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Selenium compounds as promising antiviral agents

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The past two decades of this century have faced half a dozen pandemics caused by different kinds of viruses, affecting millions of people globally. Among them three zoonotic viruses of the beta-coronavirus family, namely SARS-CoV-1, MERS-CoV and SARS-CoV-2, have emerged in a span of 17 years. Prevention of viral infection using vaccines is the foremost strategy to combat viral infection, but development of a vaccine for a virus remains a challenging task due to high genetic diversity and high mutation rates. Treatment of viral disease at different stages by antiviral drugs is another strategy. Several antiviral agents, primarily aiming to disrupt the viral life cycle, have been developed. Extensive research in different labs across the globe has shown that selenium deficiency has been associated with pathogenicity of several viruses like influenza viruses, HIV, HBV, HSV-1, *etc.* While dietary selenium supplementation has been practiced clinically, researchers are now focusing on developing new synthetic organoselenium compounds as novel antiviral agents. In this essay we have covered different classes of selenium compounds, *viz.* N-heterocyclic selenium derivatives, ebselen and its derivatives, organoselenium compounds, and selenium nanoparticles, which have been evaluated for effective management of viral infections.

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1. Introduction

The world has faced several pandemics from time to time over the millennia. These tend to appear at the intervals of centuries and/or several decades. However, the frequency of their occurrence during the past few decades has increased, and the outbreaks now appear at a cycle of 3–5 years (Table 1). The first two decades of the 21st century have faced half a dozen pandemics, caused by viruses, affecting millions of people globally. The recent outbreak of the COVID-19 (SARS-CoV-2) has not only paralyzed the functioning of normal life but also posed a great threat to the existence of mankind. The problem was further aggravated with the rapid appearance of new variants like delta, omicron, *etc.* and more recently Eris of SARS-CoV-2. The dynamic changes in genomic levels are evident from the emergence of three zoonotic viruses (SARS-CoV-1, MERS-CoV and SARS-CoV-2) in the beta coronavirus family in the past 17 years. From the current trends of the viral endemic/pandemic, it is likely that viral infections from different strains may emerge, apart from re-emergence from the previously responsible viruses, leading to public health emergencies in the future. Therefore, there is an urgent need for robust viral management strategies.

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There are primarily two strategies to combat viral diseases, *i.e.*, (a) prevention using vaccines for a specific viral strain and (b) treatment by anti-viral drugs.[1] In the former strategy, development of vaccines in several cases has been a challenging task due to the high genetic diversity of the virus, *e.g.*, vaccine for HIV. The latter strategy started emerging in the early 1960s with the approval of idoxuridine by the FDA in 1963 for treatment of herpes.[2] So far, the FDA has approved more than 90 antiviral drugs for treatment of human viral diseases.[3] Antiviral agents primarily aim to disrupt the viral life cycle. Several approaches have been adopted for the design and development of antiviral agents. These include protease inhibitors, polymerase inhibitors, nucleoside reverse transcriptase inhibitors, *etc.*[2]

Nutrition and a healthy lifestyle are some of the important features for good immunity. Good nutrition consists of a balanced diet with enough supplements of macro- and micronutrients. The micronutrients like vitamins (A, C, D, E, B6, B9, B12), magnesium, iron, copper, zinc and selenium play an important role in immunity and viral protection.[4–6] Among them selenium has been recently identified and extensively verified for its role in immune functions.[7–11]

Selenium, is generally recommended to be obtained from the dietary products, however other natural inorganic and organic forms of selenium, can be used as a supplement when necessary. The results from clinical observations have shown strong correlation of selenium with the ability to fight viral

Table 1 Different kinds of pandemics that emerged in the last few decades

Year of origin	Viral endemic/pandemic
1981	Human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS)
1996	Bird flu (avian flu) H5N1 (since 1997) H7N9 (since 2013) H5N6 (since 2014) H5N8 (since 2016)
1998-1999	Nipah virus (NiV)
2002	Severe acute respiratory syndrome (SARS-Cov-1)
2009	Swine flu (H1N1 or A(H1N1)pdm09)
2012	Middle east respiratory syndrome (MERS-Cov)
2014	Ebola virus disease (EVD)
2015	Zika virus disease
2019	COVID-19 (corona virus disease-2019) (SARS-CoV-2)

infections. This led the researchers around the globe to explore novel synthetic organoselenium compounds as antiviral agents, with the hope to develop more effective antiviral agents. Accordingly, different forms of selenium, incorporated into organic compounds and nanoparticles, have been examined for their antiviral activity, under *in vitro* and *in vivo* conditions and a few have been examined in the clinic. In this essay we have covered some of these new aspects of selenium compounds, N-heterocyclic selenium derivatives, ebselen and its derivatives, non-N-heterocyclic organoselenium compounds, and selenium nanoparticles, which are being evaluated for effective management of viral infections. The current status of research on development of such selenium compounds for treatment of viral diseases is discussed here.

2. Human pathogenic viruses

Most of the human pathogenic viruses are ribonucleic acid (RNA) viruses having RNA as their genetic material and the majority of them generally have single-stranded RNA (ssRNA). They are classified into, depending on polarity, negative-sense (*e.g.*, influenza A (IAV), influenza B (IBV), H1N1, Nipah virus (NiV), Ebola) and positive-sense (*e.g.*, SARS-CoV 1, SARS-CoV 2, MERS-COV, hepatitis A (HAV), hepatitis C (HCV), human immunodeficiency virus (HIV), Zika, *etc.*) viruses. Their infection in humans causes several diseases like common cold, influenza, dengue, *etc.* Retroviruses also have RNA as the genetic material, but use DNA intermediates in their life cycle; examples of these viruses are human immunodeficiency virus (HIV), correlated with the disease called acquired immunodeficiency syndrome (AIDS).[7,10–12]

RNA viruses undergo genetic recombination when at least two viral genomes are present in the same host cell. The resulting recombinant viruses are capable of causing an outbreak of infection in humans. Since the evolution, RNA viruses have shown the ability to mutate very fast under infective stages and this accounts for the high lethality rate associated with the pathologies caused by these viruses. The faster mutation is an adaptive strategy acquired by RNA viruses to survive under adverse conditions or to overcome the immune response of the host cells. Due to the large genetic diversity and very high mutation rates, it is difficult to make effective vaccines against RNA viruses.

There is an inverse correlation between viral infections and inherent immunity. Living beings have a natural tendency to protect themselves from viral diseases and pathogenic microorganisms by developing immunity against them. The immune response is mainly of two types, *viz.* (i) nonspecific immunity, also known as innate immunity, through mononuclear phagocytes (monocytes/macrophages), polymorphonuclear cells (such as neutrophils), and natural killer (NK) cells, and (ii) specific immunity mediated by T (cellular immunity) and B (antibody response) cells. These two types of immune response coordinately function for protection of the systems from viral/pathogen attack. The loss in the functionality of any of the above cell types decreases the immune response and increases the vulnerability for viral and bacterial infections.[7,8,10–12]

3. Selenium in biology

3.1. Role of dietary and synthetic selenium compounds in biological functions

Selenium as a micronutrient has been identified to play a crucial role in several biological functions like reducing oxidative stress, suppressing inflammation and strengthening immunity. Selenium plays its biological functions predominately through selenoproteins.[13] In selenoproteins, selenocysteine (Sec), the 21st amino acid, is specifically incorporated into one or more peptide chains. Briefly, selenium from diet or selenium supplementation is metabolized through a systematic process and converted into the active selenide (Se^{2-}) form. This is converted into selenophosphate by intracellular metabolic pathways. Its subsequent reaction with phosphoseryl-tRNA (PSer-tRNA) gives Sec-tRNA. The Sec residue is then co-translationally inserted into selenoproteins (Fig. 1). The resulting selenoproteins with the availability of highly redox active selenium can help in fine-tuning the redox status of cells, which is important in controlling cell proliferation/differentiation, *etc.*[14] Additionally, the strong nucleophilicity of selenocysteine can contribute directly to antioxidant systems of selenoproteins by scavenging reactive oxygen species (ROS).[15] In humans, 25 selenoproteins have been identified. Different selenoproteins involved in important cellular functions are glutathione peroxidases (GPXs) associated with antioxidant activity (GPX1, GPX2, GPX3, GPX4), thioredoxin reductases (TXNRD1, TXNRD2, TXNRD3) and SELENOH, SELENOM, and SELENOW associated with redox regulation, deiodinases (DIOs), which regulate thyroid hormone metabolism, SELENOP, which is involved in selenium transport and storage, *etc.* GPXs such as GPX1 (cytosolic GPX), GPX2 (gastrointestinal GPX), and GPX4 (phospholipid hydroperoxide GPX) catalyse the decomposition of unwanted hydroperoxides and hydrogen peroxide into alcohols and water, respectively, through the Sec moiety.

