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1′-Homocarbocyclic Nucleoside Analogues with an Optically Active Substituted Bicyclo[2.2.1]Heptane Scaffold †

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Abstract: An optically active bicyclo[2.2.0]heptane fragment was introduced in the molecule of new 1′-homonucleosides on a 2- 6-chloro-amino-purine scaffold to obtain 6-substituted carbocyclicnucleozide analogues as antiviral compounds. The synthesis was realized by a Mitsunobu reaction of the base with the corresponding bicyclo[2.2.0]heptane intermediate and then the nucleoside analogues were obtained by substitution of the 6-chlorime with selected pharmaceutically accepted amines. A molecular docking study of the compounds on influenza, HSV and low active Coronavirus was realized. Experimental screening of the compounds on the same viruses are developing and soon will be finished.

Keywords: bicyclo[2.2.0]heptane; 1′-homonucleoside; guanine; 2-amino-6-substituted purine; antiviral; influenza; herpes simplex virus; molecular docking

1. Introduction

Nucleosides are a recognized class of antiviral and anticancer drugs. The resistance acquired in time and the toxicity are the most factors which motivated the discovery of new more active and selective analogs. The modifications were realized on the nucleobase and/or on the sugar moiety. With guanine and 2-amino-6-substituted purine as nucleobase, recognized *carbocyclic nucleoside* drugs or active compounds studied in different clinical phases, like carbovir and its prodrug abacavir (with 6-cyclopropylamino substituent), entecavir, lobucavir, cyclohexenyl G, and also recognized *acyclic nucleosides* like acyclovir, ganciclovir, penciclovir and their valine esters valaciclovir, valganciclovir and Famciclovir became recognized are milestone compounds in the treatment of antiviral and anticancer deseases [1,2].

1′-Homonucleosides, due to the methylene group between nucleobase and sugar moiety, are structurally a class of compounds more closely to acyclicnucleosides than nucleosides. In this class there are also compounds with guanine, 6-chloro-2-aminopurine or 6-substituted-2-aminopurine as nucleobase with potential antiviral or anticancer activity (Figure 1):

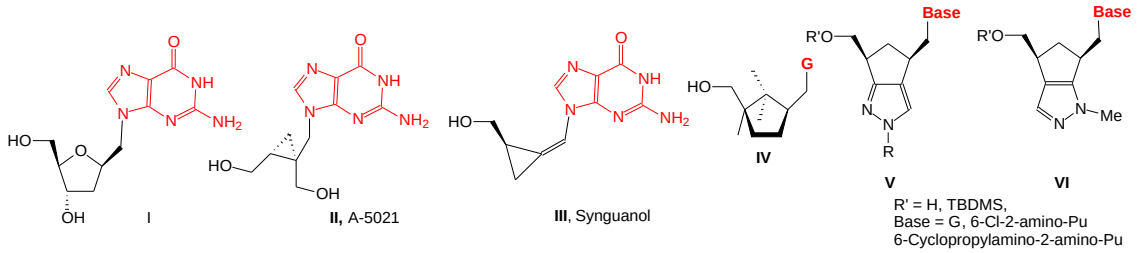


Figure 1. 1′Homonucleosides with guanine and 6-substituted-2-aminopurine as nucleobases.

Figure 1. and HSV-2), compound **II** has antiherpetic activity, compound **III** has activity against HCMV and EpsteinBarr virus, compound **IV**, with a 2,2,3-trimethylcyclopentanol, is active against HIV-1 and HIV-2, compound **V**, with a cyclopenta[*c*]pyrazole moiety, is very active against VZV/TK- strain.

Previously we used an optically active bicyclo[2.2.1]heptane moiety to obtain new L-type carbocyclic nucleosides, **VII** (Figure 2), and some of them presented antiviral activity against influenza virus or coxsackievirus B4 [3]. Then new HSV-1 1′-homocarbanucleoside analogs, **VIII**, were synthesized with nucleobase: U, 5-FU, T, C, Ad, 6-Cl-purine, 6-substituted purine and two compounds had lower IC_{50} (15 ± 2 and 21 ± 4 μM) and one equal to that of acyclovir (IC50: 28 ± 4 μM). In the present paper we present the synthesis, molecular docking study and antiviral activity of a number of compounds **VIII**, in which base is 2-amino-6-chloropurine, guanine, 2,6-diaminopurine and 2-amino-6-substitutedpurine.

