

# Recent Advances and Future Perspectives of Triazole Analogs as Promising Antiviral Agents

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**Abstract:** Emergence of severe viral infections in recent years and limited availability of antiviral chemotherapeutic agents for prevention and treatment of these infections are among the most common causes of human illness and death. Therefore, there is an urgent need to develop antiviral drugs that have a potentially critical role in the prevention and treatment of various fatal and debilitating viral infections. Triazole derivatives occupy a pivotal position in modern medicinal chemistry and several have found applications as medicines. A large volume of research has been carried out on triazole and their derivatives for antiviral activity and pharmacological importance of this scaffold has been well established. This review is primarily addressed to description of the recent advances in the synthesis and evaluation of triazole derivatives as antiviral agents which may facilitate the development of more potent and effective antiviral agents.

**Keywords:** Antiviral activity, HAART, ribavirin, 1,2,3-triazole, 1,2,4-triazole.

## INTRODUCTION

Viruses are among the smallest infecting agents, varying in size from 0.02-0.40 micrometer. They are about 1/100<sup>th</sup> of the size of bacteria [1]. Viruses consist of a nucleic acid core that contains either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), which constitutes the genetic material and provides a basis for classification of viruses. A protein coat known as capsid surrounds the nucleic acid core and the entire structure is called as nucleocapsid. The nucleocapsid may or may not be covered by another protein coat called an envelope. The envelope is composed of glycoproteins and arrangement of coat proteins defines the overall shape of the viruses. Spheres, rods, filaments, bullets, rectangles, triangles, and elongated tubes are some of the shapes of viruses. The complete infectious virus particle is called a virion [2].

Since the initial discovery of tobacco mosaic virus by Martinus Beijerinck in 1898, more than 5,000 types of viruses have been described in detail, although most of the types of viruses remain undiscovered [3, 4]. Viruses are ubiquitous, as they are found in almost every ecosystem on earth, and are the most abundant types of biological entities on the planet. They can not reproduce on their own, so they are propagated by utilizing a host cell to produce copies of themselves, thus producing the next generation. Viruses infect all types of organisms including animals, plants and bacteria etc [5].

Viruses are known to cause many serious diseases in humans like acquired immune deficiency syndrome (AIDS),

hepatitis, influenza, small pox, chicken pox, polio, genital herpes, measles and common cold etc. Most of the viral infections eventually culminate in the death of the host cell. The causes of death include cell lysis, alterations to the cell's surface membrane and apoptosis. Often cell death is caused by cessation of its normal activities due to suppression by virus-specific proteins [6]. However, the interest in antiviral chemotherapy has been boosted considerably since the identification, now more than 17 years ago, of HIV (human immunodeficiency virus) as the causative agent of the AIDS. Various facts which demonstrate the severity of viral infections are:

- AIDS is now a pandemic. In 2007, it was estimated by world health organisation (WHO) that 33.2 million people lived with the disease worldwide, and that AIDS killed an estimated 2.1 million people, including 330,000 children. Over three-quarters of these deaths occurred in sub-Saharan Africa, retarding economic growth and destroying human capital [7].
- In April 2009, a novel flu strain evolved that combined genes from human, pig, and bird flu, initially dubbed swine flu and also known as influenza A (H1N1), emerged in Mexico, the United States, and several other nations. The WHO officially declared the outbreak to be a pandemic on June 11, 2009. As per WHO's report, there are three to five million cases of this severe illness and up to 500,000 deaths worldwide due to influenza [8].
- Hepatitis B has caused epidemics in parts of Asia and Africa, and it is endemic in China. About one third of the world's population, more than 2 billion people, have been infected with the hepatitis B virus. This includes 350 million chronic carriers of the virus [9].

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Viral populations being acellular, do not grow through cell division, instead, they use the machinery and metabolism of a host cell to produce multiple copies of themselves, and they assemble in the cell. Life cycle of virus differs greatly between species, however, there are six basic stages that occur only in virus infected cells. Antiviral drugs act by blocking the viral life cycle at various points and five important therapeutic strategies include:

- 1) Inhibition of DNA polymerase.
- 2) Inhibition of reverse transcriptase activity (RNA viruses and Hepatitis B).
- 3) Inhibition of viral proteases.
- 4) Inhibition of attachment to, or penetration into the host cell.
- 5) Inhibition of protein translation and assembly of the virus [10].

