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Efficient Synthesis and Docking Studies of Novel Benzothiazole-Based Pyrimidinesulfonamide Scaffolds as New Antiviral Agents and Hsp90α Inhibitors

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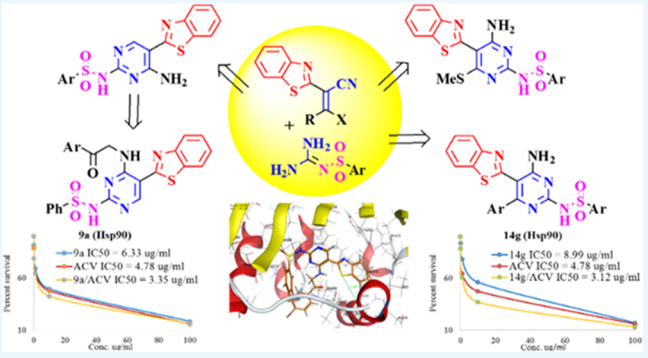
ABSTRACT: A series of novel substituted 2-pyrimidylbenzothiazoles incorporating either sulfonamide moieties or the amino group at C2 of the pyrimidine ring were synthesized and evaluated for its antiviral potency. The novel synthesis of the ring system was carried out by reacting guanidine or *N*-arylsulfonated guanidine with different derivatives of ylidene benzothiazole based on Michael addition pathways. The antiviral activity of the newly synthesized compounds was examined by a plaque reduction assay against HSV-1, CBV4, HAV HM 175, HCVcc genotype 4 viruses, and HAdV7. In the case of HSV-1, it was determined that 5 out of the 21 synthesized compounds exhibited superior viral reduction in the range of 70−90% with significant IC_{50}, CC_{50}, and SI values as compared with acyclovir. In the case of CBV4, nine compounds have shown more than 50% reduction. Comparable results were obtained for seven of these synthesized compounds when evaluated against HAV with only a couple of them showing 50% reduction or more against HCVcc genotype 4. Remarkably, one compound, **9a**, has shown broad action against all five examined viruses, rendering it as potentially an effective antiviral agent. The five potent compounds **9a**, **9b**, **14b**, **14g**, and **14h** against HSV-1 have also presented inhibitory activity against the Hsp90α protein with IC_{50} in the range of 4.87−10.47 μg/mL. Interestingly, a combination of the potent synthesized compounds with acyclovir led to IC_{50} values lower than that of acyclovir alone. The potent compounds **9a**, **9b**, **14b**, **14g**, and **14h** were also docked inside the active site of Hsp90α to assess the interaction pattern between the tested compounds and the active site of the protein.



INTRODUCTION

Viral infections are considered to be one of the major threats to human health. Herpes simplex virus type 1 (HSV-1), a member of the Herpesviridae family, is considered to be the cause of a range of diseases from mild uncomplicated mucocutaneous infections to more serious infections such as cold sores and encephalitis.[1] Acyclovir (ACV) is normally used in treating infections caused by HSV-1.[2] The most common side effects caused by ACV are nausea, diarrhea, headache, and vomiting. Coxsackievirus B4 (CBV4) virus, a member of the *Picornavirus* genus, has been involved in the development of insulin-dependent diabetes mellitus (IDDM) normally caused by virus-induced pancreatic cell damage.[3] Thus far, there is no specific treatment or vaccine available for CBV4 infections. However, the only available treatment is directed only toward relieving the symptoms resulting from the viral infections.[4] Hepatitis A (HAV) virus normally causes inflammation and may affect the liver functions[5] but hardly results in serious liver damage. Hepatitis C virus (HCV), however, causes chronic viral infection and is recognized to be one of the leading causes of liver impairment such as cirrhosis and hepatocellular carcinoma.[6] Remarkably, sofosbuvir (Sovaldi) is used for the treatment of HCV in combination with other medications such as ribavirin, peginterferon-alfa, simeprevir, ledipasvir, daclatasvir, or velpatasvir to reduce the amount of HCV in the effected body and thus help the liver to recover.[7] However, sofosbuvir may cause some unwanted effects such as fatigue, headache, nausea, and anemia. Infections caused by human adenovirus type 7 (HAdV7) may include acute respiratory disease syndrome, pneumonia, pharyngoconjunctival fever, and diseases of the central nervous system.[8] Such as the case with CBV4, there is no direct treatment against the viral infection.[9] Therefore, the development of novel drugs with superior activity against these drug-resistant viruses will most certainly require extensive synthetic research and clinical assessment.

Of interest here, it has been shown on many occasions that pyrimidine, benzothiazole, and sulfonamide structural units

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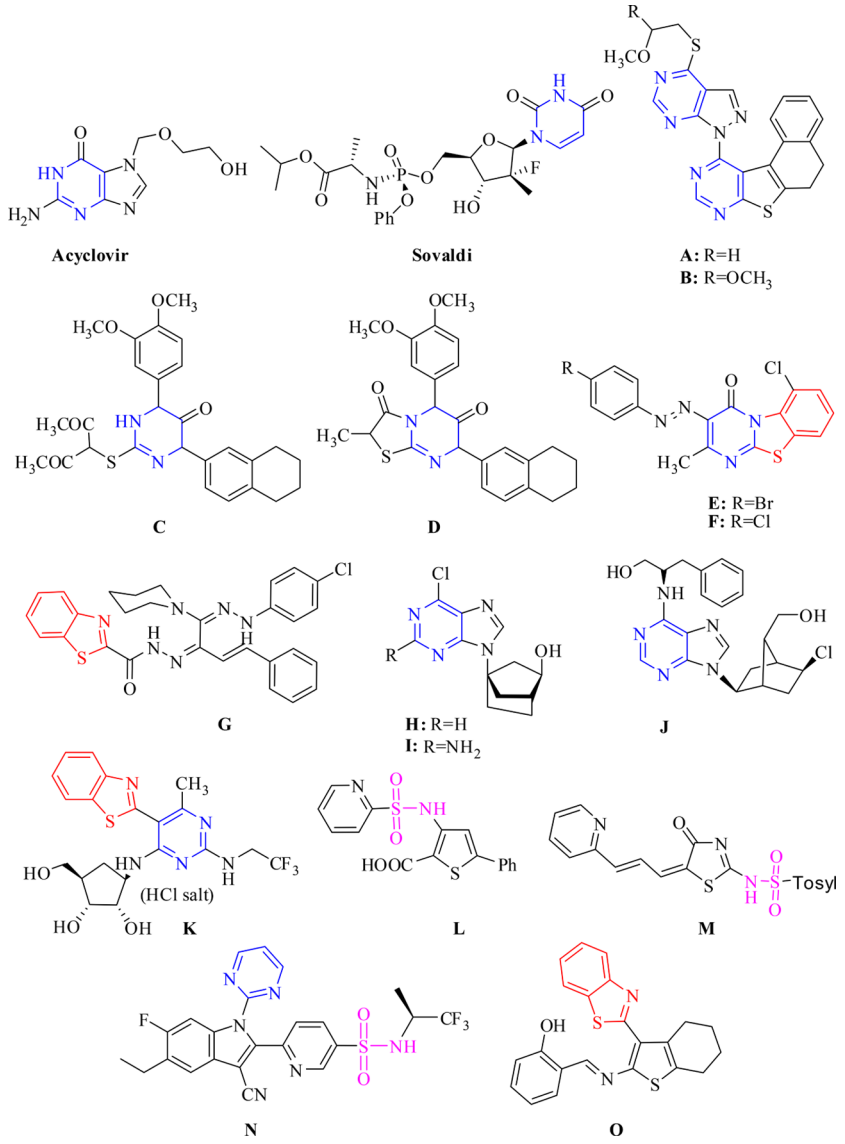

Figure 1. Pyrimidine, benzothiazole, and sulfonamide compounds as antiviral agents.

present within various molecules have exhibited interesting antiviral activities. The pyrimidine ring, for example, is the base unit of both ACV and Sovaldi, which are used for the treatment of HSV-1 and HCV, respectively. Assessing the antiviral potency of several published compounds, shown in Figure 1, has indicated their promising activity against HSV-1.[10] For example, pyrazolo[3,4-*d*]pyrimidine derivatives **A** and **B** as well as both dimethoxyphenylpyrimidin-5(4*H*)-one **C** and thiazolopyrimidine **D** all showed good antiviral activities against HSV-1 compared to ACV.[11] Moreover, pyrimido[2,1-*b*]benzothiazole derivatives **E** and **F** showed antiviral activity against HSV-1 with 61 and 50% reduction in the viral plaques, respectively.[12] The piperidinyl amidrazone derivative **G** was shown to have diminished the quantity of HSV-1 viral plaques by 62% as compared to the reference drug aphidicolin.[13]

Inasmuch, pyrimidine nucleoside analogues, compounds **H** and **I** (Figure 1), exhibited antiviral activities against CVB4 with an EC_{50} value of 9.0 μg/mL in addition to compound **J** that showed an impressive EC_{50} of 1 μM and a selectivity index of 141.[14,15]

The pyrimidine ring bearing the benzothiazole moiety at the C5 position, compound **K** (Figure 1), was found to produce the optimal inhibition for HCV replication with an EC_{50} value of 0.03 μM in addition to a selectivity index greater than 550.[16,17] Acyl sulfonamide **L**, thiazolone-based sulfonamides **M**, and 6-(indol-2-yl) pyridine-3-sulfonamide derivative **N** have all demonstrated remarkable inhibition against HCV.[18−20] Some of the cycloalkylthiopheneimine derivatives bearing a benzothiazole moiety were also synthesized and assessed for their antiviral activities against ADV7. In this regard, compound **O** (Figure 1) exhibited high potent antiviral activities with an EC_{50} value of 10.8 μg/mL, which was better than the control compound ribavirin with an EC_{50} value of 27.8 μg/mL.[21] This clearly demonstrated that the structural units of pyrimidine, benzothiazole, and sulfonamide present within the various molecules are a common factor among the active compounds for combating different infectious viruses.

Based on our experience in developing new synthetic approaches for the synthesis of novel benzothiazole, pyrimidine, and sulfonamide compounds in high yield,[22−33] novel compounds with potent antiviral activities are planned to

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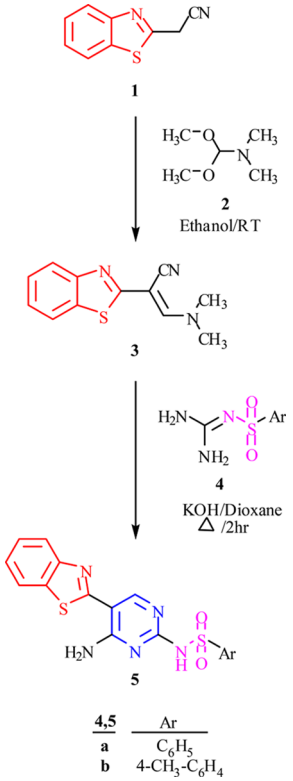
be further developed and assessed as potential antiviral drugs in this work. To achieve the target compounds in high yield, the pronounced reactivity of guanidine and its derivatives will also be utilized for the development of competent strategies for the synthesis of a novel benzothiazole pyrimidine sulfonamide ring system. This will be done through the reaction of ylidenes of benzothiazoles with either guanidine or sulfaguanidine derivatives. The study will be further extended to evaluate the exceptional characteristics of these compounds as antiviral agents.

