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Full Paper

Novel Antiviral Compounds against Gastroenteric Viral Infections

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Viral gastroenteritis is a serious viral infection which affects a large number of individuals around the world, most of them being children. The infection may occur due to different viruses, for example, coxsackievirus, adenovirus, and rotavirus. There is no available cure for such infections, and the treatment mainly depends on hospitalization and administration of nutritional supports. A new antiviral agent against gastroenteritis viral infection will be a breakthrough in healthcare. Pyrrole and pyrrolopyrimidine derivatives are well known for their biological activity as antibacterial, antifungal, and anticancer agents. These compounds also proved to possess antiviral activity. Here, we synthesized novel pyrrole and pyrrolopyrimidine compounds and examined their antiviral activity. We synthesized several new pyrrole, pyrrolo[2,3-*d*]pyrimidine, and pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives. The characterization of all synthesized compounds was based on microanalysis and spectral data. Moreover, we determined the non-toxic doses of these compounds on BGM, Hep-2, and MA-104 cells. We tested all the synthesized compounds for their antiviral activities against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Several compounds exhibited significant activities as antiviral agents.

Keywords: Adenovirus / Coxsackievirus B4 / Direct antiviral agents / Pyrrole / Pyrrolo[2,3-*d*]pyrimidine / Pyrrolo[3,2-e][1,2,4]triazolo[1,5-*c*]pyrimidine / Rotavirus / Viral gastroenteritis

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Introduction

Viral gastroenteritis is one of the most common infections in the world. Due to its significant morbidity, it constitutes a major threat especially to vulnerable individuals such as children, elderly, and immunocompromised individuals. The viral gastroenteritis constitutes around 21–40% of infectious diarrhea cases in developed countries [1]. Viral gastroenteritis represents not only a health risk but also an economical and financial burden through direct and indirect costs. Hospitalization costs ranged from US$ 1.8 to 4.6 million annually in the Middle East and North African countries [2] and approaches US$ 1 billion per year in USA [3].

Children are frequently affected by gastroenteritis due to the mode of transmission (oral–fecal route). This condition is exacerbated by the close contact between children and poor hygiene [4]. Annually, about two billion cases of diarrheal diseases occur among children under the age of five globally. About 1.5 million of the affected children die from diarrheal diseases, mostly in developing countries. This makes diarrheal diseases the second most common cause of death among children under the age of five following pneumonia [5].

Several viruses can cause gastroenteritis; the most common are coxsackievirus, adenovirus, and rotavirus. Rotavirus is the leading cause of severe gastroenteritis in the pediatric population worldwide [6], adenovirus is the second most common virus causing gastroenteritis in young children [7], and coxsackievirus is considered the least dangerous cause of gastroenteritis among the three viruses [8].

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Acute viral gastroenteritis infections require hospitalization but treatment is mainly palliative. To the best of our knowledge, there is no available specific antiviral treatment for these infections. Few recent reports described synthesis of antiviral agents against norovirus [9, 10]. There is a need to develop antiviral drugs that can be used for treatment of different viral gastroenteritis infections.

Several synthetic chemical compounds showed antiviral activity. It is noted that the pyrrole nucleus plays a vital role in many biological activities [11–15]. Also, pyrrolo[2,3-*d*]pyrimidines as 7-deaza analogs of biogenic purines are of considerable interest both from chemical and biological points of view. This heterocyclic system is widely distributed in nature, being a part of the antibiotics [16] tubercidin, toyocamycin, sangivamycin, and cadeguomycin. Many synthetic pyrrolo[2,3-*d*]pyrimidine derivatives show antibacterial [17–19], antifungal [20], anti-inflammatory [21–23], anticancer [24, 25], antiviral [26–29], analgesic [30], antihyperglycemic [31], and anticonvulsant [32] activities.

In this study, we report synthesis and biological activity of novel pyrrole and pyrrolopyrimidine derivatives as antiviral drugs for gastroenteritis viral infections.

Results and discussion

Chemistry

Condensation of benzoin and primary amines in refluxing toluene resulted in the formation of α-aminoketone intermediates, which were condensed, without isolation, with malononitrile to yield 2-amino-pyrrole-3-carbonitriles **1a–d** [17, 18]. Synthesis of pyrroles **1c**,**d** was previously reported [19].

Refluxing of pyrroles **1a**,**b** and acetic anhydride gave acetylated products **2a**,**b**, while refluxing with a mixture of HCl/acetic acid (1:3) made 2-methyl-pyrrolo[2,3-*d*]pyrimidines **3a**,**b** [33]. We obtained pyrrolo[2,3-*d*]pyrimidin-4-ones **4a**,**b** through the reaction of **1a**,**b** with formic acid [12, 21, 28], which were then refluxed with phosphorus oxychloride [17, 18] to produce the respective 4-chloro derivatives **5a**,**b**. Compounds **5a**,**b** were refluxed with thiourea, which resulted in the corresponding 4-thiones **6a**,**b** [34]. 4-Aryl amino derivatives **7a–h** were prepared via a reaction of 4-chloropyrrolopyrimidines **5a**,**b** with some aryl amines in the presence of catalytic amount of triethylamine [18] as revealed in Scheme 1.

We refluxed pyrroles **1c**,**d**, individually, with triethylorthoformate to produce 2-(ethoxymethylene)amino-pyrroles **8a**,**b**, which upon stirring with hydrazine hydrate gave 3-amino-4-imino-pyrrolo[2,3-*d*]pyrimidines **9a**,**b** [35–39], not 4-hydrazinylpyrrolopyrimidines as reported before [40–43]. The structures of these compounds were proven chemically in our previous publication [19] by unambiguous synthesis of 4-hydrazinylpyrrolopyrimidine derivatives by condensation of 4-chloro analogs with hydrazine hydrate and second by microanalysis and spectral data. The formation of 3-amino-4-imino-pyrrolo[2,3-*d*]pyrimidines, rather than the formation of 4-hydrazinylpyrrolo[2,3-*d*]pyrimidines, was confirmed through the difference in their melting points, difference in finger print region in IR spectra of both and also chemical shift of NH- proton in ^{1}H NMR as it is for 4-hydrazinopyrrolo[2,3-*d*]pyrimidines appears at the aromatic region (6.7–7.9 ppm) but in 3-amino-4-imino-pyrrolo[2,3-*d*]pyrimidines it appears in range $>$9.2 ppm.

We used pyrrolo[2,3-*d*]pyrimidines **9a**,**b** as starting materials for synthesis of several pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]-pyrimidines **10a**,**b–14a**,**b** via refluxing with triethylorthoformate, acetic anhydride, carbon disulfide, ethyl cyanoacetate, and chloroacetylchloride, respectively [35–39, 44–46], as revealed in Scheme 2. The structure of these compounds was proven by microanalysis and spectral data. We ruled out the isomeric analogs of pyrrolo[3,2-*e*][1,2,4]triazolo[4,3-c]-pyrimidine on the ground of chemical shift magnitude of the triazole and pyrimidine ring protons. The triazolo[4,3-c]-pyrimidine protons (C3-H and C5-H) appeared at δ 8–8.9 ppm, but that of triazolo[1,5-*c*]pyrimidines (C2-H and C5-H) appeared at 9.5–9.8 ppm [40].

Biological results

Cytotoxicity

The assessment of the cytotoxicity of any chemotherapeutic agent (including antiviral compounds) is an essential part of the biological evaluation of any new therapeutic compounds, as it should determine that neither acute nor long-term toxicity should occur to the host. Cytotoxicity of all synthesized compounds was examined on three different cell lines: BGM, Hep-2, and MA-104. There were variations in the susceptibilities of the cell lines to the toxicity of the tested compounds, where the BGM showed the highest resistance, followed by Hep-2 and the least resistant was the MA-104 cell line. This may be due to the differences between the origins of these cell lines. Table 1 shows the CC_{50} for all the tested compounds. The CC_{50} was determined using two different techniques: by counting the number of viable cells and by examining the effect on cell morphology. The CC_{50} for the compounds which exhibited antiviral activity: **5b**, **7f**, **10a**, and **12a**, were 144, 144, 125, and 115 μM, respectively.