Most of the antiviral drugs now available are designed to deal with HIV, herpes viruses (best known for causing cold sores and genital herpes, but actually causing a wide range of diseases), the hepatitis B and C viruses, which can cause liver cancer, and influenza A and B viruses [11-14].

Antiretroviral drugs are medications for the treatment of infections by retroviruses, primarily HIV which causes AIDS [15, 16]. When several such drugs, typically three or four, are taken in combination, the approach is known as highly active antiretroviral therapy, or HAART. There are different classes of antiretroviral drugs that act at different stages of the HIV life cycle like nucleoside and nucleotide reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors etc [17]. Recent optimal HAART options consist of combinations consisting of a minimum of three drugs belonging to at least two types, or classes, of antiretroviral agents. Typical regimens consist of two NRTIs along with either a protease inhibitor or NNRTI [18].

On the other hand, organic compounds have an enormous diversity of structure. Many of these structures contain ring systems. If the ring system is made up of atoms of carbon and at least one other element, the compound can be classified as heterocyclic compound. The elements that occur most commonly, together with carbon, in ring systems are nitrogen, oxygen and sulphur. About half of the known organic compounds have structures that incorporate at least one heterocyclic component. Small ring heterocycles containing nitrogen, oxygen and sulphur have been under investigation for a long time because of their important medicinal properties [19, 20]. Five-membered rings containing more than one nitrogen as heteroatom are the heterocycles which represent the largest and the most diverse group of the heterocyclic compounds. Most of these ring systems are derived from furan, pyrrole or thiophene by replacement of one or more of the CH groups by  $sp^2$ -hybridized nitrogen. It is the variation in the number and positions of these nitrogen atoms which lead to the structural diversity of this group of heterocycles, resulting in the formation of some important members of this group like diazoles (pyrazole and imidazole), triazoles (1,2,3- and 1,2,4-triazole) and tetrazoles containing two,

three and four nitrogen atoms in the rings respectively. Heterocyclic compounds are common and integral structural feature of a variety of natural products and medicinal agents, having a wide range of applications such as pharmaceuticals, agrochemicals and veterinary products [21].

Triazole, also known as pyrotriazole is a class of organic heterocycles containing a five membered diunsaturated ring structure composed of three nitrogen atoms and two carbon atoms at non adjacent positions. Triazoles occur (Fig. (1): **1** and **2**) as a pair of isomeric chemical compounds 1,2,3-triazole (**1**) and 1,2,4-triazole (**2**) [22]. The most common and promising approaches which are used in the synthesis of various triazole derivatives includes Pellizzari reaction, Einhorn –Brunner reaction, 1,3-dipolar cyclo addition of wide variety of organic azides  $XN_3$  ( $X$ = alkyl, vinyl, aryl, acyl, arene, sulphonyl, etc.) to acetylenes, reaction of azides with enolate anions, enol ethers, enamines and alpha-acyl phosphorus ylides and azide addition reactions by the oxidative cyclization by copper(II) salts of the bis-phenyl hydrazones of 1,2-diketones [23, 24].

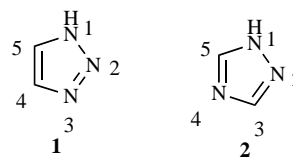


Fig. (1). Molecular scaffold of 1,2,3-triazole and 1,2,4-triazole.

## ANTIVIRAL ACTIVITY PROFILE

The pharmacologically important heterocycles derived from triazole scaffold paved the way toward active research in triazole chemistry. The expanding list of emerging and re-emerging viral infections that require treatment have invoked significant attention, moreover, resistance to antiviral drugs has also become a serious concern [25, 26]. As a result, a variety of new and improved antiviral compounds have been added to this field every year. A number of attempts were made to improve the activities of the compounds by varying the substitution on the triazole nucleus. This nucleus has been incorporated into a wide variety of therapeutically interesting medicinal compounds e.g. antiviral, tranquilizer, hypnotic, sedative, anxiolytic, diuretic, hypotensive, antidepressant, antineoplastic, non-steroidal aromatase inhibitors and antifungal agents etc [27, 28].