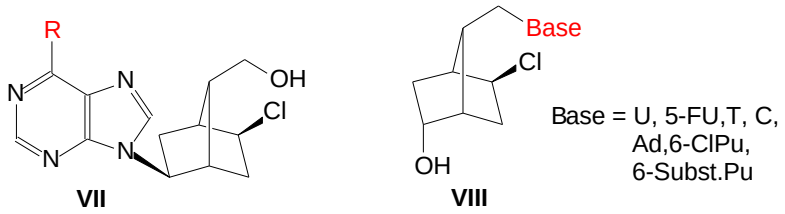
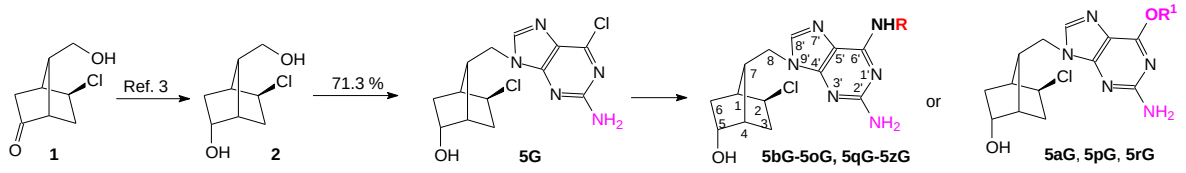


Figure 2. L-Carbanucleosides and 1′-Homocarbanucleosides with a bicyclo[2.2.1]heptane skeleton.

2. Results

Synthesis of the new 1′-Homocarbanucleosides with an optically active bicyclo[2.2.1]heptane fragment as sugar moiety and guanine, 6-O-alkyl-guanine, 2,6-diaminopurine, 2-amino-6-N-substitutedpurine as nucleobase started from the diol **2**, obtained crystallized by $NaBH_4$ reduction of the keto-compound **1** [3]. The unprotected diol **2** was used in the Mitsunobu reaction with 2-amino-6-chloropurine, taking into account that the primary alcohol will react more quickly than the secondary one. Indeed, the primary alcohol reacted selectively to give the key nucleoside intermediate **5G** isolated by simple crystallization in 71.3 % yield (Scheme 1). For comparison, 6-chloropurine reacted with diol **2** in the same Mitsunobu reaction in 67.6 % yield [4,5].



Scheme 1. Synthesis of new 1′-homocarbanucleoside analogues with guanine and 2-amino-6-substituted purine as nucleobase.

The following 1′-homocarbocyclic nucleosides were synthesized by substitution of the chlorine atom with ammonia, with primary or secondary pharmacological amines, with methoxide or ethoxide or by substitution with hydroxyl (acid hydrolysis) to the guanine, all in good yield [6]. 24

New compounds were obtained, fully characterized and used for antiviral screening against influenza, herpex simples virus and low active coronavirus.

Molecular docking study has been realized using CLC Drug Discovery Workbench Software, on *24 compounds* to obtained accurate predictions about structure and interactions of the studied compounds in complex with a protein/enzyme receptor to evaluate the biological activity.

In these study it have been used some proteins/enzymes receptors who have been imported from protein data bank (http://www.rcsb.org/:PDB):

Herpes simplex type-1 thymidine kinase (PDB ID 2KI5).
Wild-type influenza N2 neuraminidase (PDB ID 4H52).
SARS coronavirus main protease (PDB ID:3TNT).