Antiviral assay

The non-toxic doses of the compounds were tested against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Considerable antiviral activity was observed with some of the tested compounds. Four compounds among the tested ones showed activity against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain with different degrees of activity and specificity. Figure 1 showed the activity of compound **5b**, which was highly specific against coxsackievirus B4, the viral inhibition reached 76.6%. Compound **7f** achieved considerable activity against all tested viruses ranged from a maximum inhibition of coxsackievirus B4 (83.3%) followed by rotavirus Wa strain (60%) and the least activity toward adenovirus type 7 (50%) as demonstrated in Fig. 2. Moreover, compound **7f** showed significantly higher activity against coxsackievirus B4 compared to its activity against rotavirus Wa strain or adenovirus type 7. Compound **10a** showed antiviral activity

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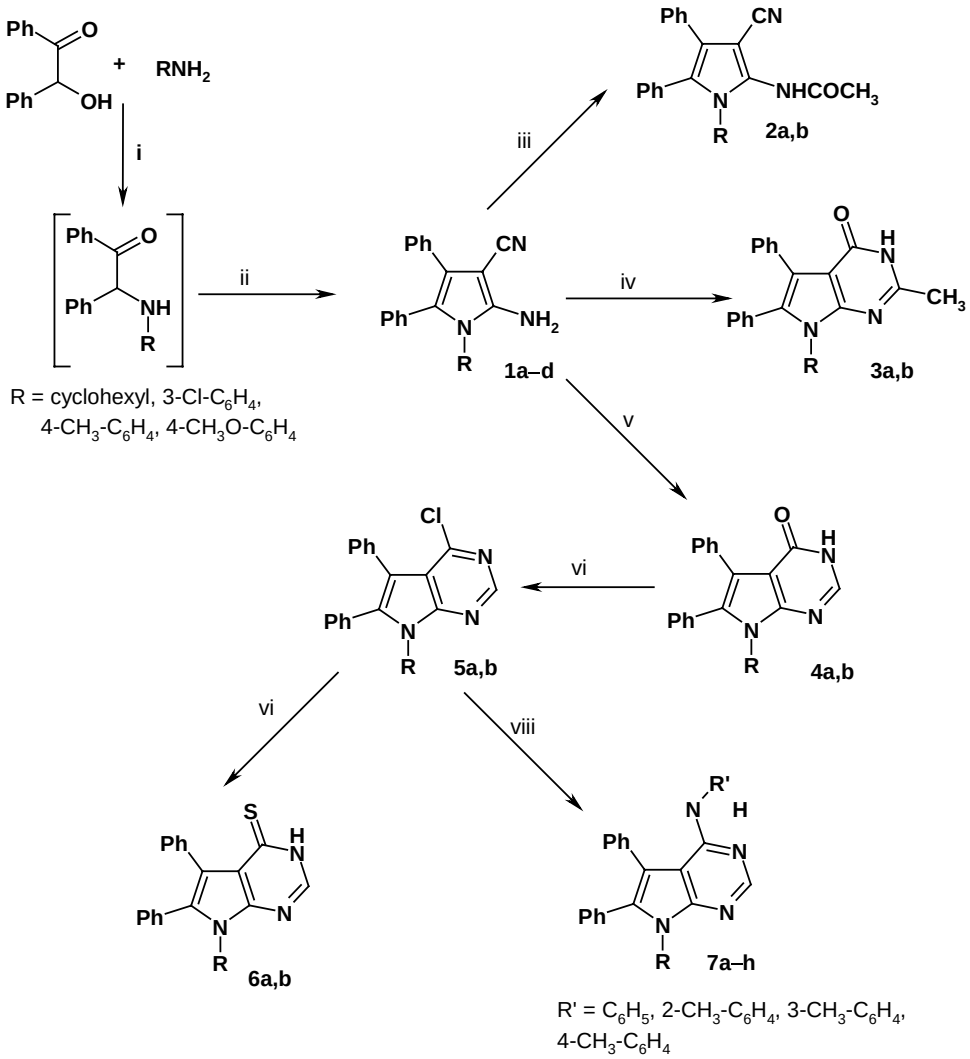


Scheme 1. Synthesis of compounds **1a–d** to **7a–h**. Reaction conditions: (i) HCl, toluene; (ii) $CH_2(CN)_2$; (iii) Ac_2O; (iv) HCl/AcOH (1:3); (v) HCOOH; (vi) $POCl_3$; (vii) $CS(NH_2)_2$; (viii) $R'NH_2$, TEA.

against both coxsackievirus B4 (63.3%) and rotavirus Wa strain (53.3%) as shown in Fig. 3. Statistical analysis did not show any significant difference in activity against the two viruses. Finally, compound **12a** showed considerable antiviral activity against all tested viruses ranged, as it appears in Fig. 4, from maximum inhibition of coxsackievirus B4 (80%) followed by rotavirus Wa strain (66.7%) and adenovirus type 7 (53.3%). Statistical analysis showed significant difference in activity against coxsackievirus B4 compared to the activity against rotavirus Wa strain and adenovirus type 7. The other tested compounds did not show antiviral effect against the tested viruses.

If we compare the activity of the different compounds against coxsackievirus B4, as shown in Fig. 5, there was no significant difference in potency between compounds **5b**, **7f**, **10a**, and **12a**, which showed the highest potency.

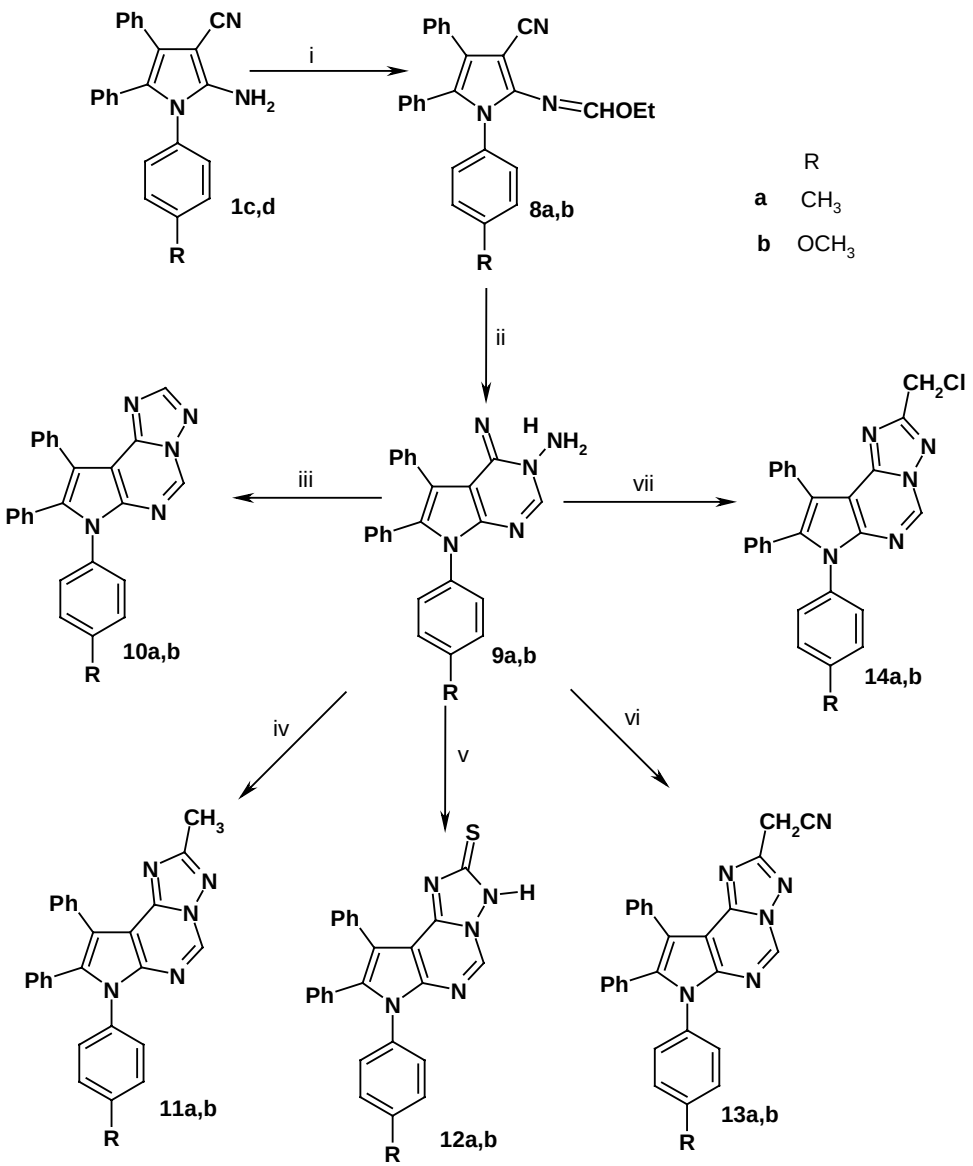
Structure-activity relationship

In the present study, we described a straight forward and efficient synthesis of novel pyrrole, pyrrolo[2,3-*d*]pyrimidine and pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives as antiviral agents.

