Novel Synthesis and Antiviral Evaluation of New Benzothiazole-Bearing N-Sulfonamide 2-Pyridone Derivatives as USP7 Enzyme Inhibitors

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ABSTRACT: In this article, a series of benzothiazole-bearing N-sulfonamide 2-pyridone derivatives were synthesized via the reaction of benzothiazole sulfonylhydrazide with sodium salts of both (hydroxymethylene) cycloalkanones and unsaturated ketones, as well as ethoxymethylene derivatives. The structures of the resultant compounds were confirmed using IR, 1H NMR, 13C NMR, 1H−1H correlation spectroscopy (COSY), 1H−13C heteronuclear multiple bond coherence (HMBC), and 1H−13C heteronuclear multiple quantum coherence (HSQC) spectral analysis and elemental analysis. The newly synthesized compounds were evaluated in vitro for their antiviral activities against the HSV-1, HAV HM175, HCVcc genotype 4, CBV4, and HAdV7 viruses. Additionally, the compounds were examined for their cytotoxic effect on five normal cell lines. It was observed that five compounds were found to possess viral reduction of 50% or more against CBV4 with significant IC50, CC50, and SI values. In the case of HSV-1 and HAV HM175 viruses, three compounds have shown more than 50% reduction, while in the case of HCVcc genotype 4 and HAdV7 viruses, only two compounds demonstrated more than 50% reduction. Furthermore, the physicochemical properties of the most active compounds were evaluated. The two most potent compounds against HSV-1 virus, 7e and 13a, were evaluated for their inhibitory activity against USP7. Docking studies using Molecular Operating Environment (MOE) were used to identify the interactions between 7e and 13a compounds and the active site of the USP7 enzyme.

INTRODUCTION

Having no specific treatment or vaccine for the majority of viral infections is a major public health threat worldwide, and discovering new drugs remains a demanding task.1 Among the wide variety of heterocycles, compounds containing a 2-pyridone or benzothiazole ring have attracted great attention in the research area of synthetic and pharmaceutical chemistry due to the large number of biologically active molecules containing these structures.2,3 Some natural compounds containing a 2-pyridone ring have demonstrated potency as antitumor and antiviral agents such as Fredericamycin A and camptothecin (CTP), Figure 1.4,5 Furthermore, it was also found that compound ABT-719 has broad-spectrum activity against various quinolone-resistant Gram-negative bacteria including anaerobes and Gram-positive bacteria including methicillin-resistant Staphylococcus aureus, Figure 1.6 Moreover, synthetic compounds based on the 2-pyridinone ring have been reported to possess varieties of biological activities, such as antimicrobial,7−10 antiviral,10−12 anticancer,11,12 cardiotonic,12 antituberculosis,13 and antidiabetic.14 Synthesis of the 2-pyridone ring can be achieved by two main synthetic approaches. The first approach is based on other heterocycle systems, and the second approach is via condensation of acyclic systems followed by cyclization. The latter is the most general approach for the synthesis of substituted 2-pyridone derivatives, which often includes Michael addition as the key step.2,15
via the condensation of an ortho-amino thiophenol with a substituted aromatic aldehyde, carboxylic acid, acyl chloride or nitrile. Encouraged by the above findings and based on our experiences in the synthesis of 2-pyridone and benzothiazole derivatives, our target was to synthesize new compounds having both benzothiazole and 2-pyridone rings substituted with an arylsulfonamide moiety at position 2 in a single molecular framework and to then investigate the antiviral and enzyme inhibition activities of the newly synthesized compounds.

**RESULTS AND DISCUSSIONS**

**Chemistry.** Novel arylsulfonohydrazides 4a–c were used as starting compounds to synthesize different derivatives of N-aryl sulfonylpyridones incorporating a benzothiazole moiety. First, acetohydrazide 2 was synthesized through the reaction of benzothiazole acetate 1 with hydrazine hydrate at room temperature. Compounds 4a–c were then formed in high yield via the sulfonation of acetohydrazide 2 with arylsulfon chloride 3a–c in pyridine at room temperature, Scheme 1. An X-ray single-crystal structure of the sulfonyl hydrazide compound 4c confirmed its proposed structure.

The reaction of N-arylsulfonylhydrazides 4a–c with the sodium salt of 2-(hydroxymethylene)-1-cycloalkanones 5a,b in piperidine acetate resulted in the formation of compounds 7a–f, through the formation of intermediates 6a–f, in good yield, Scheme 1. It was noticed that the yields of compounds containing 2-pyridone fused with a cyclohexane ring, 7a–c, were higher than that with 2-pyridone fused with the cyclooctane ring, 7d–f. The reaction was suggested to proceed via an initial nucleophilic addition of the active methylene carbon atom of arylsulfonylhydrazide 4a–c to the unhindered formyl group of cycloalkanones 5a,b forming intermediates 6, followed by the intramolecular cyclization and elimination of one molecule of water to give the final isolable kinetically and thermodynamically controlled product cycloalkane ring-fused N-sulfonyl aminated pyridones 7a–f. Similar reactions and proof of product structures by X-ray measurements have been previously reported by us. Concerning the role of piperidine acetate and the mechanism of this reaction, the literature proposed that in the presence of piperidine acetate, piperidinium cation attack the formyl group of 5, forming an iminium ion. Acetate anion deprotonates the active methylene of 4, forming an anion that attacks the iminium ion and leads to an addition intermediate. The final step involves elimination of piperidine as a piperidine acetate catalyst. A mechanism in detail has been provided in the Supporting Information. The structures of compounds 7a–f were determined based on spectral data (IR, 1H NMR, and 13C NMR) and elemental analysis. According to the IR spectra of compounds 7a–f, the appearance of a broad absorption band at 3438–3445 cm⁻¹ confirmed the presence of an NH group. In addition, the IR spectra showed a sharp band at a range of
1635–1656 cm⁻¹, which corresponds to a C═O group. The ¹H NMR spectrum of compound 7c, as an example, showed two multiplet signals at a range of δ 1.70–2.86 ppm, which could be assigned for three CH₂ groups, and two singlet signals, which could be assigned to the pyridone-CH and NH groups, at δ 8.42 and 11.32 ppm, respectively. Additionally, the ¹H NMR spectrum of compound 7c displayed four characteristic signals corresponding to the four protons of the benzothiazole ring, two apparent triplet signals at δ 7.39 and 7.51 ppm, as well as two doublets at δ 7.97 and 8.07 ppm. The ¹³C NMR spectrum of 7c confirmed the presence of three CH₂ carbons at a range of δ 21.7–27.0 ppm and the C═O carbon at δ 161.5 ppm. Additionally, 2D NMR spectra confirmed the structure of compound 7d (see the Supporting Information).