RESULTS AND DISCUSSION

Chemistry. A series of 2-pyrimidylbenzothiazole derivatives were synthesized starting with the facile preparation of benzothiazol-2-yl-acetonitrile **1**, which was allowed to react with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) **2** in ethyl alcohol at room temperature for 10 min to afford the 2-(benzo[*d*]thiazol-2-yl)-3-(dimethylamino)acrylonitrile **3** intermediate in high yield (Scheme 1).[34] This intermediate was

Scheme 1. Synthesis of *N*-(4-Amino-5-(benzo[*d*]thiazol-2-yl)pyrimidin-2-yl)arylsulfonamide 5a,b

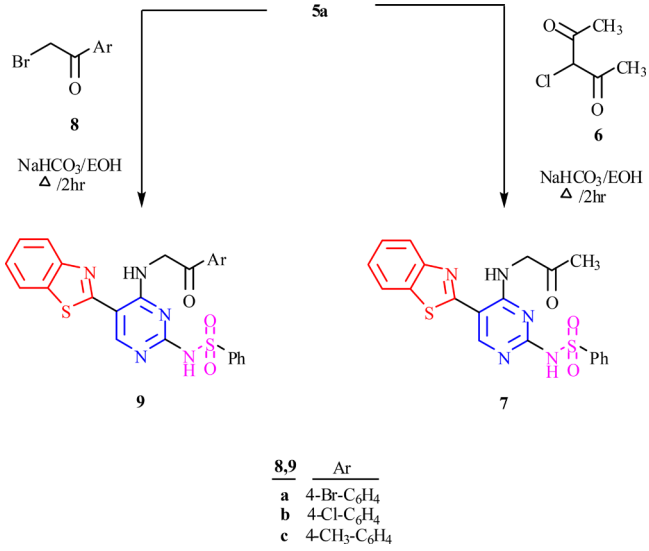


allowed to react further with *N*-arylsulfonated guanidine **4a,b** under basic conditions using potassium hydroxide, forming *N*-(4-amino-5-(benzo[*d*]thiazol-2-yl)pyrimidin-2-yl)-arylsulfonamide **5a,b** (Scheme 1).

Structures **5a,b** were elucidated on the basis of their IR, ^{1}H NMR, and ^{13}C NMR spectral analysis. The IR spectra of compound **5a** showed characteristic absorption bands of the NH_2 and NH groups in the vicinity of 3429 and 3278 cm^{-1}, respectively. The ^{1}H NMR spectrum revealed a multiplet signal at δ 7.30−7.99 ppm assigned to the aromatic protons, a broad band at δ 7.67 ppm assigned to the protons of the NH_2 group, and a characteristic singlet signal at δ 8.32 ppm assigned to the CH proton. The ^{13}C NMR showed 15 signals, which were attributed to the aromatic carbons of both the benzothiazole ring and phenylsulfonyl group. The reaction proceeded via Michael addition of the amino group of the *N*-arylsulfonated guanidine **4a,b** to the double bond of the enamine with elimination of $NH(CH_3)_2$ on the first instance followed by the intramolecular cyclization through the addition of the amino group to the cyano group as to provide the pyrimidine derivatives **5a,b**.

In an attempt to prepare the *N*-(8-(benzo[*d*]thiazol-2-yl)-3-methylimidazo[1,2-*c*]pyrimidine-5-yl)benzenesulfonamide fused-ring structure through the reaction of compound **5a** with 3-chloropentane-2,4-dione **6** catalyzed by sodium hydrogen carbonate in refluxing ethanol, a different open structure compound was obtained instead. The product was analyzed spectrally and confirmed to be *N*-(5-(benzo[*d*]thiazol-2-yl)-4-(2-oxopropyl)amino)pyrimidin-2-yl)benzenesulfonamide **7** (Scheme 2). The singlet signal in the ^{1}H NMR spectra at

Scheme 2. Synthesis of *N*-(5-(Benzo[*d*]thiazol-2-yl)-4-(2-oxopropyl)amino) and 4-(2-Aryl-2-oxoethyl)amino) pyrimidin-2-yl)benzenesulfonamide 7 and 9a−c



4.94 ppm assigned to the two protons of the CH_2 group along with the existence of the carbonyl signature at 1645 cm^{-1} in the IR data confirmed the structure of compound **7**.

Coincidentally, *N*-(3-aryl-8-(benzo[*d*]thiazol-2-yl)imidazo-[1,2-*c*]pyrimidine-5-yl) benzenesulfonamide was also not prepared by reacting **5a** with 2-bromo-4-substituted acetophenone **8a−c** using similar conditions as above. ^{1}H NMR and IR data revealed that the products of this reaction were *N*-(5-(benzo[*d*]thiazol-2-yl)-4-(2-aryl-2-oxoethyl)amino)pyrimidin-2-yl)benzenesulfonamide **9a−c** (Scheme 2). The absence of the imidazole proton signal in the ^{1}H NMR and the presence of the singlet signal in the range of 5.61−5.63 ppm as well as a signal at 57.0 ppm in the ^{13}C NMR assigned to the two protons of the CH_2 group in addition to a band at 1650 cm^{-1} of the carbonyl group in the IR spectra were all consistent with the chemical structures **9a−c** (Scheme 2).

To further the study, a new series of 5-(benzo[*d*]thiazol-2-yl)-6-arylpyrimidine-2,4-diamine **13a−c** have also been synthesized by reacting 2-(benzo[*d*]thiazol-2-yl)-arylacrylonitrile **11c−e** (prepared by reacting arylaldehyde **10a−e** with benzothiazol-2-yl-acetonitrile **1**) with guanidine

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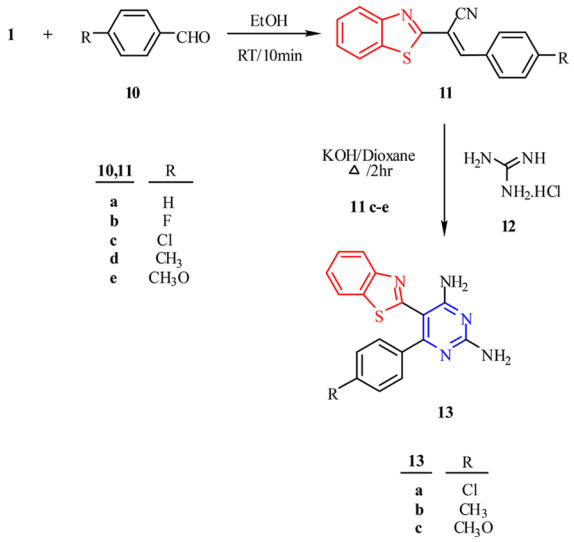
hydrochloride **12** in the presence of potassium hydroxide in 1,4-dioxane under reflux conditions (Scheme 3). The structure

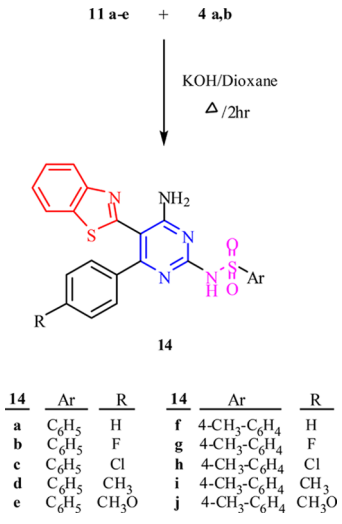
Scheme 3. Synthesis of 5-(Benzo[*d*]thiazol-2-yl)-6-arylpyrimidine-2,4-diamine 13a−c



of the resulting compounds was confirmed by spectral and elemental analysis. IR spectra of compound **13b**, for example, showed a band for the amino group in the vicinity of 3432 cm^{-1}. In addition, the ^1H NMR spectra showed a characteristic signal for protons of the methyl group at δ 2.41 ppm and aromatic protons at δ 7.07−7.65 ppm.

Replacement of guanidine hydrochloride in the previous reaction with *N*-arylsulfonated guanidine **4a,b** under the same conditions afforded the *N*-(4-amino-5-(benzo[*d*]thiazol-2-yl)-6-(4-alkylbenzene)pyrimidin-2-yl)arylsulfonamide **14a−j** (Scheme 4). Spectral data of compounds **14a−j** were consistent with their proposed structures. The ^1H NMR spectrum of **14e** revealed a singlet band at δ 3.78 ppm assigned to the protons of the OCH_3 group, a multiplet at δ 6.96−8.10 ppm assigned to the aromatic protons, a broad band at δ 8.59

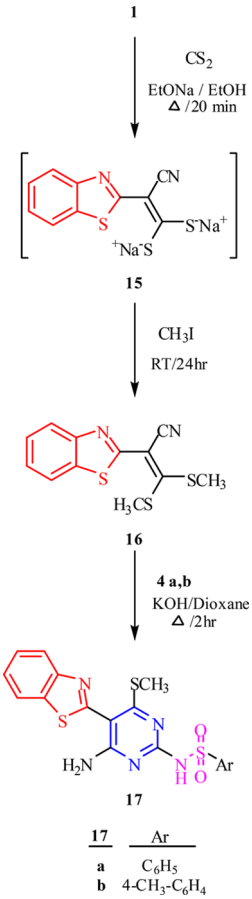
Scheme 4. Synthesis of *N*-(4-Amino-5-(benzo[*d*]thiazol-2-yl)-6-(4-substituted benzene)pyrimidin-2-yl)arylsulfonamide 14a−j



ppm assigned to the protons of the NH_2 group, and a broad band at δ 11.93 ppm characteristic of the NH proton. Additionally, ^{13}C NMR showed a signal at δ 55.7 ppm, which was attributed to the OCH_3 carbon.

Finally, benzothiazol-2-yl-acetonitrile **1** was reacted with carbon disulfide in the presence of sodium ethoxide for 20 min, which afforded the disodium salt that was further reacted with methyl iodide at room temperature to yield 2-(benzo[*d*]-thiazol-2-yl)-3,3-bis(methylthio)acrylonitrile **16**. The latter was reacted with the *N*-arylsulfonated guanidine **4a,b** to afford *N*-(4-amino-5-(benzo[*d*]thiazol-2-yl)-6-(methylthio)pyrimidin-2-yl)arylsulfonamide **17a,b**[35] (Scheme 5). Spectral data of

Scheme 5. Synthesis of *N*-(4-Amino-5-(benzo[*d*]thiazol-2-yl)-6-(methylthio)pyrimidin-2-yl)arylsulfonamide 17a,b



compound **17b** were consistent with the proposed structure. IR spectra of the latter showed characteristic absorption bands of the NH_2 and NH groups at wavenumbers 3375 and 3214 cm^{-1}, respectively. The ^1H NMR spectrum revealed two singlet signals at δ 2.37 and 2.39 ppm for the protons of CH_3 and SCH_3 groups, respectively, a multiplet at δ 7.41−8.12 ppm for the aromatic protons, a broad band at δ 8.49 ppm for the protons of the NH_2 group, and a broad band at δ 11.50 ppm characteristic of the NH proton.