In order to analyze structure-activity relationship, two structural components were considered: the nature of the heterocyclic nucleus and the nature of the substituent.

First, regarding the influence of the heterocyclic nucleus nature, we observed that pyrrolo[2,3-*d*]pyrimidine and pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives acquired significant antiviral activity over all the synthesized pyrrole derivatives **1a–d**, **2a**,**b**, and **8a**,**b**, which did not exhibit significant activity.

As to the nature of the substituent: the pyrrolo[2,3-*d*]-pyrimidine nucleus, 2-methyl-pyrrolo[2,3-*d*]pyrimidin-4-ones



Scheme 2. Synthesis of compounds **8a**,**b**–**14a**,**b**. Reaction conditions: (i) $CH(OEt)_3$; (ii) NH_2NH_2; (iii) $CH(OEt)_3$; (iv) Ac_2O; (v) C_2S; (vi) $CNCH_2CO_2C_2H_5$; (vii) $ClCH_2COCl$.

3a,**b**, pyrrolo[2,3-*d*]pyrimidin-4-ones **4a**,**b**, pyrrolo[2,3-*d*]pyrimidine-4-thiones **6a**,**b** and 3-amino-4-imino-pyrrolo[2,3-*d*]-pyrimidines **9a**,**b** were inactive. Substitution by 4-chloro group of pyrrolo[2,3-*d*]pyrimidine induced the selective and potent antiviral (against coxsackievirus) compound **5b**, its IC_{50} was 108 μM (Table 2), while substitution with *N*-aryl group resulted in an active compound against all viral strains, compound **7f**, its IC_{50} for coxsackievirus, adenovirus, and rotavirus were 82, 144, and 123 μM respectively. While comparing the activity of compounds **5b** and **7f**, it can be inferred that replacement of 4-chloro group of compound **5b** with 4-(*o*-tolyl)-amino group (compound **7f)** leads to increasing the antiviral activity against coxsackievirus B4 from (IC_{50}: 108 μM) to (IC_{50}: 82 μM) in addition to its activity against rotavirus Wa strain and adenovirus type 7.

On the other hand for substituents on position 2 of pyrrolo-[3,2-e][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus, 2-methyl-, 2-cyanomethyl-, and 2-chloromethyl-pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines **11a**,**b**, **13a**,**b** and **14a**,**b** were inactive. The activity of these derivatives appeared only for pyrrolo[3,2-e][1,2,4]triazolo[1,5-*c*]pyrimidine **10a** against coxsackievirus B4 and rotavirus Wa strain (IC_{50} were 137 and 162 μM

Table 1. CC_{50} values of the tested compounds on BGM, Hep-2 and MA-104 cell lines.

Compounds	CC_{50} for BGM cell line (μM)	CC_{50} for Hep-2 cell line (μM)	CC_{50} for MA-104 cell line (μM)
1a	175.716	175.716	146.430
1b	189.261	189.261	162.224
1c	200.321	200.321	171.703
1d	191.550	191.550	191.550
2a	182.529	182.529	182.529
2b	145.673	145.673	145.673
3a	130.378	130.378	104.302
3b	121.395	97.116	97.116
4a	189.466	189.466	189.466
4b	150.807	150.807	150.807
5a	180.459	154.679	128.899
5b	168.148	168.148	144.127
6a	155.634	129.695	129.695
6b	169.115	144.956	96.637
7a	157.456	157.456	112.468
7b	152.638	152.638	130.833
7c	130.833	130.833	130.833
7d	152.638	152.638	130.833
7e	148.001	148.001	148.001
7f	143.740	143.740	143.740
7g	123.206	102.672	102.672
7h	123.206	123.206	123.206
8a	98.644	123.305	98.644
8b	118.624	142.349	118.624
9a	153.319	153.319	153.319
9b	171.847	171.847	147.297
10a	124.542	149.451	124.542
10b	167.677	167.677	143.723
11a	144.404	144.404	144.404
11b	162.225	162.225	162.225
12a	138.396	138.396	115.329
12b	155.715	155.715	155.715
13a	136.206	136.206	113.505
13b	153.337	153.337	153.337
14a	111.123	133.348	88.899
14b	128.769	150.231	107.308

respectively as shown in Table 2) and pyrrolo[3,2-*e*][1,2,4]-triazolo[1,5-*c*]pyrimidine-2-thione **12a** against all the tested viruses.

These compounds may inhibit the rotavirus, adenovirus, and coxsackievirus through inhibition of their viral polymerases based on what has been reported regarding pyrrolopyrimidine derivatives [47–48]. Further investigation is required to study the mechanism of action of these new compounds.

Conclusion

In this study, we synthesized novel pyrrole, pyrrolo[2,3-*d*]-pyrimidine and pyrrolo[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives. Some of the synthesized compounds were potent inhibitors for gastroenteric viruses such as adenovirus, coxsackievirus, and rotavirus.

Among these compounds, compound **5b** exhibited very high specificity toward coxsackievirus B4, which renders this molecule as a lead compound for drug discovery of antiviral agents against coxsackievirus B4.

Experimental

Chemistry

All chemicals used as starting materials and reagents in this study were reagent grade and were purchased from Merck (Darmstadt, Germany). All melting points were uncorrected and measured using Electro-thermal IA 9100 apparatus (Shimadzu, Japan); IR spectra were recorded as potassium bromide pellets on a Perkin-Elmer 1650 spectrophotometer (USA), Faculty of Science, Cairo University, Cairo, Egypt. ^{1}H NMR spectra were determined on a Varian Mercury (300 MHz)

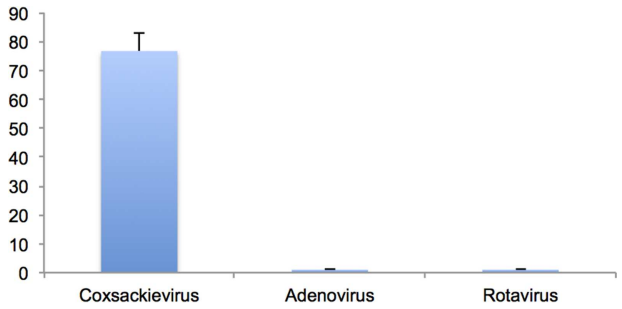


Figure 1. Antiviral activity of non-toxic doses of tested compound **5b** against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Compound **5b** showed high specificity toward coxsackievirus B4 and it exhibited no activity against adenovirus type 7 and rotavirus Wa strain.