Ribavirin (RBV) (**3**) and taribavirin (**4**) are triazole based clinically used antiviral drugs (Fig. (2)) which are active agents against a number of DNA and RNA viruses. These are indicated for severe respiratory syncytial virus (RSV) infection, hepatitis C infection and other viral infections like West Nile virus and dengue fever [29,30]. Experimental data indicates that ribavirin may have useful activity against many viruses of interest, including avian influenza, hepatitis B, polio, measles, canine distemper and smallpox [31]. RBV (**3**) is the only known treatment for a variety of viral hemorrhagic fevers, including Lassa fever, Crimean-Congo hemorrhagic fever, and Hanta virus infection [32]. Taribavirin (also known as viramidine) is an antiviral drug in Phase III human trials, but not yet approved for pharmaceutical use. It is a prodrug of RBV, active against a number of DNA and RNA

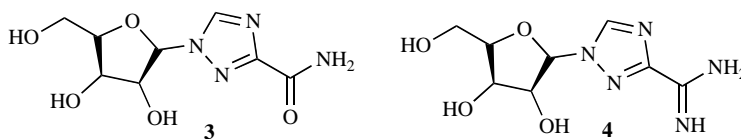


Fig. (2). Chemical structure of ribavirin and taribavirin.

viruses. Taribavirin has better liver-targeting than RBV, and has a shorter life in the body due to less penetration and storage in red blood cells. It is expected eventually to be the drug of choice for viral hepatitis syndromes in which RBV is active. These include hepatitis C and also perhaps hepatitis B and yellow fever. Taribavirin is as active against influenza as RBV in animal models, with slight less toxicity, so it may also eventually replace RBV as an anti-influenza agent [33].

In past decades, triazole and its analogs have proved their potential for their pharmacological interventions and in development of several clinically important medicinal agents [34]. Literature is enriched with plenty of information on diverse pharmacological activities but only limited number of reports have been published on antiviral activities of triazole. Thus keeping in view this fact, the literature survey of the most relevant and recent studies reporting effectiveness of triazole derivatives as potent and promising antiviral agents have been summarized in the following text:

AIDS is characterized by abnormal host defense mechanism that predisposes to infections with opportunistic microorganisms [35]. A novel series of sulfanyltriazoles (Fig. (3): 5-8) was identified by Wang *et al* as HIV-1 NNRTIs *via* high throughput screening (HTS) using a cell-based assay for inhibiting HIV-1 replication and promising activities against selected NNRTI resistant mutants such as Y181L, Y181C, K103N, and L100I were observed. Among these analogs (5-7) exhibited good overall antiviral profile. In particular, the 50% effective concentration ( $EC_{50}$ ) values of 82 nM and 6 nM for 6 and 7 respectively against the K103N mutant are of interest considering that K103N is a key mutation associated with the current NNRTI resistance. Compound 8 showed significant antiviral activity against HIV-1 NNRTI resistant mutants like L100I, Y181 and WT having  $EC_{50}$  values of 186, 440 and 20 nM respectively when compared with standard antiviral drugs like efavirenz (EFV) and nevirapine (NVP). Double mutants containing K103N are generally problematic for EFV. For example, the  $EC_{50}$  of standard drug EFV against K103/L100I is only 2.5  $\mu$ M. On the other hand, sulfanyltriazoles 5 and 7 exhibited  $EC_{50}$  values of 182 and 24 nM, respectively suggesting the potential of these sulfanyltriazoles to overcome the K103 related NNRTI resistant mutants. These novel and potent sulfanyltriazoles could serve as advanced leads promising great potential in overcoming these and other NNRTI resistant mutants [36].

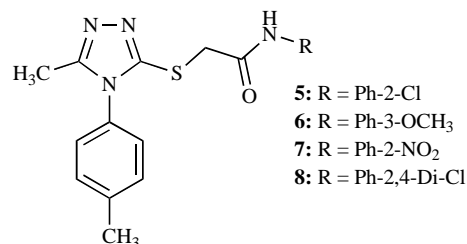
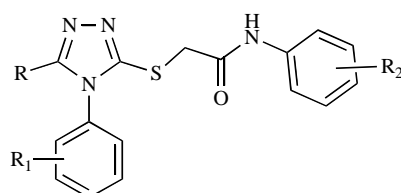


Fig. (3). Chemical structures of sulfanyltriazoles.

