RESEARCH ARTICLE

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The Synthesis and Docking Study of Novel Compounds for Variola Virus

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ABSTRACT

Three novel compounds, namely, 4-amino-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one SG-3, 3-methyl-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-(2H)-ylamino)butanoic acid SG-4 and 2-(4-oxo-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-2-phenylacetic acid SG-6 were synthesized from easily available, inexpensive, environmentally friendly starting materials (glucose, amino acid and thiobarbutric acid) and characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. the molecular modelling of these compounds was studied in Crystal structure of vaccinia virus thymidylate kinase , for the treatment of variola. Their binding motifs and drug-like properties were investigated. Results show that all compounds confirmed appropriate binding free energies; between –9.71 and –10.11 Kcal/mol . Since the novel molecules have high ligand-receptor binding interactions, they can be a powerful alternative to FDA approved drug Cidofovir.

Keywords: amino acids, glucose, molecular docking, multi component reaction, thiobarbutric acid, virola virus.

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I. Introduction

The variola virus is one of the DNA virus that causes smallpox [1]. The transmission ways of the virus between humans can be through respiratory droplets, cutaneous lesions, infected body fluids, or fomites [2]. After 12 days incubation period [3], the eruptive stage begins especially on the face and the extremities of the limbs after 3-4 days [4]. Secondary infections accompanying the disease lead to fatal complications [5]. The smallpox mortality rates are between 30% and 40%. Humanity's struggle with smallpox has continued almost throughout human history. Although the earliest books about smallpox were found in China (4th century), India (7th century), and Asia (10th century) [6], the history of smallpox disease dates back to Egypt mummies. Smallpox was eradicated in 1980 by the public vaccination campaigns organized by World Health Organization (WHO) [7]. However, there is always the danger of the virus to return due to inequalities in access to vaccines and vaccine hesitancy across the world. In addition, after the cessation of vaccination, the unvaccinated young population rapidly increased, and the protective immunity in previously vaccinated people decreased [8]. Factors such as climate changes, increased international mobility, and profound demographic shift may cause virus-induced pandemics to start again at any time. Pathogenic viruses can also be used as weapons by bioterrorism. Since people with immunosuppressive diseases, skin diseases, and those used organ transplantation medications, pregnant women and heart patients are not suitable for vaccination, the discovery of drugs that can be used in a possible smallpox pandemic is very important research area in chemistry and pharmacy.

There are two U.S. Food and Drug Administration (FDA) -approved drugs for the treatment of smallpox; Cidofovir or CDV-Vistide (an acyclic nucleoside phosphonate) and Tecovirimat. CDV inhibits poxvirus replication in cell culture by interfering with viral DNA polymerase and is active in lethal poxvirus challenge models in mice and monkeys [9]. CDV has limited use in an emergency because it must be administered by intravenous infusion and it shows a high level of nephrotoxicity in humans [10]. The dose selection of Tecovirimat could not be detected because of the absence of efficacy data in humans [11]. In addition to these drugs, there are several drug candidates with some advantages and disadvantages are described in the literature [12]-[26].

An ideal drug should have some properties such as it can be orally administered, safe for special populations (i.e., children, immunocompromised individuals, etc.), inexpensive to allow for large stockpiles, and stable over long periods even under adverse conditions [27].

Here we reported the synthesis and docking study of three novel molecules, 4-amino-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one, 3-methyl-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-(2H)-ylamino)butanoic acid and 2-(4-oxo-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-2-phenylacetic acid which can be valuable alternatives to present drugs.

The molecular skeleton of novel molecules consists of three main parts: amino acid, glucose and thiobarbutric acid moiety (Fig. 1).