Biological Evaluation. *Antiviral Activity.* The antiviral activities of the newly synthesized 2-pyrimidylbenzothiazoles were evaluated in vitro against a wide variety of viruses such as HSV-1, CBV4, HAV HM 175, ED-43/SG-Feo (VYG) replicon of HCV genotype 4a, and HAdV7. As it is well known that there is no specific cure available for CBV4, HCVgenotype4 and HAdV7 viruses and that available commercial remedies are

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Table 1. Antiviral Mean Percent of Reduction of Nontoxic Doses of Synthesized Compounds against Herpes Simplex Virus, Coxsackievirus B4, Hepatitis A Virus HM 175, HCVcc Genotype 4, and Adenovirus Type 7[a]

	mean % of reduction				
compd no.	herpes simplex virus	coxsackievirus B4	hepatitis A virus HM 175	HCVcc genotype 4	adenovirus type 7
5a	20 ± 0.9	60 ± 1.5	50 ± 1	30 ± 0.8	20 ± 0.5
5b	40 ± 1.4	50 ± 1.2	40 ± 1.0	20 ± 0.8	20 ± 0.3
7	10 ± 0.5	13.3 ± 0.4	10 ± 0.2	10 ± 0.3	10 ± 0.2
9a	90 ± 2.5	70 ± 1.4	70 ± 1.1	50 ± 0.9	50 ± 0.9
9b	70 ± 1.5	70 ± 1.6	60 ± 1.2	60 ± 1.2	40 ± 0.8
9c	30 ± 1.0	50 ± 1.1	40 ± 1.2	10 ± 0.6	10 ± 0.2
13b	10 ± 0.1	10 ± 0.1	10 ± 0.1	10 ± 0.2	10 ± 0.1
13c	10 ± 0.3	10 ± 0.2	10 ± 0.1	10 ± 0.1	10 ± 0.2
14a	10 ± 0.2	10 ± 0.1	10 ± 0.1	10 ± 0.1	10 ± 0.2
14b	70 ± 1.3	60 ± 1.1	50 ± 1.1	10 ± 0.6	30 ± 0.5
14c	30 ± 0.8	60 ± 1.2	50 ± 1.0	30 ± 0.7	30 ± 0.6
14d	10 ± 0.1	30 ± 0.2	20 ± 0.2	10 ± 0.1	10 ± 0.1
14e	16.7 ± 0.1	20 ± 0.2	13.3 ± 0.2	10 ± 0.3	10 ± 0.1
14f	10 ± 0.1	10 ± 0.2	10 ± 0.1	10 ± 0.1	10 ± 0.2
14g	80 ± 2.4	70 ± 1.5	60 ± 1.0	20 ± 0.7	40 ± 0.8
14h	70 ± 2.0	66.7 ± 1.1	60 ± 0.9	20 ± 0.5	40 ± 0.7
14i	10 ± 0.1	10 ± 0.1	10 ± 0.2	10 ± 0.2	10 ± 0.1
14j	10 ± 0.1	10 ± 0.1	10 ± 0.1	10 ± 0.3	10 ± 0.1
17a	10 ± 0.3	10 ± 0.1	10 ± 0.2	10 ± 0.1	10 ± 0.2
17b	10 ± 0.2	20 ± 0.2	10 ± 0.1	10 ± 0.1	10 ± 0.1
acyclovir	99.6 ± 2.8	NT	NT	NT	NT

[a]NT = not tested.

only used to treat the symptoms but not the illness itself. For this particular reason, acyclovir was used as a commercial standard for HSV-1 against which our new compounds are compared. In order to study the antiviral activities, the newly synthesized compounds were first subjected to a cytotoxicity evaluation using cell lines FRHK-4, Hep2, BGM, Vero, and Huh 7.5 as described clearly in the Supporting Information. No significant difference was observed between the amounts of the nontoxic doses of the various synthesized compounds, which ranged between 60 and 120 μg/mL. The synthesized compounds showed an apparent effect on the infected viral cell lines having different types of genome. The percentage of viral replication was assessed by measuring the viral load in treated cells as compared to the untreated cell line (Table 1).

The results indicated that nine compounds, **5a**, **5b**, **9a**, **9b**, **9c**, **14b**, **14c**, **14g**, and **14h**, had remarkable antiviral effects that exceeded 50% reduction against the studied viruses (Figure 2). Error bars in the figure represent the standard deviation of the measured data. The 50% maximum cytotoxicity concentration (CC_{50}) and the 50% maximal inhibitory concentration (IC_{50}) as well as the selectivity index (SI), CC_{50}/IC_{50} ratio, were evaluated for the nine compounds that exhibited greater than 50% viral reduction against the aforementioned tested viruses (Tables 2−4 and Figures 3−7).

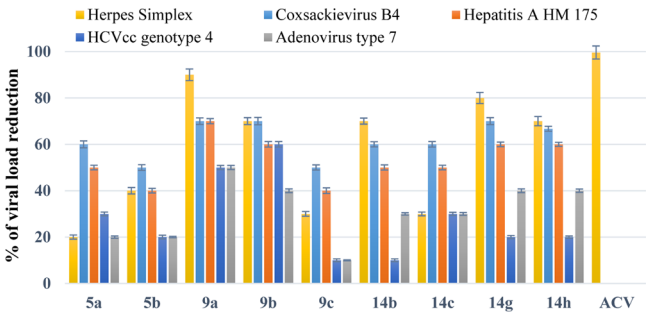

Figure 2. Comparison between the percent of viral load reduction of most potent compounds **5a,b**, **9a−c**, **14b,c**, and **14g,h**.

Table 2. Antiviral Activity against Herpes Simplex Virus of Compounds with Viral Reduction 50% or More in Terms of CC_{50}, IC_{50} (μg/μL), and SI

compd No.	CC_{50} (μg/μL)	IC_{50} (μg/μL)	SI
9a	0.27	0.063	4.29
9b	0.24	0.074	3.24
14b	0.25	0.066	3.79
14g	0.24	0.05	4.80
14h	0.23	0.071	3.24
acyclovir	0.028	0.007	4.00

Five of the synthesized compounds, **9a**, **9b**, **14b**, **14g**, and **14h**, showed a high level of potency against HSV-1 (Figure 2). While the viral reduction of ACV hit the 99.6% mark, compound **9a** reached 90%, compound **14g** reached 80%, and compounds **9b** and **14h** reached 70% (Figure 2). Furthermore, as shown in Table 2, the five compounds also had IC_{50} values ranging from 0.05 to 0.074 μg/μL, while that of ACV was 0.007 μg/μL. Although these compounds showed high performance against HSV-1when compared to the standard drug, two compounds in particular, **9a** and **14g**, showed much better performance than ACV in terms of its CC_{50}. Compound **9a** had a CC_{50} value of 0.27 μg/μL, and compound **14g** had a CC_{50} value of 0.24 μg/μL, whereas ACV had a value of 0.028 μg/μL (Table 2). With respect to the SI factor, compound **9a** gave an SI value of 4.29, and compound **14g** had an SI value of 4.8, whereas ACV had an SI value of 4. These selectivity indices and cytotoxicity results as well as the viral reduction

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Table 3. Antiviral Activity against Coxsackievirus B4 and Hepatitis A Virus HM 175 of Compounds with Viral Reduction 50% or More in Terms of CC_{50}, IC_{50} ($\mu g/\mu L$), and SI[a]

	coxsackievirus B4			hepatitis A virus HM 175		
compd no.	CC_{50} ($\mu g/\mu L$)	IC_{50} ($\mu g/\mu L$)	SI	CC_{50} ($\mu g/\mu L$)	IC_{50} ($\mu g/\mu L$)	SI
5a	0.22	0.085	2.59	0.22	0.10	2.20
5b	0.20	0.11	1.82	NT	NT	
9a	0.22	0.084	2.62	0.24	0.078	3.08
9b	0.22	0.092	2.39	0.23	0.086	2.67
9c	0.17	0.09	1.89	NT	NT	
14b	0.18	0.093	1.93	0.22	0.010	2.20
14c	0.23	0.091	2.53	0.23	0.010	2.30
14g	0.19	0.082	2.32	0.23	0.081	2.84
14h	0.19	0.074	2.57	0.20	0.074	2.70

[a]NT = not tested.

Table 4. Antiviral Activity against HCVcc Genotype 4 and Adenovirus Type 7 of Compounds with Viral Reduction 50% or More in Terms of CC_{50}, IC_{50} ($\mu g/\mu L$), and SI[a]

	HCVcc genotype 4			adenovirus type 7		
compd no.	CC_{50} ($\mu g/\mu L$)	IC_{50} ($\mu g/\mu L$)	SI	CC_{50} ($\mu g/\mu L$)	IC_{50} ($\mu g/\mu L$)	SI
9a	0.25	0.12	2.08	0.26	0.12	2.17
9b	0.25	0.086	2.91	NT	NT	

[a]NT = not tested.

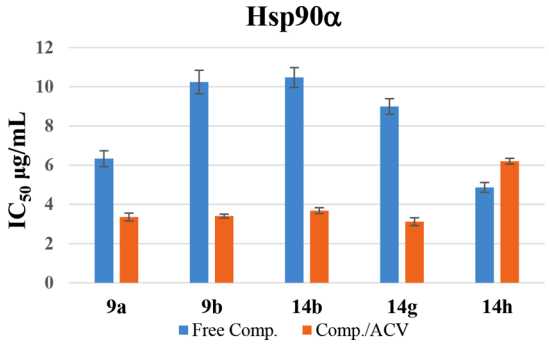

Figure 3. Relative IC_{50} of tested compounds and their combination with acyclovir on the Hsp90α protein.

percentages indicate clearly the antiviral potency of these newly synthesized compounds, **9a** and **14g**, especially when compared to the standard drug, ACV.

In the case of coxsackievirus B4, nine compounds, **5a**, **5b**, **9a**, **9b**, **9c**, **14b**, **14c**, **14g**, and **14h**, showed more than 50% viral reduction (Figure 2). The IC_{50} values of these compounds ranged from 0.074 to 0.093 $\mu g/\mu L$ with CC_{50} values ranging from 0.23 to 0.17 $\mu g/\mu L$ (Table 3). Among these nine compounds, compound **14c** (CC_{50} = 0.23 $\mu g/\mu L$) showed the highest value for the cytotoxicity, whereas compound **14h** showed the lowest inhibition concentration (IC_{50} = 0.074 $\mu g/100\ \mu L$). Furthermore, compound **9a** showed the highest SI value of 2.62.

Seven compounds, namely, **5a**, **9a**, **9b**, **14b**, **14c**, **14g**, and **14h**, have shown more than 50% reduction against HAV as clearly indicated in the supplementary tables with compound **9a** exhibiting the highest level of inhibitory activity among all others against HAV with 70% viral reduction. An IC_{50} value of 0.078 $\mu g/\mu L$, a CC_{50} value of 0.24 $\mu g/\mu L$, and an SI value of 3.08 were observed of **9a** against the virus as indicated in Table 3. Although compounds **14g** and **14h** revealed the same average reduction around 60%, compound **14g** showed higher CC_{50} than compound **14h**.

Compound **9a** showed apparent activity against both hepatitis C virus genotype 4a and adenovirus type 7, while compound **9b** showed slightly higher activity against hepatitis C virus genotype 4a (Figure 7). As shown in Table 4, both compounds **9a** and **9b** have the same cytotoxicity concentrations (CC_{50} = 0.25 $\mu g/\mu L$) but different inhibition concentrations (IC_{50}) with values of 0.12 and 0.086 $\mu g/\mu L$, respectively.