Several synthetic selenium compounds, which are in the form of diselenides (RSeSeR), monoselenides (RSeR) or selenol

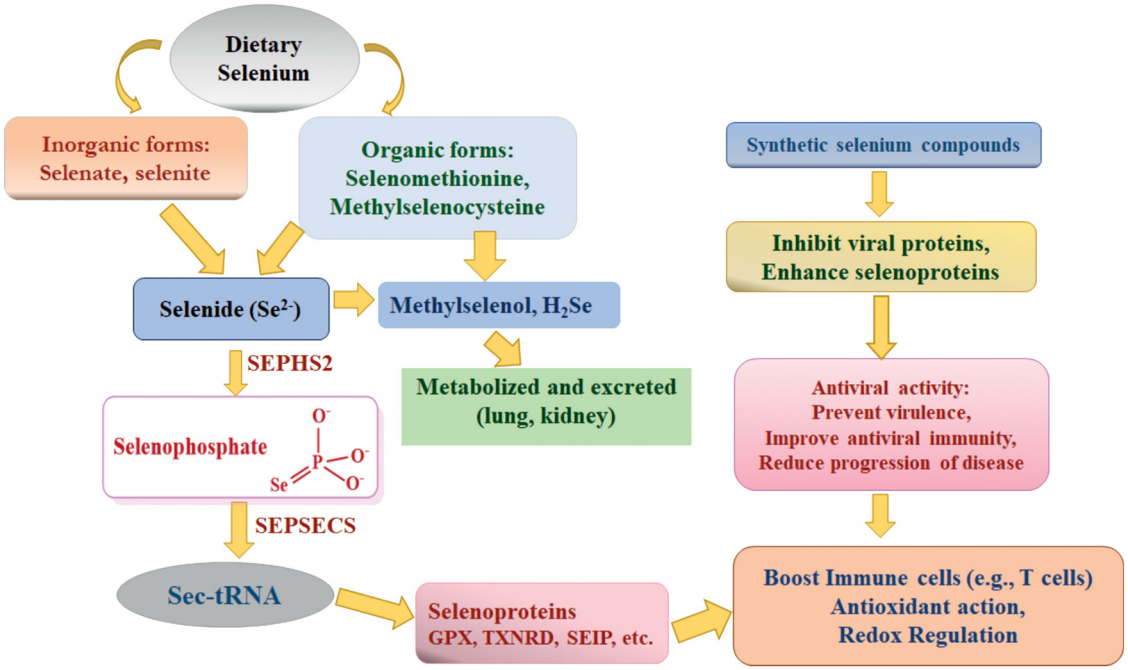


Fig. 1 Biochemical role of dietary and synthetic selenium compounds in antiviral activity (SEPHS2 = selenophosphate synthetase; SEPSECS = *o*-phosphoserine tRNA).

Fig. 2 GPx enzyme like activity of synthetic selenium compounds.

(RSeH), have been evaluated for GPx activity. When such compounds are fed to animals, a significant increase in the GPx enzyme levels has been observed. During the GPX like activity of synthetic selenium compounds, the selenol (RSeH) form is first oxidized by hydroperoxide to form selenenic acid (RSeOH), which is reduced by glutathione (GSH) into selenylsulfide (RSeSG). RSeSG regenerates the selenol back by subsequent reduction using another molecule of GSH. The oxidized form, GSSG, produced during this process is regenerated with the help of the glutathione reductase enzyme (Fig. 2).[16]

3.2. Biochemical role of selenium in viral infection

The pathogenesis of viral disease is marked by three distinct phases: (i) viral entry into the host cells, (ii) viral replication and (iii) oxidative stress and inflammation followed by death of host cells. Selenium in various chemical forms has been postulated to block the viral pathogenesis at all the above three stages. The entry of the virus into host cells is mediated through the interaction of viral capsid proteins with host cell receptors. Generally, selenium compounds being redox active in nature establish covalent interaction with the cysteine residue of the viral capsid proteins and thereby prevent the entry of the virus into host cells. Some synthetic selenium compounds have also been shown to inhibit the activity of viral proteins involved in the replication of the viral genome. Further, the selenium status of the cells has been shown to influence viral replication. For instance, recent findings have established that some of the human pathogenic viruses possess genetic sequences for synthesis of selenoproteins.[17,18] Therefore, it has been hypothesized that inadequate selenium status of host cells acts as an unfavorable or escape signal for the virus and this increases the transmissibility of viruses. Another line of thought is that inadequate selenium status compromises with the cellular antioxidants leading to higher levels of ROS in the host cells.[19,20] This promotes oxidative damage of the viral genome leading to mutation. The gain of function mutations lead to the evolution of more virulent or pathogenic viral strains. Thus, maintaining selenium status helps to control viral replication within host cells. Accordingly, selenium supplementation has also been explored for the symptomatic treatment of viral diseases by modulating innate and adoptive immune responses and preventing oxidative stress and cell death pathways.[8,9,13,21] Apart from inducing selenoproteins, various selenium compounds can also mimic the GPx-like activity and thereby affect the cellular redox status during viral infections. Thus, supplementation either through dietary forms or through synthetic selenium compounds can not only help in reducing the virulence but can also protect from disease progress and death. Fig. 1 briefly describes the antiviral role of dietary selenium and synthetic selenium compounds.

4. Observed clinical and *in vivo* effects of dietary selenium on viral infections

Humans obtain their regular supply of selenium through dietary products, like Brazilian nuts, broccoli, garlic, *etc.*, along with milk products and sea food. However, in selenium deficient areas like China, selenium is supplemented either in the form of selenium enriched agricultural products or in some cases in the form of selenite or selenate. Additionally, selenomethionine (in plant products) or selenium yeast (having organic forms like selenomethionine, and small amounts of methylselenocysteine and gamma-glutamyl Se-methylselenocysteine) is provided as a direct supplement. The recommended daily dose of selenium is 55 μg and can vary up to 200 μg depending on the selenium status in the region.

Considering the role of selenium in immune functions, its deficiency in humans or animals is linked with increased incidences of severity (virulence) and/or progression of viral infections.[7,8,10,11,19–24] The first evidence of involvement of selenium in the progression of viral infections came from the findings in human subjects with Keshan disease in China, which is a pathological condition characterized by cardiomyopathy, who were found to be infected by Coxsackievirus B4 (isolated from a victim of Keshan disease) and were also found to have significant selenium deficiency. This suggested that selenium deficiency is associated with increased infection of Coxsackievirus and the onset of the pathogenesis of Keshan disease. Furthermore, Beck and colleagues observed that when inoculated in selenium deficient hosts, the amyocarditic strain of Coxsackievirus B3 (CVB3/0) mutated to a virulence strain (CVB3/0).[8,19] Supplementation of selenium enriched food in mouse models indicated that mice with an adequate selenium level showed high expression of the GPX level and activated immune response and were prevented from the viral disease. With regular selenium supplementation in the disease affected areas of China,[8] the incidence fell from 1.16% to 0.06%. Similar observations have been reported with influenza virus infections, where mice deficient in selenium were found to have a decrease in the GPX level and were more susceptible to higher lung infections as compared to those with adequate selenium.[8,22] In fact, selenium deficiency has also been reported with the pathogenicity of several other viruses like HIV, HBV, HSV-1, Ebola, *etc.*[6,10,20,23,25–28]

Selenium deficiency has been recognized to progressively increase in virulence of HIV and its supplementation helps in reducing HIV infections.[29–31] It has been confirmed that the progression of infection leading to mortality in selenium deficient HIV patients is several times more than that of those with adequate selenium levels. Daily supplementation with selenium rich foods, in HIV infected subjects, has been reported to be effective in suppressing the viral burden. Synthetic organoselenium compounds such as diselenodibenzamides have been reported to inhibit nucleocapsid protein 7 (NCp7), a conserved retroviral protein of HIV that exerts essential and multiple functions in both early and late stages of viral replication, thereby reducing HIV disease progression. Similarly in West Africa, the outbreak of Ebola virus has also been linked with the deficiency of selenium in the populations. A clinical study indicated that selenium supplementation could effectively be used to treat an Ebola-like hemorrhagic fever.[32,33] Patients infected with hantavirus, also characterized by hemorrhagic fever, when treated with sodium selenite, showed 80% reduction in mortality.[8] Clearly, these reports suggest that the antiviral effects exerted by selenium supplementation are by redox regulation which in turn influences the immune functions of host cells through antioxidant selenoproteins and thereby affects the viral infection and progression of disease. Increased selenium levels have also been reported to increase responsiveness to vaccines. One study reported that in polio vaccinated subjects, selenium supplementation helped in rapid clearance of poliovirus after immunization.[7]

Recently, SARS-CoV-2 caused a severe pandemic worldwide associated with multi-organ disease and high morbidity leading to loss of millions of lives and also disturbing the economic status of several countries. Like in other viral infections, SARS-CoV-2 also showed an inverse correlation between selenium status and immunity, rate of infection, virulence and morbidity.[8,11,24,34–37] Studies from China indicated that provinces with high selenium status showed a high cure rate, while those with very low selenium status had a much lower cure rate from COVID-19 infections.[37] Further, research by several groups has indicated that supplementation with selenium at different stages of COVID-19 infection could provide significant clinical benefits.[8,11] It has been noticed that SARS-CoV-2 during replication within the host induces selenium deficiency, thereby reducing the capability of the infected host to synthesize selenoproteins, necessary for their immunity. In one such analysis of actual Covid-19 patients from European countries, significant deficiency in total serum selenium was identified in non-survivors.[36] Subsequently several groups have performed multi-disciplinary research on development of selenium-based compounds as possible drugs to cure COVID-19 infection, and ebselen, an organoselenium compound, showed promise.[38] Mainly such compounds like ebselen act as potent inhibitors of the activity of main protease (M^{pro}). The apparent role of dietary and synthetic selenium compounds in viral diseases is summarized in Fig. 3.[11,21,22,33,35,39–44]

5. Organoselenium-based antiviral agents

5.1. N-Heterocyclic selenium derivatives

N-Heterocyclic scaffolds such as pyridine,[45,46] quinolone,[47,48] pyridazine,[49] pyrimidine,[50] pyrazine,[51] *etc.* (Scheme 1) are the most extensively employed pharmaceutical motifs in the field of medicinal chemistry due to their profound effect on pharmaceutical activity. The structural significance of these scaffolds in small molecule drug design and discovery is evident from the FDA database which reveals that more than 60% small molecule drugs are composed of N-heterocycles.[46,52] These scaffolds are found in nature in several biologically active

