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First discovery of novel 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones as specific anti-vaccinia and adenovirus agents via 'privileged scaffold' refining approach

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ABSTRACT

A series of 1,2,3-triazolyl 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones was constructed utilizing Cu(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) method. The biological significance of the novel synthesized quinazolines was highlighted by evaluating them in vitro for antiviral activity, wherein several compounds exhibited excellent activity specifically against vaccinia and adenovirus. Especially, **24b11** displayed the most potent inhibitory activity against vaccinia with an EC_{50} value of 1.7 μM, which was 15 fold than that of the reference drug Cidofovir (EC_{50} = 25 μM). **24b13** was the most potent compound against adenovirus-2 with an EC_{50} value of 6.2 μM, which proved lower than all the reference drugs. Preliminary structure–activity relationships were also discussed. To the best of our knowledge, no data are present in the literature on antiviral activity of 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones against DNA-viruses. Thus, these findings warrant further investigations (library expansion and compound refinement) on this novel class of antiviral agents.

Poxvirus-associated diseases are a major threat for human health.[1] Smallpox, a highly transmissible and infectious disease with high morbidity and mortality, was the most dangerous human pathogen of poxviruses group.[2] Although smallpox was declared eradicated in 1980 by the World Health Organization (WHO) after an intensive immunization campaign with the vaccinia virus vaccine of the global, there are stocks of VARV were kept in two WHO-approved laboratories: the U.S. Center for Disease Control and Prevention (CDC) in Atlanta and the Russian State Research Center of Virology and Biotechnology in Novosibirsk.[3,4] By the fact that the vaccinia virus vaccines have substantial side effects,[5] the vaccination programs have been terminated since the last century, which led to the human population today more susceptible to a smallpox disaster. In addition, the emergence of zoonotic poxvirus infections such as the monkeypox virus in both the US and Western Africa in human populations aggravated the people's panic.[6] For all of these reasons, special attention has been paid to establish efficient safe therapies for dealing with poxvirus infections.

In spite of number of potential antipoxviral agents have been reported recently, there have no approved drugs by US Food and Drug Administration (FDA) for the prevention and treatment of smallpox infections available on the market currently. Cidofovir (CDV, **1**) is a potent and selective anti-DNA virus agent and can inhibit viral DNA replication (Fig. 1), so it has a broad-spectrum activities and has been approved for the treatment of smallpox virus. But the low oral bioavailability of CDV and potential nephrotoxicity accompany with its intravenous administration limited the clinical application of the CDV. Recently, the lipophilic prodrug of CDV, hexadecyloxypropyl ester (HDP-CDV, **2**), was demonstrated that have improved bioavailability and equivalent effectiveness against orthopoxvirus infections and is in phase I/II clinical studies currently.[7] Moreover, Tecovirimat (ST-246, **3**), an orally bioavailable compound that targets the F13L protein of the virus, which inhibit the growth of multiple orthopoxviruses and has significant antiviral activity in various poxvirus disease animal models, was demonstrated favorable safety, tolerability, and pharmacokinetics in phase I clinic trial.[8,9] In 2010, Tecovirimat was received both orphan drug designation and fast-track status from

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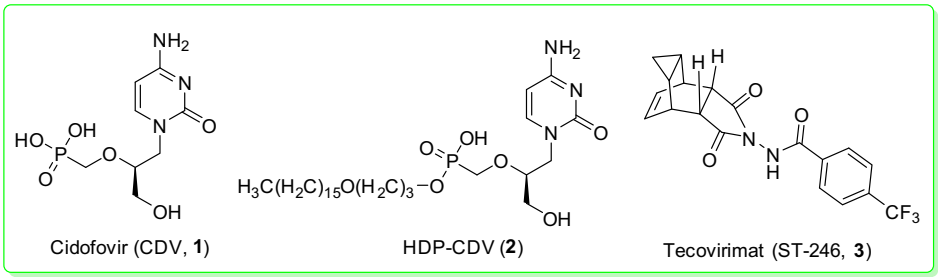


Figure 1. Structures of CDV, HDP-CDV and tecovirimat.

the US FDA and with the hope that it can be approved for the prevention and treatment of smallpox infections.