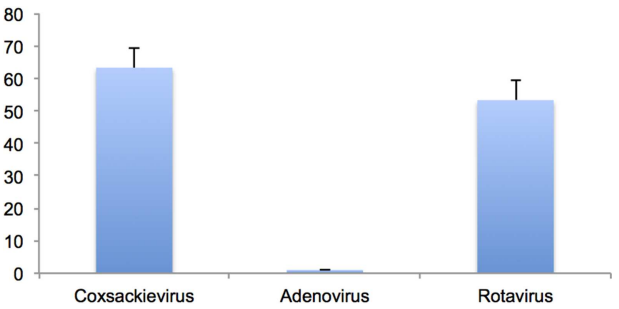


Figure 3. Antiviral activity of non-toxic doses of tested compound **10a** against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Compound **10a** was active against coxsackievirus B4, and rotavirus Wa strain, it did not exhibit any activity against adenovirus type 7. There was no significant difference in the activity of compound **10a** toward coxsackievirus compared to its activity against rotavirus.

spectrometer (Varian UK) and chemical shifts were expressed as ppm against TMS as internal reference (National Research Center, Dokki, Cairo, Egypt). Mass spectrometric analysis is carried out using a TSQ Quantum Access MAXtriplequadrupole system. Data acquisition and processing is performed using Thermo Scientific Xcalibur 2.1 software (Faculty of Pharmacy, Helwan University, Cairo, Egypt). Microanalyses were operated using Vario, Elmentar apparatus (Shimadzu, Japan), Organic Microanalysis Unit, Faculty of Science, Cairo University, Cairo, Egypt. Column chromatography was performed on (Merck) silica gel 60 (particle size 0.06–0.20 mm). All new compounds yielded spectral data consistent with the proposed structure and microanalysis within $\pm 0.4\%$ of the theoretical values. Compounds **1c**,**d** were prepared before [19], the other synthesized compounds are new and were confirmed with spectral data.

*General procedure for the synthesis of 2-amino-4,5-diphenyl-1-substituted-1H-pyrrole-3-carbonitrile (**1a,b**)*

A mixture of benzoin (2 g, 0.01 mol), the appropriate amine (0.01 mol) and conc. HCl (6–8 drops) in toluene (50 mL) was heated under reflux for 36 h, then cooled. Malononitrile (0.66 mg, 0.01 mol) was added, followed by a catalytic amount of pyridine (1.5 mL) portion-wise and left to reflux until a solid

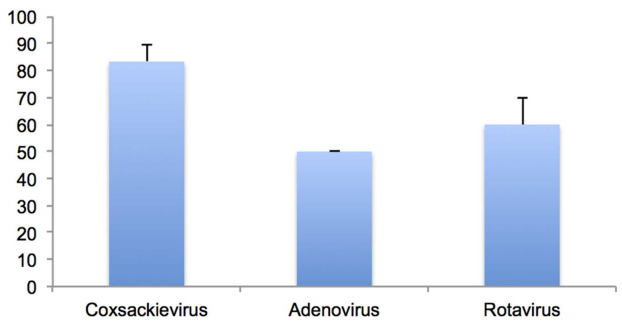


Figure 2. Antiviral activity of non-toxic doses of tested compound **7f** against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Compound **7f** was active against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Compound **7f** showed higher selectivity toward coxsackievirus, there was significant difference in activity toward coxsackievirus compared to its activity against adenovirus and rotavirus with $p < 0.001$ and $p < 0.05$, respectively.

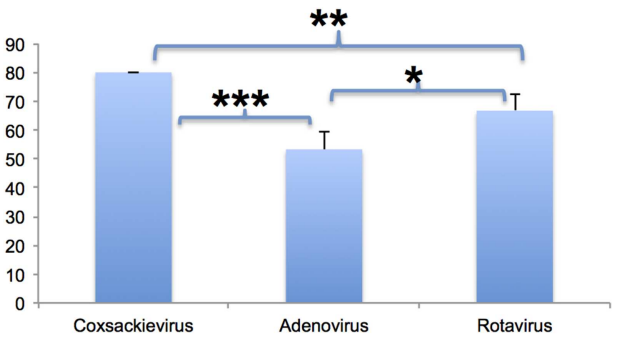


Figure 4. Antiviral activity of non-toxic doses of tested compound **12a** against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Compound **12a** was active against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain. Compound **7f** showed higher selectivity toward coxsackievirus, there was significant difference in activity toward coxsackievirus compared to its activity against adenovirus and rotavirus with $p < 0.001$ and $p < 0.01$, respectively. Compound **12a** was more active against rotavirus than adenovirus, there was significant difference in activity with $p < 0.05$.

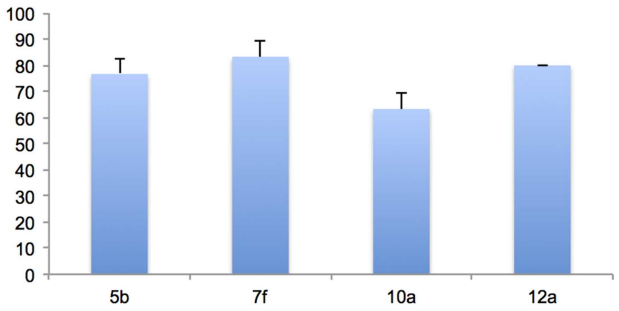


Figure 5. Comparison of the activity of the different compounds against coxsackievirus B4. There was no significant difference in the activity of the different compounds (**5b**, **7f**, **10a**, and **12a**) toward the coxsackievirus B4.

was formed. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give compounds **1a**,**b**.

*2-Amino-1-cyclohexyl-4,5-diphenyl-1H-pyrrole-3-carbonitrile (**1a**)*
Brown solid in 65%; m.p.: 283–285°C; IR (KBr) υ (cm^{-1}): 3439, 3315 (NH_2), 2217 (C≡N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.4–2.1 (m, 10H, cyclohexyl), 3.5 (m, 1H, CH-N cyclohexyl), 5.04 (s, 2H, NH_2, D_2O exchangeable), 7.05–7.75 (m, 10H, Ar-H); ESI-MS *m/z*: 341.25 (M^+, 6%); Anal. calcd. for $C_{23}H_{23}N_3$ (341.46): C, 80.90; H, 6.79; N, 12.31%. Found: C, 81.11; H, 6.91; N, 12.12%.

*2-Amino-1-(3-chlorophenyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile (**1b**)*
Light brown solid in 78%; m.p.: 275–277°C; IR (KBr) υ (cm^{-1}): 3441, 3340 (NH_2), 2209 (C≡N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.07 (s, 2H, NH_2, D_2O exchangeable), 7.04–7.9 (m, 14H, Ar-H); ESI-MS *m/z*: 369.10 (M^+, ^{35}Cl, 2%), 371.23 (M^++2, ^{37}Cl, 0.66%); Anal. calcd. for $C_{23}H_{16}ClN_3$ (369.86): C, 74.69; H, 4.36; N, 11.36%. Found: C, 74.50; H, 4.13; N, 11.52%.

*General procedure for the synthesis of N-(3-cyano-4,5-diphenyl-1-substituted-1H-pyrrol-2-yl)acetamide (**2a,b**)*
The appropriate aminopyrrole (**1a**,**b**) (0.01 mol) in acetic anhydride (40 mL) was heated under reflux for 36 h, then cooled, poured onto ice water, and neutralized with ammonia to give precipitate, which was filtered off, dried, and recrystallized from methanol, to give compounds **2a**,**b**.

*N-(3-Cyano-1-cyclohexyl-4,5-diphenyl-1H-pyrrol-2-yl)-acetamide (**2a**)*
Brown solid in 62%; m.p.: 203–205°C; IR (KBr) υ (cm^{-1}): 3427 (NH), 2217 (C≡N), 1672 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.19–2.00 (m, 10H, cyclohexyl), 2.3 (s, 3H, CH_3-CO), 3.88 (m, 1H, CH-N cyclohexyl), 7.01–8.6 (m, 10H, Ar-H), 11.6 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 383.23 (M^+, 3.25%); Anal. calcd. for $C_{25}H_{25}N_3O$ (383.50): C, 78.30; H, 6.57; N, 10.96; O, 4.17%. Found: C, 78.53; H, 6.29; N, 10.73; O, 4.35%.

*N-(1-(3-Chlorophenyl)-3-cyano-4,5-diphenyl-1H-pyrrol-2-yl)acetamide (**2b**)*
Light brown solid in 72%; m.p.: 190–192°C; IR (KBr) υ (cm^{-1}): 3350 (NH), 2219 (C≡N), 1699 (C=O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.1 (s, 3H, CH_3-CO), 7.27–8.36 (m, 14H, Ar-H), 11.73 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 411.26 (M^+, ^{35}Cl, 7.6%), 413.32 (M^++2, ^{37}Cl, 2.5%); Anal. calcd. for $C_{25}H_{18}ClN_3O$ (411.88): C, 72.90; H, 4.40; Cl, 8.61; N, 10.20; O, 3.88%. Found: C, 72.68; H,4.21; Cl, 8.40; N, 9.95; O, 4.05%.