Structure–Activity Relationships. Based on the above results, the preliminary structure–activity relationships (SAR) have been established. In the case of pyrimidine substituted with phenylsulfonamide at C2, **5a**, the compound exhibited higher activity when compared to pyrimidine substituted with tosylamide group, **5b** (Figure 2). Additionally, the presence of a variety of amino arylethanone groups on the pyrimidine ring, **9a–c**, has increased its antiviral activities when compared to that of the starting compound **5a** (Figure 2). It is also clear that compound **9a** with a bromide substituent at the para position of the aryl moiety showed higher activity against HSV-1, CBV4, HAV, and HAdV7 (Figure 8) than that of **9b** with a chloride substituent. However, replacement of the bromide substituent on the aryl moiety with an electron-donating group such as a methyl group, **9c**, has lowered its activity (Figure 2). Similar observations can be also made in the case of compounds **14a–h**. The presence of electron-withdrawing groups such as a fluoride or chloride group on the para position of the aryl group (position C6 of the pyrimidine ring), **14b**, **14c**, **14g**, and **14h**, showed higher activities than those of compounds containing electron-donating groups such as a methyl or methoxy group, **14d**, **14e**, **14i**, and **14j**. Compounds with the methylthio group at C6 of the pyrimidine ring **17a,b** showed very low activities against all tested viruses.

Hsp90α Inhibition Assay. Heat shock protein 90 (Hsp90α) present in most cell types is important for viral protein folding, assembly, and replication.[36] During infection, HSV-1, for example, uses the Hsp90α chaperone system, and the viral polymerase could be a client protein of Hsp90α.[37] The possibility that inhibitors of Hsp90α would also be inhibitors of HSV-1 infection has led us to examine our newly synthesized compounds as possible novel Hsp90α inhibitors.

In order to investigate the effect of the potent HSV-1 antiviral compounds **9a**, **9b**, **14b**, **14g**, and **14h** against Hsp90α, the Hsp90α (C-Terminal) inhibitor screening assay kit was used. A combination of each potent compound with ACV (reference drug), in a 1:1 ratio, was also tested. Results for all compounds were calculated as IC_{50} and are displayed in Figure 3. The data obtained during this study were used to graph a dose–response curve. The concentration of tested compounds that is required to inhibit 50% of the virus cell population, IC_{50}, was evaluated (Figure 4). Error bars in the various figures represent the standard deviation of the measured data. By comparing the performance of these compounds against that of the standard drug ACV, several observations are noted. All five compounds exhibited a potent inhibitory effect toward Hsp90α and were active in the microgram per milliliter solution (Table 5).

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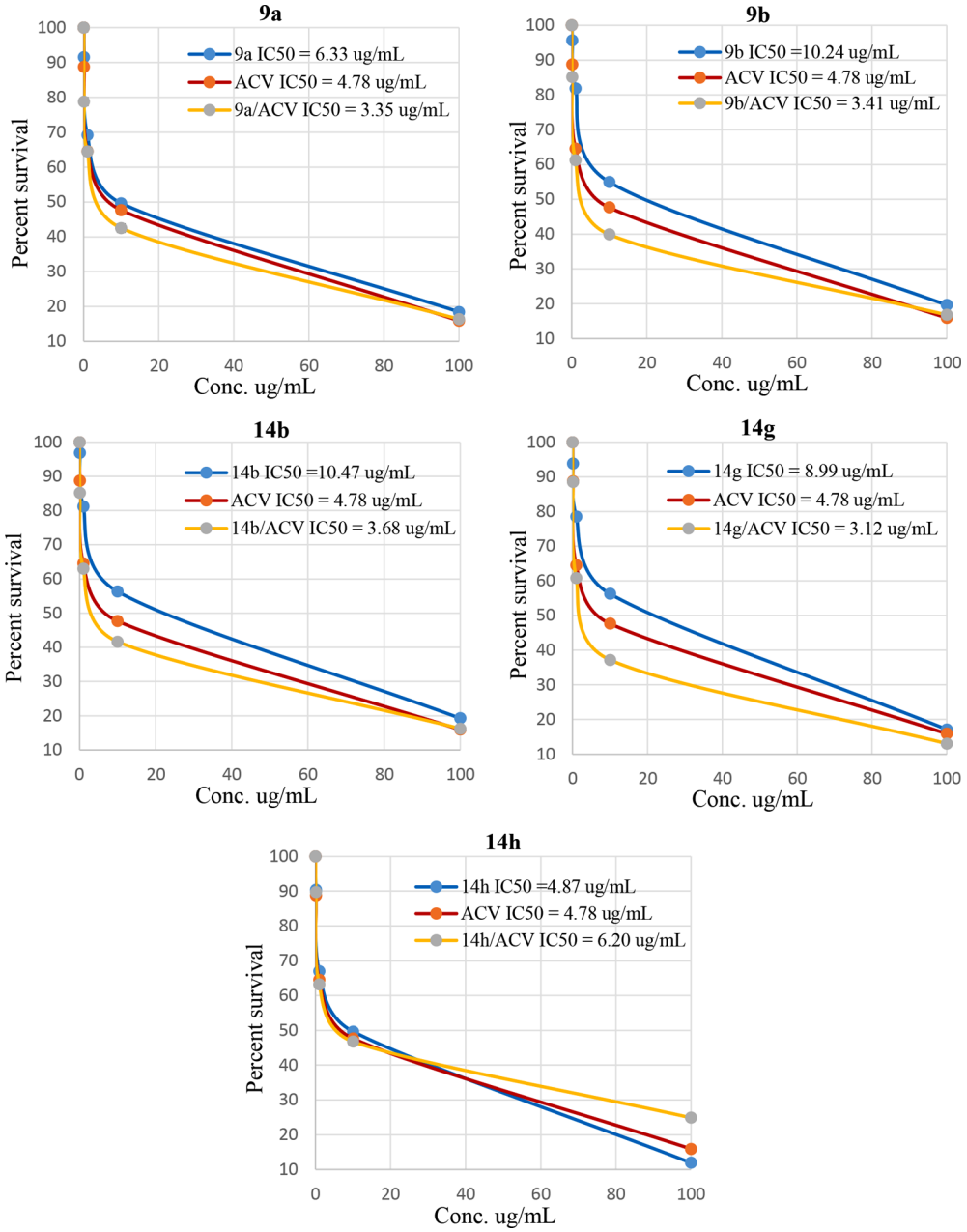

Figure 4. Different concentrations of **9a,b**, **14b**, and **14g,h** and their combination with acyclovir versus the corresponding percent of cell survival using Hsp90α (C-Terminal) inhibitor screening assay. Each point is the mean (standard deviation) of three independent experiments.

Table 5. Mean ± SD of IC_{50} Values (the Drug Concentrations That Inhibited 50% of Cell Proliferation) and the Different Concentrations Used of the Tested Compounds and Their Combination with Acyclovir on the Hsp90α Protein

compd no.	100 (μg/mL)	10 (μg/mL)	1 (μg/mL)	0.1 (μg/mL)	IC_{50} (μg/mL)
9a	81.5553	50.3722	30.80014	8.45589	6.33 ± 0.4
9b	80.37387	45.04568	18.17787	4.320374	10.24 ± 0.6
14b	80.73341	43.68688	18.83649	3.165316	10.47 ± 0.5
14g	82.85524	43.74068	21.44356	6.136187	8.99 ± 0.4
14h	88.05286	50.34291	33.03409	9.589642	4.87 ± 0.25
ACV	84.06329	52.33982	35.451	11.2422	4.78 ± 0.2
9a/ACV	83.63504	57.47486	35.49681	21.20627	3.36 ± 0.2
9b/ACV	83.20892	60.08167	38.78328	14.9151	3.41 ± 0.1
14b/ACV	83.78818	58.34788	37.00635	14.80698	3.68 ± 0.2
14g/ACV	86.96226	62.80885	39.23257	11.46644	3.13 ± 0.2
14h/ACV	75.0849	53.2219	36.75174	10.26611	6.20 ± 0.2

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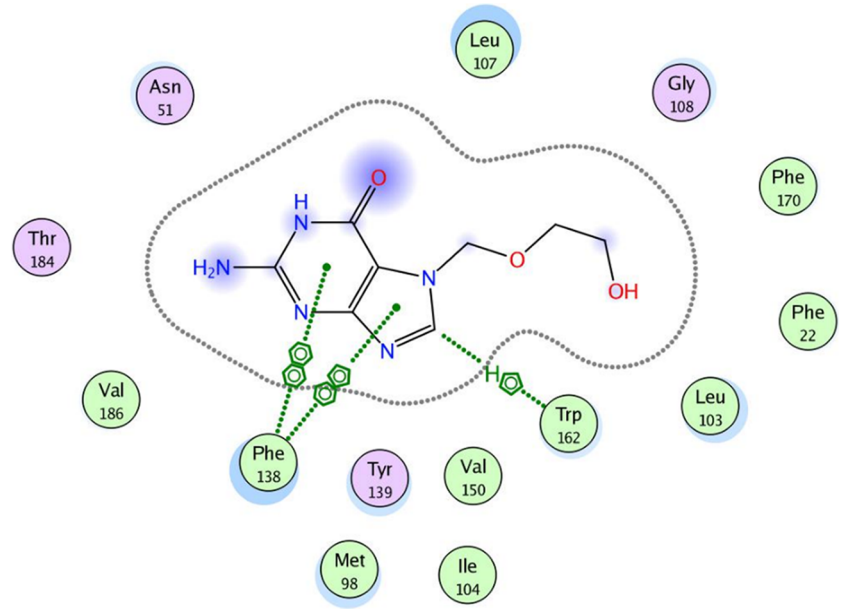

Figure 5. Best docked pose of acyclovir inside the binding pocket of Hsp90α (PDB ID 3b25) with a docking score of −5.6421 kcal/mol.

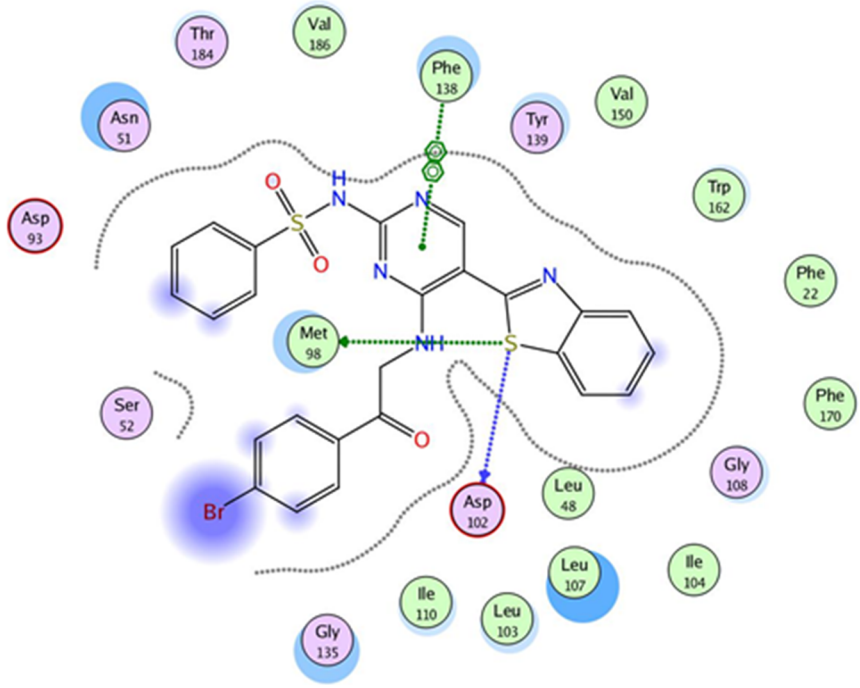

Figure 6. Best docked pose of **9a** inside the binding pocket of Hsp90α (PDB ID 3b25) with a docking score of −8.6521 kcal/mol.