Adenoviruses (AdVs) are double-stranded DNA viruses (about 60–100 nm in size) with a nonenveloped icosahedral capsid and a genome of 26–45 kb.[9] AdVs comprise more than 50 human Ad serotypes, which have been identified and classified into six species (A–F) in terms of their biological, physiochemical and genetic properties.[10] AdVs are opportunistic pathogens and associated with a wide variety of severe clinical symptoms in healthy individuals, such as respiratory illness, renal disease, gastroenteritis, hemorrhagic cystitis, and so on.[11–13] However, they are generally not considered to be highly pathogenic viruses for the reason that the adenovirus infections are most often self-limited in immunocompetent individuals. But an adenovirus infection might led to severe and life-threatening disease (multiple organ failure) in the immunocompromised individuals.[14] During the last two decades, a number of adenovirus serotypes that largely from species A, B, and C were isolated from immunocompromised patients successfully. Ad2, a species A serotype that has been most detailed studied of adenovirus so far, are often associated with respiratory illness with a lethal outcome occasionally. Currently, there have no available drugs for treatment of AdVs infections. Cidofovir was proved to be the most promising anti-adenoviral agent of those currently used in clinical settings, but the outcome in the hematopoietic stem cell recipients has been found to be poor adenovirus infections.[15] Therefore, there is compelling need for the discovery of new antiviral drugs of vaccinia and adenovirus that possess an improved safety property and oral bioavailability.

Over the past few decades, serendipitous or high-throughput screen (HTS) campaign of heterocyclic compound collections continues to remain a major paradigm for antiviral hits or leads discovery.[16,17] Due to their chemical and biological importance, hydroxy-(iso)quinazoline-2,4(1,3)-dione and its analogues are attractive 'privileged structures' in antiviral medicinal chemistry. Recently, 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-dione and 3-hydroxypyrimidine-2,4-dione derivatives were reported as miscellaneous inhibitors targeting bridged dinuclear metalloenzymes, such as HIV RNase H and integrase (IN) dual inhibitors **4**–**7**[18], **8**, **9**[19], **10**[20], HIV RNase H active-site inhibitors MB-76 (**11**)[21–23] and **12**[24], as well as Hepatitis C virus (HCV) NS5B polymerase inhibitor **13**[25] (Fig. 2), suggesting that these divalent metal ion chelators could be useful inhibitor scaffolds with a broad range of biological activity via various modifications.[26]

Evolved from the concept of drug repositioning, 'privileged structure'-guided scaffold refining is a very effective strategy to exploit undescribed bioactivites by making full use of readily derivatized scaffolds with well-established synthetic methods.[27] In this context, in view of the above fact and to discover completely new anti-vaccinia and adenovirus agents with a novel skeleton and unique mode of action, a relatively small library of 6-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-3-hydroxyquinazoline-2,4(1*H*,3*H*)-dione compounds (the general formula in Scheme 1) was constructed via the copper(I)-catalyzed azidealkyne cycloaddition (CuAAC) reaction[28] and the biological significance of the novel synthesized quinazolines was highlighted by evaluating them in cell culture-based antiviral high-throughput screening (HTS) assays against a broad panel of DNA viruses, retroviruses and several RNA viruses.