Docking evaluation against Herpes simplex type-1 thymidine kinase. Docking studies have been performed to achieve accurate predictions on the optimized conformations for both ligand and protein target to form a stable complex. The all ligands were docked on the crystal structure of the *Herpes simplex type-1 thymidine kinase* (PDB ID: 2KI5). The docking pose of the co-crystallized AC2 interacting with amino acid residues of the active site is shown in Figure 3. The oseltamivir and ribavirin have been taken as reference ligands to compare the docking results of the all studied compounds. The docking studies revealed that the **5jG** compound has the best docking score −66.42 (RMSD: 0.55) (Table 1, Figure 12). The docking pose of the **5jG** compound is shown in Figure 4.

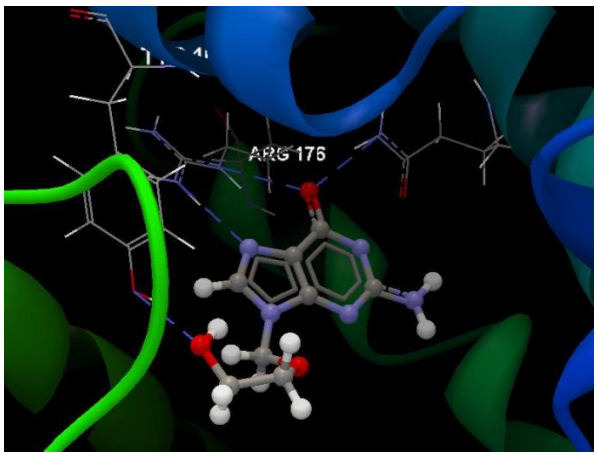


Figure 3. Hydrogen bond (blue dotted lines) between AC2 and amino acids residues from binding site of 2KI5**.**

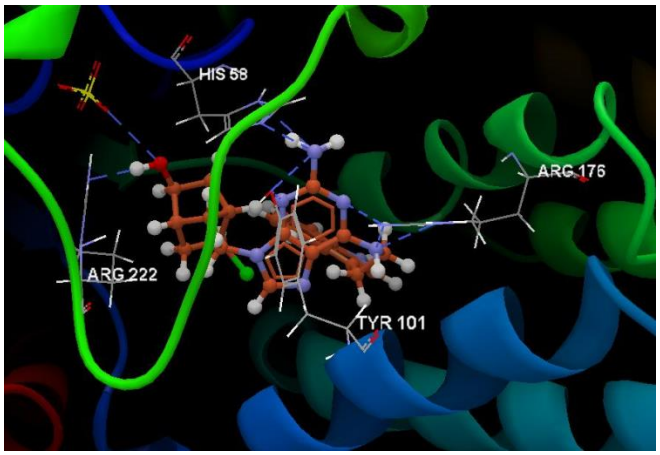


Figure 4. Hydrogen bond (blue dotted lines) between 5jG compound and amino acids residues from binding site of 2KI5**.**

After analyzing the data obtained from the docking study, it was observed that the all compounds were placed in the same binding site of 2KI5 as the co-crystallized (Figure 5).

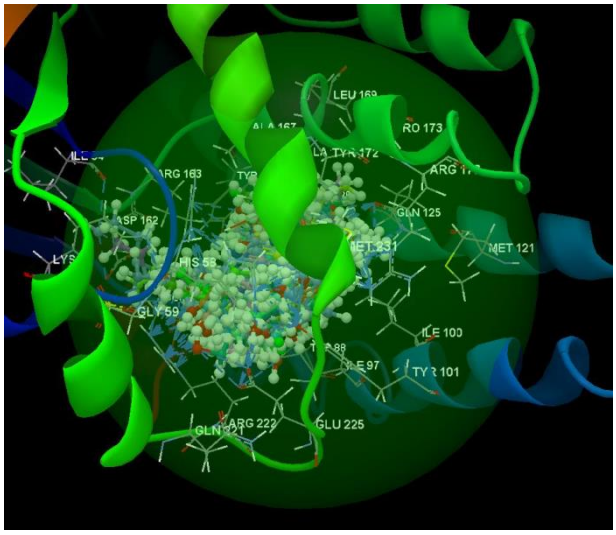


Figure 5. Docking pose of the co-crystallized AC2, of the oseltamivir, ribavirin and of the studiedcompounds in the binding site of 2KI5**.**