Consistent with the calculated IC_{50}, compound **14h** was the most potent Hsp90α inhibitor with an IC_{50} value of 4.87 μg/mL followed by **9a** and **14g** with IC_{50} values of 6.33 and 8.99 μg/mL, respectively, as compared to the ACV reference drug IC_{50} value of 4.78 μg/mL. It is clear that the combination of four out of the five tested compounds, namely, **9a**, **9b**, **14b**, and **14g**, with ACV has increased the potency of the compounds, which was reflected as a marked reduction in the IC_{50} values of the original single compounds. Notwithstanding, these combinations have also shown lower IC_{50} values than that of ACV itself. For example, the IC_{50} values of compounds **9a**, **9b**, **14b**, and **14g** have dropped from 6.33, 10.24, 10.47, and 8.99 μg/mL to 3.35, 3.41, 3.68, and 3.12 μg/mL, respectively, when combined with ACV in a 1:1 ratio. The resulting data indicated that a combination of compounds **9a**, **9b**, **14b**, and **14g** with ACV is highly recommended for use as possible potent inhibitors for Hsp90α and, consequently, inhibitors for HSV-1.

Molecular Modeling and Docking Study. To evaluate the underlying principles behind the action of these new compounds in inhibiting Hsp90α, a molecular docking study using a molecular modeling environment (MOE) was performed on the reference drug ACV and the potent compounds **9a**, **9b**, **14b**, **14g**, and **14h**. The compounds were docked with the crystal structure of the Hsp90α protein (PDB ID 3b25) through the removal of the bound ligand, B2K

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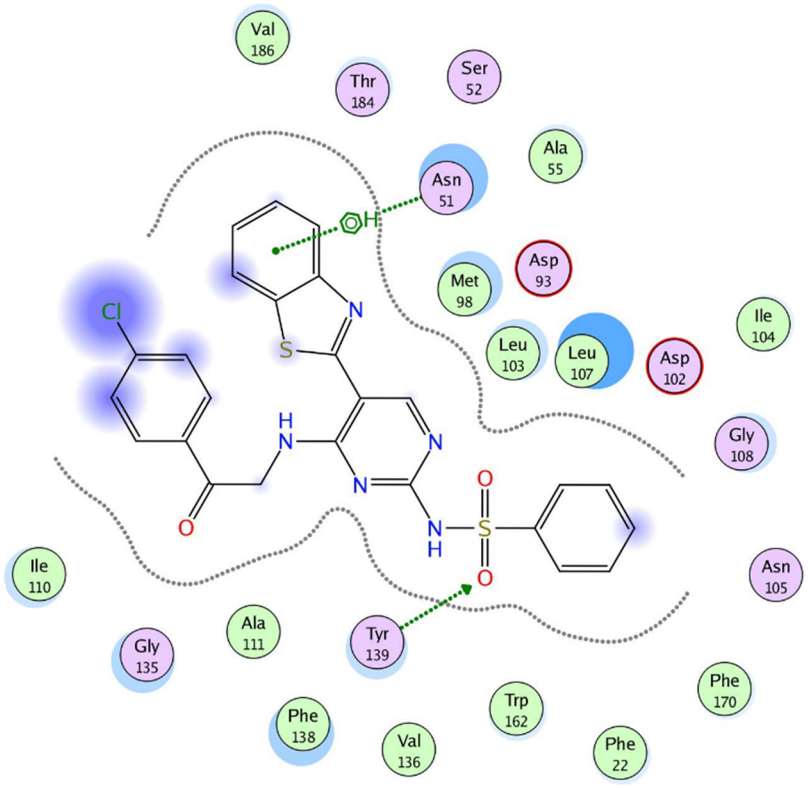

Figure 7. Best docked pose of **9b** inside the binding pocket of Hsp90α (PDB ID 3b25) with a docking score of −8.3103 kcal/mol.

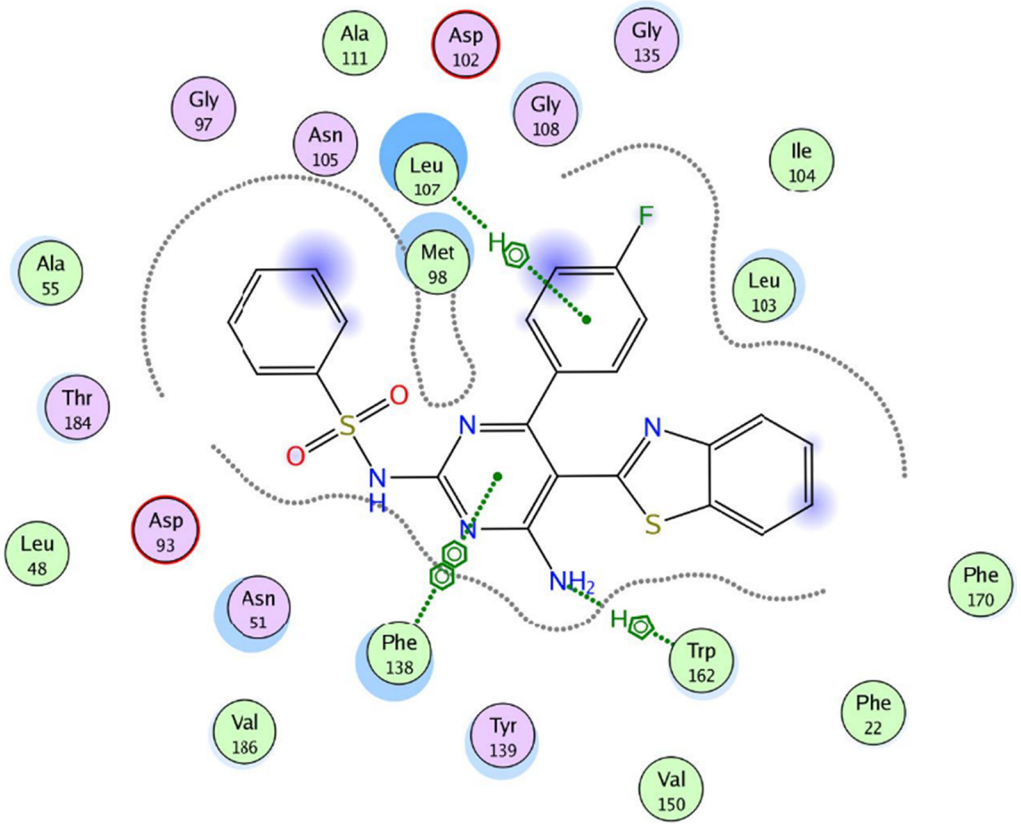

Figure 8. Best docked pose of **14b** inside the binding pocket of Hsp90α (PDB ID 3b25) with a docking score of −9.7282 kcal/mol.

(4-methyl-6-(toluene-4-sulfonyl)-pyrimidin-2-ylamine), to uncover the binding pattern of these compounds with the receptor. The docking study revealed that the molecules had good binding energy in the range of −5.6421 to −9.7282 kcal/

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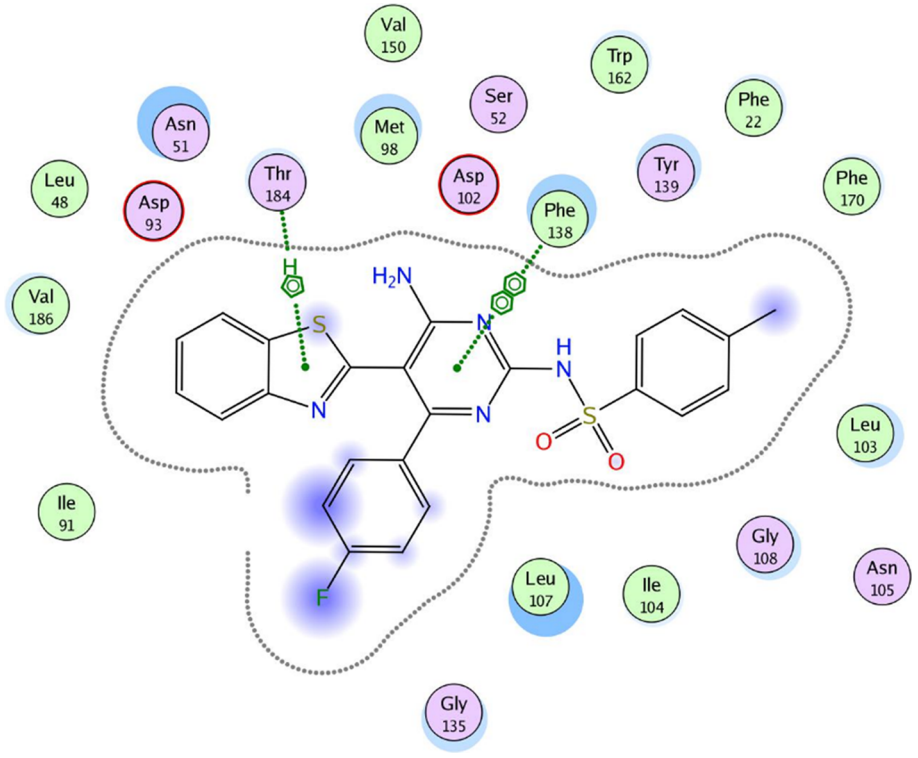

Figure 9. Best docked pose of **14g** inside the binding pocket of Hsp90α (PDB ID 3b25) with a docking score of −8.1512 kcal/mol.

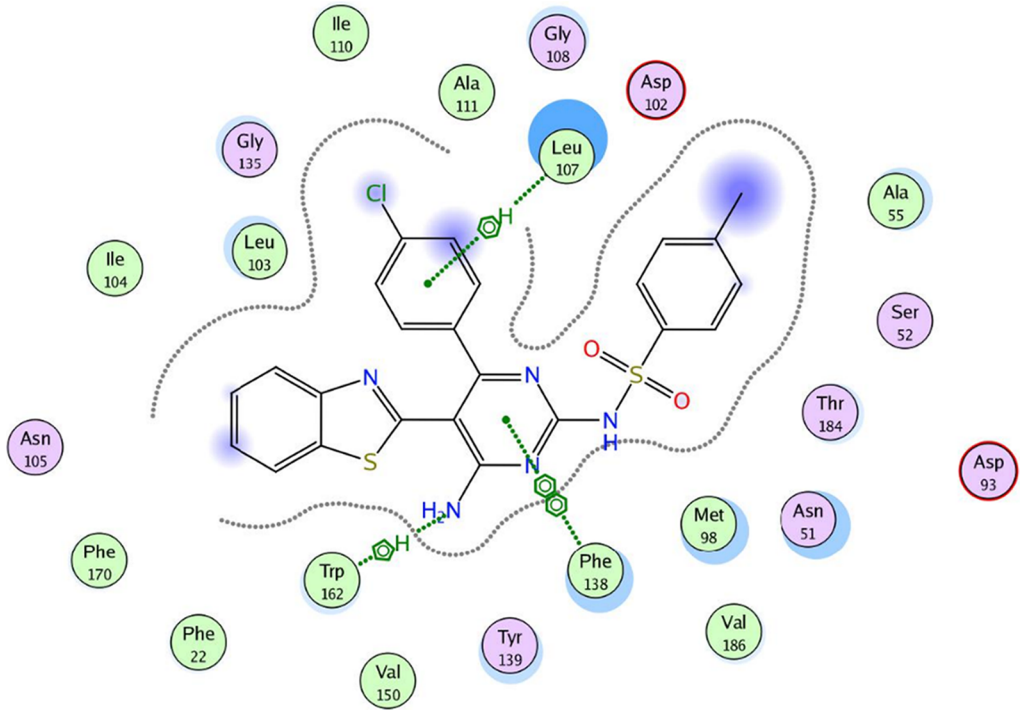

Figure 10. Best docked pose of **14h** inside the binding pocket of Hsp90α (PDB ID 3b25) with a docking score of − 9.1024 kcal/mol.