To further study the reactivity of N-arylsulfonylhydrazides compounds 4a–c toward the sodium salt of an alkanone, reactions involving sodium salts of unsaturated keto compounds 8a,b were investigated. The reaction of compounds 4a–c with the sodium salt of 4-hydroxybut-3-en-2-one 8a or 4-hydroxy-3-methylbut-3-en-2-one 8b in piperidine acetate afforded N-arylsulfonylpyridones derivatives 10a–f, through the formation of the intermediate 9a–f, in reasonable yields. All of the spectral data (IR, ¹H NMR, and ¹³C NMR) and elemental analyses confirmed the structure of compounds 10a–f, Scheme 2. The IR spectra of compounds 10a–f revealed the presence of NH and C═O groups at ranges of 3432–3444 and 1636–1666 cm⁻¹, respectively. The ¹H NMR spectra clearly revealed that compounds 10a–c had two doublet signals in ranges of δ 6.56–6.55 and 8.55–8.56 ppm, corresponding to the two pyridone-CH groups, while compounds 10d–f showed one singlet signal in the range of δ 8.49–8.52 ppm, corresponding to one pyridone-H. Additionally, in the case of compounds 10d–f, the presence of two singlet signals at δ 2.23–2.32 and 2.45–2.51 ppm revealed the presence of two CH₃ groups, while in the case of compounds 10a–c, the presence of one singlet signal at δ 2.49–2.54 ppm revealed the presence of one CH₃ group. Moreover, the ¹³C NMR spectra of compounds 10f and 10c, as an example of this series, showed a signal at δ 161.8 and 166.5 ppm, respectively, corresponding to the carbon of the C═O group. Furthermore, the structure of compound 10f was characterized by 2D NMR, such as heteronuclear multiple bond correlation (¹H–¹³C heteronuclear multiple bond correlation (HMBC)), Figure 2, homonuclear correlation spectroscopy (¹H–¹H correlation spectroscopy (COSY)), and heteronuclear single quantum coherence (¹H–¹³C heteronuclear multiple quantum coherence (HSQC)) (see the Supporting Information). Based on the 2D NMR measurements, the chemical shifts of both protons and carbons for compound 10f were assigned, Figure 3.

Furthermore, our study on the reactivity of N-arylsulfonylhydrazides was extended to involve the reaction of arylsulfonylhydrazides 4a–c with ethoxymethylene compounds, 2-(ethoxymethylene)malononitrile 11a and (E)-ethyl-2-cyano-3-ethoxyacrylate 11b, Scheme 2. The reaction was carried out using ethanolic sodium ethoxide and afforded the correspond-
ing N-arylsulfonylpyridones 13a−f. It was noticed that all derivatives of this series were formed in high yields, ranging between 80 and 85%.

The reaction occurred first via the Michael addition of N-arylsulfonylhydrazide with the ethoxymethylene compounds, which is followed by the elimination of ethanol, forming intermediates 12a−f. Finally, intramolecular cyclization occurred through the addition of the NH group to the N-arylsulfonylpyridone products. The elemental analysis and spectral data confirmed the proposed structures of compounds 13a−f. The IR spectra clearly showed that compounds 13a−f had NH2 groups, as evident by the absorption bands at the range of 3430−3407 cm−1. According to the 1H NMR spectra of compounds 13a−f, the appearance of a singlet signal at the range of δ 8.8−8.4 ppm confirmed the existence of the pyridone-H proton. In the case of compounds 13d−f, 1H NMR spectra showed a triplet signal at δ 1.3 ppm and a quartet at δ 4.3 ppm for CH3 and CH2, respectively, of the COOCH2CH3 group. Furthermore, the 13C NMR spectrum of 13c showed a signal at δ 117.6 ppm for the CN group, while in the case of 13d, two signals appeared at δ 14.5 and 59.9 ppm, corresponding to the carbons of the CH3 and CH2 groups, respectively, in addition to two signals at δ 163.6 and 165.9 ppm, corresponding to the carbons of the C==O groups. Moreover, 2D NMR such as 1H−1H COSY, 1H−13C HSQC, and 1H−13C HMBC confirmed the structures of both compounds 13c and 13d (see the Supporting Information). Based on the 1H−1H COSY spectrum of compound 13c, there is a strong correlation between the proton of the benzothiazole ring at 7.29 ppm (app. t, dd, 1H) and both protons at 7.43 ppm (app. t, dd, 1H) and 7.98 ppm (dd, 1H), as well as a strong correlation between the proton at 7.43 ppm (app. t, dd, 1H) and both protons at 7.29 ppm (app. t, dd, 1H) and 7.86 ppm (d, 1H), Figure 4.