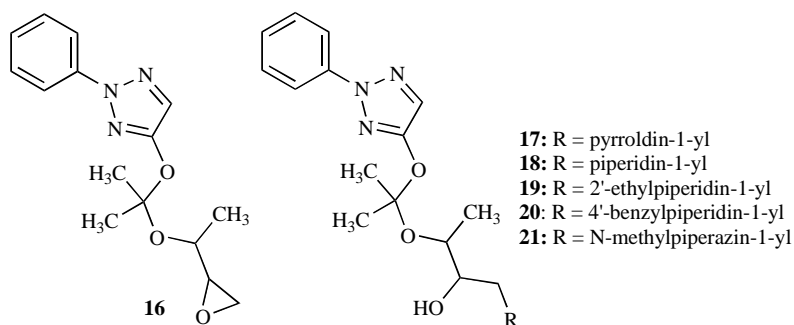
A new series of tri-substituted triazoles (Fig. (4): 9-15) was evaluated by De La Rosa *et al* for their antiviral activity against viruses carrying wild type (WT) and NNRTI-resistant K103N-Y181C HIV-1 reverse transcriptase (RT) using a cell-based assay system employing VSV-G pseudotyped pNL4-.Luc.R-E reporter virus and HeLa-JC53 cells. Several of these compounds exhibited potent antiviral activities against EFV and NVP-resistant viruses, containing K103N and/or Y181C mutations or Y188L mutation. First compound 9 was discovered by screening a library of 87,000 compounds using a cell-based assay which showed moderate activity against viruses carrying WT and K103N-Y181C HIV-1 RT with  $EC_{50}$  values of 0.1 and 1.3  $\mu$ M respectively. Compound 9 was also active in an enzymatic assay against the purified WT HIV-1 RT. Compounds 10 and 11 showed a very strong inhibition of the WT virus ( $EC_{50}$  = 0.019 and 0.008  $\mu$ M) along with an improved inhibition of the double mutant K103N-Y181C ( $EC_{50}$  = 0.23 and 0.1  $\mu$ M) when compared with standard drug EFV having  $EC_{50}$  values of 0.0004 and 0.011  $\mu$ M against viruses carrying WT and K103N-Y181C respectively. The presence of an ortho or para R<sup>1</sup> substituent improved activities against both viruses, with the biggest effect being observed when R<sup>1</sup> was in para position. Compounds 12 and 13 showed the strongest HIV-1 inhibition, and shared the presence of an ortho halogen substitution on the aniline. Para substituents like methyl and methyl carboxylate showed similar sort of good activity, while trifluoromethoxy and isopropyl exhibited a reduced activity. The range of substitutions allowed at the position 5 of triazole proved to be narrow as seen in compounds 14 and 15; only methyl, ethyl, and trifluoromethyl depicted considerable activity on the wild type virus ( $EC_{50}$  = 0.070 and 0.0001  $\mu$ M) [37].

Triazolylacyclonucleosides containing unnatural bases (Fig. (5): 16-21) were synthesized by Himanshu *et al*. These



- 9: R = CH<sub>3</sub>; R<sub>1</sub> = H; R<sub>2</sub> = 2-Br-4-CH<sub>3</sub>  
10: R = CH<sub>3</sub>; R<sub>1</sub> = 4-CF<sub>3</sub>; R<sub>2</sub> = 2-Cl-4-CH<sub>3</sub>  
11: R = CH<sub>3</sub>; R<sub>1</sub> = 2-Cl; R<sub>2</sub> = 2-Cl-4-CH<sub>3</sub>  
12: R = CH<sub>3</sub>; R<sub>1</sub> = 4-CH<sub>3</sub>; R<sub>2</sub> = 2-Br-4-CH<sub>3</sub>  
13: R = CH<sub>3</sub>; R<sub>1</sub> = 4-CH<sub>3</sub>; R<sub>2</sub> = 2-Cl  
14: R = H; R<sub>1</sub> = 4-CH<sub>3</sub>; R<sub>2</sub> = 2-Br-4-CH<sub>3</sub>  
15: R = C<sub>2</sub>H<sub>5</sub>; R<sub>1</sub> = 4-CH<sub>3</sub>; R<sub>2</sub> = 2-Br-4-CH<sub>3</sub>

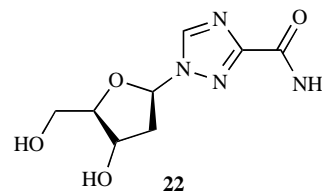
Fig. (4). Chemical structures of tri-substituted triazoles.