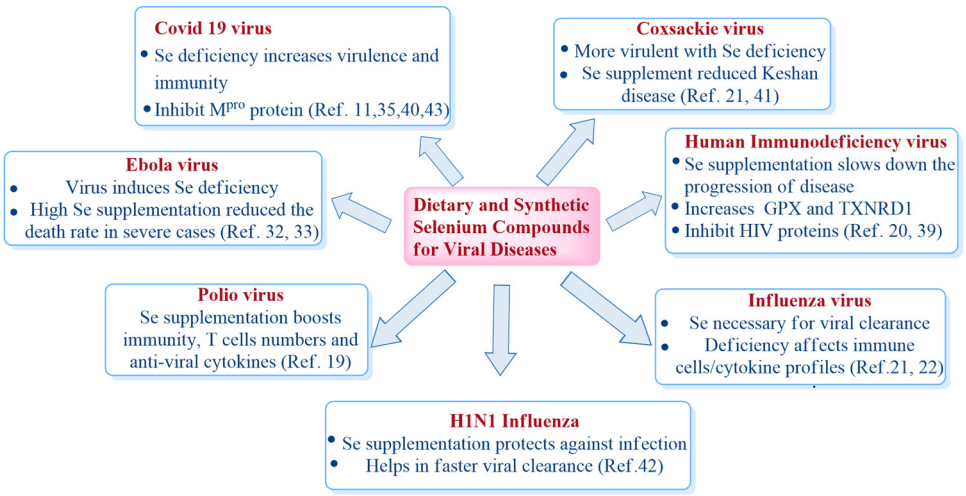
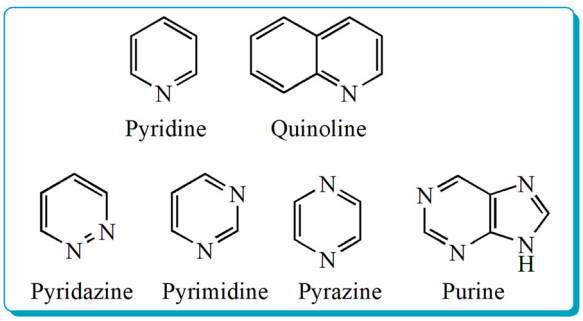


Fig. 3 Role of dietary and synthetic selenium compounds in viral diseases.

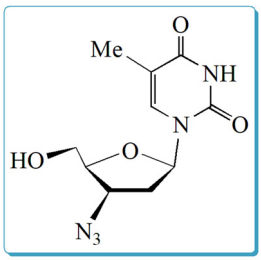


Scheme 1

molecules and are also responsible for a wide range of biological activities like redox reactions (*e. g.* NAD ⇌ NADH; $NADP^+$ ⇌ NADPH). Being endogenous in nature their derivatives can easily interact with bio-components in the cell. The initial antiviral drug development was based on these motifs, *e.g.*, idoxuridine, the first antiviral drug approved by the FDA in 1963 for the treatment of Herpes.[2]

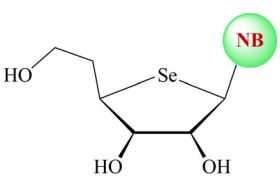
A number of selenium-containing compounds, both diselenides and seleno ethers, based on these scaffolds have been synthesized.[53–55] These compounds, besides their antioxidant properties, show promising antimicrobial and antitumor activities,[55–57] and their use as antiviral agents is emerging.[57–59] Sancineto and co-workers evaluated 2,2′-diselenobisbenzamides (DISeBAs) as HIV retroviral NCp7 inhibitors.[39] The pyridine containing derivative, 2,2′-diselanediyl bis(4-sulfamoylphenyl)nicotinamide, exhibited good antiviral activity against HIV-1 (III_B) and HIV-2 (ROD) with the median effective concentration (EC_{50}) values of 3.77 ± 0.63 and ≥3.11 μM, respectively.[39]

Inspired by the success of a nucleoside-based molecule, AZT (3′-azido-3′-deoxythymidine, also known as zidovudine) (**1**), approved as a drug by FDA in 1987 for treatment of HIV infections, numerous nucleoside analogues have been designed and developed as antiviral agents for more selective and effective action.[60,61] Among them selenium-based nucleosides with modifications made either in the nucleo-base or sugar moiety or both were of significant interest as they also provided selenium to HIV infected patients known for selenium deficiency.



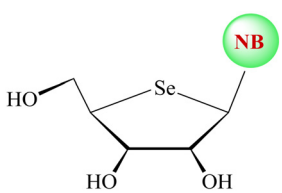
1 (AZT)

Accordingly, several selenium derivatives of nucleosides (**2** and **3**),[62–69] deoxynucleosides (**4**)[70] and 2′3′-dideoxynucleosides (**5**)[63,71] have been synthesized and assayed for their antiviral activities, in particular for anti-HIV activity. Neither **4** nor **5** exhibited anti-HIV-1 activity up to 100 μM.[70] The 5′-homo-4′-selenonucleosides **2** are however active against herpes simplex virus (HSV-1) with EC_{50} values of 2.9 μM for **2a** and 2.3 μM for **2b**.[68] Surprisingly, the 4′-selenonucleosides **3** are devoid of any antiviral activity against HSV-1 up to 100 μM.[62,68] The absence



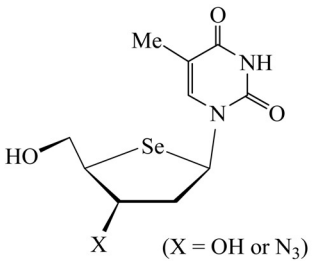
(2)

(NB = adenine (**2a**), uracil (**2b**)



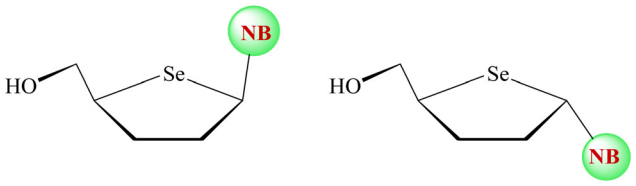
(3)

(NB = adenine (**3a**), guanine (**3b**), uracil (**3c**), cytosine (**3d**)



(X = OH or N_3)

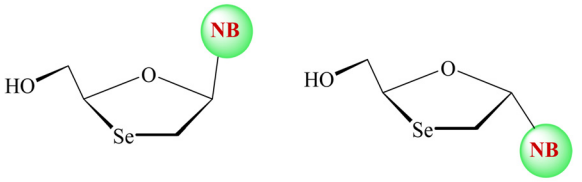
(4)



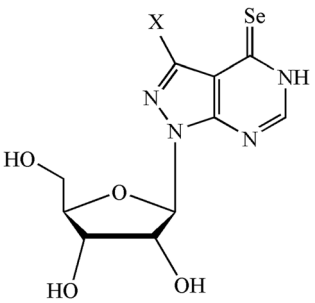
(5)
(NB = Thymine, uracil or cytosine)

of antiviral activity has been attributed to their inability to undergo phosphorylation by cellular kinases.[68] The antiviral activity of some representative organoselenium compounds is given in Table 2.[39,40,68,72–78]

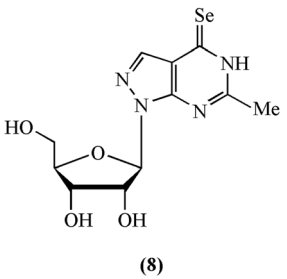
Racemic forms as well as α- and β-anomers of oxaselenolane nucleosides (**6**) have been evaluated for their anti-HIV type-I and anti-hepatitis B virus (HBV) activity in peripheral blood mononuclear (PMB) cells.[72,73] Racemic **6a** and **6b** exhibit potent anti-HIV activity (EC_{50} 0.88 and 0.51 μM, respectively) and anti-HBV activity (EC_{50} 1.2 μM for both the compounds). These compounds hardly showed any toxicity up to 100 μM in various cell lines (PBM, CEM and Vero).[72] The (−) form is more potent than the corresponding (+) counterparts.[73] For instance, EC_{50} values for the anti-HIV activity of (−)**6a** and (+)**6a** are 0.9 and 3.4 μM, respectively.[73] Anti-HIV and anti-HSV-2 activities of O, S, Se substituted compounds are given in Table 3 for comparison purpose.[73,74,79–83] Pyrazolo pyrimidine seleno-nucleosides (**7**, **8**) have been synthesized and evaluated for their antiviral activity against herpes simplex virus type-2 (HSV-2), vaccinia (VV) and measles virus. These compounds showed marked activity against these viruses with ED_{50} values of 5–9 × 10^{-6} M.[74]



(6)
(NB = cytosine (**6a**), 5-fluorocytosine (**6b**); uracil (**6c**), thymine (**6d**), adenine (**6e**), guanine (**6f**) and hypoxanthine (**6g**))



(7) (X = H (**7a**), Br (**7b**)

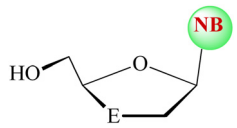


(8)

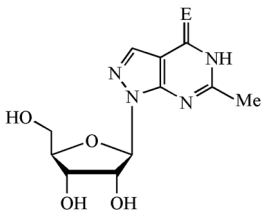
Table 2 Antiviral activity of some representative organoselenium compounds against different viruses