*General procedure for the synthesis of 5,6-diphenyl-2-methyl-7-substituted-3,7-dihydro-pyrrolo[2,3-d]-pyrimidin-4-one (**3a,b**)*
The appropriate aminopyrrole (**1a**,**b**) (0.01 mol) was refluxed in a mixture of hydrochloric acid (5 mL) and acetic acid (15 mL) for 18 h. The reaction mixture was cooled, poured onto ice, and the formed solid was filtered off, dried, and recrystallized from methanol to afford **3a**,**b**.

*7-Cyclohexyl-5,6-diphenyl-2-methyl-3H-pyrrolo[2,3-d]-pyrimidin-4(7H)-one (**3a**)*
Grey solid in 62%; m.p.: 250–252°C; IR (KBr) υ (cm^{-1}): 3425 (NH), 1685 (C=O), 1582 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.41–2.00 (m, 10H, cyclohexyl), 2.22 (s, 3H, CH_3), 3.88 (m, 1H, CH-N cyclohexyl), 7.11–8.20 (m, 10H, Ar-H), 11.85 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 383.33 (M^+, 3%); Anal. calcd. for $C_{25}H_{25}N_3O$ (383.50): C, 78.30; H, 6.57; N, 10.96; O, 4.17%. Found: C, 78.11; H, 6.71; N, 11.19; O, 3.99%.

*7-(3-Chlorophenyl)-5,6-diphenyl-2-methyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**3b**)*
Greyish brown solid in 72%; m.p.: 245–247°C; IR (KBr) υ (cm^{-1}): 3395 (NH), 1707 (C=O), 1589 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.1 (s, 3H, CH_3), 7.1–8.2 (m, 14H, Ar-H), 11.42 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 411.32 (M^+, ^{35}Cl, 2%), 413 (M^++2, ^{37}Cl, 0.67); Anal. calcd. for $C_{25}H_{18}ClN_3O$

Table 2. **IC_{50} (μM) of compounds 5b, 7f, 10a, and 12a against coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain.**

Compounds	IC_{50} for coxsackievirus B4 (μM)	IC_{50} for adenovirus type 7 (μM)	IC_{50} for rotavirus Wa strain (μM)
5b	108	—	—
7f	82	144	123
10a	137	—	162
12a	97	138	115

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(411.88): C, 72.90; H, 4.40; Cl, 8.61; N, 10.20; O, 3.88%. Found: C, 72.73; H, 4.11; Cl, 8.89; N, 10.52; O, 3.62%.

*General procedure for the synthesis of 5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidin-4(3H)-one (**4a,b**)*
The appropriate aminopyrrole (**1a**,**b**) (0.01 mol) in formic acid (20 mL, 85%) was heated under reflux for 36 h, cooled, poured onto ice water to give a precipitate **4a**,**b**, which was filtered off, dried, and recrystallized from ethanol.

*7-Cyclohexyl-5,6-diphenyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one (**4a**)*
Yellowish brown solid in 65%; m.p.: >300°C; IR (KBr) υ (cm^{-1}): 3365 (NH), 1698 (C=O), 1595 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.1–2.3 (m, 10H, cyclohexyl), 3.3 (m, 1H, CH-N cyclohexyl), 7.1–8.0 (m, 10H, Ar-H), 8.3 (s, 1H, C2-H), 11.9 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 369.30 (M^+, 11%); Anal. calcd. for $C_{24}H_{23}N_3O$ (369.46): C, 78.02; H, 6.27; N, 11.37; O, 4.33%. Found: C, 78.24; H, 6.03; N, 11.52; O, 4.18%.

*7-(3-Chlorophenyl)-5,6-diphenyl-3H-pyrrolo[2,3-d]-pyrimidin-4(7H)-one (**4b**)*
Yellowish brown solid 69%; m.p.: >300°C; IR (KBr) υ (cm^{-1}): 3382 (NH), 1680 (C=O), 1587 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.0–8.0 (m, 14H, Ar-H), 9.1 (s, 1H, C2-H), 11.6 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 397.23 (M^+, ^{35}Cl, 3%), 399 (M^++2, ^{37}Cl, 1.10%); Anal. calcd. for $C_{24}H_{16}ClN_3O$ (397.86): C, 72.45; H, 4.05; Cl, 8.91; N, 10.56; O, 4.02%. Found: C, 72.24; H, 4.28; Cl, 8.70; N, 10.31; O, 4.29%.

*General procedure for the synthesis of 4-chloro-5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidine (**5a,b**)*
The appropriate pyrrolopyrimidinone (**4a**,**b**) (0.01 mol) was heated under reflux in phosphorus oxychloride (30 mL) for 15 h, then cooled and poured onto ice water to yield precipitate, which was recrystallized from ethanol to give compounds **5a**,**b**.

*4-Chloro-7-cyclohexyl-5,6-diphenyl-7H-pyrrolo[2,3-d]-pyrimidine (**5a**)*
Dark grey solid in 73%; m.p.: 267–269°C; IR (KBr) υ (cm^{-1}): 3059, 2929 (CH), 1620 (C=C), 1585 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.3–2.1 (m, 10H, cyclohexyl), 3.6 (m, 1H, CH-N cyclohexyl), 7.0–8.0 (m, 10H, Ar-H), 8.4 (s, 1H, C2-H); ESI-MS *m/z*: 387.26 (M^+, ^{35}Cl, 2%), 389.14 (M^++2, ^{37}Cl, 0.65%); Anal. calcd. for $C_{24}H_{22}ClN_3$ (387.90): C, 74.31; H, 5.72; Cl, 9.14; N, 10.83%. Found: C, 74.49; H,5.46; Cl, 9.30; N, 10.61%.

*4-Chloro-7-(3-chlorophenyl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (**5b**)*
Dark grey solid in 79%; m.p.: 278–280°C; IR (KBr) υ (cm^{-1}): 3045, 2960 (CH), 1615 (C=C), 1571 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.8–7.9 (m, 14H, Ar-H), 8.3 (s, 1H, C2-H); ESI-MS *m/z*: 416.20 (M^+, ^{35}Cl, 2%), 418.22 (M^++2, ^{37}Cl, 1.3%); Anal. calcd. for $C_{24}H_{15}Cl_2N_3$ (416.30): C, 69.24; H, 3.63; Cl, 17.03; N, 10.09%. Found: C, 69.67; H, 3.41; Cl, 16.85; N, 10.41%.

*General procedure for the synthesis of 5,6-diphenyl-7-substituted-3,7-dihydro-pyrrolo[2,3-d]pyrimidine-4-thione (**6a,b**)*
A mixture of 4-chloropyrrolopyrimidine (**5a**,**b**) (0.01 mol) and thiourea (1.5 g, 0.02 mol) was heated under reflux in dry ethanol (30 mL) for 10 h, then cooled, poured onto ice water, to give precipitate, which were filtered off, dried, and recrystallized from methanol to give compounds **6a**,**b**.

*7-Cyclohexyl-5,6-diphenyl-3H-pyrrolo[2,3-d]pyrimidine-4(7H)-thione (**6a**)*
Brown solid in 64%; m.p.: 205–207°C; IR (KBr) υ (cm^{-1}): 3445 (NH), 1605 (C=N), 1260 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.4–2.0 (m, 10H, cyclohexyl), 3.61 (m, 1H, CH-N cyclohexyl), 6.9–7.8 (m, 10H, Ar-H), 9.2 (s, 1H, C2-H), 11.7 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 385.11 (M^+, 2.5%); Anal. calcd. for $C_{24}H_{23}N_3S$ (385.52): C, 74.77; H, 6.01; N, 10.90; S, 8.32%. Found: C, 74.89; H, 6.27; N, 10.63; S, 8.15%.