**Fig. (5).** Chemical structures of triazolylacyclonucleosides.

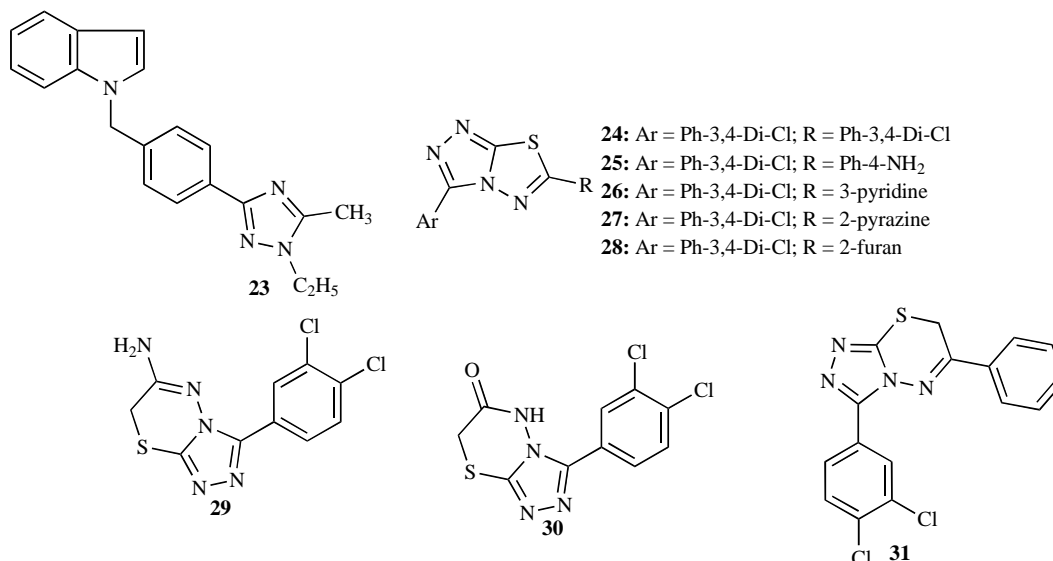
nucleosides **17-21** and their precursor **16** were evaluated for antiviral efficacy against two strains of HIV-1 (HIV-1<sub>IIB</sub> and HIV-1<sub>RF</sub>) in the standard CEM-SS cell-based anti-HIV assay. In this assay system, none of these six compounds reached an IC<sub>50</sub> value at the highest test concentration evaluated (100 mM), most of the compounds were cytotoxic at higher concentrations, except compound **16**. The overall assay performance was validated by the multiplicity of infection (MOI) sensitive positive control compound azidothymidine (AZT), exhibiting the expected level of antiviral activity (IC<sub>50</sub> = 0.003 μM against HIV-1<sub>IIB</sub> and 0.006 μM against HIV-1<sub>RF</sub>) [38].

Novel therapy approaches have been developed and considered to combat with the emergence of HIV strains resistant or cross-resistant to nearly all antiretroviral regimens. In order to study various viral mutagenic compounds, Vivet-Boudou *et al* tested the nucleoside mutagenic activity of 1-(2'-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (2'-deoxy-ribavirin) **22** (Fig. (6)) and its 5'-triphosphate derivative on HIV-1 NL4-3 in CEMx174 cell culture. No reduction on HIV-1 viability was observed after 2.5 months. On the other hand, *in vitro* experiments with purified HIV-1 RT demonstrated that the triphosphate analog can be incorporated opposite to several natural nucleosides to develop some novel antiviral agents [39].



**Fig. (6).** Chemical structure of 1-(2'-deoxy-β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide (2'-deoxy-ribavirin).