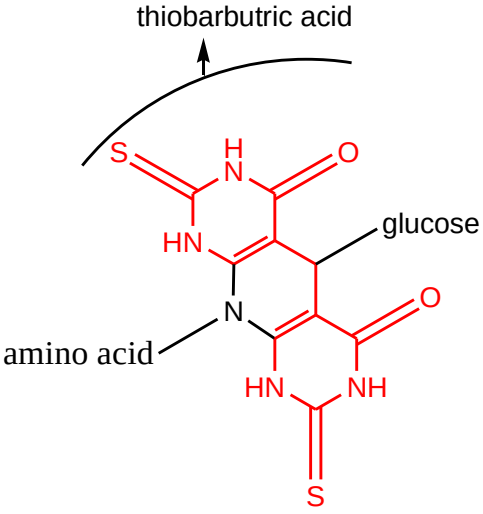


Fig. 1. General structure of molecules.

Carbohydrates are well-known starting material due to their several key characteristics including, availability, highly water-solubility and optical activity. Carbohydrate derivatives have been used in medicinal chemistry as antibiotics, antiviral drugs, protein glycosylation and glycosylation inhibitors [28].

Amino acids are the building blocks of proteins. Functional groups of amino acids can be modified by convenient chemistry [29]-[38]. Drugs with amino acids moieties have some unique properties such as high bioavailability, low toxicity, decrease fast metabolism.

The thiobarbutric acid moiety can be found in antibacterial [39], antiviral [40], anticancer [41], anticonvultstant [42], antifungal [43] drugs.

II. Experimental

A. Materials and Instruments

All reagents and solvents were of commercial origin and used without further purification unless otherwise noted. The 1H NMR spectra were recorded with Bruker Ultra Shield Plus ultra-long-hold-time spectrometer using DMSO-d_6 as the solvent. All chemical shifts are given in ppm relative to tetramethylsilane (TMS) standard. Mass spectrums are taken from Waters SYNAPT G1 MS at (ESI-TOF-MS) mode.

B. Chemical Syntheses

A mixture of thiobarbituric acid (2 mmol), glucose (1 mmol), amino acid (1 mmol), and p-toluenesulfonic acid (0.1 g) in ethanol (5 mL) was stirred for 24 h at 50 °C. Completion of the reaction was confirmed by TLC (eluent EtOAc/MeOH). Then the reaction mixture was cooled to room temperature. Solid was filtered and the remaining washed with ethanol (3 x 5 mL). Removal of the solvent under reduced pressure gave almost pure products which were further purified by recrystallization from EtOH.

SG-3: 4-amino-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one: ^{1}H NMR (DMSO-d_6, 600 MHz;δ, ppm): 12.17, s, 2H, 12.02, s, 7H, 5.7, d, 1H, J= 3.33 Hz, 4.8, s, 1H, 4.3, d, 1H, J= 3.3 Hz, 4.0, s, 1H, 3.8, d, 1H, J= 8.5 Hz, 3.6, t, 1H, 2.3, d, 1H, J= 4.3 Hz, 2.0, s, 2H, 1.3, s, 3H, 1.2, s, 3H. 13C NMR (DMSO-d_6, 150MHz;δ, ppm): 206, 181, 175, 166, 162, 110, 104, 85, 82, 80, 73, 68, 64, 31, 27, 26. MS: m/z: 547 (M^+). Mp: 245-247 °C.

SG-4: 3-methyl-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-(2H)-ylamino)butanoic acid :^{1}H NMR (DMSO-d_6, 600 MHz;δ, ppm): 12.1, s, 6H, 5.7, d, 1H, J= 3.42 Hz, 4.8, s, 1H, 4.3, d, 1H, J= 3.3 Hz, 4.2, m, 1H, 4.0, s, 1H, 3.8, d, 1H, J= 8.5 Hz, 3.6, m, 1H, 3.53, s, 1H, 3.5 m, 2H, 2.0, s, 2H, 1.3, s, 3H, 1.2, s, 3H. ^{13}C NMR (DMSO-d_6, 150MHz;δ, ppm): 206, 175, 166, 162, 110, 104, 85, 82, 80, 73, 68, 64, 31, 27, 26. MS: m/z: 531 (M^+). Mp: 164-165°C. Orange solid. 45% yield.