**Antiviral Evaluation.** The newly synthesized (3-benzo[d]thiazole-2-yl)-2-pyridones were tested in vitro against both DNA viruses, such as herpes simplex virus type 1 (HSV-1) and adenovirus type 7 (HAdV7), and RNA viruses, such as coxsackievirus B4 (CBV4), hepatitis A virus (HAV) HM175, and HCVcc genotype 4.

**Cytotoxicity Evaluation.** To study the antiviral activities of the synthesized compounds, the cytotoxicity against different normal cell lines was first evaluated, as presented in Table 1. The normal cell lines Vero, FRHK-4, Huh 7.5, BGM, and Hep-2 were used because they are the specific hosts to the HSV-1, HAV HM175, HCVcc genotype 4, CBV4, and HAdV7 viruses, respectively, Table 1. The resultant data showed no significant...
The difference between the nontoxic doses, which ranged between 100 and 140 μg/mL, of the synthesized compounds on tested normal cell lines. It was notable that compounds 7d and 13f showed the highest safety profile as compared to other tested compounds that could participate in a relatively wider therapeutic index. Compound 7d exhibited the highest nontoxic dose among all of the tested compounds with 140 μg/mL against all of the tested cell lines. On the other hand, compound 13b showed the least safe dosing profile with 100 μg/mL nontoxic dose value against all of the tested cell lines.

**Plaque Assay for Antiviral Activities.** The synthesized compounds exhibited a noticeable effect on viruses having various types of genomes, that is, either DNA or RNA, Table 2. The plaque assay was used to determine the mean percentage reduction of viral titers ($1 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$), see the Supporting Information, by mixing the nontoxic dilutions of

| compound no. | Vero | Hep-2 | BGM | FRHK-4 | Huh 7.5 |
|--------------|------|-------|-----|--------|--------|
| 7a           | 110  | 110   | 110 | 110    | 100    |
| 7b           | 130  | 130   | 130 | 130    | 130    |
| 7c           | 130  | 130   | 130 | 130    | 130    |
| 7d           | 140  | 140   | 140 | 140    | 140    |
| 7e           | 130  | 130   | 120 | 130    | 130    |
| 7f           | 130  | 130   | 120 | 130    | 130    |
| 13a          | 110  | 110   | 110 | 100    | 100    |
| 13b          | 100  | 100   | 100 | 100    | 100    |
| 13c          | 110  | 110   | 110 | 110    | 100    |
| 13d          | 110  | 110   | 100 | 110    | 100    |
| 13e          | 130  | 130   | 130 | 130    | 130    |
| 13f          | 140  | 140   | 130 | 140    | 140    |
the tested compounds with 0.1 mL of HSV-1, HADV7, CBV4, HAV, and HCVcc, Table 2. According to our literature review, there is not an effective medication available for HCVcc genotype 4, HADV7, and CBV4 viruses, while acyclovir is considered the most active drug used for the treatment of HSV-1. Five compounds, in particular, 7a, 7c, 7e, 7f, and 13a, showed promising antiviral effects (50% reduction or more) against the studied viruses, Table 2. Both CC50 (the concentration in μg/mL required to reach 50% cytotoxicity of the uninfected cell) and IC50 (the concentration in μg/mL required to inhibit 50% of the tested viruses) and SI (the selectivity index, CC50/IC50 ratio) were evaluated for those compounds that presented greater than 50% viral reduction, Table 3.

The results showed that three compounds, 7e, 7f, and 13a, revealed a viral reduction percentage higher than 50% against HSV-1. Both compounds 7e and 13a reached 70% viral reduction, while compound 7f reached 63.3%, Table 2. Moreover, as shown in Table 3, these three compounds also had IC50 values ranging from 75 to 98 μg/mL and CC50 values ranging from 280 to 320 μg/mL. Although these three compounds showed a moderate performance against HSV-1 when compared to acyclovir, they displayed SI values comparable to that of the standard drug. The CC50 values, the selectivity indices, and the viral reduction percentages indicated the potency of these compounds as antiviral agents, Table 3.

In the case of HAV, two compounds, 7f and 13a, revealed 60% viral reduction and one compound, 7c, revealed 50% viral reduction, Table 2. While compound 7c has the highest CC50 value of 320 μg/mL, compound 13a has the lowest IC50 value of 90 μg/mL. It was also observed that compound 7f has the highest SI among these three compounds with a value of 2.94, Table 3.

Compound 13a exhibited some potency against both HCVcc and HADV7 with viral reduction percentage values of 56.7% and 50%, respectively. While compound 7e showed a viral reduction of more than 50% against HCVcc genotype 4, compound 7f showed 53% viral reduction against HADV7, Table 2.

In the case of the activities against CBV4, five compounds, 7a, 7c, 7e, 7f, and 13a, showed a viral reduction of more than 50%, Table 2. The IC50 values of these compounds ranged from 84 to 101 μg/mL with CC50 values ranging from 240 to 310 μg/mL. Among these five compounds, compound 7e showed the highest SI value of 3.23 followed by compound 7a with a SI value of 2.95, Table 3.