Several novel 1,5-dialkyl-1,2,4-triazole derivatives of acetic acid alkylidene hydrazides, the acid, 1,5-dialkyl-3-(5-mercapto-4-N-aryl-1*H*-[1,2,4]-triazol-3-ylmethylene)-1*H*-[1,2,4] triazoles, their 1,3,4-oxadiazole analogues, as well as the 1,2,4-triazolo-indoles (Fig. (7)) were investigated by Al-Soud *et al* for their anti-HIV activity *in vitro* using IIB strain for HIV-1 and the ROD strain for HIV-2, and activity was monitored by the inhibition of the virus-induced cytopathic effect in MT-4 cells. None of these compounds were found to inhibit HIV-1 or HIV-2 replication, *in vitro*, at IC<sub>50</sub> lower than the CC<sub>50</sub>, in comparison to standard antiviral agent delaviridine except compound **23** which showed activity against HIV-1 and HIV-2 at IC<sub>50</sub> values of >0.48 and 0.436 μg/mL respectively [40]. Further they tested a series of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (Fig. (7): **24-28**) and the thiadiazine analogues **29-31** for their *in-vitro* anti-HIV-1



**Fig. (7).** Chemical structures of 1,5-dialkyl-1,2,4-triazolo-indole derivative and [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles.

(strain IIB) and HIV-2 (strain ROD) activity in human T lymphocyte (MT-4) cells using the MT-4/MTT assay, as well as their activity against other viruses, such as human T-cells containing an integrated HTLV-1 genome, MDBK (Madin-Darby bovine (normal) kidney cells) with BVDV (bovine viral diarrhea virus), BHK (hamster normal kidney fibroblast), BHK (kidney fibroblast) cells from the YFV (yellow fever virus) and Reo (reovirus 1), mockinfected VERO-76 (monkey normal kidney), HSV-1 (herpes virus 1), VV (vaccinia virus), VSV (vesicular stomatitis virus), CVB-2 (coxsackievirus B2), Sb-1 (poliovirus 1) and RSV. All the compounds were inactive except for **27** and **31** which showed  $EC_{50} = 2.11$  and  $1.97 \mu\text{g/mL}$ , respectively in comparison to standard drugs EFV and capravirine. Compounds **27** and **31** were found equipotent against HIV-1 and HIV-2 replication *in vitro* and, therefore, most probably they are no NNRTI's. Regarding other viruses, the CVB-2 was the only virus inhibited by compounds **24-26** and **28** with  $EC_{50} = 12, 20, 31,$  and  $22 \mu\text{g/mL}$ , respectively ( $CC_{50} > 100 \mu\text{g/mL}$ ), meanwhile, all other compounds demonstrated lack of activity against the other screened viruses. The results recommend that only selected compounds may be considered as new leads in the development of novel antiviral agents [41].

Johns *et al* developed a series of HIV-1 integrase inhibitors (Fig. (8): **32-39**) containing a novel metal binding moiety consisting of 8-hydroxy-1,6-naphthyridine core and either an oxadiazole or triazole rings. Inhibitory activity of the compounds initially measured using a recombinant HIV-1 integrase strand transfer assay. At several points, these results were followed up with assessment of antiviral activity in a whole cell pseudo-type HIV assay (PHIV). The triazole containing compounds showed similar potency against the enzyme system for the benzyl and 4-fluorobenzyl substituted analogs **32** and **33**, however, moving beyond the small halogens in the 4-position appeared to be detrimental to both enzyme related as well as antiviral activities (compounds **34-36**). The methyl substituted triazole analogs **37** and **38** while not extremely potent do shed some light on the metal binding motif present within these scaffolds. The lack of any measurable activity in compound **38** was found consistent with the methylated nitrogen removing an obligatory chelation group from coordination of the active site magnesium. A

modest improvement in antiviral potency was observed for triazole **39** compared to protio derivative **33**. However, more noteworthy is the improved therapeutic index observed in the C5-substituted analog ( $>104$ -fold for **39** vs. 7-fold for **33**). Compound **39** showed the highest HIV-1 integrase inhibition activity with  $IC_{50}$  of  $0.011 \mu\text{M}$  as compared to that of the validated clinical candidate L-870,810 ( $IC_{50} = 0.013 \mu\text{M}$ ) [42].