The library of 3-hydroxyquinolin-2(1*H*)-one compounds was constructed by the following general synthetic route, which was straightforward and depicted in Scheme 1. The starting material 2-hydroxyisoindoline-1,3-dione (**14**) was firstly reacted with benzyl bromide to obtain 2-(benzyloxy)isoindoline-1,3-dione (**15**). Then **15** was treated with hydrochloric acid and acetic acid via an acidulation reaction to form the key intermediate *O*-benzylhydroxylamine hydrochloride (**16**).[29] Meanwhile, the commercially available material 2-amino-5-iodobenzoic acid (**17**) was treated with methanol in the presence of concentrated H_2SO_4 gave the intermediate methyl 2-amino-5-iodobenzoate (**18**) via an esterification reaction. The intermediate 3-(benzyloxy)-6-iodoquinazoline-2,4(1*H*,3*H*)-dione (**19**) was obtained by ring closure of **18** with carbonyldimidazole (CDI) and **16** under the condition of sodium hydroxide.[30,31] Then this heterocyclic scaffold was alkylated with iodomethane and iodoethane in DMF afforded the N_1-methylation product **20a** and N_1-ethylation product **20b** respectively. The key alkyne building block **22a** or **22b** was prepared from **20a** or **20b** via the Sonogashira cross-coupling reaction and trimethylsilyl-removal reaction successfully. Copper(I) catalyzed click reaction (CuAAC) of the alkyne key intermediate **22a** or **22b** with different azido substituent groups generated the corresponding key 1,2,3-triazole intermediates (**23a01–23a13** and **23b01–23b13**), which were deprotected under basic condition affording two series of target compounds 1,2,3-triazole-substituted 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones **24a01–24a13** and **24b01–24b13**. Their structures were determined by their ^{1}H NMR, ^{13}C NMR, and MS (ESI) spectra. Notably, the ^{1}H NMR spectrum showed a singlet at 8.50 corresponding to the triazolyl proton while the ^{13}C NMR spectrum showed peaks at 125.73–125.90 and 149.12–149.65 corresponding to CH and qC characteristic to the triazole core unit.

The newly synthesized 1,2,3-triazole-linked 3-hydroxy-quinazoline-2,4 (1*H*,3*H*)-diones were performed to evaluate against their antiviral activity against a broad panel of DNA virus, including Herpes simplex virus-1 (KOS), Herpes simplex virus-2 (G), Herpes simplex virus-1 TKKOS ACVr, vaccinia virus and Adeno virus-2 (evaluated in infected human embryonic lung fibroblast (HEL) cells). In addition, all the compounds were also examined against retroviruses [i.e., human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2)] and several RNA viruses [i.e., human coronavirus and influenza virus]. The results were expressed as EC_{50} and MCC (Minimum cytotoxic concentration) (Table 1).

As shown in Table 1, some compounds exhibited remarkable inhibitory efficacy against vaccinia with EC_{50} values ranging from 1.7 μM to 15 μM and adenovirus with EC_{50} values ranging from 6.2 μM to 13 μM respectively in HEL cell cultures. Most of the