*7-(3-Chlorophenyl)-5,6-diphenyl-3H-pyrrolo[2,3-d]-pyrimidine-4(7H)-thione (**6b**)*
Dark brown solid in 69%; m.p.: 215–217°C; IR (KBr) υ (cm^{-1}): 3375 (NH), 1585 (C=N), 1255 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.2–8.0 (m, 14H, Ar-H), 8.8 (s, 1H, C2-H), 11.8 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 413.23 (M^+, ^{35}Cl, 3%); 415.31 (M^++2, ^{37}Cl, 1%); Anal. calcd. for $C_{24}H_{16}ClN_3S$ (413.92): C, 69.64; H, 3.90; Cl, 8.57; N, 10.15%. Found: C, 69.41; H, 3.70; Cl, 8.73; N, 10.36%.

*General procedure for the synthesis of 5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidin-4-yl)-arylamine (**7a–h**)*
A mixture of 4-chloro pyrrolopyrimidine (**5a,b)** (0.01 mol), the appropriate amine (0.01 mol) and few drops of triethylamine was heated under reflux in absolute ethanol for 8 h, then cooled, and poured onto ice water to give precipitate, which were filtered off, dried, and recrystallized from methanol to give compounds **7a–h**.

*7-Cyclohexyl-N,5,6-triphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**7a**)*
Brown solid in 62%; m.p.: 200–202°C; IR (KBr) υ (cm^{-1}): 3337 (NH), 1612 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.2–1.7 (m, 10H, cyclohexyl), 3.9 (m, 1H, CH-N cyclohexyl), 7.0–7.9 (m, 15H, Ar-H), 8.9 (s, 1H, C2-H), 10.9 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 444.23 (M^+, 2%); Anal. calcd. for $C_{30}H_{28}N_4$ (444.57): C, 81.05; H, 6.35; N, 12.60%. Found: C, 81.31; H,6.10; N, 12.85%.

*7-Cyclohexyl-5,6-diphenyl-N-o-tolyl-7H-pyrrolo[2,3-d]-pyrimidin-4-amine (**7b**)*
Light brown solid in 67%; m.p.: 190–192°C; IR (KBr) υ (cm^{-1}): 3359 (NH), 1585 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.1–1.9 (m, 10H, cyclohexyl), 2.2 (s, 3H, CH_3), 3.7 (m, 1H, CH-N cyclohexyl), 6.8–7.8 (m, 14H, Ar-H), 9.0 (s, 1H, C2-H), 11.5 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 458.25 (M^+, 2.5%); Anal.

calcd. for $C_{31}H_{30}N_4$ (458.60): C, 81.19; H, 6.59; N, 12.22%. Found: C, 80.90; H, 6.30; N, 12.41%.

*7-Cyclohexyl-5,6-diphenyl-N-m-tolyl-7H-pyrrolo[2,3-d]-pyrimidin-4-amine (**7c**)*
Brown solid in 69%; m.p.: 217–219°C; IR (KBr) υ (cm^{-1}): 3359 (NH), 1585 (C=N); ESI-MS *m/z*: 458.38 (M^+, 4%); Anal. calcd. for $C_{31}H_{30}N_4$ (458.60): C, 81.19; H, 6.59; N, 12.22%. Found: C, 81.42; H, 6.42; N, 12.39%.

*7-Cyclohexyl-5,6-diphenyl-N-p-tolyl-7H-pyrrolo[2,3-d]-pyrimidin-4-amine (**7d**)*
Brown solid in 71%; m.p.: 221–223°C; IR (KBr) υ (cm^{-1}): 3359 (NH), 1585 (C=N); ESI-MS *m/z*: 458.34 (M^+, 2.4%); Anal. calcd. for $C_{31}H_{30}N_4$ (458.60): C, 81.19; H, 6.59; N, 12.22%. Found: C, 81.01; H, 6.77; N, 12.00%.

*7-(3-Chlorophenyl)-N,5,6-triphenyl-7H-pyrrolo[2,3-d]-pyrimidin-4-amine (**7e**)*
Brown solid in 61%; m.p.: 211–213°C; IR (KBr) υ (cm^{-1}): 3435 (NH), 1607 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.9–8.0 (m, 19H, Ar-H), 9.2 (s, 1H, C2-H), 11.9 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 472.27 (M^+, ^{35}Cl, 6%), 474.30 (M^++2, ^{37}Cl, 2%); Anal. calcd. for $C_{30}H_{21}ClN_4$ (472.97): C, 76.18; H, 4.48; Cl, 7.50; N, 11.85%. Found: C, 76.34; H, 4.21; Cl, 7.32; N, 11.57%.

*7-(3-Chlorophenyl)-5,6-diphenyl-N-o-tolyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**7f**)*
Brown solid in 60%; m.p.: 223–225°C; IR (KBr) υ (cm^{-1}): 3448 (NH), 1598 (C=N); ESI-MS *m/z*: 486.90 (M^+, ^{35}Cl, 2.6%), 489 (M^++2, ^{37}Cl, 0.87%); Anal. calcd. for $C_{31}H_{23}ClN_4$ (486.99): C, 76.46; H, 4.76; Cl, 7.28; N, 11.50%. Found: C, 76.62; H, 4.91; Cl, 7.41; N, 11.23%.

*7-(3-Chlorophenyl)-5,6-diphenyl-N-m-tolyl-7H-pyrrolo-[2,3-d]pyrimidin-4-amine (**7g**)*
Brown solid in 61%; m.p.: 229–231°C; IR (KBr) υ (cm^{-1}): 3448 (NH), 1598 (C=N); ESI-MS *m/z*: 487.30 (M^+, ^{35}Cl, 2%), 489.42 (M^++2, ^{37}Cl, 0.86%); Anal. calcd. for $C_{31}H_{23}ClN_4$ (486.99): C, 76.46; H, 4.76; Cl, 7.28; N, 11.50%. Found: C, 76.20; H, 4.85; Cl, 7.55; N, 11.72%.

*7-(3-Chlorophenyl)-5,6-diphenyl-N-p-tolyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (**7h**)*
Brown solid in 63%; m.p.: 213–215°C; IR (KBr) υ (cm^{-1}): 3448 (NH), 1598 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.0 (s, 3H, CH_3), 7.1–7.9 (m, 18H, Ar-H), 9.6 (s, 1H, C2-H), 11.7 (s, 1H, NH, D_2O exchangeable); ESI-MS *m/z*: 486.27 (M^+, ^{35}Cl, 3%), 488.68 (M^++2, ^{37}Cl, 1%); Anal. calcd. for $C_{31}H_{23}ClN_4$ (486.99): C, 76.46; H, 4.76; Cl, 7.28; N, 11.50%. Found: C, 76.71; H, 4.53; Cl, 7.01; N, 11.35%.

*General procedure for the synthesis of 2-(ethoxymethylene)amino-4,5-diphenyl-1-substituted-1H-pyrrole-3-carbonitrile (**8a,b**)*
Pyrroles (**1c,d**) (0.01 mol) were heated under reflux in triethylorthoformate (30 mL) for 8 h. The solvent was removed under reduced pressure to give precipitates which were recrystallized from methanol to yield compounds **8a,b**.

*2-(Ethoxymethylene)amino-1-(4-methylphenyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile (**8a**)*
Brown solid in 61%; m.p.: 172–174°C; IR (KBr) υ (cm^{-1}): 2207 (C≡N), 1566 (C=N), 1226 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.12 (t, 3H, CH_3*-CH_2), 2.4 (s, 3H, CH_3), 3.5 (q, 2H, CH_3-CH_2*), 6.8–7.9 (m, 15H, Ar-H, CH); ESI-MS *m/z*: 405 (M^+, 22%); Anal. calcd. for $C_{27}H_{23}N_3O$ (405.50): C, 79.97; H, 5.72; N, 10.36; O, 3.95%. Found: C, 79.68; H, 5.52; N, 10.48; O, 4.23%.