Compound	Virus	Model/culture	Result	Ref.
2a	HSV-1	*In vitro*/Vero cells	EC_{50} = 2.9 μM	68
2b	HSV-1	*In vitro*/Vero cells	EC_{50} = 2.3 μM	68
(±)**6a** (β)	HIV	*In vitro*/PBM and CEM cells	EC_{50} = 0.88 μM Non-toxic up to 100 μM in PBM, CEM and Vero cell lines	72
	HBV	*In vitro*/2.2.15 cells	EC_{50} = 1.2 μM	72
(±)**6b** (β)	HIV	*In vitro*/PBM and CEM cells	EC_{50} = 0.51 μM	72
	HBV	*In vitro*/2.2.15 cells	EC_{50} = 1.2 μM	72
(±)**6a** (α)	HIV	*In vitro*/PBM and CEM cells	EC_{50} = 2.4 μM	72
(−)**6a**	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.9 μM	73
(+)**6a**	HIV-1	*In vitro*/PBM cells	EC_{50} = 3.4 μM	73
7a	HSV-2	*In vitro*/Vero cell lines	ED_{50} = 5 μM	74
7b	HSV-2	*In vitro*/Vero cell lines	ED_{50} = 9 μM	74
8	HSV-2	*In vitro*/Vero cell lines	ED_{50} = 5 μM	74
9b	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.96 μM	75
10	HIV-1	*In vitro*/PBM cells	EC_{50} = 2.8 μM	75
11c	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.017 μM	76
13 with R = NH_2, R′ = NHMe, X = CH_2OH	HCMV	*In vitro*/Vero cells	EC_{50} = 32.1 μM	77
15a	HIV-1	*In vitro*/MT-4 cells	EC_{50} = 2.45 ± 1.08 μM	78
15b	HIV-1	*In vitro*/MT-4 cells	EC_{50} = 5.42 ± 2.41 μM	78
Ebselen	SARS-CoV-2	*In vitro*/Vero cells	IC_{50} = 0.67 μM	40
25 with R= H, R′ = Et	HIV-1	*In vitro*/MT-4 cells	EC_{50} = 0.91 ± 0.14 μM	39
25 with R= H, R′ = Et	HIV-2	*In vitro*/MT-4 cells	EC_{50} = 0.74 ± 0.12 μM	39

Table 3 Comparison of antiviral activity of some oxygen and sulfur analogues of organoselenium compounds

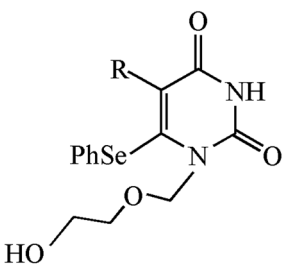


E	NB	Isomer	Virus	Model/culture	Result	Ref.
O	Adenine	(−)β	HIV	*In vitro*/PBM cells	EC_{50} = 0.5 μM	79
O	Adenine	(+)α	HIV	*In vitro*/PBM cells	EC_{50} = 6.2 μM	79
O	2-Fluoro adenine	(−)β	HIV	*In vitro*/PBM cells	EC_{50} = 0.3 μM	79
O	Cytosine	(−)β	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.016 μM	80
O	Cytosine	(+)α	HIV-1	*In vitro*/PBM cells	EC_{50} = 2.4 μM	80
S	Cytosine		HIV-1	*In vitro*/MT-4 and PBM cells	ID_{50} = 0.73 μM	81
S	Cytosine		HIV-1 RF	*In vitro*/MT-4 and PBL cells	IC_{50} = 0.67 ± 0.21 μM	82
S	5-Fluoro cytosine	(−)	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.008 μM	83
S	5-Fluoro cytosine	(+)	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.84 μM	83
Se	Cytosine	(−)β	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.9 μM	73
Se	Cytosine	(+)β	HIV-1	*In vitro*/PBM cells	EC_{50} = 3.4 μM	73
Se	5-Fluoro cytosine	(−)β	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.2 μM	73
Se	5-Fluoro cytosine	(+)β	HIV-1	*In vitro*/PBM cells	EC_{50} = 41.9 μM	73

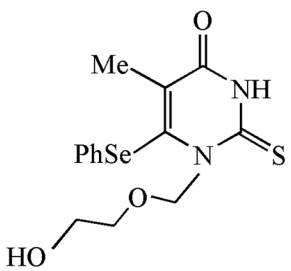


E	Virus	Model/culture	Result	Ref.
O	HSV-2	*In vitro*/Vero cells	ED_{50} = 50 μM	74
S	HSV-2	*In vitro*/Vero cells	ED_{50} = 320 μM	74
Se	HSV-2	*In vitro*/Vero cells	ED_{50} = 5 μM	74
AZT	HIV-1	*In vitro*/PBM cells	EC_{50} = 0.009 μM	80

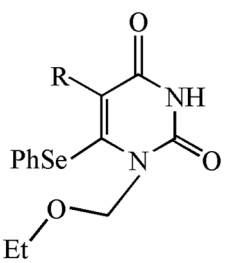
Several 5-phenylselenyl derivatives of pyrimidine nucleosides[84] and selenium substituted acyclouridine derivatives (**9**, **10**)[75] have been synthesized and evaluated for their antiviral activity against HIV-1, HIV-2 and herpes simplex virus type-1



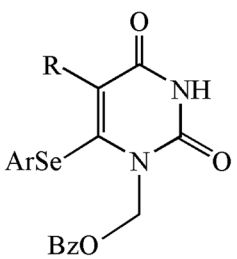
(9)
(R = H (**9a**), Me (**9b**), F (**9c**), Cl (**9d**), Br (**9e**))



(10)



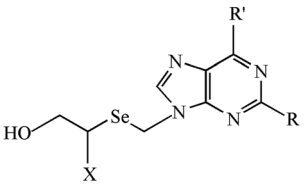
(11)
(R = H (**11a**), Me (**11b**), Et (**11c**), F (**11d**))



(12)
(R/Ar = H/Ph (**12a**); Me/Ph (**12b**); Et/Ph (**12c**); Et/2-py (**12d**))

(HSV). EC_{50} values of these compounds (**9, 10**) ranged from 0.96 to 13.0 and 2.0 to 25.6 μM for HIV-1 and HIV-2, respectively. None of these compounds showed any activity against HSV-1.[75] The 1-(ethoxymethyl)-6-(phenylselenenyl)uracils (**11**) showed activity against HIV-1 and HIV-2 in primary human lymphocytes, the most potent compound being **11c** with a median effective concentration of 17 nM.[76] No discernible cytotoxicity of **11c** in either primary human lymphocytes or Vero cells was observed.[76] Pharmacokinetics revealed that **11c** can act as an effective antiviral agent in low concentrations.[85] This compound was further studied for its activity against several other HIV-1 mutants.[86] Compounds **12** were evaluated as anti-HIV-1 (HTLV-III) and for their cytotoxicity in MT-2 cell lines. The compounds with R/Ar = Et/Ph (**12c**) and Et/2-py (**12d**) (EC_{50} = 0.02 ± 0.005 and 0.06 ± 0.02 μM, respectively) are highly active against HIV-1, being even more potent than clinically used AZT.[87]

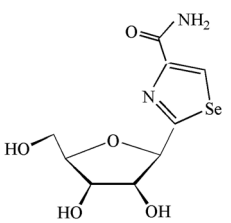
Selenium derivatives of acyclic nucleoside based antiviral drugs, acyclovir and ganciclovir, have been synthesized (**13**) and assayed for their antiviral activity against several herpes viruses such as HSV-1 (strain F, VR-733), HSV-2 (strain MS, VR-540), VZV (Ellen, VR-1367), and HCMV (Davis, VR-807).[77,88] Selenoacyclovir (R = NH_2, R′ = OH, X = H, **13a**) showed activity against HSV-1 and HSV-2 with EC_{50} of 1.47 and 6.34 μM. Selenoganciclovir (R = NH_2, R′ = OH, X = CH_2OH, **13b**) exhibited moderate activity against HCMV with an EC_{50} of 53.1 μM,[77,88] whereas the compound with R = NH_2, R' = NHMe and X = CH_2OH showed better performance (EC_{50} = 32.1 μM).[77]



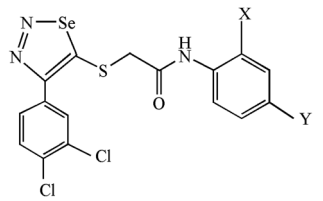
R	R'	X
H	OH	H or CH_2OH
NH_2	OH	H or CH_2OH
H	NHMe	H or CH_2OH
NH_2	NHMe	H or CH_2OH
NH_2	NH_2	H or CH_2OH

(**13**)

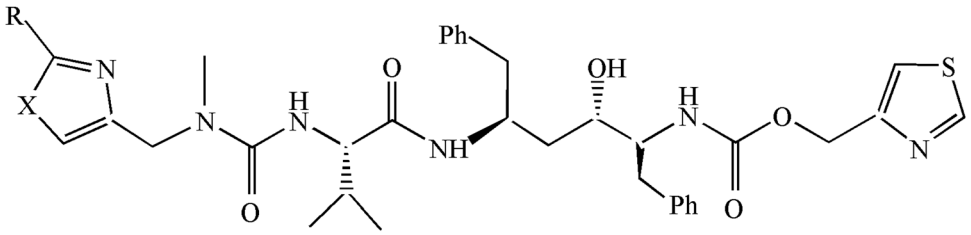
A selenazole carboxamide nucleoside (selenazofurin) (**14**) has been reported to have *in vitro* activity against a broad spectrum of RNA and DNA viruses, but is more effective against RNA viruses and is significantly more potent than antiviral drugs – thiazofurin (sulfur analogue of selenazofurin) and ribavirin.[89–92] Selenazofurin is in fact considered as the first successful selenium containing antiviral agent. **14** is active against both influenza A and B viruses in MDCK cells with



(**14**)

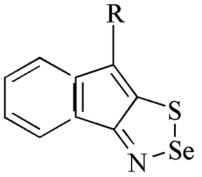


(**15**)
(X/Y = Br/COMe (**15a**); NO_2/Me (**15b**); Me/H (**15c**); H/Me (**15d**); H/Cl (**15e**))