SG-6: 2-(4-oxo-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-2-phenylacetic acid :^{1}H NMR (DMSO-d_6, 600 MHz;δ, ppm): 12.1, s, 4H, 5.7, d, 1H, J= 3.33 Hz, 4.8, s, 1H, 4.3, d, 1H, J= 3.3 Hz, 4.0, s, 1H, 3.8, d, 1H, J= 8.5 Hz, 3.6, t, 1H, 2.3, d, 1H, J= 4.3 Hz, 2.0, s, 2H, 1.3, s, 3H, 1.2, s, 3H. ^{13}C NMR (DMSO-d_6, 150MHz;δ, ppm): 206, 178, 173, 163, 162, 160, 157, 154, 147, 144, 134, 133, 124, 119, 118, 116, 115, 114, 111, 104, 96, 85, 80, 73, 68, 64, 56, 55, 31, 27. MS: m/z: 489 (M^+). Mp: 231-232 °C. Dark pink solid. 54% yield.

C. MolecularDocking Studies

Antiviral effects of these novel compounds have been investigated with molecular docking studies and the best-docked poses of the molecules have been thoroughly evaluated. The best binding affinity and receptor-ligand interaction of every compound have been assessed and well- established good interactions of compounds withinside the receptor's active pocket of the target receptor proteins have been demonstrated in Table I. Based on the previous research of similar structures, we have decided to look for possible binding motifs for smallpox virus to investigate their anti-viral activity for variola [44]. The results were compared with FDA approved cidofovir.

III. Results and Discussion

Three novel molecules, SG-3, SG-4 and SG-5, were synthesized by a multicomponent reaction (MCR) involving glucose, different amino acids and thiobarbutric acid. Here envorimentally friendly starting materials were converted to polyhydroxy compounds incorporating pyrimidine-fused heterocycles (PFHs).

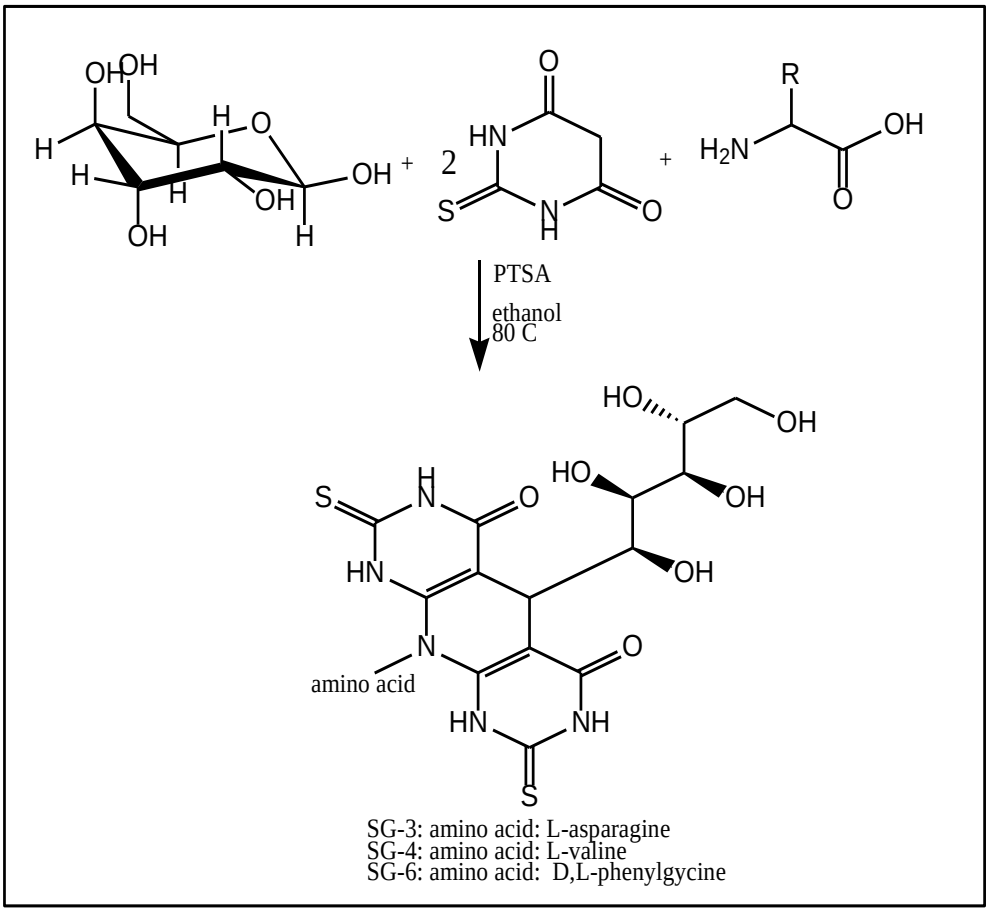
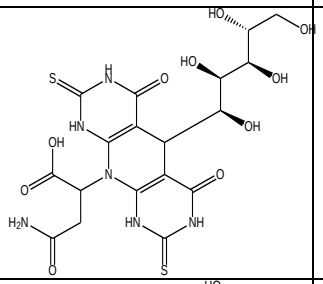
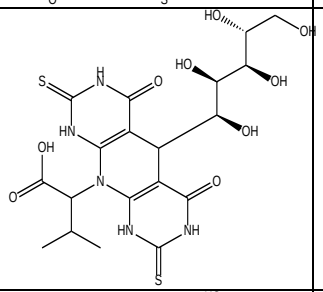
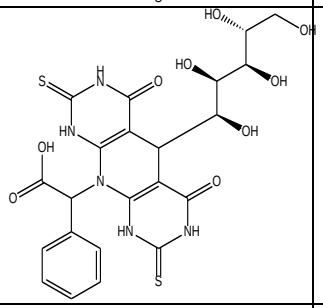
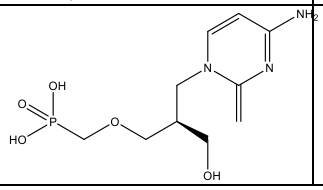