Docking evaluation against Wild-type influenza N2 neuraminidase**.** Docking studies have been performed to achieve accurate predictions on the optimized conformations for both ligand and protein target to form a stable complex. All ligands wer docked on the crystal structure of the *Wild-type influenza N2 neuraminidase* (PDB ID: 4H52). The docking pose of the co-crystallized **FSI A 508** interacting with amino acid residues of the active site is shown in Figure 6. Oseltamivir and ribavirin have been taken as reference ligands to compare the docking results of the all studied compounds. The docking studies revealed that the **5kG** compound has the best docking score −67.57 (RMSD: 0.58) (Table 1, Figure 12). The docking pose of the **5kG** compound is shown in Figure 7.

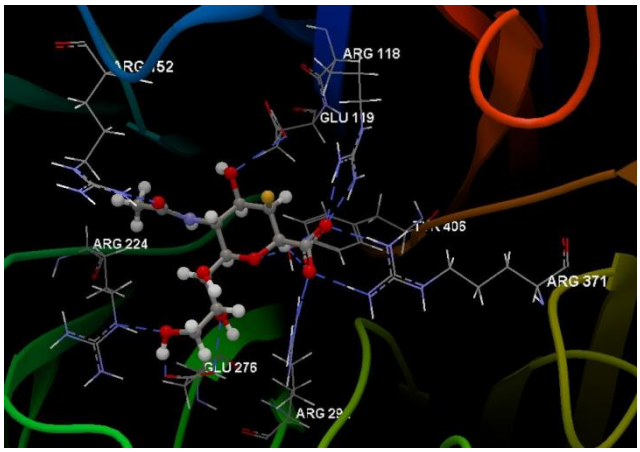


Figure 6. Hydrogen bond (blue dotted lines) between FSI A 508 and amino acids residues from binding site of 4H52**.**

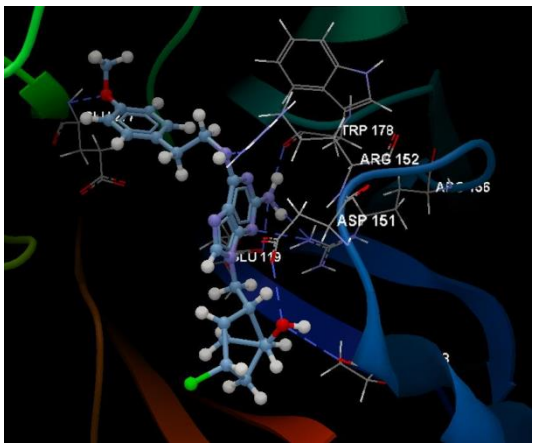


Figure 7. Hydrogen bond (blue dotted lines) between 5kG compound and amino acids residues from binding site of 4H52**.**

After analyzing the data obtained from the docking study, it was observed that the all compounds were placed in the same binding site of 4H52 as the co-crystallized (Figure 8).

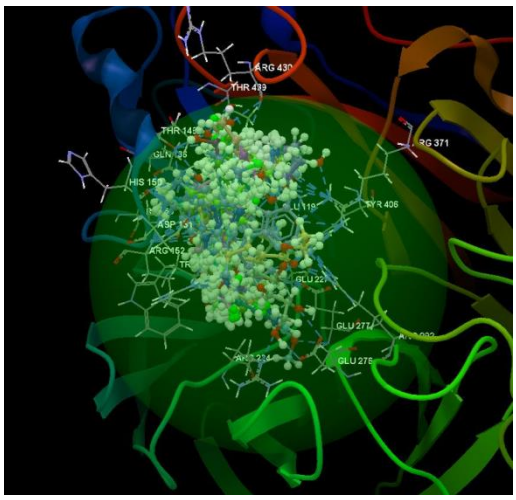


Figure 8. Docking pose of the co-crystallized **FSI A 508**, of the oseltamivir, ribavirin and of the **studied** compounds in the binding site of 4H52.