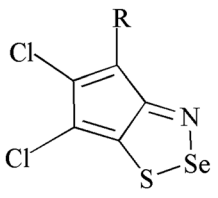


X = S, R = isopropyl (Ritonavir)
X = Se, R = isopropyl or Phenyl

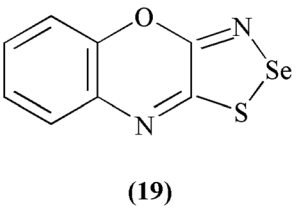
(**16**)



(**17**)
(R = Ph (**17a**); Cl (**17b**)



(**18**)
(R = Cl (**18a**); CN (**18b**); COOEt (**18c**)



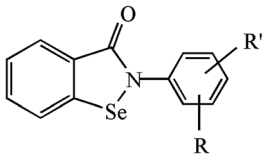
(**19**)

ED_{50} of 1.0 and 1–3.2 mg ml^{-1}, respectively (ED_{50} = 50% effective dose).[89] When the compound is administered intraperitoneally (ip) to influenza A and B infected mice, reduction in lung consolidation is noted and also prolonged mean day of death. It is well tolerated at a dose of 50 mg/kg administered twice a day for three days.[90] Another series of amide derivatives, 1,2,3-selenadazole thioacetanilides (**15**), have been designed as non-nucleoside reverse transcriptase inhibitors which display anti-HIV-1 activity with the EC_{50} value varying between 2.45 and 6.65 μM.[78] Anti-HIV-1 activities of the selenium analogue of ritonavir (an anti-HIV drug) (**16**) have been evaluated in acutely infected MT-4 cells. These compounds showed good antiviral activity with EC_{50} values of 550 and 170 nM for R = Pr^i and Ph, respectively.[93] A series of 1,2,3-thiaslenazoles (**17**–**19**) have been synthesized and evaluated against feline immunodeficiency virus (FIV) which has similarity to HIV.[94] These compounds are potent FIV nucleocapside protein (NCp7) inhibitors and act through zinc ejection. The EC_{50} values varied in the range 0.24 to 0.028 μM with **18c** being the most potent compound (EC_{50} = 0.028 μM).[94]

5.2. Ebselen and its derivatives

Among numerous organoselenium compounds, ebselen (2-phenylbenzo[*d*][1,2]-selenazol-3(2*H*)-one) has emerged as the most potent antimicrobial agent in cell assays.[45] Its antiviral activity was first reported by Baba in 1997 against HIV.[95] Ebselen selectively inhibited replication of HIV-1. It has been shown recently by time-resolved fluorescence resonance energy transfer assay that ebselen inhibits dimerization of the HIV capsid protein by binding covalently cys198 and cys218 fragments of the capsid C-terminal domain with inhibitory concentration for HIV-1 replication in the nano-molar range (IC_{50} = 46.1 nM).[96]

Ebselen has been shown to inhibit non-structural protein 3 (nsp3) helicase of hepatitis C virus (HCV).[97] It is a far more potent inhibitor (IC_{50} = 1.4 ± 0.2 μM) of HCV than other derivatives containing substituted phenyl rings. It is able to bind covalently all the 14 cysteine residues present in HCV helicase.[97] Ebselen treatment significantly alleviates the Zika virus induced testicular oxidative stress and also prevents sexual transmission of the virus.[98] Ebselen and its derivatives (**20** with R/R′ = H/H; H/4-Me; H/3-MeO; H/4-MeO; 2-Me/4-MeO; 2-Me/3-Cl; 2-Me/5-Cl; 3-Me/4-Cl; 2-MeO/5-Me; 2-MeO/5-Bu^t; 2-MeO/5-Cl; 2-Cl/4-Me; 2-Cl/5-Me)) exhibit inhibitory activity against herpes virus type 1 (HHV-1) with the minimal inhibitory concentration (MIC) value in the range of 2–8 μg ml^{-1},[99,100] herpes simplex virus (HSV-1) with a MIC of 2 μg ml^{-1} for ebselen[101,102] and encephalomyocarditis virus (MVC).[99]

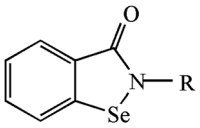


(20)

Having earned an impressive reputation as an antiviral agent, ebselen and its derivatives have emerged as potential candidates as inhibitors for the recent pandemic caused by SARS-CoV-2 infection. The first report by Jin *et al.* revealed the antiviral activity of ebselen against SARS-CoV-2.[40] They screened more than 10 000 compounds including FDA approved drugs, clinical trial/pre-clinical trial drug molecules and natural products as M^{Pro} (3 chymotrypsin like protease or $3CL^{PRO}$) inhibitors by fluorescence resonance energy transfer assay. Among all the screened compounds only six including ebselen showed promise as M^{Pro} inhibitors. Ebselen showed the highest inhibitory activity against purified M^{Pro} *in vitro* with an IC_{50} value of 0.67 μM.[40] Quantitative real time RT-PCR showed that ebselen is the strongest antiviral compound in SARS-CoV-2 infected Vero cells.[40] Ebselen covalently binds the thiol group of the cys145 residue in the catalytic dyad (His and Cys) of the protease. The selenylsulfide thus formed is sufficiently stable to inhibit the enzyme activity irreversibly leading to viral inhibition. However, the addition of reducing agents like 1,4-dithiothreitol (DTT) reverses the inhibition of M^{Pro} of SARS-CoV-2 indicating non-specific binding of ebselen.[103,104]

Ebselen and its phenyl substituted derivatives have been screened as inhibitors for SARS-CoV-2 M^{Pro}, PL^{Pro} and other non-structural proteins (nsps).[105–110] Biological assays employed to evaluate their anti-SARS-CoV-2 activity in Vero cells revealed that some of these compounds are superior to ebselen as antiviral agents.[110] Like M^{Pro}, the papain-like protease (PL^{Pro}) of SARS-CoV-2 is also a cysteine protease and is pivotal for viral replication. The IC_{50} value for most of the ebselen derivatives against SARS-CoV-2 M^{Pro} inhibition lies in the 1–7.4 μM range.[111] Compounds **20** with R/R′ = H/4-NO_2 and 2-F/5-Cl are found to be superior M^{Pro} inhibitors compared to ebselen with IC_{50} values of 15.24 ± 4.58 and 27.95 ± 5.10 nM.[105] The 6-hydroxypryridyl substituted compound is a better PL^{Pro} inhibitor than ebselen with an IC_{50} value of 0.578 ± 0.04 μM.[105] Ebselen and its derivatives (**21** with R = H, Me, Ph, 4-MeC_6H_4, 4-$Bu^tC_6H_4$, 2-HOC_6H_4, 2-$MeOeC_6H_4$, and CH_2CH_2Ph) through the selenium atom bind the cys112 residue in the catalytic triad (Asp-His-Cys) of the protease.[106,108] The inhibition constant of ebselen for suppressing the PL^{Pro} of SARS-CoV-2 is approximately 2 μM, and the most effective derivatives are 2-HOC_6H_4 and 2-$MeOC_6H_4$ with IC_{50} values of 236 ± 107 and 256 ± 35 nM, respectively.[106,108] These compounds are also effective in inhibiting the PL^{Pro} activity of SARS-CoV-1, but binding is weaker than that of PL^{Pro} of SARS-CoV-2.[107] The IC_{50} values for M^{Pro} and PL^{Pro} are in the nano-molar and micro-molar range, respectively.[110] A number of **21** derivatives with R = substituted phenyl and pyridyl groups at 0.3 μM concentration have been screened as M^{Pro} and PL^{Pro} inhibitors by HPLC and FRET assays. Compounds **21** with R = 2-methoxyphenyl and 3-fluoropydridyl have been found to be six-fold and three-fold more potent than ebselen in inhibiting SARS-CoV-2 viral replication.[112] Compounds **21** also exhibit high antiviral activity against HHV-1 and EMCV while the non-selenium analogues of **21** (Se replaced by either CH_2 or CO group) are either inactive or show substantially low activity.

This observation substantiates the role of selenium in antiviral activity.[113]



(21)

Zinc sites in non-structural proteins (nsps) (nsp3 (PL^{Pro}), nsp10, nsp12 (RNA-dependent RNA polymerase), nsp13 (helicase), nsp14) of corona viruses play a vital catalytic role. The zinc ejecting agents are capable of inhibiting enzyme activity. Ebselen given in combination with such agents like disulfiram and remdesivir could synergistically inhibit SARS-CoV-2 replication in Vero cells. The half maximal inhibitory activity (IC_{50}) of ebselen in inhibiting nsp13 and nsp14 of SARS-CoV-2 is 291.9 and 3.18 nM, respectively.[114] It also inhibits the *N*7-methyltransferase activity of SARS-CoV-2 nsp14.[113] Besides cys145 of M^{Pro}, other binding sites localized between II and III domains of proteins have been identified. Ebselen exerts a pronounced allosteric effect that regulates catalytic sites which results in inhibition of the enzymatic function of M^{Pro} of SARS-CoV-2.[115]

Molecular docking,[116–118] density functional theory (DFT)[118–121] and the solvent assisted proton exchange method[119] have been used to understand the mode of antiviral action of ebselen and its derivatives. The molecular docking analysis has revealed that a nucleophilic attack of the thiol group of cys from the protease active site takes place at the electrophilic centers of organoselenium compounds, *i.e.*, C═O···HS and Se···HS. Both these interactions may lead to the formation of adducts of protease and organoselenium compounds.[118] The DFT calculations indicate that the energetics of nucleophilic attack of thiol at the selenium centre rather than at the carbonyl oxygen atom is more favourable.[118] Calculations indicate that the inhibition process takes place in two steps, *viz.*, (i) activation of cys145 by His41 to generate the cys-His ion pair, and (ii) nucleophilic attack of deprotonated cys145 on selenium with the formation of a selenylsulfide bond and simultaneous ring opening which is mediated by a water molecule.[121] This has been demonstrated experimentally by mass spectrometry measurements by Xu *et al.*[122] It has been shown that biological thiols, like cysteine, rapidly (in a few seconds), effectively and selectively cleave the Se–N bond of ebselen to form a new Se–S bond.[122] This reactivity and ability of ebselen and related derivatives to bind covalently numerous thiol dependent enzymes in various pathogens is responsible for their antiviral activity.