Fig. 2. The general synthesis route.

A. MolecularDocking Studies

Antiviral effects of novel compounds have been investigated with molecular docking studies and the best-docked poses of the molecules have been thoroughly evaluated. The best binding affinity and receptor-ligand interaction of every compound have been assessed and well- established good interactions of compounds with inside the receptor's active pocket of the target receptor proteins have been demonstrated in Table I. Based on the previous research of similar structures, we have decided to look for possible binding motifs for smallpox virus to investigate their anti-viral activity for variola [44].The results were compared with FDA approved Cidofovir.

TABLE I: MOLECULAR DOCKING RESULTS

Comp No.	Structures	Docked aminoacid residues (vdW interactions)	Energy Score	RMSD Value	H bond (distance Å)
SG-3		THR18, ARG41, GLU142	-9.85	0.45	O of OH with NH of LYS14 (2.081) H of NH with Carbonyl of ASP92 (2.167) O of Carbonyl with NH of ARG93 (1.91)
SG-4		THR18, ARG41, GLU145	-9.71	0.99	O of Carbonyl with NH of ARG93 (1.746)
SG-6		ARG41, LEU53, GLU142	-10.11	0.64	O of OH with NH of ARG93 (2.214) H of OH with O of SER97 (2.144) H of NH with O of PRO39 (1.912)
Cidofovir		ARG41, LEU53, ARG72,	-10.51	1.18	H of OH with Carbonyl of ASP13 (1.928) O of OH with NH of LYS17 (1.958) O of OH with NH of ARG93 (2.058)

Since the lowest negative binding energy values indicate the most powerful binding capability of the ligand withinside the target, the conformation with the reasonably low docking energy scores is chosen. All the compounds confirmed appropriate binding free energies towards vaccinia virus thymidylate kinase, which have been acquired in a selection between –9.71 and –10.11 Kcal/mol.

As shown in the Fig. 3-4, compounds bonded to the active site and overlapped with reference compounds. Our preliminary results showed that these compounds demonstrate reasonably good ligand-receptor binding interactions.

Compound SG-6 has the lowest binding energy score with the small RMSD score in target. It also showed strong hydrogen bonds with similar amino acid residues as explained in the tables. Figures Show that active conformations of each compound bonded active site and overlapped with each other. The results verified these compounds have high ligand-receptor binding interactions.