Docking evaluation against SARS coronavirus main protease. Docking studies have been performed to achieve accurate predictions on the optimized conformations for both ligand and protein target to form a stable complex. All ligands were docked on the crystal structure of the *SARS coronavirus main protease* (PDB ID: 3TNT). The docking pose of the co-crystallized **G85A 501** interacting with amino acid residues of the active site is shown in Figure 9. Oseltamivir and ribavirin have been taken as reference ligands to compare the docking results of the all studied compounds. The docking studies revealed that the **5jjG** compound has the best docking score −81.11 (RMSD: 1.90) (Table 1, Figure 12). The docking pose of the **5jjG** compound is shown in Figure 10.

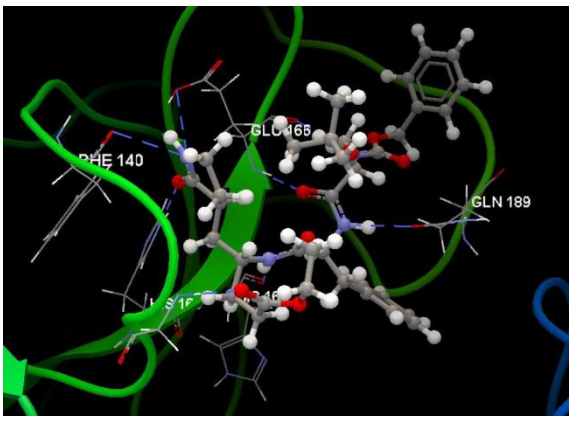


Figure 9. Hydrogen bond (blue dotted lines) between G85A 501and amino acids residues from binding site of 3TNT.

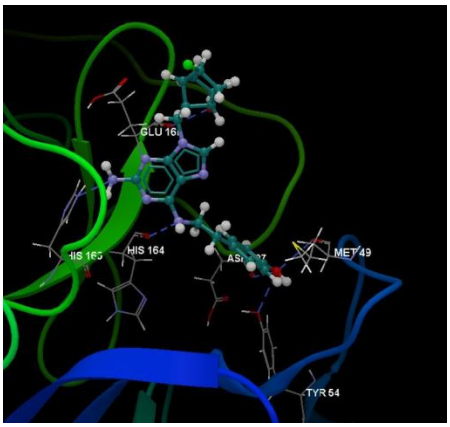


Figure 10. Hydrogen bond (blue dotted lines) between 5jjG compound and amino acids residues from binding site of 3TNT.

After analyzing the data obtained from the docking study, it was observed that the all compounds were placed in the same binding site of 3TNT as the co-crystallized (Figure 11).

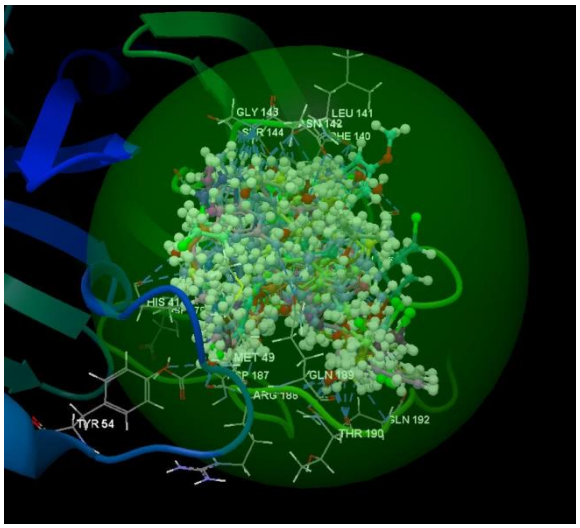


Figure 11. Docking pose of the co-crystallized **G85A 501**, of the oseltamivir, ribavirin and of the **studied** compounds in the binding site of 3TNT.

Table 1. Docking score of ligands.