The synthesis of a number of novel 1,2,4-triazoles derivatives of 5-(N-aryl-aminomethyl-1,3,4-oxadiazol-2-yl)hydrazines and their sugars (Fig. (9): **40-45**) was reported by Abdel-Aal *et al.* Some of the synthesized compounds were tested for their antiviral activity against HSV-1 and hepatitis-A virus (HAV) including three possibilities for antiviral activity: virucidal effect, virus adsorption, and effect on virus replication for both HAV and HSV-1. The antiviral activity against HSV revealed that compound **41** showed little activity at concentration  $20 \mu\text{g/mL}$  in comparison with standard drug acyclovir. The results revealed that sugar hydrazones showed superior antiviral activity when compared to other hydrazones and their acetylated derivatives [43].

The antiviral evaluation of new N-amino-1,2,3-triazole derivatives (Fig. (10): **46-55**), 1-(substitutedphenylamino)-5-methyl-1H-[1,2,3]-triazole-4-carboxylic acid ethyl esters, (Fig. (10): **46-50**) and 1-(4-substituted-phenylamino)-5-methyl-1H-[1,2,3]-triazole-4-carboxylic acid hydrazides, (Fig. (10): **51-55**) on replication of Cantagalo virus was described by Jordao *et al.* in BSC-40 cells. Among ten compounds tested, three compounds **48, 49** and **55** inhibited the formation of viral plaques by more than 30% at 50 mM after 48 hours post-infection and treatment. Compound **48** had higher activity than the less lipophilic, unsubstituted compound **46**. At a higher dose level 100 mM, **48** induced 84.62% inhibition of viral plaque formation. Unfortunately, this compound was found to be toxic to the cells, altering cell morphology and reducing cell viability to 52.6% and so was not further studied. The dichloro derivative **49** had a greater activity than did the monochloro derivative **47**. Except for compound **50**, the change from a 4-carboxytriazole (**46-49**) to the corresponding acylhydrazides (**51-54**) resulted in a decreasing inhibitory response on Cantagalo virus replication. For compound **55**, the electronic withdrawing effect of fluorine group and the hydrogen donor and ac-

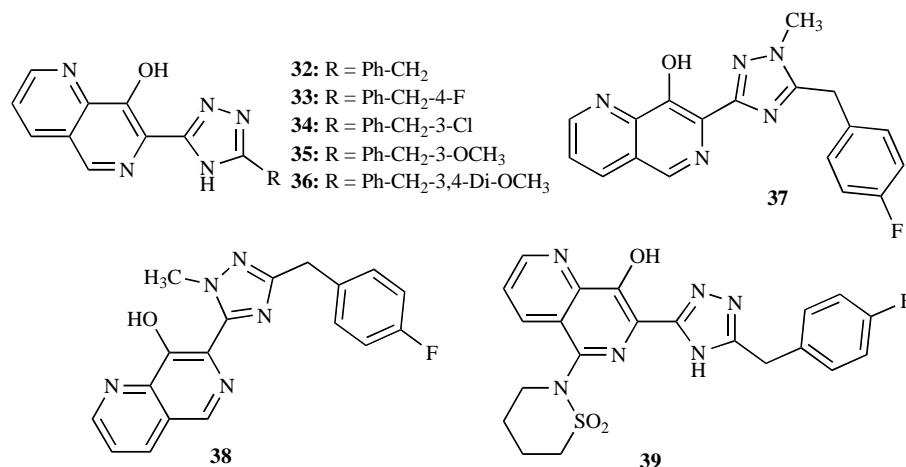
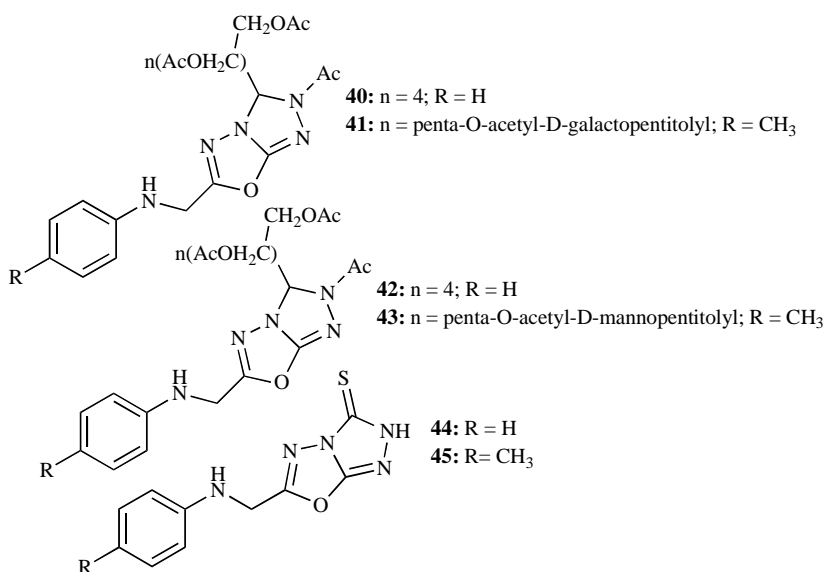