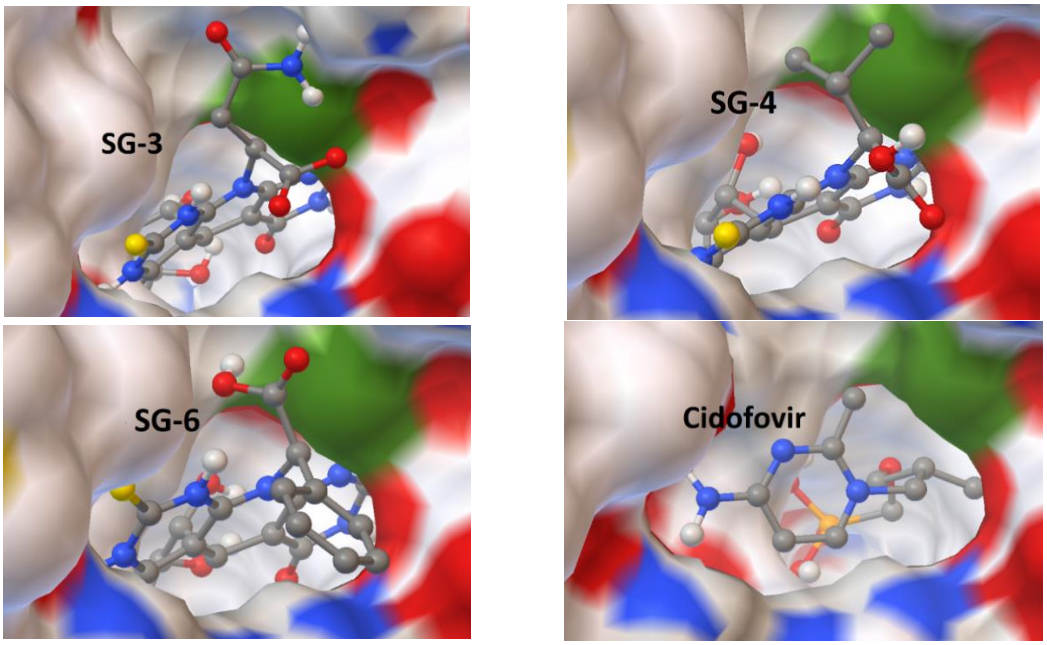


Fig. 3. Interaction of the best-docked poses of compounds SG-3, SG-4, SG-6 and reference drug Cidofovir to 2V54 target.