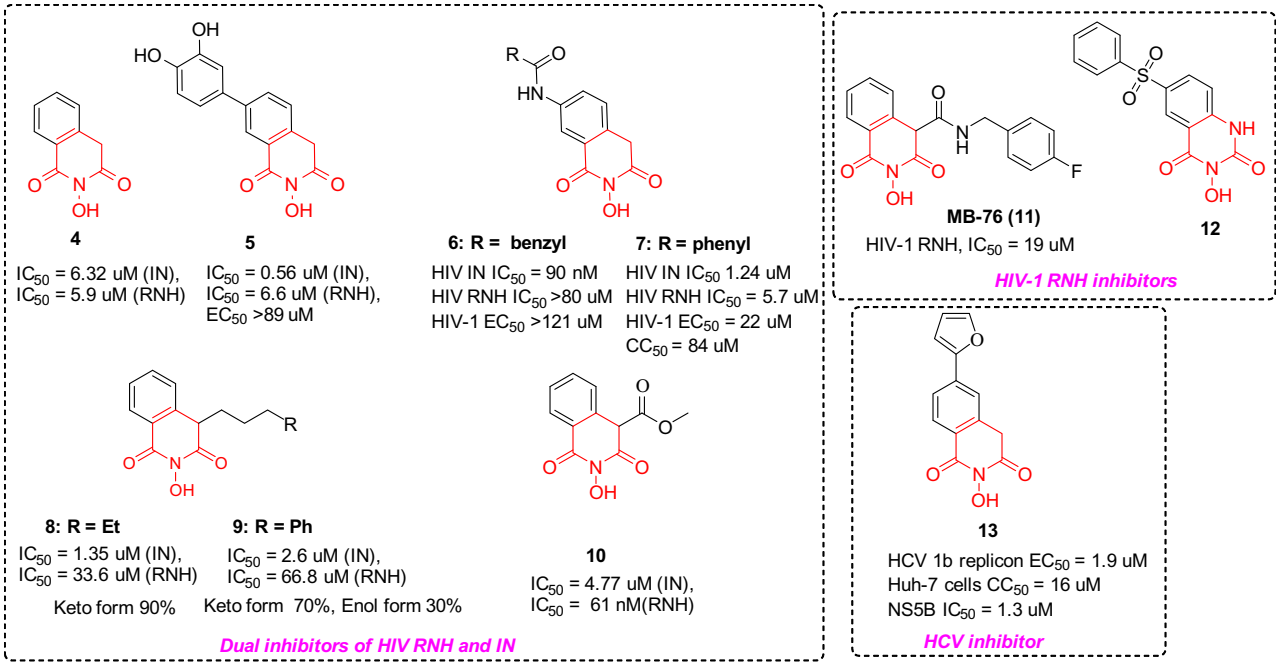
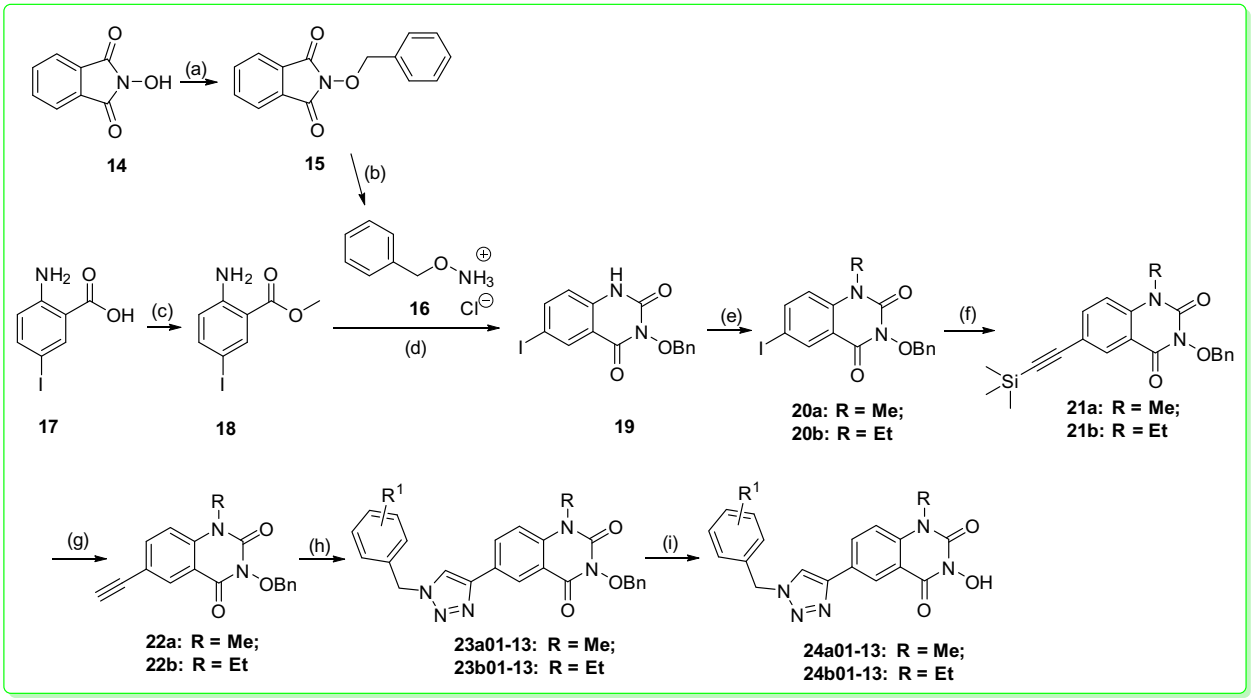


