaiser M., Brun R., Ruiz-Pérez L. M., Johansson N. G., González-Pacanowska D., Gilbert I. H.: *J. Med. Chem.* **2005**, *48*, 5942.
5. Stankova I., Simeonov M. F., Maximova V., Galabov A. S., Golovinsky E. V.: *Z. Naturforsch., C: Biosci.* **1999**, *54*, 75.
6. Zhivkova Zv., Stankova I.: *Int. J. Pharm.* **2000**, *200*, 181.
7. Le Questel J., Berthlot M., Laurence Ch.: *J. Chem. Soc., Perkin Trans. 2* **1997**, 2711.
8. Raczynska E. D.: *Anal. Chim. Acta* **1997**, *348*, 431.
9. Gawinecki R., Raczynska E. D., Rasala D., Styrcz S.: *Tetrahedron* **1997**, *53*, 17211.
10. Raczynska E. D.: *J. Chem. Res., Synop.* **1998**, 704.
11. http://www.cachesoftware.com/mopac/index.shtml
12. http://www.hyper.com/products/Professional/index.htm
13. Saenger W.: *Principles of Nucleic Acid Structure*. Springer-Verlag, New York 1984.
14. Cornell W. D., Cieplak P., Bayly C. I., Gould I. R., Merz K. M., Ferguson D. M., Spellmeyer D. C., Fox Th., Caldwell J. W., Kollman P. A.: *J. Am. Chem. Soc.* **1995**, *117*, 5179.
15. Weiner S. J., Kollman P. A., Case D. A., Singh U. C., Ghio C., Alagona G., Profeta S., Weiner P.: *J. Am. Chem. Soc.* **1984**, *106*, 765.
16. Weiner S. J., Kollman P. A., Nguyen D. T., Case D. A.: *J. Comput. Chem.* **1986**, *7*, 230.
17. Pearlman D. A., Case D. A., Caldwell J. W., Ross W. S, Cheatham T. E., DeBolt S., Ferguson D., Seibel G., Kollman P.: *Comput. Phys. Chem.* **1995**, *91*, 1.
18. Mulliken R. S.: *J. Chem. Phys.* **1955**, *23*, 1833.
19. Martin F., Zipse H.: *J. Comput. Chem.* **2005**, *26*, 97.
20. Ghose A. K., Pritchett A., Crippen G. M.: *J. Comput. Chem.* **1988**, *9*, 80.