Ligand	PDB ID: 2KI5		PDB ID: 4H52		PDB ID: 3TNT	
	Score	RMSD (Å)	Score	RMSD (Å)	Score	RMSD (Å)
Co-crystallized	−49.29	0.71	−49.94	0.21	−82.61	2.41
Oseltamivir	−46.52	0.02	−54.44	0.62	−48.75	1.96
Ribavirin	−47.48	0.009	−49.66	1.02	−44.73	0.47
5G	−56.24	0.02	−51.08	0.27	−48.31	0.02
5aG	−63.14	0.01	−42.49	0.01	−48.31	0.04
5bG	−60.18	0.01	−48.23	0.19	−51.18	0.14
5cG	−50.98	0.05	−47.88	0.87	−59.63	0.28
5dG	−35.83	0.03	−54.81	0.72	−57.15	0.15
5eG	−43.80	0.23	−50.95	1.04	−60.92	0.35
5fG	−27.82	0.02	−46.04	0.28	−60.85	0.08
5gG	−13.47	0.18	−46.27	0.10	−62.98	0.50
5hG	+1.06	0.03	−45.50	1.72	−65.84	0.04
5iG	−65.51	0.19	−64.95	0.28	−64.07	1.46
5jG	**−66.42**	0.55	−51.86	0.79	−75.00	0.77
5jjG	−61.71	0.04	−57.33	1.37	**−81.11**	1.90
5kG	−59.01	0.60	**−67.57**	0.58	−65.73	0.55
5lG	−51.11	1.16	−54.69	1.14	−73.07	0.29
5mG	−65.46	0.45	−59.33	0.78	−62.74	1.21
5nG	−65.07	0.05	−59.18	0.96	−63.65	0.50
5oG	+1.37	0.04	−50.88	0.19	−62.38	0.45
5pG	−56.25	0.007	−41.01	0.25	−48.16	0.02
5rG	−53.58	0.22	−48.02	0.09	−53.66	0.06
5qG	−51.17	0.06	−46.27	0.10	−61.64	0.22
5sG	−58.31	0.02	−50.72	0.99	−62.51	0.54
5tG	−54.19	0.04	−55.52	0.80	−67.67	0.75
5uG	−58.66	0.13	−53.11	0.03	−68.96	0.42
5zG	−56.23	0.07	−42.23	0.26	−56.91	0.16

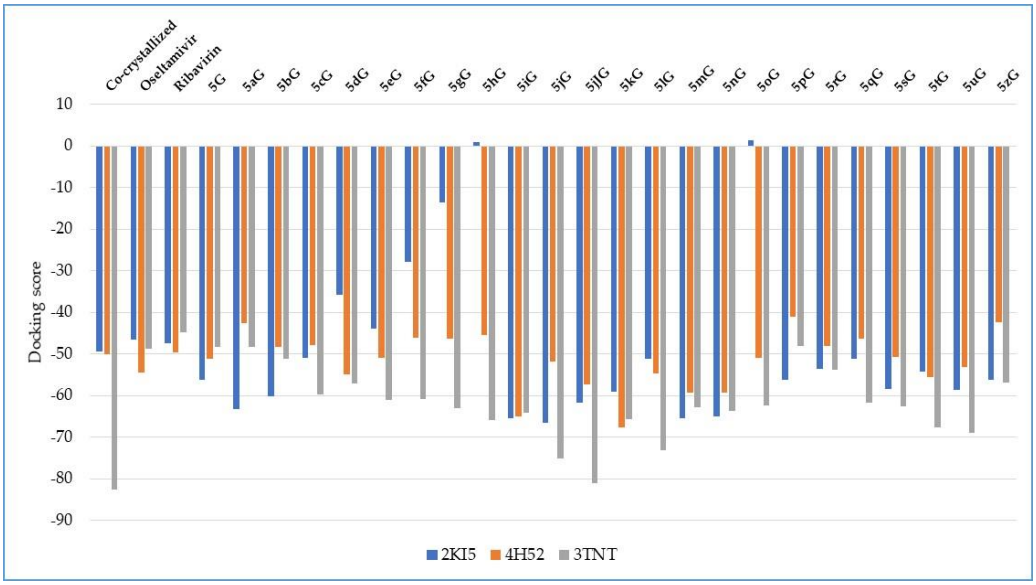


Figure 12. Docking score of the compounds, comparative with the docking score of the co-crystallized and with the docking score of the reference drugs oseltamivir and ribavirin.