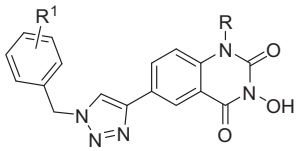
Figure 2. The reported 2-hydroxyisoquinoline-1,3(2*H*,4*H*)-diones (**4**–**11**,**13**) and 3-hydroxypyrimidine-2,4-dione **12** as antiviral agents.



Scheme 1. Synthetic route to 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones. Reagents and condition: (a) (bromomethyl)benzene, K_2CO_3, DMSO, 92%; (b) CH_3COOH, HCl, 78%; (c) CH_3OH, H_2SO_4, 94%; (d) (i) CDI, THF, 2 h; (ii) NH_2OBn, reflux, 2 h; (iii) NaOH, EtOH, reflux, 2 h; (iv) CH_3 COOH, 62%; (e) CH_3I or C_2H_5I, DMF, rt, 98% and 96%; (f) CuI, $PdCl_2(PPh_3)_2$, Et_3N, trimethylsilylacetylene, THF, 76% and 78%; (g) K_2CO_3, CH_3OH, rt, 88% and 90%; (h) VcNa, $CuSO_4.5H_2O$, H_2O/*t*-BuOH, 65 °C,67 % to 90 %; (i) HBr, CH_3COOH, reflux; or, Pd/C, H_2, MeOH, rt.

active compounds exhibited higher inhibitory activity than those of the reference drugs Brivudin, Cidofovir, Zalcitabine and Alovudine. Especially, **24b11** displayed the most potent inhibitory activity against vaccinia with an EC_{50} of 1.7 μM, which was 8–15 fold than that of Brivudin (EC_{50} = 15 μM) and Cidofovir (EC_{50} = 25 μM). **24b13** was the most potent compound against adenovirus with EC_{50} values of 6.2 μM, which proved lower than all the reference drugs. In addition, most of the compounds showed no cytotoxicity (as determined by microscopy) at the highest concentration (MCC > 100 μM) tested in HEL cells. In addition, none of the compounds showed considerable activity against the rest of the DNA virus and any of the RNA viruses tested at nontoxic concentrations. To the best of our knowledge, no data are present in the literature on antiviral activity of 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones

Table 1
Antiviral activity, cytostatic activity and selection index of the test compounds against vaccinia and adenovirus in HEL cell cultures



Compd.	R	R^1	EC_{50}[a] (μM)		MCC[b] (μM)
			Vacciniavirus	Adeno virus-2	
24a01	Me	2-F	>100	>100	>100
24a02	Me	4-F	>100	>100	>100
24a03	Me	3-F	>100	>100	>100
24a04	Me	H	>100	>100	>100
24a05	Me	4-CN	>100	>100	>100
24a06	Me	2,6-diF	>100	>100	100
24a07	Me	3,4-diF	**5.0 ± 0.75**	>100	100
24a08	Me	2,5-diF	>100	>100	>100
24a09	Me	2,4-diF	>100	>100	100
24a10	Me	2-CH_3	>100	>100	100
24a11	Me	4-NO_2	**2.4 ± 0.55**	>100	>100
24a12	Me	2,6-diCl	>100	>100	>100
24a13	Me	4-OCH_3	>100	**12 ± 8**	>100
24b01	Et	2-F	>100	**6.5 ± 2.5**	>100
24b02	Et	4-F	>100	>100	100
24b03	Et	3-F	>100	**13 ± 7**	>100
24b04	Et	Ph	>100	**12 ± 8**	>100
24b05	Et	4-CN	**15 ± 5**	>100	>100
24b06	Et	2,6-diF	>100	**8 ± 4**	>100
24b07	Et	3,4-diF	**1.9 ± 0.1**	>100	20
24b08	Et	2,5-diF	>100	>100	20
24b09	Et	2,4-diF	>100	>100	20
24b10	Et	2-CH_3	>100	>100	20
24b11	Et	4-NO_2	**1.7 ± 0.3**	>100	>100
24b12	Et	2,6-diCl	>100	>100	>100
24b13	Et	4-OCH_3	>100	**6.2 ± 3.8**	>100
Brivudin			15	—	>250
Cidofovir			25	10	>250
Zalcitabine			—	7.2	>250
Alovudine			—	10	>250