*2-(Ethoxymethylene)amino-1-(4-methoxyphenyl)-4,5-diphenyl-1H-pyrrole-3-carbonitrile (**8b**)*
Dark brown solid in 68%; m.p.: 189–191°C; IR (KBr) υ (cm^{-1}): 2205 (C≡N), 1554 (C=N), 1246 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.2 (t, 3H, CH_3*-CH_2), 3.42 (q, 2H, CH_3-CH_2*), 3.5 (s, 3H, OCH_3), 6.7–8.0 (m, 15H, Ar-H, CH); ESI-MS *m/z*: 421 (M^+, 17.4%); Anal. calcd. for $C_{27}H_{23}N_3O_2$ (421.50): C, 76.94; H, 5.50; N, 9.97; O, 7.59%. Found: C, 77.21; H, 5.83; N, 10.14; O, 7.28%.

*General procedure for the synthesis of 3-amino-4-imino-5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidine (**9a,b**)*
A mixture of the appropriate 2-(ethoxymethylene)amino-pyrrole (**8a,b**) (0.01 mol) and hydrazine hydrate (5 mL, 80%) in ethanol (20 mL) was stirred at room temperature for 6 h. The solid formed was recrystallized from methanol to yield compounds **9a,b.**

*3-Amino-4-imino-7-(4-methylphenyl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (**9a**)*
Grey solid in 89%; m.p.: 188–190°C; IR (KBr) υ (cm^{-1}): 3353, 3312 (NH_2), 3166 (NH), 1576 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.25 (s, 3H, CH_3), 5.36 (s, 2H, NH_2, D_2O exchangeable), 6.7–7.9 (m, 14H, Ar-H), 8.27 (s, 1H, C2-H), 9.21 (s, 1H, NH); ESI-MS *m/z*: 391 (M^+, 18%); Anal. calcd. for $C_{25}H_{21}N_5$ (391.34): C, 76.73; H, 5.37; N, 17.90%. Found: C, 76.37; H, 5.59; N, 17.64%.

*3-Amino-4-imino-7-(4-methoxyphenyl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidine (**9b**)*
Dark grey solid in 93%; m.p.: 196–198°C; IR (KBr) υ (cm^{-1}): 3402, 3326 (NH_2), 3158 (NH), 1616 (C=N), 1234 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.54 (s, 3H, OCH_3), 5.4 (s, 2H, NH_2, D_2O exchangeable), 6.7–7.6 (m, 14H, Ar-H), 8.3 (s, 1H, C2-H), 9.3 (s, 1H, NH); ESI-MS *m/z*: 407 (M^+, 29.5%); Anal. calcd. for $C_{25}H_{21}N_5O$ (407.34): C, 73.71; H, 5.16; N, 17.19; O, 3.63%. Found: C, 73.38; H, 4.82; N, 17.34; O, 3.87%.

*General procedure for the synthesis of 8,9-diphenyl-7-substituted-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]-pyrimidine (**10a,b**)*
The appropriate 4-imino-pyrrolopyrimidine (**9a,b**) (0.01 mol) was heated under reflux for 8 h in triethylorthoformate (15 mL), the solvent was removed under reduced pressure to

give precipitates which were recrystallized from methanol to yield compounds **10a,b.**

*7-(4-Methylphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e][1,2,4]-triazolo[1,5-c]pyrimidine (**10a**)*
Light brown solid in 59%; m.p.: 214–216°C; IR (KBr) υ (cm^{-1}): 1588 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.4 (s, 3H, CH_3), 7.0–7.6 (m, 14H, Ar-H), 9.71 (s,1H, C3-H), 9.73 (s, 1H,C5-H); ESI-MS *m/z*: 401 (M^+, 24%); Anal. calcd. for $C_{26}H_{19}N_5$ (401.47): C, 77.79; H, 4.77; N, 17.44%. Found: C, 77.55; H, 4.98; N, 17.13%.

*7-(4-Methoxyphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e]-[1,2,4]triazolo[1,5-c]pyrimidine (**10b**)*
Brown solid in 63%; m.p.: 221–223°C; IR (KBr) υ (cm^{-1}): 1609 (C=N), 1235 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.42 (s, 3H, OCH_3), 6.8–7.6 (m, 14H, Ar-H), 9.35 (s,1H, C3-H), 9.63 (s, 1H, C5-H); ESI-MS *m/z*: 417 (M^+, 2.5%); Anal. calcd. for $C_{26}H_{19}N_5O$ (417.47): C, 74.80; H, 4.59; N, 16.78; O, 3.83%. Found: C, 74.55; H, 4.32; N, 16.99; O, 4.12%.

*General procedure for the synthesis of 2-methyl-8,9-diphenyl-7-substituted-7H-pyrrolo[3,2-e][1,2,4]triazolo-[1,5-c]pyrimidine (**11a,b**)*
The appropriate 4-imino-pyrrolopyrimidine (**9a,b**) (0.01 mol) was heated under reflux for 8 h in acetic anhydride (30 mL), cooled, poured onto ice water and neutralized with ammonia to give precipitates which were filtered off, dried, and recrystallized from ethanol to yield compounds **11a,b.**

*2-Methyl-7-(4-methylphenyl)-8,9-diphenyl-7H-pyrrolo-[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**11a**)*
Brown solid in 68%; m.p.: 205–207°C; IR (KBr) υ (cm^{-1}): 1612 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.31 (s, 3H, CH_3-Ph), 2.4 (s, 3H, CH_3-triazole), 7.0–7.9 (m, 14H, Ar-H), 9.5 (s,1H, C5-H); ESI-MS *m/z*: 415 (M^+, 30.9%); Anal. calcd. for $C_{27}H_{21}N_5$ (415.50): C, 78.05; H, 5.09; N, 16.86%. Found: C, 77.84; H, 5.33; N, 17.09%.

*2-Methyl-7-(4-methoxyphenyl)-8,9-diphenyl-7H-pyrrolo-[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**11b**)*
Brown solid in 58%; m.p.: 216–218°C; IR (KBr) υ (cm^{-1}): 1615 (C=N), 1212 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.42 (s, 3H, CH_3-triazole), 3.5 (s, 3H, OCH_3-Ph), 6.8–7.8 (m, 14H, Ar-H), 9.45 (s,1H, C5-H); ESI-MS *m/z*: 431 (M^+, 28%); Anal. calcd. for $C_{27}H_{21}N_5O$ (431.50): C, 75.16; H, 4.91; N, 16.23; O, 3.71%. Found: C, 75.41; H, 5.17; N, 16.55; O, 3.63%.

*General procedure for the synthesis of 8,9-diphenyl-7-substituted-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]-pyrimidine-2-thione (**12a,b**)*
The appropriate 4-imino-pyrrolopyrimidine (**9a,b**) (0.01 mol), CS_2 (0.76 g, 0.01 mol) and KOH (0.01 mol) in absolute ethanol (15 mL) were refluxed for 11 h. After removal of ethanol precipitates were formed which were filtered off, dried, and recrystallized from ethanol to yield compounds **12a,b.**

*7-(4-Methylphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e][1,2,4]-triazolo[1,5-c]pyrimidine-2-thione (**12a**)*
Light brown solid in 44%; m.p.: 223–225°C; IR (KBr) υ (cm^{-1}): 3228 (NH), 1607 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.32 (s, 3H, CH_3), 6.8–8.0 (m, 14H, Ar-H), 9.2 (s, 1H, C5-H), 9.33 (s, 1H, NH); ESI-MS *m/z*: 433 (M^+, 10.7%); Anal. calcd. for $C_{26}H_{19}N_5S$ (433.54): C, 72.03; H, 4.42; N, 16.15; S, 7.40%. Found: C, 72.32; H, 4.15; N, 15.98; S, 7.67%.

*7-(4-Methoxyphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e]-[1,2,4]triazolo[1,5-c]pyrimidine-2-thione (**12b**)*
Dark brown solid in 54%; m.p.: 229–231°C; IR (KBr) υ (cm^{-1}): 3123 (NH), 1576 (C=N), 1234 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.4 (s, 3H, OCH_3), 6.7–7.7 (m, 14H, Ar-H), 9.3 (s,1H, C5-H), 9.38 (s, 1H, NH); ESI-MS *m/z*: 449 (M^+, 26%); Anal. calcd. for $C_{26}H_{19}N_5OS$ (449.54): C, 69.47; H, 4.26; N, 15.58; S, 7.13 O, 3.56%. Found: C, 69.76; H, 4.11; N, 15.33; S, 7.43 O, 3.88%.