According to the above-mentioned data, we observed that compounds 7e and 7f, which contained cyloocta[b]pyrid-2-one rings, exhibited some activities against all tested viruses when compared to compounds 7b and 7c, which contained cyclohexa[k]pyrid-2-one rings. Additionally, the presence of a cyano (CN) group at C5 on the 2-pyridone ring had increased the activities of compounds 13a and 13b when compared to

Table 2. Mean % of Reduction of Nontoxic Doses of Synthesized Compounds against Tested DNA and RNA Viruses

| compound no. | DNA viruses | mean % of reduction | RNA viruses |
|--------------|-------------|---------------------|-------------|
|              | herpes simplex virus type-1 | adenovirus type 7 | coxsackievirus B4 | hepatitis A virus HM175 | HCVcc genotype 4 |
| 7a           | 40.0        | 10.0                | 50.0        | 30.0                   | 30.0             |
| 7b           | **a**       | -                   | -           | -                      | -                |
| 7c           | 40.0        | 30.0                | 50.0        | 50.0                   | 50.0             |
| 7d           | -           | -                   | -           | -                      | -                |
| 7e           | 70.0        | 20.0                | 66.7        | 23.3                   | 56.7             |
| 7f           | 63.3        | 53.3                | 60.0        | 60.0                   | 60.0             |
| 13a          | 70.0        | 50.0                | 63.3        | 60.0                   | 60.0             |
| 13b          | 26.7        | 16.7                | 30.0        | 26.7                   | 26.7             |
| 13c          | 10.0        | 3.3                 | 10.0        | 6.7                    | 10.0             |
| 13d          | -           | -                   | -           | -                      | -                |
| 13e          | -           | -                   | -           | -                      | -                |
| 13f          | 23.3        | 16.7                | 20.0        | 20.0                   | 20.0             |
| acyclovir    | 99.6        | NT                  | NT          | NT                     | NT               |

“a” indicates no inhibition.

**Table 3. Antiviral Activity of Compounds with Viral Reduction of 50% or More in Terms of CC50 (μg/mL), IC50 (μg/mL), and SI against Tested Viruses**

| compound no. | CC50 (μg/mL) | IC50 (μg/mL) | SI  |
|--------------|--------------|--------------|-----|
|              | Herpes Simplex Virus | | |
| 7e           | 320          | 89           | 3.56 |
| 7f           | 300          | 98           | 3.06 |
| 13a          | 280          | 75           | 3.73 |
| acyclovir    | 2.8          | 0.7          | 4.00 |
|              | Hepatitis A Virus HM175 | | |
| 7c           | 320          | 130          | 2.46 |
| 7f           | 300          | 102          | 2.94 |
| 13a          | 260          | 90           | 2.89 |
|              | HCVcc Genotype 4 | | |
| 7e           | 310          | 111          | 2.79 |
| 13a          | 260          | 84           | 3.09 |
|              | Adenovirus Type 7 | | |
| 7f           | 330          | 118          | 2.79 |
| 13a          | 270          | 110          | 2.45 |
|              | Coxsackievirus B4 | | |
| 7a           | 260          | 88           | 2.95 |
| 7c           | 240          | 90           | 2.66 |
| 7e           | 310          | 96           | 3.23 |
| 7f           | 280          | 101          | 2.77 |
| 13a          | 240          | 84           | 2.86 |

The data is expressed as the mean ± SD of three independent experiments.
those of comparable compounds with an ethoxycarbonyl group at Cs, such as compounds 13d and 13e.

Physicochemical Properties of the Potent Antiviral Synthesized Compounds. Both drug absorption and appropriate drug delivery are considered to be the essential parameters in the development of drugs intended for oral use. To predict the oral bioavailability of the most potent antiviral compounds, MedChem Designer 3.0 was used to evaluate their in silico physicochemical properties and drug-likeness. The drug-likeness for the investigated compounds was checked using Lipinski’s rule of 5. This rule states that an orally active drug should have at least three of the following four criteria: no more than 500 Da molecular weight (MW), no more than 5 octanol/water partition coefficient (MlogP), no more than 10 hydrogen bond acceptors (MNO), and no more than 5 hydrogen bond donors (HBDH). The octanol/water partition coefficient was determined to estimate the level of hydrophobicity and hydrophilicity of the molecules. Moreover, the topological polar surface area (TPSA) was also evaluated to predict the drug transport properties since compounds with TPSA values ranging between 60 and 140 Å² have good oral bioavailability. The physicochemical parameters of the investigated compounds are listed in Table 4. The results of the physicochemical properties of the investigated compounds showed that all compounds expected to exhibit TPSA values within the range of 81–130 Å². These indicate that all compounds should be able to penetrate the cell easily if they are to be used as potential drug candidates. Moreover, compounds 7a, 7c, 7f, and 13a displayed MW less than 500 and MlogP values smaller than 5, which indicate the potential for good bioavailability by oral administration.

(USP7) Enzymatic Assay. The herpes simplex virus (HSV) is a viral infection that affects the mucous membranes of the mouth, lips, genitals, and nose. There are two types of herpes viruses that affect humans: HSV-1 and HSV-2. HSV-1 is usually responsible for cold sores and fever blisters around the mouth and on the mucous membranes of the mouth, lips, genitals, and nose. There are two types of herpes infections that affect humans: HSV-1 and HSV-2. HSV-1 is usually responsible for cold sores and fever blisters around the mouth and on the mucous membranes of the mouth, lips, genitals, and nose.

The most potent compounds against HSV-1, 7e and 13a, were examined for their ability to inhibit the USP7 enzyme. A summary of the inhibition percentages and IC₅₀ values against the USP7 enzyme is listed in Table 5. Acyclovir was used as a positive control in this study. The data obtained from this study were used to graph a dose–response curve. Error bars in the figure represent the standard deviation of the measured data, Figure 5. The data showed that compound 13a that contains a CN group at Cs of the 2-pyridone ring has an IC₅₀ value of 4.80 μg/mL, while compound 7e with a cycloocta[β]pyrid-2-one ring has an IC₅₀ value of 10.37 μg/mL. This indicated that compound 13a is the most potent one as compared to acyclovir (IC₅₀ = 2.48 μg/mL).