mol with the receptor within the active site. The pyrimidine ring of ACV and the tested compounds **9b**, **14b**, **14g**, and **14h** showed hydrophobic interaction with the active site of Phe 138 (Figures 5−10). Only one compound, **9a**, displayed one hydrogen-bonding interaction at a distance of 3.84 Å between the sulfur atom of the benzothiazole ring and amino acid residue Asp 102 (Figure 6). Moreover, compounds **9a** and **9b** interacted with the amino acid residues Met 98 and Tyr 139, respectively, through polar bonds (Figures 6 and 7). In addition, two aren-H interactions of the benzene ring and the amino group of compounds **14b** and **14h** were observed to bind to Leu 107 and Trp162, respectively (Figures 8 and 10), while compounds **9b** and **14g** showed one aren-H interaction with Asn 51 and Thr 184, respectively (Figures 7 and 9). The

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docking study revealed a docking score of −9.1024 kcal/mol for compound **14h**, −8.6521 kcal/mol for compound **9a**, and a docking score of −5.6421 kcal/mol for the reference drug ACV.

CONCLUSIONS

In conclusion, 21 new compounds of 2-pyrimidylbenzothiazoles bearing either the amino group or sulfonamide moieties at the C2 position of the pyrimidine ring were synthesized by reacting guanidine or *N*-arylsulfonated guanidine with different derivatives of ylidene benzothiazole. The structures of all the compounds were confirmed spectroscopically and via elemental analyses. The newly synthesized compounds were evaluated for their antiviral activity against HSV-1, COB4, HAV HM 175, ED-43/SG-Feo (VYG) replicon of HCV genotype 4a, and HAdV7. Nine compounds exhibited remarkable activities against these viruses with high cytotoxicity concentration and more than 50% viral reduction. The most active five compounds against HSV-1 have been also evaluated against Hsp90α with their activities compared to that of the reference drug acyclovir. A combination of the tested compounds and acyclovir in a 1:1 ratio, amazingly, led to increased potency for these compounds with IC_{50} values lower than that of acyclovir. The work confirms that the newly synthesized novel compounds, 2-pyrimidylbenzothiazole derivatives, exhibit exceptional antiviral activities and inhibitory effect on the Hsp90α protein and thus can be useful as highly effective broad spectrum antiviral agents.

EXPERIMENTAL SECTION

Chemistry. Melting points were measured using an SMP3 apparatus. IR spectra, using KBr discs, were measured on either a Pye Unicam SP-1000 or FTIR plus 460 spectrophotometer. Both ^{1}H and ^{13}C spectra were recorded on a Bruker Avance (III)-400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) at the Ain Shams University, Cairo, Egypt, using DMSO-d_6 with $Si(CH_3)_4$ as an internal standard. Thin-layer chromatography (TLC), aluminum sheets coated with silica gel F254 (Merck), and an ultraviolet (UV) lamp were used to monitor the progress of the reactions. The elemental analyses were done at the Microanalytical Data Unit at the Cairo University and performed on Vario EI III Elemental CHNS analyzer.

General Procedure for the Synthesis of 5a,b. 2-(Benzo[*d*]thiazol-2-yl)-3-(dimethylamino)acrylonitrile **3** (2.30 g, 0.01 mmol) was added to a stirred solution of the *N*-carbamimidoylarylsulfonamide **4a,b** (0.01 mol) in dry dioxane (20 mL) containing potassium hydroxide (0.56 g, 0.01 mol). The reaction mixture was heated under reflux for 2 h. After completion of the reaction (TLC), the reaction mixture was cooled and poured into ice water. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from DMF.

*N-(4-Amino-5-(Benzo[d]thiazol-2-yl)pyrimidin-2-yl)-benzenesulfonamide (**5a**).* Yellow crystals; (DMF); yield 74.3%; m.p. 345−346 °C; IR (KBr, cm^{-1}): ν 3429 and 3278 (NH, NH_2), 3059 (ArCH), 1599 (C═C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.30−7.45 (m, 5H, 3Ar-H & 1benzothiazole-H), 7.67 (br s, 2H, NH_2), 7.85−7.99 (m, 4H, 2Ar-H & 2benzothiazole-H), 8.32 (s, 1H, CH pyrimidine). ^{13}C NMR (100 MHz, DMSO-d_6): δ 99.9, 121.5, 122.0, 124.7, 126.7, 127.6, 127.7, 129.7, 131.7, 147.2, 153.2, 158.2, 160.1, 164.5, 166.9 (Ar-C). Anal. Calcd for $C_{17}H_{13}N_5O_2S_2$ (383.45): C% 53.25; H% 3.42; N% 18.26. Found: C% 53.33; H% 3.40; N% 18.20.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)pyrimidin-2-yl)-4-methylbenzenesulfonamide (**5b**).* Yellow crystals; (DMF); yield 85.7%; m.p. 336−337 °C; IR (KBr, cm^{-1}): ν 3397 and 3288 (NH, NH_2), 3061 (ArCH), 1605 (C═C). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.31 (s, 3H, CH_3), 7.21 (d, 2H, J = 6.8 Hz, Ar-H), 7.36 (t, 1H, J = 8 Hz, benzothiazole-H), 7.46 (t, 1H, J = 8 Hz, benzothiazole-H), 7.86 (d, 2H, J = 8 Hz, Ar-H), 7.92 (d, 1H, J = 8 Hz, benzothiazole-H), 8.01 (d, 1H, J = 8 Hz, benzothiazole-H), 8.19 (br s, 2H, NH_2), 8.49 (s, 1H, CH pyrimidine). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.3 (CH_3), 100.8, 121.9, 122.1, 125.3, 126.8, 127.9, 128.6, 132.1, 140.3, 142.7, 153.0, 156.0, 160.2, 161.0, 166.0 (Ar-C). Anal. Calcd for $C_{18}H_{15}N_5O_2S_2$ (397.47): C% 54.39; H% 3.80; N% 17.62. Found: C% 54.30; H% 3.87; N% 17.55.

Procedure for the Synthesis of 7. To a solution of 3-chloropentane-2,4-dione **6** (1.20 mL, 0.01 mol) in 30 mL of ethanol, **5a** (3.83 g, 0.01 mol) was added. After being refluxed for 1 h, sodium bicarbonate (1.30 g, 0.015 mol) was added, and the mixture was heated for additional 2 h. After completion of the reaction (TLC), the solid precipitate was filtered off, washed with water, and dried.

*N-(5-(Benzo[d]thiazol-2-yl)-4-((2-oxopropyl)amino)-pyrimidin-2-yl)benzenesulfonamide (**7**).* White crystals; (DMF); yield 80%; m.p. 291−292 °C; IR (KBr, cm^{-1}): ν 3423 (NH), 2921 (ArCH), 1645 (C═O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.21 (s, 3H, CH_3), 4.94 (s, 2H, CH_2), 7.46−7.53 (m, 5H, 3Ar-H & 2benzothiazole-H), 7.96−8.12 (m, 4H, 2Ar-H & 2benzothiazole-H), 8.64 (s, 1H, CH pyrimidine), 8.82 (br s, 1H, NH), 9.31 (br s, 1H, NH). Anal. Calcd for $C_{20}H_{17}N_5O_3S_2$ (439.51): C% 54.65; H% 3.90; N% 15.93. Found: C% 54.60; H% 3.82; N% 15.89.

General Procedure for the Synthesis of 9a−c. To a solution of substituted phenacyl bromide **8a−c** (0.01 mol) in 30 mL of ethanol, **5a** (3.83 g, 0.01 mol) was added, and the mixture heated under reflux for 1 h. Sodium bicarbonate (1.30 g, 0.015 mol) was then added, and the mixture was refluxed for additional 2 h. After completion of the reaction (TLC), the solid precipitate was filtered off, washed with water, and dried.

*N-(5-(Benzo[d]thiazol-2-yl)-4-((2-(4-bromophenyl)-2-oxoethyl)amino)pyrimidin-2-yl)benzenesulfonamide (**9a**).* White needles; (DMF); yield 80%; m.p. 294−295 °C; IR (KBr, cm^{-1}): ν 3440 (NH), 3047 (ArCH), 1651 (C═O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.61 (s, 2H, CH_2), 7.39 (t, 2H, J = 8 Hz, Ar-H), 7.45−7.49 (m, 2H, Ar-H, benzothiazole-H), 7.55 (t, 1H, J = 8 Hz, benzothiazole-H), 7.83 (d, 2H, J = 8 Hz, Ar-H), 7.89 (d, 2H, J = 8 Hz, Ar-H), 8.00 (d, 2H, J = 8 Hz, Ar-H), 8.07 (d, 1H, J = 8 Hz, benzothiazole-H), 8.13 (d, 1H, J = 8 Hz, benzothiazole-H), 8.78 (s, 1H, CH pyrimidine), 8.88 (br s, 1H, NH), 9.37 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 57.0 (CH_2), 101.3, 122.1, 122.3, 125.9, 126.7, 127.4, 127.7, 128.3, 130.0, 130.9, 132.0, 132.1, 133.2, 143.2, 149.7, 151.9, 152.1, 159.0, 162.9, 191.8 (Ar-C). Anal. Calcd for $C_{25}H_{18}BrN_5O_3S_2$ (580.48): C% 51.73; H% 3.13; N% 12.06. Found: C% 51.65; H% 3.19; N% 12.14.

*N-(5-(Benzo[d]thiazol-2-yl)-4-((2-(4-chlorophenyl)-2-oxoethyl)amino)pyrimidin-2-yl)benzenesulfonamide (**9b**).* White needles; (DMF); yield 80%; m.p. 292−293 °C; IR (KBr, cm^{-1}): ν 3365 (NH), 3057 (ArCH), 1649 (C═O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.63 (s, 2H, CH_2), 7.41−7.53 (m, 5H, 3Ar-H, 2benzothiazole-H), 7.67 (d, 2H, J = 8 Hz, Ar-

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H), 7.92 (d, 2H, J = 8 Hz, Ar-H), 8.01−8.09 (m, 4H, 2Ar-H & 2benzothiazole-H), 8.75 (s, 1H, CH), 8.88 (br s, 1H, NH), 9.36 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 57.0 (CH_2), 101.3, 122.0, 122.3, 125.8, 126.7, 127.4, 127.7, 129.1, 129.9, 130.9, 132.1, 132.9, 139.0, 143.2, 149.7, 151.9, 152.1 and 159.0 (Ar-C), 162.9 (C═O), 191.8 (Ar-C). Anal. Calcd for $C_{25}H_{18}ClN_5O_3S_2$ (536.03): C% 56.02; H% 3.38; N% 13.07. Found: C% 56.10; H% 3.30; N% 13.10.