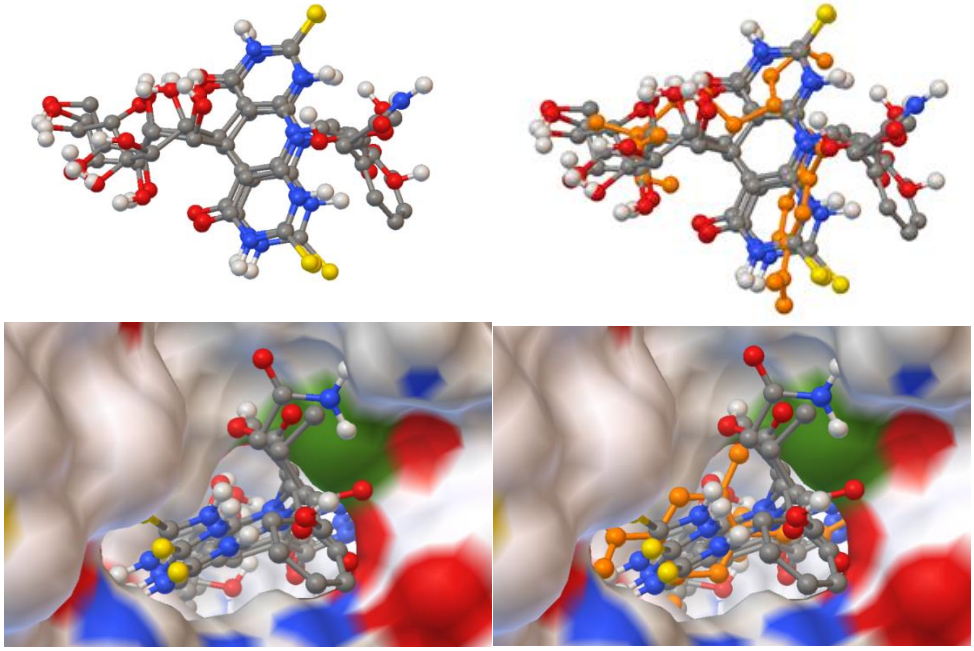


Fig. 4. Superimposing poses of the best scored compounds with and without reference drug Cidofovir against 2V54.

B. Drug-Like Properties

Swiss ADME Calculation program was used to determine the structure-activity relationships of compounds and drug-likeness rankings. The molecular weight, logP, TPSA, crossing the BBB, GI absorption properties and sort of CYPP450 inhibition type of compounds are provided in Table II.

TABLE II: DRUG LİKENESS OF SG COMPOUNDS

Comp. No	MW (g/mol)[a]	LogP [b]	TPSA [c]	BBB [d]	GI Abs.[e]	Type of CYP Inh.[f]	Rule of Five[g]
SG-3	548.55	-2.77	356.06	No	Low	None	No
SG-4	533.58	-1.23	321.97	No	Low	None	No
SG-6	567.59	-1.25	321.97	No	Low	None	No
Cidofovir	279.19	-2.11	157.71	No	Low	None	Yes
Brincidofovir	561.69	4.16	155.94	No	Low	CYP2C19	Yes

Drug-like properties of **SG compounds** were calculated by Swiss ADME online software program

[a]Molecular weight (recommended value <500).

[b] Logarithm of the partition coefficient of the compound between n-octanol and water (recommended value <5).

[c] Polar surface area (recommended value ≤140Å^2).

[d] Indicates whether the compound pass blod Brain Barrier or not.

[e] Degree of Gastrointestinal Absorption.

[f] Represent the ınhıbıtıon of CYP450 subtypes.

[g] Indicates whether the compound obeys Lipinski's Rule of Five or not.

IV. CONCLUSIONS

The last pandemic showed us that we must always be equipped against new virus outbreaks. Viruses that have not been seen for a long time may reappear, or some forces may use them as bioweapons. Smallpox is a deadly viral disease that humanity has struggled with throughout history. Due to effective vaccination it has not been seen since 1980. However, despite the possibility of a new pandemic at any moment, new drugs have been designed and synthesized by taking into account the people who cannot use the vaccine.

Three novel compounds, namely, 4-amino-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one SG-3, 3-methyl-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-(2H)-ylamino)butanoic acid SG-4 and 2-(4-oxo-5-(2,3,4,5,6-pentahydroxy-1-(4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-yl)hexyl)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one-2-phenylacetic acid SG-6 were synthesized, characterized and their docking calculations were performed in this research. Easily available, inexpensive, environmentally friendly starting materials (glucose, amino acid and thiobarbutric acid) were converted target molecules with moderate chemical yields by MCR.

In order to determine the antiviral activity of compounds, the in vivo study could not be performed because smallpox was eradicated and, virus strains have only been kept in two repositories: the State Research Centre for Virology and Biotechnology VECTOR, Koltsovo, Russian Federation, and the Centers for Disease Control and Prevention (CDC), Atlanta, United States, so in-silico work was preferred.

Results show that all compounds confirmed appropriate binding free energies towards vaccinia virus thymidylate kinase, between –9.71 and –10.11 Kcal/mol. The highest value was observed for SG-6. The comparison of these values with Cidofovir (-10.51Kcal/mol) concluded that the novel molecules have high ligand-receptor binding interactions. These molecules can be a powerful alternative to FDA approved drug Cidofovir.

The high binding constants demonstrated by these three novel compounds have opened up a valuable avenue in the synthesis of new drugs that can be used in the treatment of smallpox. The proposed drug structure can be modified by using different sugars and different amino acids.

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