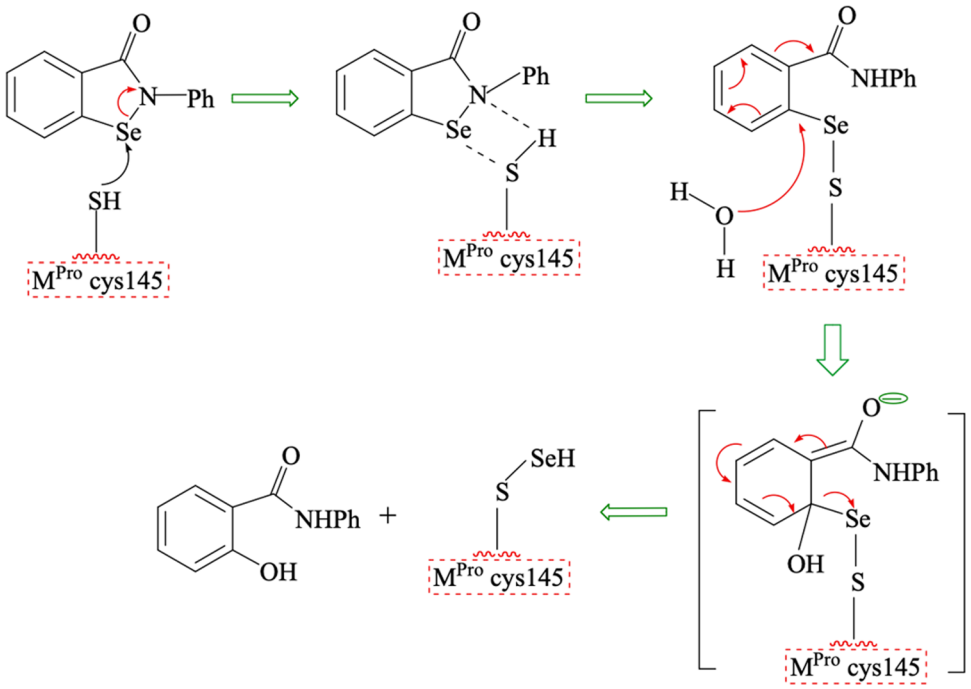
Enzyme kinetics and fluorescent labelling measurements suggest that these molecules bind M^{Pro} covalently and irreversibly.[104] High resolution co-crystallography analysis of the M^{Pro}–ebselen adduct has shown that the selenium atom lies at a distance of 2.2 Å from cys145 and His41 supporting the formation of a Se–S bond with cys145.[109] Interestingly, this interaction hardly affects the conformation of surrounding amino acids within the active site. The conserved water molecule which forms hydrogen bonds with His164 is 3.6–4.0 Å away from His41. This water molecule comes closer in the M^{Pro}–ebselen covalent adduct. His41 assists a water-mediated attack on the Se–N cleaved intermediate containing the covalent Se–S bond. This hydrolysis reaction, akin to peptide hydrolysis, generates selenylated cys145 and a phenolic product (salicylanilide in the case of ebselen) (Scheme 2).[100] The formation of these phenolic compounds has been confirmed by mass spectrometry.[100]

5.3. Non-N-heterocyclic organoselenium compounds

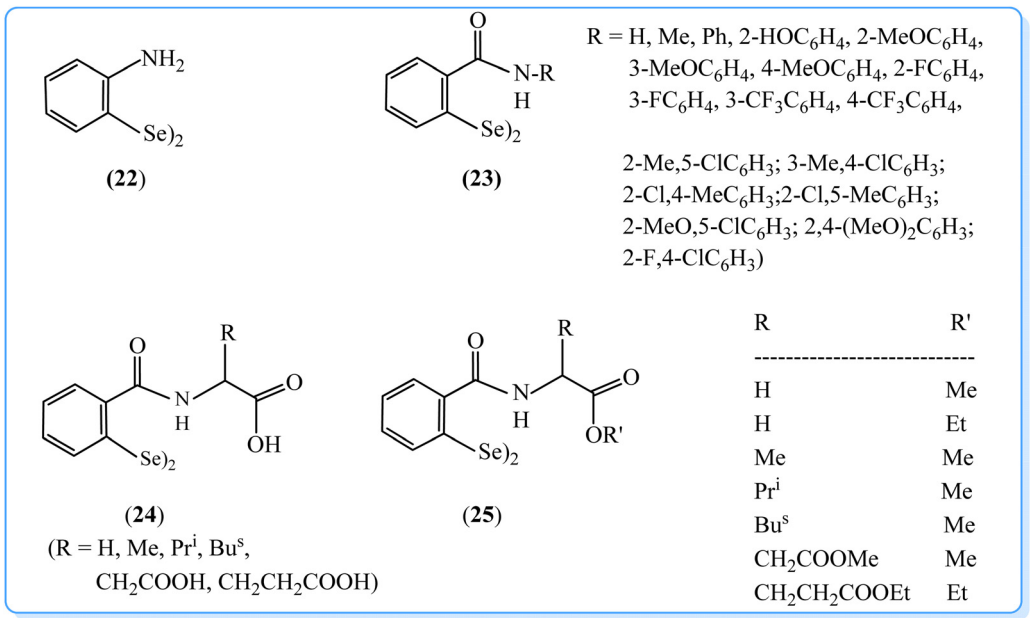
Selenium supplementation in the form of sodium selenite (Na_2SeO_3), selenomethionine and selenized yeast given alone or in combination with other micronutrients/antiviral drugs results in suppression of viral infection as well as reduction of viral mutation rates.[20,23,123,124] It has been hypothesized that selenite used in supplementation oxidizes sulfhydryl groups in the active site of viral protein disulfide isomerase to generate inactive disulfide, thereby preventing viral entry to healthy cells.[125] Sodium selenite (2.5 μM concentration) alone or in combination with other antiviral drugs has been shown to suppress hepatitis B virus (HBV) protein expression, transcription and genome replication in human hepatoma cell lines.[124]

Sartori *et al.* examined the antiviral and virucidal properties of diphenyldiselenide against herpes simplex virus-2 (HSV-2) in female BALB/c mice.[126,127] Diphenyldiselenide was able to significantly reduce replication of HSV-2 in the mouse model. It was effective in reducing renal and hepatic oxidative stress and toxicity in HSV-2 infected BALB/c mice.[127] At a dose of 5 mg kg^{-1} day^{-1}, it could reduce lesions and histological damage in the vaginal tissue of the mice.[126] Its antiviral action has been attributed to its immunomodulatory, antioxidant and anti-inflammatory properties. Bis(2-aminophenyl)diselenide (**22**, Scheme 3) exhibits moderate inhibitory activity with a MIC of 40 mg ml^{-1} (MIC = minimal inhibitory concentration) against herpes simplex virus (HSV-1) whereas ebselen is highly active with a MIC of 2 mg ml^{-1} under similar conditions.[101] Another series of diselenides, bis(2-carbamoylaryl)diselenides (**23**, Scheme 3), have been examined as antiviral agents against herpes virus-1 (HHV-1) and encephalomyocarditis virus (EMCV)[100] and they showed activities similar to **21**.[113] Some of these are active against HHV-1 (*e.g.*, MIC values for 3-Me,4-ClC_6H_3 and 5-Me,2-ClC_6H_3 are 10 and 20 mg ml^{-1}, respectively), but exhibit either poor or no activity against EMCV.[100] Compound **23** with R = 4-$NH_2SO_2C_6H_4$ exhibited low micromolar anti-HIV-1 and anti-HIV-2 activity with pronounced cytotoxicity.[39]

Sancinato *et al.*[39] have synthesized a series of diseleno bis(benzamides) (**24** and **25**, Scheme 3). These compounds have been identified as HIV-1 and HIV-2 retroviral nucleocapsid protein 7 (NCP 7) inhibitors. Some of these compounds exhibit activity at micro-molar concentrations while others are devoid of antiviral activity and also did not show any cytotoxicity. The amino acid derivatives (**24**, Scheme 3) possess very low cytotoxicity and good antiviral activity against HIV-1 and HIV-2 with EC_{50} values ranging between 3.15 and 13.19 μM.[39] The ester



Scheme 2 Mechanism of selenylation of M^{Pro} cys145 by ebselen.