*N-(5-(Benzo[d]thiazol-2-yl)-4-((2-(p-tolyl)-2-oxoethyl)-amino)pyrimidin-2-yl)benzenesulfonamide (**9c**).* White needles; (DMF); yield 80%; m.p. 305−307 °C; IR (KBr, cm^{-1}): ν 3436 (NH), 3038 (ArCH), 1648 (C═O). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.44 (s, 3H, CH_3), 5.62 (s, 2H, CH_2), 7.37−7.50 (m, 6H, 5Ar-H & benzothiazole-H), 7.56 (t, 1H, J = 8 Hz, benzothiazole-H), 7.88 (d, 2H, J = 8 Hz, Ar-H), 7.98 (d, 2H, J = 8 Hz, Ar-H), 8.08 (d, 1H, J = 8 Hz, benzothiazole-H), 8.14 (d, 1H, J = 8 Hz, benzothiazole-H), 8.79 (s, 1H, CH pyrimidine), 8.85 (br s, 1H, NH), 9.35 (br s, 1H, NH). Anal. Calcd for $C_{26}H_{21}N_5O_3S_2$ (515.61): C% 60.57; H% 4.11; N% 13.58. Found: C% 60.65; H% 4.03; N% 13.63.

General Procedure for the Synthesis of 13a−c. To a stirred solution of guanidine hydrochloride (1.43 g, 0.015 mol) **12** in dry dioxane (30 mL) containing potassium hydroxide (0.84 g, 0.015 mol), 2-(benzo[d]thiazol-2-yl)-3-arylacrylonitrile **11c−e** (0.01 mol) was added, and the mixture was refluxed for 2 h. After completion of the reaction (TLC), the reaction mixture was then cooled and poured into ice water. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from DMF.

*5-(Benzo[d]thiazol-2-yl)-6-(4-chlorophenyl)pyrimidine-2,4-diamine (**13a**).* Orange crystals; (DMF); yield 76%; m.p. >350 °C; IR (KBr, cm^{-1}): ν 3431 (NH_2), 2913 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.09 (t, 1H, J = 8 Hz, benzothiazole-H), 7.26 (t, 1H, J = 8 Hz, benzothiazole-H), 7.50−7.58 (m, 5H, 4Ar-H, benzothiazole-H), 7.69 (d, 1H, J = 8 Hz, benzothiazole-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 66.83, 81.58, 120.61, 121.30, 123.26, 125.98, 129.26, 133.87, 153.22, 167.59 (Ar-C). Anal. Calcd for $C_{17}H_{12}ClN_5S$ (353.83): C% 57.71; H% 3.42; N% 19.79. Found: C% 57.77; H% 3.35; N% 19.84.

*5-(Benzo[d]thiazol-2-yl)-6-p-tolylpyrimidine-2,4-diamine (**13b**).* Orange crystals; (DMF); yield 77%; m.p. >350 °C; IR (KBr, cm^{-1}): ν 3432 (NH_2), 2919 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.41 (s, 3H, CH_3), 7.07 (t, 1H, J = 8 Hz, benzothiazole-H), 7.24−7.28 (m, 3H, 2Ar-H & benzothiazole-H), 7.44 (d, 2H, J = 5.6 Hz, Ar-H), 7.57 (d, 1H, J = 8 Hz, benzothiazole-H), 7.65 (d, 1H, J = 8 Hz, benzothiazole-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.61 (CH_3), 66.83, 81.60, 120.45, 121.18, 123.10, 125.87, 129.84, 153.18, 168.15 (Ar-C). Anal. Calcd for $C_{18}H_{15}N_5S$ (333.41): C% 64.84; H% 4.53; N% 21.01. Found: C% 64.90; H% 4.48; N% 21.08.

*5-(Benzo[d]thiazol-2-yl)-6-(4-methoxyphenyl)pyrimidine-2,4-diamine (**13c**).* Orange crystals; (DMF); yield 79%; m.p. >350 °C; IR (KBr, cm^{-1}): ν 3441 (NH_2), 2915 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 7.01−7.09 (m, 3H, 2Ar-H & benzothiazole-H), 7.25 (t, 1H, J = 8 Hz, benzothiazole-H), 7.48 (d, 1H, J = 8 Hz, benzothiazole-H), 7.58 (d, 2H, J = 8 Hz, Ar-H), 7.65 (d, 1H, J = 8 Hz, benzothiazole-H). Anal. Calcd for $C_{18}H_{15}N_5OS$ (349.41): C% 61.87; H% 4.33; N% 20.04. Found: C% 61.94; H% 4.26; N% 20.10.

General Procedure for the Synthesis of 14a−j. A mixture of 2-(benzo[d]thiazol-2-yl)-3-arylacrylonitrile **11a−c** (0.01 mol) and *N*-carbamimidoylarylsulfonamide **4a,b** (0.01 mol) in dry dioxane (20 mL) containing potassium hydroxide (0.56 g, 0.01 mol) was refluxed for 2 h. After completion of the reaction (TLC), the reaction mixture was then cooled and poured into ice water. The resulting precipitate was filtered off, washed with water, dried, and recrystallized from appropriate solvent.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-phenylpyrimidin-2-yl)benzenesulfonamide (**14a**).* Buff crystals; (Ethanol); yield 83%; m.p. 260−261 °C; IR (KBr, cm^{-1}): ν 3419 and 3366 (NH, NH_2), 3224 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.33−7.60 (m, 10H, 8Ar-H & 2benzothiazole-H), 7.83 (d, 1H, J = 8 Hz, benzothiazole-H), 8.02 (d, 1H, J = 8 Hz, benzothiazole-H), 8.11 (d, 2H, J = 8 Hz, Ar-H), 8.6 (br s, 2H, NH_2), 11.9 (br s, 1H, NH). Anal. Calcd for $C_{23}H_{17}N_5O_2S_2$ (459.54): C% 60.11; H% 3.73; N% 15.24. Found: C% 60.18; H% 3.68; N% 15.30.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(4-fluorophenyl)-pyrimidin-2-yl)benzenesulfonamide (**14b**).* Yellow needles; (DMF); yield 76%; m.p. 281−282 °C; IR (KBr, cm^{-1}): ν 3405 and 3368 (NH, NH_2), 3191 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.27−7.59 (m, 9H, 7Ar-H & 2benzothiazole-H), 7.90 (d, 1H, J = 8 Hz, benzothiazole-H), 8.03 (d, 1H, J = 8 Hz, benzothiazole-H), 8.11 (d, 2H, J = 8 Hz, Ar-H), 8.62 (br s, 2H, NH_2), 11.90 (br s, 1H, NH). Anal. Calcd for $C_{23}H_{16}FN_5O_2S_2$ (477.53): C% 57.85; H% 3.38; N% 14.67. Found: C% 57.94; H% 3.32; N% 14.71.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(4-chlorophenyl)-pyrimidin-2-yl)benzenesulfonamide (**14c**).* Yellow needles; (DMF); yield 81%; m.p. 298−299 °C; IR (KBr, cm^{-1}): ν 3410 and 3362 (NH, NH_2), 3181 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.37−7.63 (m, 9H, 7Ar-H & 2benzothiazole-H), 7.91 (d, 1H, J = 8 Hz, benzothiazole-H), 8.03 (d, 1H, J = 8 Hz, benzothiazole-H), 8.09 (d, 2H, J = 7.6 Hz, Ar-H), 8.50 (br s, 2H, NH_2), 11.81 (br s, 1H, NH). Anal. Calcd for $C_{23}H_{16}ClN_5O_2S_2$ (493.99): C% 55.92; H% 3.26; N% 14.18. Found: C% 55.98; H% 3.21; N% 14.26.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-p-tolylpyrimidin-2-yl)benzenesulfonamide (**14d**).* Yellow needles; (Ethanol); yield 72%; m.p. 258−259 °C; IR (KBr, cm^{-1}): ν 3385 and 3309 (NH_2, NH), 3179 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 7.08−7.22 (m, 5H, 4Ar-H & benzothiazole-H), 7.34−7.43 (m, 3H, Ar-H), 7.52 (t, 1H, J = 7.6 Hz, benzothiazole-H), 7.67−7.70 (m, 3H, 2Ar-H & benzothiazole-H), 7.81−7.84 (m, 3H, benzothiazole-H, NH_2), 10.05 (br s, 1H, NH). Anal. Calcd for $C_{24}H_{19}N_5O_2S_2$ (473.57): C% 60.87; H% 4.04; N% 14.79. Found: C% 61.00; H% 3.98; N% 14.84.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)benzenesulfonamide (**14e**).* Buff crystals; (DMF); yield 76%; m.p. 286−287 °C; IR (KBr, cm^{-1}): ν 3405 and 3358 (NH, NH_2), 3169 (ArCH). ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 3H, OCH_3), 6.96 (d, 2H, J = 7.6 Hz, Ar-H), 7.30−7.36 (m, 3H, 2Ar-H & benzothiazole-H), 7.45−7.60 (m, 4H, 3Ar-H & benzothiazole-H), 7.87 (d, 1H, J = 7.6, benzothiazole-H), 8.02 (d, 1H, J = 6.8 Hz, benzothiazole-H), 8.10 (d, 2H, J = 7.6 Hz, Ar-H), 8.59 (br s, 2H, NH_2), 11.93 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 55.7 (OCH_3), 114.5, 122.0, 122.9, 125.8, 126.6, 128.5, 128.7, 131.8, 135.1, 151.7, 161.6, 161.9 (Ar-C). Anal. Calcd for $C_{24}H_{19}N_5O_3S_2$ (489.57): C% 58.88; H% 3.91; N% 14.31. Found: C% 58.99; H% 3.97; N% 14.40.