Docking Studies. To understand the binding mode and the interaction of pyridone compounds, 7e and 13a, the possible binding site of the compounds to the USP7 enzyme was investigated. Docking studies were carried out using a Molecular Operating Environment (MOE 2014) program and the crystal structure of USP7 (PDB ID: 4MSW). The docking results revealed that both compounds 7e and 13a occupied the ubiquitin-binding pocket of USP7 and the top-ranked poses were selected, Figures 6 and 7. The docking studies showed that compounds 7e and 13a have binding energies of −6.6760 and −6.5958 kcal/mol, respectively. The benzene ring of the arylsulfonyl amide moiety in compounds 7e and 13a hydrophobically interacts with residues Val296. As shown in Figures 6 and 7, both compounds exhibited three hydrogen bonds, two donor and one acceptor hydrogen bonds. The acceptor hydrogen bond was between the oxygen of the SO₂ group and Tyr465 with bond lengths of 2.16 and 2.03 Å, respectively.

Table 4. Physicochemical Properties of the Most Potent Antiviral Compounds

| compound no. | MW   | MlogP | MNO | HBDH | TPSA |
|--------------|------|-------|-----|------|------|
| 7a           | 437.53 | 3.830 | 6   | 1    | 81.06 |
| 7c           | 451.56 | 4.045 | 6   | 1    | 81.06 |
| 7e           | 500.03 | 4.463 | 6   | 1    | 81.06 |
| 7f           | 479.61 | 4.463 | 6   | 1    | 81.06 |
| 13a          | 423.47 | 1.991 | 8   | 3    | 130.87 |
| acyclovir    | 223.21 | −0.940 | 8 | 4    | 119.05 |

Table 5. Inhibition Percentages and IC₅₀ Values of Compounds 7e, 13a, and Acyclovir against the USP7 Protease Enzyme

| compound no. | 100 (μg/mL) | 10 (μg/mL) | 1 (μg/mL) | 0.1 (μg/mL) | IC₅₀ (μg/mL) |
|--------------|-------------|------------|-----------|-------------|-------------|
| 7e           | 71.86568    | 54.97389   | 20.32922  | 3.146768    | 10.37 ± 0.75 |
| 13a          | 81.51579    | 53.95502   | 35.75837  | 11.84952    | 4.80 ± 0.19  |
| acyclovir    | 84.54831    | 71.65506   | 34.95775  | 18.65208    | 2.48 ± 0.09  |

The data is expressed as the mean ± SD of three independent experiments.
hydrogen donor bond between the NH of the sulfonamide group and the C=O group of His294 amino acid with a bond length of 2.27 Å, while the other one was between the sulfur atom of the benzothiazole ring and the C=O group of Met292 amino acid with a bond length of 3.85 Å.

■ CONCLUSIONS

In summary, a new class of functionalized benzothiazole-bearing N-sulfonamide 2-pyridone derivatives was synthesized and its antiviral potency was exhibited. The synthesis was carried out through the reaction of benzothiazole sulfonylhydrazide with sodium salts of both (hydroxymethylene) cycloalkanones and unsaturated ketones as well as ethoxymethylene derivatives. Both spectral and elemental analyses confirmed the structures of the synthesized compounds. The in vitro antiviral studies against HSV-1, HAV HM175, HCVcc genotype 4a, CBV4, and HAdV7 revealed that five of the newly synthesized compounds, 7a, 7c, 7e, 7f, and 13a, showed antiviral activities with more than 50% reduction. The CC_{50} and IC_{50} values for the five promising compounds were measured, and their respective selective indices/indexes were determined. The in silico physicochemical properties showed that all five of the compounds might have good bioavailability by oral administration and could penetrate the cell easily. The two most potent compounds against HSV-1, 7e and 13a, have shown some inhibitory activity against the USP7 enzyme. Additionally, docking studies revealed that both compounds occupied the protein-binding pockets of USP7 with strong binding interactions.

■ EXPERIMENTAL SECTION

Chemistry. Melting points were measured on an SMP3 melting point apparatus. The ¹H and ¹³C NMR spectra were recorded on a Bruker Advance III 400 Spectrometer (400 and 100 MHz, respectively) in DMSO-d₆ using Si(CH₃)₄ as an internal standard at the Ain Shams University, Cairo, Egypt. Chemical shifts are expressed in δ values (ppm). All coupling constants (J) values are given in hertz. The abbreviations used are as follows: s, singlet; d, doublet and m, multiplet. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel-precoated F₂₅₄ plates (Merck) and using a UV lamp.

General Procedure for the Synthesis of N-(2-(Benzo[d]thiazole-2-yl)acetyl)arylsulfonohydrazide (4a–c).
a stirred solution of 2-[(benzo[d]thiazol-2-yl)acetohydrazide (1 mmol) in pyridine (5 mL), a solution of arylsulfonyl chloride (1.2 mmol) in pyridine (2 mL) was added gradually at 0 °C. The reaction mixture stirred at room temperature for 3h. After completion, the reaction mixture poured onto ice water with stirring and a solution of 1N HCl was added dropwise until the pH reaches 7. The precipitate was filtrated off, washed with water, and recrystallized from ethanol to give colorless crystals.

N-(2-[(B enzo[d]th i azol-2-yl) acetyl]benzenesulfonylhydrazide (4a))
N-(2-[B enzo(d)thiazol-2-yl)acetyl]-4-methylbenzenesulfonylhydrazide (4c))

**General Procedure for the Synthesis of N-(3-B enzo[d]thiazol-2-yl)-2-oxo-(1H)-arylsulfonamide (7a–f)**

To a stirred solution of the sodium salt of 2-(hydroxy-methylene)-1-cycloalkanone (10 mmol) in ethanol (15 mL) containing piperidine acetate (1 mL), N-(2-(benzo[d]thiazole-2-yl)acetyl)arylsulfonhydrazide 4a–c (5 mmol) was added and the reaction mixture was refluxed for 10 min. Acetic acid (1.5 mL) was added to the hot solution; after completion, the solvent was allowed to evaporate and the solid obtained was stored in desiccator and recrystallized from ethanol.