Scheme 3

derivatives (**25**, Scheme 3) are more potent than the acid counterparts (**24**, Scheme 3). Among them, compound **25** with R = H and R′ = Et is the most effective with EC_{50} values of 0.91 and 0.74 μM for HIV-1 and HIV-2, respectively.[39]

More recently, mono substituted bis(2-carbamoylaryl)diselenides (**23**, Scheme 3) have been examined for their antiviral activities against SARS-CoV-2. Molecular docking studies on a series of organoselenium compounds have revealed a strong interaction, with binding affinity ranging from −3.1 to −7.0 kcal mol^{-1}.[116] Docking results have shown that out of 19 compounds, aliphatic compounds have lower binding as compared to aromatic derivatives. Compound **23** with R = H and Ph showed better binding (−6.6 and −7.0 kcal mol^{-1}, respectively) than that of ebselen (−5.4 kcal mol^{-1}).[116] The protease (both M^{Pro} and PL^{Pro} of SARS-CoV-2) activity of these compounds has also been evaluated. The compounds with

R = 2-HOC_6H_4 and 2-$MeOC_6H_4$ bind favourably to PL^{Pro} of SARS-CoV-2 with IC_{50} values of 339 ± 109 and 263 ± 121 nM, respectively.[106,108] Some of these compounds have also been identified as potent inhibitors of both the proteases, PL^{pro} and M^{pro}, as well as the nsp14 protein of SARS-CoV-2.[110]

The antiviral activity of naturally occurring selenoamino acids has also been investigated.[128] The antiviral activity of selenomethionine (Se-Met) (at concentrations of 2, 4, 8, and 16 μM) against porcine deltacoronavirus (PDCoV) using pig kidney epithelial (LLC-PK) cells has been examined. Se-Met could inhibit the replication of PDCoV in a dose-dependent manner and also improved the intracellular production of IFNα/β and antioxidant capacity of cells.[129] Selenomethionine combined with curcumin, vitamin C, vitamin K2-7 and zinc in SSV-003 formulation showed potent antiviral activities against influenza A-H1N1-VR219 with no cytopathic effect and with IC_{50} value significantly less than that of ribavirin. This formulation is also active against human beta coronavirus with an IC_{50} of 2.26 μg ml^{-1} which is comparable to the IC_{50} value of ribavirin (2.25 μg ml^{-1}).[130] *In vitro* studies in Vero cells have shown that selenomethionine at a concentration of 50 μM can inhibit single-stranded RNA viruses, like Coxsackie virus and herpes simplex 1 virus (HSV-1).[130]

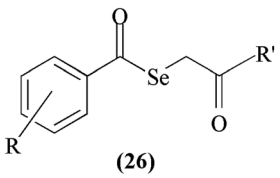
A number of selenoesters (**26–28**) exhibit antiviral activities against different viruses.[131,132] The antiviral activity of **26** was evaluated in HSV-2 infected Vero cells using real-time polymerase chain reaction.[131] Among these compounds, **26f** emerged as the most potent anti-HSV-2 inhibitor as it could inhibit replication of HSV-2 at 1.25 μM concentration whereas under similar conditions acyclovir could not inhibit the replication of the virus completely in the concentration range of 1.25 to 2500 μM.[131] Unsaturated selenoesters (**27**, **28**), obtained by $TiCl_4$ promoted aldol condensation of Se-phenylselenoacetate (PhSeC(O)Me) with an appropriate aldehyde, have been evaluated for their antiviral activities against HIV-1, SARS-CoV-2 and hepatitis B virus (HBV) using MTT, WST and PCR assays, respectively.[132] All these compounds showed antiviral activities against these viruses with EC_{50} values ranging from 1.6 to 32.2 μM. Compounds of series **27** have better activity than those of **28**. The HIV-1 and SARS-CoV-2 antiviral activities of these compounds have been compared with those of standard antiviral agents, *viz.* abacavir and lamivudine in the case of HIV-1 and remdesivir in the case of SARS-CoV-2. **27a** was found to be the most or the second most potent molecule. This compound also showed comparable activity against Alpha and Delta variants of SARS-CoV-2, but exhibited lower activity against other variants of concern (such as Beta, Gamma and Omicron).[132]

6. Antiviral activity of selenium nanoparticles (SeNPs)

During the past decade, selenium nanoparticles (SeNPs) have emerged as promising candidates for a wide range of biomedical applications.[133–136] Their interesting and favourable *in vitro* and *in vivo* biological properties like low toxicity, biocompatibility, high bioavailability and degradability compared with organic and inorganic selenium compounds have made them suitable for several therapeutic applications such as anticancer, antibacterial, antifungal, anti-parasitic, antioxidant, anti-inflammatory and immune-stimulatory.[133–142] Of late their antiviral activities have also been explored.[135,136,143,144]

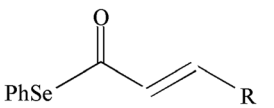
SeNPs in the bare form are highly unstable and tend to aggregate and precipitate and finally turn into grey/black selenium – an inactive form. Therefore, to improve stability and therapeutic effectiveness, SeNPs are stabilized by encapsulation into a suitable matrix/substance like peptides,[141] chitosan,[145] *etc.* The surface functionalization of SeNPs can influence their physical (size, surface charge) and chemical (toxicity) properties and pharmacokinetics.[138,146]

SeNPs are synthesized primarily by chemical, physical and biological techniques.[133,134,147] Vitamin C, sodium thiosulfate, sodium sulfite, and hydrazine are some common reagents employed for the reduction of selenium salts like sodium selenite (Na_2SeO_3) to Se^0 nanoparticles. Stabilizers or capping agents are needed to stabilize chemically synthesized SeNPs. The biological approach for the synthesis of SeNPs utilizes natural components from plants,[148] bacteria, fungi, algae, food or agricultural waste to reduce selenate (Na_2SeO_4) or selenite (Na_2SeO_3) to elemental selenium.[133,134,149] These agents also serve as stabilizing or capping substances.[133,150] Biosynthesized SeNPs are deep orange to red in colour. This route being environmentally friendly is often referred to as the 'green



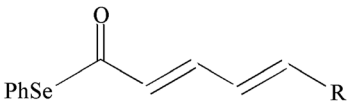
(26)

R	R'	
H	NH_2	**(26a)**
H	OPh	**(26b)**
4-Cl	OMe	**(26c)**
4-Cl	Me	**(26d)**
4-Cl	Bu^t	**(26e)**
3,5-$(MeO)_2$	Bu^t	**(26f)**



(27)

R = Ph (**27a**);
4-HOC_6H_4 (**27b**);
3-MeO,4-HOC_6H_3 (**27c**)



(28)

R = Ph (**28a**);
4-MeC_6H_4 (**28b**)
4-$MeOC_6H_4$ (**28c**)
4-BrC_6H_4 (**28d**)
4-$NO_2C_6H_4$ (**28e**)
3-MeO,4-HOC_6H_3 (**28f**)

Table 4 Antiviral activity of functionalized and non-functionalized SeNPs against different viruses

SeNPs/size	Virus	Model/culture	Result	Ref.
Non-functionalized SeNPs				
SeNPs/av. 60 nm	Hepatitis B	Oral feeding to female BALB/c mice at a dose of 200 μg	Increased Th 1 (type 1T helper) immune response	151
SeNPs/—	Enterovirus 71 (EV 71)	*In vitro*/human astrocyte U251 cells	Cell viability 38.6%	152
SeNPs/av. 10–25 nm	H5N1	Chickens fed with the diet incorporating SeNPs	Lower viral shedding and milder inflammation in the lung, spleen and liver	153
SeNPs/av. 200 nm	H1N1	*In vitro*/MDCK cells	Cell viability 60%	154
SeNPs/av. 200 nm	H1N1	*In vitro*/MDCK cells	Cell viability 67%	155
SeNPs/av. 200 nm	H1N1	*In vitro*/MDCK cells	• 82.5% survival of MDCK cells using 0.25 μM SeNPs • Prevented chromatin condensation and DNA fragmentation	156
SeNPs/142 nm	H1N1	*In vitro*/MDCK cells	Cell viability 41.4%	157
SeNPs/av. 100 nm	H1N1	*In vitro*/MDCK cells	Cell viability 60%	158
Supported SeNPs				
Se@CS/90 nm	Porcine reproductive and respiratory syndrome virus (PRRSV)	*In vitro*/African green monkey kidney epithelial cell lines	IC_{50} = 99.35 μM for Marc-145 cells	145
Se@CS	H3N2	*In vitro*/MDCK cells	Cell viability 94.8%	159
Se-actinobacterium/ 100–250 nm	Type 1 dengue virus	*In vitro*/Vero cloned cell lines	Inhibited viral growth at 700 ppm	160
SeNPs (40–80 nm) printed on fabric	SARS-CoV 2		Inhibition percentage 87.5%	161
SeNP conjugate of antiviral drugs				
Se@ZNV/av. 82 nm	H1N1	*In vitro*/MDCK cells	Inhibited caspase-3 activity, cell viability 72.7%	157
Se@OTV/av. 100 nm	Enterovirus 71 (EV 71)	*In vitro*/human astrocyte U251 cells	• Prevented host cell apoptosis induced by EV 71 infection • Cell viability 83.2%	152
Se@OTV/av. 100 nm (concentration of Se = 125 μM and OTV = 0.3 μM)	H1N1	*In vitro*/MDCK cells	• Reduction in intracellular ROS generation to 120% in Se@OTV treated cells from 380% in H1N1 infected cells • Cell viability 93%	154
Se@AM/av. 70 nm	H1N1	*In vitro*/MDCK cells	• Inhibited caspase-3 activity and significant reduction in ROS production • Cell viability 93%	162
Se@RBV/av. 65 nm	H1N1	• *In vitro*/MDCK cells • *In vivo*/female BALB/c mice	• Prevented DNA damage and protected the lung from H1N1 infection • Cell viability 80.6%	158
Se@ARB/av. 70 nm	H1N1	*In vitro* and *in vivo*/ MDCK cells	• *In vivo*: inhibited cell apoptosis by ROS modulated p53 and AKT signaling pathways, protected the lung from viral infection • *In vitro*: cell viability 86%	163
Se@TP/av. 80 nm	H1N1	*In vitro*/MDCK cells	• Rescues MDCK cells from H1N1 infection induced apoptosis • Cell viability 88%	155

synthesis' approach. There is a series of review articles dealing with the detailed methods of SeNP synthesis[133,135,136,144,147] and therefore not elaborated here.