*General procedure for the synthesis of 2-cyanomethyl-8,9-diphenyl-7-substituted-7H-pyrrolo[3,2-e][1,2,4]triazolo-[1,5-c]pyrimidine (**13a,b**)*
A mixture of the appropriate 4-imino-pyrrolopyrimidine (**9a,b**) (0.01 mol) and ethyl cyanoacetate (0.02 mol) was refluxed in absolute ethanol (15 mL) for 8 h. After removal of ethanol precipitates were formed which were filtered off, dried, and recrystallized from methanol to yield compounds **13a,b.**

*2-Cyanomethyl-7-(4-methylphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**13a**)*
Brown solid in 37%; m.p.: 212–214°C; IR (KBr) υ (cm^{-1}): 2206 (C≡N), 1583 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.3 (s, 3H, CH_3), 3.6 (s, 2H, CH_2), 6.9–8.1 (m, 14H, Ar-H), 9.42 (s,1H, C5-H); ESI-MS *m/z*: 440 (M^+, 8%); Anal. calcd. for $C_{28}H_{20}N_6$ (440.51): C, 76.35; H, 4.58; N, 19.08%. Found: C, 76.64; H, 4.91; N, 18.75%.

*2-Cyanomethyl-7-(4-methoxyphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**13b**)*
Brown solid in 44%; m.p.: 218–220°C; IR (KBr) υ (cm^{-1}): 2206 (C≡N), 1604 (C–N), 1261 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.43 (s, 2H, CH_2), 3.56 (s, 3H, OCH_3), 6.7–7.9 (m, 14H, Ar-H), 9.5 (s, 1H, C5-H); ESI-MS *m/z*: 456 (M^+, 26.3%); Anal. calcd. for $C_{28}H_{20}N_6O$ (456.51): C, 73.67; H, 4.42; N, 18.41; O, 3.50%. Found: C, 73.93; H, 4.66; N, 18.15; O, 3.81%.

*General procedure for the synthesis of 2-chloromethyl-8,9-diphenyl-7-substituted-7H-pyrrolo[3,2-e][1,2,4]triazolo-[1,5-c]pyrimidine (**14a,b**)*
A mixture of the appropriate 4-imino-pyrrolopyrimidine (**9a, b**) (0.01 mol) and chloroacetylchloride (0.02 mol) was refluxed in absolute ethanol (15 mL) for 8 h. After removal of ethanol precipitates were formed which were filtered off, dried, and recrystallized from methanol to yield compounds **14a,b**.

*2-Chloromethyl-7-(4-methylphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**14a**)*
Dark brown solid in 65%; m.p.: 198–200°C; IR (KBr) υ (cm^{-1}): 1616 (C=N); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.31 (s, 3H, CH_3), 4.2 (s, 2H, CH_2), 6.8–8.0 (m, 14H, Ar-H), 9.3 (s,1H, C5-H); ESI-MS *m/z*: 449 (M^+, ^{35}Cl, 20.5%), 451 (M^++2, ^{37}Cl, 6.8%); Anal. calcd. for $C_{27}H_{20}ClN_5$ (449.95): C, 72.08; H, 4.48; Cl, 7.88; N, 15.56%. Found: C, 71.94; H, 4.77; Cl, 8.16; N, 15.67%.

*2-Chloromethyl-7-(4-methoxyphenyl)-8,9-diphenyl-7H-pyrrolo[3,2-e][1,2,4]triazolo[1,5-c]pyrimidine (**14b**)*
Light brown solid in 70%; m.p.: 207–209°C; IR (KBr) υ (cm^{-1}): 1567 (C=N), 1237 (C–O); ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.4 (s, 3H, OCH_3), 4.4 (s, 2H, CH_2), 6.7–8.0 (m, 14H, Ar-H), 9.44 (s, 1H, C5-H); ESI-MS *m/z*: 465 (M^+, ^{35}Cl, 44.3%), 467 (M^++2, ^{37}Cl, 14.8%); Anal. calcd. for $C_{27}H_{20}ClN_5O$ (465.95): C, 69.60; H, 4.33; Cl, 7.61; N, 15.03; O, 3.43%. Found: C, 69.89; H, 4.56; Cl, 7.92; N, 14.84; O, 3.14%.

Antiviral screening

Cell culture
The cell lines BGM, Hep-2, and MA-104 were obtained from the Holding Company for Biological Products and Vaccines, VACSERA, Egypt. Cell lines were routinely cultured in 96-well plates (Greiner-Bio One, Germany) in DMEM (GIBCO BRL), at 37°C in a humidified incubator of 5% (v/v) CO_2.

Cytotoxicity test
The cytotoxicity test was performed according to methods reported [47, 48]. Briefly, all samples (50 mg) were dissolved in 1 mL DMSO containing 24 μL of 100× of antibiotic/antimycotic mixture for decontamination of the samples. One hundred milliliters of the dissolved samples were diluted by serial dilution. One hundred milliliters of each dilution were inoculated in BGM, Hep-2, and MA-104 cell lines previously cultured in 96-well plates to estimate the non-toxic dose of the tested samples. Cytotoxicity assay was done using cell morphology evaluation by inverted light microscope and cell viability test applying Trypan blue dye exclusion method.

Cell morphology evaluation by inverted light microscopy
BGM, Hep-2, and MA-104 cell cultures (2×10^5 cells/mL) were prepared separately in 96-well tissue culture plates (Greiner-Bio One, Germany) and incubated for 24 h at 37°C in humidified 5% (v/v) CO_2 atmosphere. Confluent cell monolayers were formed, the medium was removed from each well and replenished with 100 μL of serial dilution of different samples tested prepared in DMEM (GIBCO BRL). For cell controls 100 mL of DMEM without samples was added. All cultures were incubated at 37°C in a humidified 5% (v/v) CO_2 atmosphere for 72 h. Cell morphology was observed daily for microscopically detectable morphological alterations, such as loss of confluence, cell rounding and shrinking, cytoplasm granulation and vacuolization. Morphological changes were scored [49].

Cell viability assay
It was done by Trypan blue dye exclusion method [50]. BGM, Hep-2 and MA-104 cell lines were grown in 12-well tissue culture plates (2×10^5 cells/mL). After 24 h incubation, the same assay described above for tested samples cytotoxicity was followed by applying 100 μL of tested samples dilutions (serial dilutions) per well. After 72 h, the medium was removed, cells were trypsinized and an equal volume of 0.4% (w/v) Trypan blue dye aqueous solution was added to cell suspension. Viable cell were counted under the phase contrast microscope.

Determination of coxsackievirus B4, adenovirus type 7, and rotavirus Wa strain titers using plaque assay
Non-toxic dilutions were mixed (100 μL) with 100 μL of different doses of coxsackievirus B4 (1×10^5, 1×10^6, 1×10^7) and the same doses of adenovirus type 7 and rotavirus Wa strain. The infectivity of rotavirus stocks were activated with 10 μg/mL trypsin for 30 min at 37°C. The mixture was incubated for 0.5 h at 37°C. The inoculation of (100 μL) 10 fold dilutions of treated and untreated coxsackievirus B4, adenovirus type 7, rotavirus Wa strain was carried out separately into BGM, Hep-2, MA-104 cell lines respectively in 12-well plates. After 1 h of incubation for adsorption at 37°C in a 5% CO_2–water vapor atmosphere without constant rocking, the plates were rocked intermittently to keep the cells from drying. After adsorption, 1 mL of 2× media (Dulbecco's Modified Eagle Medium, Gibco-BRL (DMEM) plus 1 mL 1% agarose) was added to each well, 0.5 μg/mL was added to the media–agarose mixture in the case of rotavirus Wa strain and the plates were incubated at 37°C in a 5% CO_2–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 counted. The viral titers were then calculated, and expressed as plaque-forming units per milliliter (pfu/mL) [51].

Data analysis
IC_{50} for each compound were obtained from dose–effect curves. The IC_{50} is the concentration of the compound that causes 50% inhibition of the virus. The dose–effect curve was plotted from the average of four assays with five concentrations within the inhibitory range of the compound.

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