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*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-phenylpyrimidin-2-yl)-4-methylbenzenesulfonamide (**14f**).* Colorless needles; (Ethanol); yield 82%; m.p. 244−245 °C; IR (KBr, cm^{-1}): ν 3440 and 3350 (NH_2, NH), 3226 (ArCH). ^1H NMR (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3), 7.35−7.48 (m, 9H, 7Ar-H & benzothiazole-H), 7.85−7.97 (m, 4H, 2Ar-H & 2benzothiazole-H). Anal. Calcd for $C_{24}H_{19}N_5O_2S_2$ (473.57): C % 60.87; H% 4.04; N% 14.79. Found: C% 60.97; H% 4.00; N % 14.85.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(4-fluorophenyl)-pyrimidin-2-yl)-4-methylbenzenesulfonamide (**14g**).* Yellow needles; (Ethanol); yield 77.9%; m.p. 266−267 °C; IR (KBr, cm^{-1}): ν 3400 and 3366 (NH, NH_2), 3181 (ArCH). ^1H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3) 7.24−7.50 (m, 8H, 6Ar-H & 2benzothiazole-H), 7.89−8.04 (m, 4H, 2Ar-H & 2benzothiazole-H), 8.05 (br s, 2H, NH_2), 11.75 (br s, 1H, NH). Anal. Calcd for $C_{24}H_{18}FN_5O_2S_2$ (491.56): C% 58.64; H % 3.69; N% 14.25. Found: C% 58.73; H% 3.63; N% 14.32.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(4-chlorophenyl)-pyrimidin-2-yl)-4-methylbenzenesulfonamide (**14h**).* Yellow solid; (DMF); yield 85%; m.p. 297−298 °C; IR (KBr, cm^{-1}): ν 3404 and 3366 (NH, NH_2), 3181 (ArCH). ^1H NMR (400 MHz, DMSO-d_6): δ 2.36 (s, 3H, CH_3), 7.33−7.47 (m, 8H, 6Ar-H & 2benzothiazole-H), 7.89−8.03 (m, 4H, 2Ar-H & 2 benzothiazole-H). Anal. Calcd for $C_{24}H_{18}ClN_5O_2S_2$ (508.02): C% 56.74; H% 3.57; N% 13.79. Found: C% 56.81; H% 3.53; N% 13.84.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-p-tolylpyrimidin-2-yl)-4-methylbenzenesulfonamide (**14i**).* White crystals; (DMF); yield 78%; m.p. 323−325 °C; IR (KBr, cm^{-1}): ν 3453 and 3360 (NH_2, NH). ^1H NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 7.10−7.16 (m, 6H, Ar-H), 7.24 (t, 1H, J = 8 Hz, benzothiazole-H), 7.39 (t, 1H, J = 7.6 Hz, benzothiazole-H), 7.74−7.77 (m, 3H, 2Ar-H & benzothiazole-H), 7.88 (d, 1H, J = 8 Hz, benzothiazole-H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.3, 21.4 ($2CH_3$), 98.5, 121.5, 121.8, 124.6, 126.2, 128.0, 129.1, 130.1, 134.6, 137.2, 138.9, 139.1, 144.5, 151.9, 161.7, 167.2 (Ar-C). Anal. Calcd for $C_{25}H_{21}N_5O_2S_2$ (487.60): C% 61.58; H% 4.34; N% 14.36. Found: C% 61.68; H% 4.29; N% 14.41.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(4-methoxyphenyl)pyrimidin-2-yl)-4-methylbenzenesulfonamide (**14j**).* Yellow crystals; (Ethanol); yield 79.4%; m.p. 248−249 °C; IR (KBr, cm^{-1}) ν 3368 and 3237 (NH, NH_2), 3065 (ArCH). ^1H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 6.96 (d, 2H, J = 9.2 Hz, Ar-H), 7.32−7.37 (m, 5H, 4Ar-H & benzothiazole-H), 7.48 (t, 1H, J = 7.2 Hz, benzothiazole-H), 7.87 (d, 1H, J = 8.4 Hz, benzothiazole-H), 7.98−8.03 (m, 3H, 2Ar-H & benzothiazole-H), 8.51 (br s, 2H, NH_2), 11.67 (br s, 1H, NH). Anal. Calcd for $C_{25}H_{21}N_5O_3S_2$ (503.60): C% 59.62; H% 4.20; N% 13.91. Found: C% 59.72; H% 4.16; N% 13.97.

General Procedure for Synthesis of 17a,b. 2-(Benzo-[*d*]thiazol-2-yl)-3,3-bis(methylthio)acrylonitrile[38] **16** (2.78 g, 0.01 mol) was added to a stirred solution of the *N*-carbamimidoylarylsulfonamide **4a,b** (0.01 mol) in dry dioxane (20 mL) containing potassium hydroxide (0.56 g, 0.01 mol), and the reaction mixture was refluxed for 2 h. After completion of the reaction (TLC), the solid precipitate was filtered off and then recrystallized using an appropriate solvent.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(methylthio)-pyrimidin-2-yl)benzenesulfonamide (**17a**).* Colorless crystal; (DMF); yield 75%; m.p. 244−245 °C; IR (KBr, cm^{-1}): ν 3431 and 3874 (NH, NH_2). ^1H NMR (400 MHz, DMSO-d_6): δ 2.19 (s, 3H, CH_3), 7.32−7.39 (m, 4H, 3Ar-H & benzothiazole-H), 7.46 (t, 1H, J = 8 Hz, benzothiazole-H), 7.83−7.85 (m, 2H, Ar-H), 7.92 (d, 1H, J = 8 Hz, benzothiazole-H), 8.01 (d, 1H, J = 8 Hz, benzothiazole-H). Anal. Calcd for $C_{18}H_{15}N_5O_2S_3$ (429.54): C% 50.33; H% 3.52; N% 16.30. Found: C% 50.43; H% 3.48; N% 16.35.

*N-(4-Amino-5-(benzo[d]thiazol-2-yl)-6-(methylthio)-pyrimidin-2-yl)-4-methylbenzenesulfonamide (**17b**).* Yellow crystals; (Ethanol); yield 75%; m.p. 238−239 °C; IR (KBr, cm^{-1}): ν 3375 and 3321 (NH, NH_2), 3161 (ArCH). ^1H NMR (400 MHz, DMSO-d_6): δ 2.37 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 7.41−7.53 (m, 4H, 2Ar-H & 2benzothiazole-H), 7.94−8.12 (m, 4H, 2Ar-H & 2benzothiazole-H), 8.49 (br s, 2H, NH_2), 11.50 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 14.2 (SCH_3), 21.4 (CH_3), 122.1, 122.7, 125.7, 126.8, 127.9, 128.4, 129.2, 129.7, 134.5, 138.3, 143.7, 151.7, 154.6, 162.3, 168.5 (Ar-C). Anal. Calcd for $C_{19}H_{17}N_5O_2S_3$ (443.57): C% 51.45; H% 3.86; N% 15.79. Found: C% 51.52; H% 3.82; N% 15.86.

Biological Evaluation. *Cytotoxicity Test.* Cytotoxicity and antiviral tests were carried out at The National Research Center, Cairo, Egypt. Cytotoxicity was done according to the literature.[39−41] To prepare for the tests, 50 mg of each sample was allowed to dissolve in 1 mL of DMSO. To avoid possible contamination of the samples, 24 μL of 100× of an antibiotic−antimycotic mixture was added to 1 mL of each sample. Next, 100 μL of each sample was subjected to bi-fold dilutions followed by inoculating 100 μL of each dilution in Hep-2, Vero, BGM, FRHK4, and Huh 7.5 cell lines previously cultured in 96-multiwell plates to determine the nontoxic dose of the examined samples. The well plates were provided from Greiner Bio-One, Germany, while the cell lines were obtained from the Holding Company for Biological Products & Vaccines VACSERA, Egypt. To complete the cytotoxicity assay, cell morphology evaluation using an inverted light microscope and the cell viability test through the application of trypan blue dye exclusion method was utilized.

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 bi-fold dilutions, prepared in DMEM (GIBCO BRL), of the different tested samples. One hundred microliters of DMEM was used as the cell control 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.[40]

Cell Viability Assay. Trypan blue dye exclusion method was used in this assay.[42] The aforementioned cell cultures (2×10^5 cells/mL) were grown in 12-well tissue culture plates. One hundred microliters of bi-fold dilutions of the tested samples was applied after 24 h incubation for each well as described previously. The medium was then removed after 72 h, which is followed by trypsinizing the cells with an equal volume of 0.4%

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(w/v) of the trypan blue dye aqueous solution. Viable cells were assessed using a phase contrast microscope.

Determination of Coxsackievirus B4, HAV HM175, Adenovirus 7, and HSV-1 Titers Using Plaque Assay. One hundred microliters of nontoxic dilutions was mixed with 100 μL of the different doses of HSV-1, HAdV7, HAV HM175, and CBV4 (1×10^5, 1×10^6, and 1×10^7). Each mixture was incubated for half an hour at a temperature of 37 °C. One hundred microliters of 10-fold dilutions of the untreated and treated adenovirus 7, HAV HM175, CBV4, and HSV-1 was inoculated separately into Hep-2, FRHK4, BGM, and Vero cell lines in 12-multiwell plates, respectively. The samples were incubated without constant rocking as to allow for the adsorption for 1 h in a 5% CO_2-water vapor atmosphere at a temperature of 37 °C as to mimic the human body temperature. To keep the cells from drying, the plates were occasionally rocked. One milliliter of 2X DMEM media (Dulbecco's modified Eagle's medium supplied from Gibco-BRL) in addition to another 1 mL of 1% agarose was added to each well after the adsorption is complete. The plates were then incubated in a 5% CO_2-water vapor atmosphere at a temperature of 37 °C. Following the incubation, the sample cells were stained with 0.4% crystal violet after fixation with formalin, the number of plaques was counted, and the titers were also calculated. The latter was expressed in terms of plaque-forming units per milliliter (pfu/mL).[43,44] CC_{50} and IC_{50} were evaluated for the promising materials (viral reduction 50% or more). CC_{50} referring 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. IC_{50} refers to the concentration at which the compound plaque reduction rate reaches halfway between the baseline and the maximum. All data were taken as the average of three measurements (triplicates).

Antiviral Bioassay of Tested Compounds against ED-43/SG-Feo (VYG) Replicon of HCV Genotype 4a. To carry out the antiviral assay, a nontoxic dose of the tested compounds was used against ED-43/SG-Feo (VYG) replicon of HCV genotype 4a. To quantify HCV RNA, qRT-PCR supplied from Taqman probe kit (Qiagen) was used. This was done in algal extracts treated with Huh 7.5-infected cells. According to the literature and following the manufacturer's instructions, a dose-dependent decrease in subgenomic RNA copies was shown.[45]

Hsp90α (C-Terminal) Inhibitor Screening Assay. The Hsp90α (C-Terminal) inhibitor screening assay was used to assess the inhibition of Hsp90α binding to its target protein cyclophilin D (PPID). A solution of 3X Hsp90α assay buffer 2 was first diluted with water to 1x Hsp90α assay buffer 2. Four microliters of the diluted Hspα protein (1.5 ng/μL) was added to each well designated "Blank" and "Substrate Control". Two microliters of the same solution without an inhibitor (inhibitor buffer) was added to wells assigned to "Positive Control", "Substrate Control", and "Blank". To initiate the enzymatic reaction, 4 μL of diluted PPID was added to each well designated "Substrate control", "Positive Control", and "Test Inhibitor" and then incubated at room temperature for 30 min. The total volume for each well was 10 μL. An amount of 10 μL of diluted glutathione (250-fold with 1x detection buffer) was added to each well. Ten microliters of diluted streptavidin-conjugated donor beads was also added to wells in a 96-well plate. Alpha-counts were then read. The percentage inhibition was calculated for the different concentrations tested against the control, and the IC_{50} values against the HSPα protein were calculated from the concentration−inhibition response curve.

Molecular Modeling. Docking simulations were performed using the crystal structure of Hsp90α (PDB ID 3b25) in complex to 4-methyl-6-(toluene-4-sulfonyl)-pyrimidin-2-ylamine (B2K).[46] The PDB file was retrieved from the Protein Data Bank. The structure of chain A was processed using the Structure Preparation application in an MOE (molecular operating environment, 2014), and the ligand molecule was removed from the protein active site. Subsequently, the Protonate 3D application of the MOE was used to add the missing hydrogens and properly assign the ionization states. The default procedure in the MOE Dock application was used to find the favorable binding configurations of the studied ligands. Initial placement poses generated by the Alpha Triangle matcher were rescored and filtered using the London dG Scoring method to pick those exhibiting maximal hydrophobic, ionic, and hydrogen-bond contacts to the protein. This was followed by a refinement stage. 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.[47]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03706.

IR, ^{1}H NMR, and ^{13}C NMR spectra for the synthesized compounds, table of the nontoxic doses of tested compounds on FRHK-4, Hep2, BGM, Vero, and Huh 7.5 cell lines, and 3D interaction of acyclovir, **9a**, **9b**, **14b**, **14g**, and **14h** inside the binding pocket of Hsp90α (PDB ID 3b25) (PDF)

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Notes

The authors declare no competing financial interest.

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