Important molecular properties: molecular weight, flexible bonds, the number of hydrogen bond donors, the number of hydrogen bond acceptors and log P have been calculated (Table 2). These parameters can predict if a molecule possesses properties that might turn it into an oral active drug, according to the Lipinski's rule of five. The number of violations of the Lipinski rules allows to evaluate drug likeness for a molecule. According to the data presented in Table 2, only **5lG** compound failed respect the Lipinski Rules (Lipinski violation is 1, hydrogen bond donors >5).

Table 2. Calculated properties of ligands.

Compound	Atoms	Weight (Daltons)	Flexible Bonds	Lipinski Violations	Hydrogen Donors	Hydrogen Acceptors	Log P
AC2 A *	26	224.20	4	0	3	8	0.50
FSI A 508 **	38	310.25	5	0	5	9	−1.72
G85A 501 ***	95	652.78	20	2	4	12	3.69
Oseltamivir	50	312.40	8	0	3	6	1.70
Ribavirin	29	244.20	3	0	5	9	−3.05
5G	36	328.20	2	0	3	6	1.67
5aG	37	309.75	2	0	4	7	0.69
5bG	38	308.77	2	0	5	7	0.36
5cG	45	348.83	4	0	4	7	1.58
5dG	51	376.88	4	0	4	7	2.30
5eG	54	390.91	4	0	4	7	2.84
5fG	49	378.86	3	0	3	8	0.79
5gG	53	391.90	3	0	3	8	0.98
5hG	56	405.92	4	0	3	8	1.34
5iG	54	412.92	6	0	4	7	2.98
5jG	58	451.95	6	0	5	8	3.11
5jJG	55	428.92	6	0	5	8	2.63
5kG	58	442.94	7	0	4	8	2.95
5lG	56	444.91	6	1	6	9	2.27
5mG	54	416.91	7	0	4	9	1.10
5nG	56	405.92	6	0	4	8	1.55
5oG	58	420.94	5	0	5	9	0.04
5pG	40	323.78	3	0	3	7	1.01
5rG	43	337.80	4	0	3	7	1.38
5qG	58	442.94	7	0	5	8	2.36
5sG	50	399.88	5	0	4	8	1.49
5tG	50	399.88	5	0	4	8	1.45
5uG	50	399.88	5	0	4	8	1.45
5zG	44	336.82	3	0	3	7	1.17

* PDB ID: 2KI5; ** PDB IB: 4H52; *** PDB ID: 3TNT.

The compounds were screened against influenza virus and two compounds, **5fG** and **5gG**, had SI of 25 and 23, (IC_{50} = 8 μM and 12.8 μM) and two SI of 10 (**5kG**, IC_{50} = 24 μM and **5sG**, IC_{50} = 29 μM). The screening of the compounds against HSV and low active Coronavirus are developing and soon will be finished.

In conclusion, a number of 24 new 1'-homonucleoside with a 2-amino-6-substituted purine as nucleobase and an optically active bicyclo[2.2.0]heptane scafold were synthesized, a molecular docking study on three viruses and an experimental screening of the compounds against influenza virus were realized.

3. Patents

Tanase, C.; Pintilie, L. New 1′-homocarbanucleoside analogs with a constrained bicyclo[2.2.0]heptane fragment and 2-amino-6-substituted purine as nucleobase. Patent request A/00290/27.05.2020.

Author Contributions: Conceptualization, C.T.; methodology, C.T.; molecular docking L.P.; IR analysis, M.M.; NMR spectroscopy, C.D. and A.H.; antiviral screening, V.V.Z, A.V. and E.S.; writing—original draft preparation, C.T. and L.P. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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6. Tanase, C.; Pintilie, L. New 1′-homocarbanucleoside Analogs with a Constrained bicyclo[2.2.0]heptane Fragment and 2-amino-6-substituted Purine as Nucleobase. Patent request A/00290/27.05. 2020.