N-(3-B enzo[d]thiazol-2-yl)-2-oxo-5,6,7,8-Tetrahydroquinolin-1(2H)-yl-benzenesulfonamide (7a).
N-(3-B enzo[d]thiazol-2-yl)-2-oxo-5,6,7,8-tetrahydroquinolin-1(2H)-yl-4-chlorobenzenesulfonamide (7b).

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NH); Anal calcd for C_{23}H_{23}N_{2}O_{6}S_{2} (479.61): calcd. C% 62.16; H% 5.25; N% 8.76, found C% 62.18; H% 5.29; N% 8.71.

**General Procedure for the Synthesis of N-(3-Benzoyl-[d]thiazole-2-yl)-6-methyl-2-oxopyridin-1(2H)-yl)-arylsulfonamide (10a–f).** To a stirred solution of the sodium salt of 4-hydroxybut-3-en-2-one or 4-hydroxy-3-methylbut-3-en-2-one 8a,b (10 mmol) in ethanol (15 mL) containing piperidine acetate (1 mL), N-(2-[benzoyl[d]thiazole-2-yl]acetyl)arylsulfonhydrazide 4a–c (10 mmol) was added and the reaction mixture was refluxed for 10 min. Acetic acid (1.5 mL) was added to the hot solution; after completion, the solvent was allowed to evaporate and the solid was collected and crystallized from ethanol.

N-(3-Benzoyl[d]thiazole-2-yl)-6-methyl-2-oxopyridin-1(2H)-yl)benzensulfonamide (10a). Yellow solid (yield 60%), mp: 220–222 °C; IR (KBr, ν cm⁻¹): 3434 (NH), 2926 (Ar–CH), 1666 (C=O); 1H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_{3}), 2.45 (s, 3H, CH_{3}), 2.49 (s, 3H, CH_{3}), 4.12 (q, 2H, Ar–CH) (app. t, dd, J = 8.0 Hz, 2H, Ar–H), 7.32 (d, J = 8.0 Hz, 1H, benzothiazole-H), 7.50 (app. t, dd, J = 8.0 Hz, 1H, benzothiazole-H), 7.59 (d, J = 8.0 Hz, 2H, Ar–H), 7.73 (d, J = 8.0 Hz, 2H, Ar–H). 8.07 (d, J = 6.8 Hz, 1H, benzothiazole-H), 8.00 (d, J = 6.8 Hz, 1H, benzothiazole-H), 8.52 (s, 1H, CH-pyridine).

**N-(3-Benzoyl[d]thiazole-2-yl)-5,6-dimethyl-2-oxopyridin-1(2H)-yl)-4-chlorobenzensulfonamide (10b).** Yellow solid (yield 50%), mp: 236–238 °C; IR (KBr, ν cm⁻¹): 3444 (NH), 2924 (Ar–CH), 1637 (C=O); 1H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_{3}), 2.33 (s, 3H, CH_{3}), 2.45 (s, 3H, CH_{3}), 7.30 (d, J = 8.0 Hz, 2H, Ar–H), 7.39 (app. t, dd, J = 7.2 Hz, 1H, benzothiazole-H), 7.50 (app. t, dd, J = 7.2 Hz, 1H, benzothiazole-H), 7.61 (d, J = 8.0 Hz, 2H, Ar–H), 7.97 (d, J = 8.0 Hz, 1H, benzothiazole-H), 8.07 (d, J = 8.0 Hz, 1H, benzothiazole-H), 8.07 (d, J = 8.0 Hz, 1H, benzothiazole-H), 8.49 (s, 1H, CH-pyridine).

**N-(3-Benzoyl[d]thiazole-2-yl)-6-methyl-2-oxopyridin-1(2H)-yl)-4-chlorobenzensulfonamide (10c).** Yellow solid (yield 52%), mp: 266–268 °C; IR (KBr, ν cm⁻¹): 3436 (NH), 2924 (Ar–CH), 1636 (C=O); 1H NMR (400 MHz, DMSO-d_6): δ 2.23 (s, 3H, CH_{3}), 2.32 (s, 3H, CH_{3}), 2.45 (s, 3H, CH_{3}), 7.31 (d, J = 8.0 Hz, 2H, Ar–H), 7.39 (app. t, dd, J = 7.2 Hz, 1H, benzothiazole-H), 7.50 (app. t, dd, J = 7.2 Hz, 1H, benzothiazole-H), 7.61 (d, J = 8.0 Hz, 2H, Ar–H), 7.97 (d, J = 8.0 Hz, 1H, benzothiazole-H), 8.07 (d, J = 8.0 Hz, 1H, benzothiazole-H), 8.49 (s, 1H, CH-pyridine).

**N-(3-Benzoyl[d]thiazole-2-yl)-5,6-dimethyl-2-oxopyridin-1(2H)-yl)-4-methylbenzensulfonamide (10f).** Yellow solid (yield 52%), mp: above 350 °C; IR (KBr, ν cm⁻¹): 3410, 3293 (NH, NH_{2}), 3293 (Ar–CH), 2223 (CN), 1635 (C=O); 1H NMR (400 MHz, DMSO-d_6): δ 7.28 (app. t, dd, J = 8.0 Hz, 1H, benzothiazole-H), 7.38–7.46 (m, 4H, 3Ar–H, 1benzothiazole-H), 7.78–7.80 (m, 2H, Ar–H), 7.86 (d, J = 8.8 Hz, 1H, benzothiazole-H), 7.98 (d, J = 8.8 Hz, 1H, benzothiazole-H), 8.40 (s, 1H, CH-pyridine).