[a] Required to reduce virus-induced cytopathogenicity by 50%.
[b] Required to cause a microscopically detectable alteration of normal cell morphology.

on vaccinia and adenovirus and this study can help to relate the structural characteristics of this complexes to their antiviral activity.

Preliminary investigation of the structure–activity relationships (SARs) revealed that the nature of the N_1-R substituent and the aryl group which connected to the triazole influenced the antiviral activity remarkably. For instance, the result revealed that the antiviral activity of N_1-ethyl substituted analogues are more potent than that of the corresponding N_1-methyl substituted counterparts (EC_{50}: **24b07** > **24a07**, **24b11** > **24a11** and **24b13** > **24a13**). Especially, most of N_1-ethyl substituted analogues showed favorable activity against adenovirus, but nearly all the compounds lost their activity when the compounds with N_1-methyl substituted counterparts with an exception of compound **24a12**. Introduction of an electron-withdrawing group at the *para* substituents of the aryl can remarkable improve the anti-vaccinia activities (NO_2 > -CN > F > CH_3). To the contrary, a strong electron-withdrawing group can result in the compound lost their adenovirus activities, but the compounds with electrondrawing group (**24b13** and **24a13**) exhibited the most potent antiviral activity against adenovirus. When comparing compound **24b01** with **24b02**, **24b03**, **24b06**, **24b07** and **24b08**, only compound with fluorine substituted in the *meta* and *para* position of the aryl simultaneous can display potent inhibitory activity toward vaccinia (**24b07**, EC_{50} = 1.9 μM). But for the adenovirus, compounds with *ortho* or *meta* substituted showed moderate inhibitory activity (**24b01**, EC_{50} = 6.5 μM; **24b03**, EC_{50} = 13 μM), and *para*-substituted counterpart was inactive (**24b02**, EC_{50} > 100 μM); Moreover, changing the pattern of substitution on the aryl to disubstituted resulted in reduced activity (**24b06**, EC_{50} = 8 μM), even completely lost inhibitory activity (**24b08** and **24b09**, EC_{50} > 100 μM).

In conclusion, a series of novel 1,2,3-triazole-containing 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones has been synthesized using CuAAC reaction, and was firstly identified as potent and specific vaccinia and adenovirus inhibitors in vitro. Among them, **24b11** and **24b13** was demonstrated with the most potent vaccinia and adenovirus inhibitory activity respectively, which were promising compounds for further exploration as drug candidates for anti-poxvirus or adenovirus therapy. Preliminary SARs were discussed with the hope to provide a helpful guidance for the design of next-generation of quinolinone analogues. Obviously, the mechanism of action and precise viral target of these 1,2,3-triazolyl 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones derivatives presented here remain to be identified. Consequently, a detailed study on further elaboration of these 3-hydroxy-quinazoline-2,4(1*H*,3*H*)-diones and investigation of their prospective mechanism

of action is currently underway in our lab and would be disclosed in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.09.071.

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