Antiviral activities of both functionalized and non-functionalized SeNPs have been investigated against several viruses (Table 4).[145,151–163] These include H1N1 influenza virus,[154–156,158,162] hepatitis virus (HAV),[150,164] herpes simplex-II virus (HSV-2),[154] Cox-B4 virus,[164] enterovirus 71 (EV71)[152,165] and adenovirus strain 2.[154] For these studies SeNPs have primarily been synthesized by the chemical reduction method. The particle size of SeNPs varied between 10 and 200 nm. In general, the effective concentration for antiviral activity decreases with decreasing size of SeNPs. Conversely, as the size of SeNPs decreases, the antiviral activity against viruses increases.[136]

Biosynthesized SeNPs (particle size 100–250 nm), prepared by reduction of Na_2SeO_3 by actinobacteria – isolated from magnesite mine soil – could effectively suppress the growth of type-1 dengue virus. Inhibition of viral growth was maximum at 700 ppm dose of SeNPs.[160] Recently, chitosan (a non-toxic alkaline polysaccharide) coated selenium nanoparticles (Se@CS) (average diameter 90 ± 13 nm) were reported to inhibit the replication of porcine reproductive and respiratory syndrome virus (PRRSV).[145] Se@CS treatment enhances antioxidant activity and effectively suppresses PRRSV-induced apoptosis in Marc-145 cells *via* the ROS/JNK signalling pathway.[145] The CCK-8 assay and RT-PCR measurements showed that Se@CS effectively prevented replication of H3N2 influenza virus in MDCK cells. Se@CS can also inhibit the overproduction of ROS and inflammatory cytokines in viral

infected cells. Hardly any toxicity of Se@CS has been noted at 2, 4, 8, 16 and 32 μM concentrations.[159]

Bare SeNPs[166] and biosynthesized SeNPs[7,22] exhibit antiviral activities against hepatitis-B (HBV),[166] hepatitis-A (HAV),[138,164] Coxsackie B (Cox-B4)[164] and herpes simplex-II (HSV-2)[150] viruses. Replicating human cell line HepG2 and normal hepatocyte cells were used for evaluation of the antiviral activity of SeNPs against HBV.[166] A significant reduction in inflammatory markers as well as DNA damage was reported in the SeNP treated HBV human cell line HepG2 compared to diseased ones.[166] SeNPs synthesized using an aqueous extract of *Portulaca oleracea* L. leaves could resist proliferation of HAV and Cox-B4 viruses in Vero cells with cell viability of 84.2 and 76.5%, respectively, at the non-lethal dose (62.5 μg mL^{-1}) of SeNPs.[164]

SeNPs prepared using a brown alga, *Polycladia myrica*, have been evaluated for their antiviral activity at a MNCC of 50 μg ml^{-1} (MNCC = maximum non-cytotoxic concentration) in the Vero cell line. This formulation exhibited viral inhibition of 40.24 ± 2.61, 17.39 ± 1.45 and 8.64 ± 0.82% against HAV-10, HSV-2 and adenovirus strain-2, respectively.[150] The best antiviral activity of SeNPs was found against HAV-10 with an EC_{50} value of 63.81 ± 2.93 μM.

Li and co-workers have shown that bare SeNPs[167] and surface modified SeNPs[165] are effective against enterovirus 71 (EV71) which causes hand, foot and mouth disease. It has been shown that SeNPs can decrease apoptosis of Vero cells induced by EV71. Initial generation of ROS by EV71 is inhibited followed by inhibition of the JNK (Jun amino-terminal kinase) signalling pathway.[167] SeNPs functionalized with small interfering RNA (siRNA) and coated with polyethylenimine (PEI), isolated as Se@PEI@siRNA with an average diameter of 80 nm, show remarkable efficiency in preventing the nerve cell line SK-N-SH from apoptosis induced by EV71 and also prevent replication of EV71.[165]

Zhu and co-workers have demonstrated the effectiveness of conjugated SeNPs with standard antiviral drugs against H1N1 influenza virus.[152,154–158,162,163] Surface decorated SeNPs with antiviral drugs, *viz.* zanamivir (ZNV),[157] oseltamivir (OTV),[154] amantadine (AM),[162] ribavirin (RBV),[158] arbidol (ARB),[163] and β-thujaplicin (TP),[155] isolated as Se@AVD (AVD = ZNV, OTV, AM, RBV, ARB, TP) (Scheme 4), exhibit superior activity against H1N1 influenza virus infection. These decorated SeNPs even show high activity in the drug resistant cases. The antiviral effect of Se@AVD is markedly alleviated in comparison to either bare SeNPs or free antiviral drugs. Se@AVD essentially interferes with the interaction between the host cells and H1N1 influenza virus by suppressing the activity of virus surface glycoproteins – hemagglutinin (HA) and neuraminidase (NA) – and consequently prevents H1N1 from infecting MDCK (Madin-Darby canine kidney) cells. Further Se@AVD also showed p53 signaling and ROS mediated AKT pathways resulting in inhibition of apoptosis induced by H1N1 influenza virus.[155]

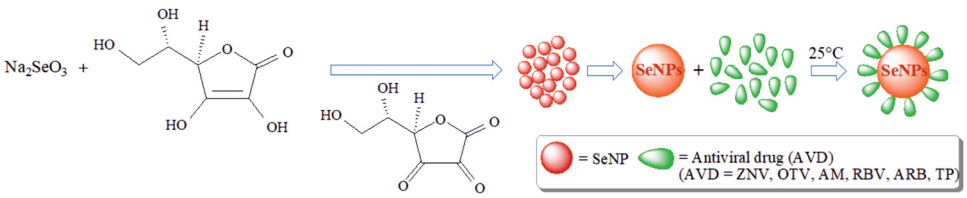
Several mechanisms have been proposed for the antiviral activity of SeNPs.[154,159,163,165] These include disruption of the functioning of viral capsid proteins and inhibition of virus-induced cell apoptosis by ROS mediated p53, Akt and MAPK signaling pathways. It should also be noted that conjugated SeNPs with antiviral drugs serve as substrates for targeted delivery of antiviral drugs. It is likely that the SeNPs get dissociated from their conjugate. The displaced SeNPs under physiological conditions enter the selenium cycle through different channels (like selenium oxide formed on oxidation is solubilized in water) and add to the selenium pool of the cell. The observed enhanced antiviral activity of conjugated SeNPs may be the outcome of complementary roles played by selenium and the antiviral drug.

Yehia *et al.* demonstrated that SeNPs (size 10–25 nm) incorporated in chicken diet enhance the efficacy of homologous vaccine against avian influenza H5N1 virus in chickens.[153]

SeNPs combined with polyester fabric were used to create fabric with multifunctional properties by flat-screen printing.[161] The size of SeNPs varied between 40 and 80 nm. The SeNP printed fabric exhibited low toxicity against the HFB4 cell line and showed high disinfection activity against SARS-CoV-2 with an inhibition percentage of 87.5%.[161]

7. Conclusion and future developments

Selenium, classified by the WHO as a micronutrient, is the only trace element specified in the genetic code and has been confirmed to be an important component in regulating immunity in living beings to fight many diseases including viral infections. Extensive research in the recent past has confirmed that selenium deficiency causes impairment of both innate and adaptive immune functions, while its supplementation improves the immune functions. This has been attributed mainly to maintaining cellular redox homeostasis through activation of selenoproteins like GPX, TXNRD, SELENOP, *etc.* Several positive outcomes have been observed in the clinic with selenium supplementation such as reduction in infectivity and



Scheme 4 Synthetic route for Se@AVD.

virulence of RNA viruses. In most of these studies, selenium has been supplemented in the form of sodium selenite, which is water soluble and readily incorporated into selenoproteins. Selenase as sodium selenite-based formulation is already in the market for several diseases. However, selenite is associated with very high and long-term toxicity at the employed doses. Another form of selenium supplement, selenium yeast, although looks very promising, is highly expensive.

Over the past two decades, synthetic chemists have designed and developed a variety of organoselenium compounds and have investigated them for many diseases including viral infections. It has been postulated that selenium in organic form can be less toxic, redox tunable and target directed. In view of this, in this article we have focused on different types of organoselenium compounds that are being explored as potential antiviral agents, along with results reported for dietary selenium compounds. The synthetic compounds include N-heterocyclic compounds, other cyclic and aromatic selenium compounds, diaryl diselenides, selenoesters and selenium nanoparticles. Undoubtedly, among the synthetic selenium compounds, ebselen is the front runner and has been identified and recommended as a promising agent even against SARS-CoV-2. Other synthetic compounds are mostly in the initial stages of evaluation for anti-viral activity, which need to be considered for detailed examination. The actual role and metabolism of such synthetic compounds are not fully understood. Interestingly, when such compounds were fed to mice, the animals showed increased expression of selenoproteins, like GPX. It is speculated that such compounds while exhibiting GPX like activity can also show good ROS scavenging ability. There are also reports that some synthetic diselenides can directly inhibit viral proteins and inactivate them. Therefore a lot needs to be understood for each class of organoselenium compounds regarding their antiviral role. Interestingly, selenium nanoparticle-conjugates of antiviral drugs showed encouraging results under *in vivo* conditions, needing further testing in other models. It may be true that no organoselenium compound has yet been approved in the clinic as an antiviral agent, but looking at the pace of research on this subject, it may not be too far to expect a new selenium based organic compound as a novel therapeutic agent. We hope that this essay would further accelerate research and development activities on new synthetic selenium compounds with reference to their utility as antiviral agents.

Conflicts of interest

There are no conflicts to declare.

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