**N-(3-Benzoyl[d]thiazole-2-yl)-6-methyl-2-oxopyridin-1(2H)-yl)-4-methylbenzenesulfonamide (10c).** Yellow solid (yield 80%), mp: above 350 °C; IR (KBr, ν cm⁻¹): 3410, 3293 (NH, NH_{2}), 3293 (Ar–CH), 2223 (CN), 1635 (C=O); 1H NMR (400 MHz, DMSO-d_6): δ 7.28 (app. t, dd, J = 8.0 Hz, 1H, benzothiazole-H), 7.38–7.46 (m, 4H, 3Ar–H, 1benzothiazole-H), 7.78–7.80 (m, 2H, Ar–H), 7.86 (d, J = 8.8 Hz, 1H, benzothiazole-H), 7.98 (d, J = 8.8 Hz, 1H, benzothiazole-H), 8.40 (s, 1H, CH-pyridine).

**N-(3-Benzoyl[d]thiazole-2-yl)-5,6-dimethyl-2-oxopyridin-1(2H)-yl)-4-chlorobenzensulfonamide (13b).** Yellow solid (yield 85%), mp: above 350 °C; IR (KBr, ν cm⁻¹): 3430, 3293 (NH, NH_{2}), 2924 (Ar–CH), 2218 (CN), 1632 (C=O); 1H NMR (400 MHz, DMSO-d_6): δ 7.29 (app. t, dd, J = 8.0 Hz, 1H, benzothiazole-H), 7.41–7.46 (m, 3H, 2Ar–H, benzothiazole-H), 7.76 (d, J = 7.2 Hz, 1H, Ar–H), 7.86 (d, J = 8.4 Hz, 1H, benzothiazole-H), 7.99 (d, J = 8.4 Hz, 1H, benzothiazole-H), 8.4 (s, 1H, CH-pyridine).

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MHz, DMSO-d$_6$): δ 71.9, 108.1 (Ar–C), 117.8 (CN), 121.5, 122.1, 123.9, 126.2, 128.0, 128.5, 134.3, 135.3, 137.8, 147.3, 152.3, 158.2, 160.1 (Ar–C), 163.2 (C=O); anal calc'd for C$_{20}$H$_{15}$ClN$_5$O$_3$S$_2$ (457.91): calcd. C% 49.84; H% 2.64; N% 15.29, found C% 49.87; H% 2.61; N% 15.31.

N-(6-Amino-3-benzo[d]thiazole-2-yl)-5-cyano-2-oxopyridine-1(2H)-yl)-4-methylbenzensulfonamide (13c). Yellow solid (yield 85%), mp: above 350 °C (IR (KBr, ν cm$^{-1}$): 3402, 3298 (NH, NH$_2$), 2925 (Ar–CH), 2217 (CN), 1636 (C=O); $^1$H NMR (400 MHz, DMSO-d$_6$): δ 2.35 (s, 3H, CH$_3$-CH$_2$), 7.19 (d, J = 8.0 Hz, 2H, Ar–H), 7.29 (app. t, dd, 1H, benzothiazole-H), 7.43 (app. t, dd, J = 8.0 Hz, 1H, benzothiazole-H), 7.68 (d, J = 8.0 Hz, 2H, Ar–H), 7.86 (d, J = 7.6 Hz, 1H, benzothiazole-H), 7.99 (d, J = 7.6 Hz, 1H, benzothiazole-H), 8.39 (s, 1H, CH-pyridine); $^{13}$C NMR (100 MHz, DMSO-d$_6$): δ 20.9 (CH$_3$), 71.3, 107.6 (Ar–C), 117.4 (CN), 120.9, 121.6, 123.4, 125.7, 126.1, 128.4, 134.8, 137.2, 138.9, 144.8, 151.8, 159.7, 159.9 (Ar–C), 162.8 (C=O); anal calc'd for C$_{21}$H$_{18}$N$_4$O$_5$S$_2$ (470.52): calcd. C% 53.61; H% 3.86; N% 11.91, found C% 53.58; H% 3.87; N% 11.90.

Ethyl-2-amino-5-(benzo[d]thiazole-2-yl)-1-(4-methylphenylsulfonamido)-1,6-dihydropyridine-3-carboxylate (13d). Buff solid (yield 80%), mp: 314 °C; IR (KBr, ν cm$^{-1}$): 3407, 3275 (NH, NH$_2$), 2968 (Ar

Cell Morphology Evaluation by Inverted Light Microscopy. As mentioned earlier, cultures of the cell lines (2 × 10$^5$ cells/mL) were prepared. The cultures were then incubated at a temperature of 37 °C for 24 h in a humidified CO$_2$ atmosphere with a 5% ratio (v/v). This is to allow for the cell monolayers to be confluent. From each well separately, the medium was then removed and subsequently replenished with 100 μL of bifold dilutions, prepared in DMEM (Gibco BRL), of the different tested samples. One hundred microliters of DMEM was used as the control cell without sample addition. All cell cultures were incubated in a humidified 5% (v/v) CO$_2$ atmosphere at a temperature of 37 °C for 72 h. On a daily basis, the cell morphology was assessed for any possible morphological alterations on a microscopic scale such as cell rounding and shrinking, loss of confluence, and cytoplasm granulation and vacuolization. The morphological changes were thus scored.

