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

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A series of 1,2,3-triazolyl 3-hydroxy-quinazoline-2,4(1H,3H)-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 EC₅₀ value of 1.7 μM, which was 15 fold than that of the reference drug Cidofovir (EC₅₀ = 25 μM). 24b13 was the most potent compound against adenovirus-2 with an EC₅₀ 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(1H,3H)-diones against DNA-viruses. Thus, these findings warrant further investigations (library expansion and compound refinement) on this novel class of antiviral agents.

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Poxvirus-associated diseases are a major threat for human health. Smallpox, a highly transmissible and infectious disease with high morbidity and mortality, was the most dangerous human pathogen of poxviruses group. Although smallpox was declared eradicated in 1980 by the World Health Organization (WHO), the smallpox 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. By the fact that the vaccinia virus vaccines have substantial side effects, 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. 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. 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. In 2010, Tecovirimat was received both orphan drug designation and fast-track status from...
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 nonenvelopedicosahedral capsid and a genome of 26–45 kb. 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. 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. 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. 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 claimed 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. 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. Due to their chemical and biological importance, hydroxy-(iso)quinazoline-2,4(1H)-dione and its analogues are attractive ‘privileged structures’ in antiviral medicinal chemistry. Recently, 2-hydroxyquinolin-2(1H)-diones were performed to evaluate against their antiviral activity via various modifications.

Evolved from the concept of drug repositioning, ‘privileged structure’-guided scaffold refining is a very effective strategy to exploit undescribed bioactivities by making full use of readily derivatized scaffolds with well-established synthetic methods. 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-1H-1,2,3-triazol-4-yl)-3-hydroxyquinazoline-2,4(1H,3H)-dione compounds (the general formula in Scheme 1) was constructed via the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction 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(1H)-one compounds was constructed by the following general synthetic route, which was straightforward and depicted in Scheme 1. The starting material 2-hydroxyisoindolone-1,3-dione (14) was firstly reacted with benzyl bromide to obtain 2-(benzoyl)isoindolone-1,3-dione (15). Then 15 was treated with hydrochloric acid and acetic acid via an acidification reaction to form the key intermediate 0-benzylhydroxylamine hydrochloride (16). Meanwhile, the commercially available material 2-amino-5-iodobenzoic acid (17) was treated with methanol in the presence of concentrated H2SO4 gave the intermediate methyl 2-amino-5-iodobenzoate (18) via an esterification reaction. The intermediate 3-(benzoyl)-6-iodoquinazoline-2,4(1H,3H)-dione (19) was obtained by ring closure of 18 with carbonimidimazolide (CDI) and 16 under the condition of sodium hydroxide. Then this heterocyclic scaffold was alkylated with iodomethane and iodoethane in DMF afforded the N1-methylation product 20a and N2-ethylation product 20b respectively. The key alkyl building block 22a or 22b was prepared from 20a or 20b via the Sonogashira cross-coupling reaction and trimethylsil-yl-reaction removal reaction successfully.

The newly synthesized 1,2,3-triazole-linked 3-hydroxy-quinazoline-2,4(1H,3H)-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 ACrV, vaccinia virus and Adenovirus-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 EC50 and MCC (Minimum cytotoxic concentration) (Table 1).

As shown in Table 1, some compounds exhibited remarkable inhibitory efficacy against vaccinia with EC50 values ranging from 1.7 μM to 15 μM and adenovirus with EC50 values ranging from 6.2 μM to 13 μM respectively in HEL cell cultures. Most of the
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 EC50 of 1.7 μM, which was 8–15 fold than that of Brivudin (EC50 = 15 μM) and Cidofovir (EC50 = 25 μM). 24b13 was the most potent compound against adenovirus with EC50 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(1H,3H)-diones.
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 N1–R substituent and the aryl group which connected to the triazole influenced the antiviral activity remarkably. For instance, the result revealed that the group which connected to the triazole influenced the antiviral activity of N1-ethyl substituted analogues are more potent than that of the corresponding N1-methyl substituted counterparts (EC50: >100). To the contrary, a strong electron-withdrawing group at the para position of the aryl simultaneous can remarkable improve the anti-vaccinia activities (NO2 > CN > F > CH3). To enhance the antiviral activity, both the compounds with electrondonating group (Me, Et) and the compounds with electron-withdrawing group (CN, NO2, F) were synthesized and evaluated in this study.

Table 1

| Compd. | R | R1 | Vaccinia Virus | Adenovirus-2 |
|--------|---|----|---------------|--------------|
| 24a01  | Me | 2-F | >100          | >100         |
| 24a02  | Me | 4-F | >100          | >100         |
| 24a03  | Me | 3-F | >100          | >100         |
| 24a04  | Me | H   | >100          | >100         |
| 24a05  | Me | 4-CN| >100          | >100         |
| 24a06  | Me | 2,6-diF | >100    | >100         |
| 24a07  | Me | 3,4-diF | 5.0 ± 0.75  | >100         |
| 24a08  | Me | 2,5-diF | >100    | >100         |
| 24a09  | Me | 2,4-diF | >100    | >100         |
| 24a10  | Me | 2-CH2 | >100    | >100         |
| 24a11  | Me | 4-NO2| 2.4 ± 0.55  | >100         |
| 24a12  | Me | 2,6-diCl | >100  | >100         |
| 24a13  | Me | 4-OCH3 | 12 ± 8  | >100         |
| 24b01  | Et | 2-F | 6.5 ± 2.5   | >100         |
| 24b02  | Et | 4-F | >100         | >100         |
| 24b03  | Et | 3-F | >100         | >100         |
| 24b04  | Et | Ph  | >100         | >100         |
| 24b05  | Et | 4-CN| 1.7 ± 0.3   | >100         |
| 24b06  | Et | 2,6-diF | >100    | 8 ± 4        |
| 24b07  | Et | 3,4-diF | 1.9 ± 0.1  | >100         |
| 24b08  | Et | 2,5-diF | >100    | >100         |
| 24b09  | Et | 2,4-diF | >100    | >100         |
| 24b10  | Et | 2-CH2 | >100    | >100         |
| 24b11  | Et | 4-NO2| 1.7 ± 0.3   | >100         |
| 24b12  | Et | 2,6-diCl | >100   | >100         |
| 24b13  | Et | 4-OCH3 | >100   | 6.2 ± 3.8   |
| Brivudin|   | —   | >100         | >250         |
| Cidofovir| | 25   | 10           | >250         |
| Zalcitabine| | —   | 7.2          | >250         |
| Alovudine| | —   | 10           | >250         |

**Notes:**

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

![Diagram](D. Kang et al. / Bioorg. Med. Chem. Lett. 26 (2016) 5182–5186)
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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