Cell Viability Assay. This was done by the trypsin blue dye exclusion method.$^{47}$ Hep-2, Vero, BGM, FRHK-4, and Huh 7.5 cell cultures (2 × 10$^5$ cells/mL) were grown in 12-well tissue culture plates (Greiner Bio-One, Germany). After 24h incubation, the same assay described above for tested samples cytotoxicity was followed by applying 100 μL of tested samples dilutions (bifold dilutions) per well. After 72 h, the medium was removed, cells were trypsinized, and an equal volume of a 0.4% (w/v) trypan blue dye aqueous solution was added to the cell suspension. Viable cells were counted under a phase-contrast microscope.

Determination of Adenovirus 7, HAV HM175, Coxackievirus B4, and Herpes Simplex Virus Type 1 Titers Using the Plaque Assay. Nontoxic dilutions were mixed (100 μL) with 100 μL of different doses of adenovirus 7, HAV HM175, coxsackievirus B4, and herpes simplex virus type 1. The mixture was incubated for 0.5 h at 37 °C. The inoculation of (100 μL) 10 fold dilutions of treated and untreated adenovirus 7, HAV HM175, coxsackievirus B4, and herpes simplex virus type 1 was carried out separately into Hep-2, FRHK-4, BGM, and Vero cell lines, respectively, in 12-multiwell plates. The samples were incubated without constant rocking to allow for
the adsorption for 1 h in a 5% CO₂—water vapor atmosphere at a temperature of 37 °C to mimic the human body temperature. To keep the cells from drying, the plates were occasionally rocked. After adsorption, 1 mL of 2X media (Dulbecco’s modified Eagle’s medium, Gibco BRL (DMEM) and 1 mL of 1% agarose were added to each well, and the plates were incubated at 37 °C in a 5% CO₂—water vapor atmosphere. After the appropriate incubation period, the cells were stained with 0.4% crystal violet after formalin fixation, and the number of plaques was counted. The viral titers were then calculated and expressed as plaque-forming units per milliliter (pfu/mL).48 CC₅₀ and IC₅₀ were evaluated for the promising materials (viral reduction 50% or more). CC₅₀ refers to the 50% cytotoxic concentration of the test extract is defined as the concentration that reduces the OD492 of the treated uninfected cells to half the OD492 of the untreated uninfected cells. IC₅₀ refers to the concentration at which the compound plaque reduction rate reaches halfway between the baseline and the maximum. All data was taken as the average of three measurements (triplicates).

**Antiviral Bioassay of Tested Materials against ED-43/SG-Feo (VYG) Replicon of Hepatitis C Virus Genotype 4a**. ED-43/SG-Feo (VYG) replicon of HCVcc genotype 4a was treated with the nontoxic dose of the tested materials. To quantify HCV RNA, qRT-PCR supplied from the Taqman Probe Kit (Qiagen) was used. This was done in algal extracts and following the manufacturer’s instructions, a dose-dependent increase in subgenomic RNA copies was observed.49

**Ubiquitin-Specific Protease (USP7) Assay**. The USP7 Inhibitor Screening Assay Kit (BPS Bioscience, Catalog no. 79256) was used for this assay. The assay for the inhibitory effect of target compounds against the USP7 enzyme was applied as instructed in the USP7 assay kit.50 USP7 provided with the kit is a human USP7 recombinant expressed in Herpes simplex virus. First, an amount of 13 μL of 0.5 M DTT was added to stabilize the used enzyme. Stock solutions of the ubiquitin-specific protease 7 (1X USP7) assay buffer were prepared by diluting 5X USP7 assay buffer in distilled water. The Ub-AMC substrate was diluted 400-fold in 1X USP7 assay buffer; then, 20 μL of Ub-AMC substrate was added to each well designated as “Test Inhibitor,” “Positive Control,” and “Blank.” An amount of 5 μL of inhibitor solution was also added to each well designated as “Test Inhibitor,” “Positive Control,” and “Blank”; then, 5 μL of the same solution without the inhibitor (inhibitor buffer) was also added. After that, an amount of 25 μL of 1X USP7 assay buffer was added into the well designated as “Blank.” The reaction was initiated by adding 25 μL of diluted USP7 enzyme (diluted to 0.4 ng/μL (10 ng/25 μL) with 1X USP7 assay buffer) to the wells labeled as “Test Inhibitor” and “Positive Control.” After incubation at room temperature for 30 min on a rotating platform, the fluorescence intensity was immediately read in a luminometer or microtiter plate reader capable of reading at 360 nm excitation and 460 nm emission. The “Blank” value was subtracted from all readings.

**Molecular Docking**. The molecular docking studies were carried out using Molecular Operating Environment (MOE 2014). All of the minimizations were performed with MOE until an rmsd gradient of 0.01 kcal/mol Å with the MMFF94X force field, and the partial charges were automatically calculated. Docking simulations were performed using the crystal structure of the USP7 enzyme (PDB ID: 4MSW) that was obtained from the Protein Data Bank. Water molecules and chloride ions were all removed. Protonate 3D application of MOE was used to add the missing hydrogens and properly assign the ionization states. The ligand molecules were constructed using the builder molecule and were energy-minimized. The active site was generated using the MOE-α site finder. Dummy atoms were created from the obtained α spheres. Ligands were docked within the active sites using the MOE-Dock. The generated poses were energy-minimized using the MMFF94x force field. Finally, the optimized poses were ranked using the GBVI/WSA DG free-energy estimates. Docking poses were visually inspected, and interactions with binding pocket residues were analyzed.

## ASSOCIATED CONTENT

### Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04424.

Spectral data such as IR, ¹H NMR, ¹³C NMR, ¹H−¹H COSY, ¹H−¹³C HMBC, and ¹H−¹³C HSQC for the synthesized compounds as well as tables of antiviral activity of nontoxic doses of tested compounds against HSV-1, HAdV7, CBV4, HAV, and HCVcc (PDF)

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