Fig. (8). Chemical structures of triazole substituted naphthyridines.

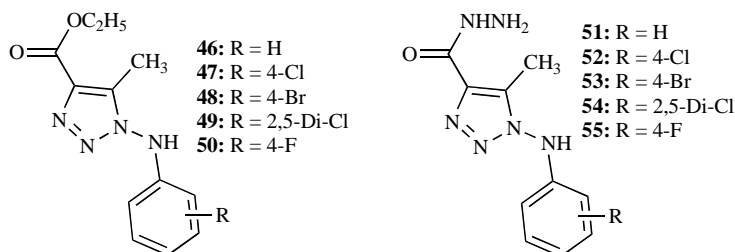


**Fig. (9).** Chemical structures of 1,2,4-triazoles derivatives of 5-(N-aryl-aminomethyl-1,3,4-oxadiazol-2-yl)hydrazines and their sugars.

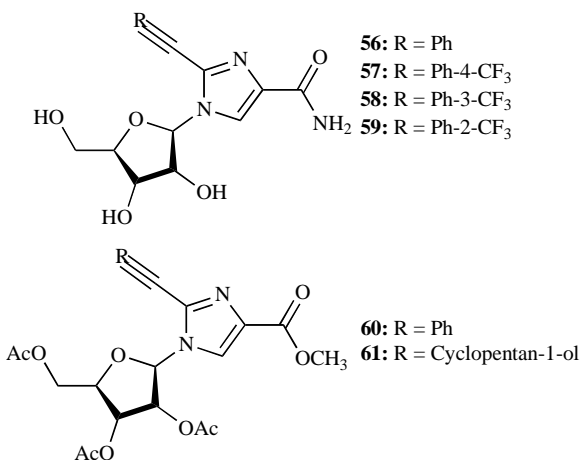
ceptor functionalities of the acylhydrazide moiety increased its biological activity. Effects of 4-carboxy-triazole **49** and acylhydrazide **55** on yield of infectious virus particles were then analyzed after 24 hours post-infection and treatment. Both compounds inhibited virus progeny production by more than 55% at 50 mM. Moreover, using the neutral red assay to evaluate cellular toxicity, it was observed that 60% of the cells were viable when treated with the derivative **49** at 50 mM for 24 hours. At a similar concentration, **55** generated 10% of non-viable cells after 24 hours of treatment and inhibited the yield of infectious virus particles by approximately 55%. Doses higher than 100 mM were toxic to BSC-40 cells, although at this concentration, the virus yield was inhibited by nearly 80% with only 30% of non-viable cells. The accumulation of viral proteins during infection was also inhibited in the presence of **55**. Western Blots, using antibodies to detect viral structural proteins, revealed normal levels of protein accumulation in infected cells treated with 0.5% DMSO (control). Virus proteins were not detected in mock-infected cells, as expected. Increasing concentrations of compound **55** gradually reduced the accumulation of virus proteins, reaching 71.2% inhibition at 100 mM, as determined by densitometric analysis of the blots. The cellular protein  $\alpha$ -tubulin was detected as a control for total protein loading and normalization for the densitometric analysis. Compound **55**, therefore, revealed a great potential as a lead structure that can be used in the development of new derivatives with anti-cantagalo virus activity [44].

Some novel ethynyltriazole ribonucleosides (Fig. (11): **56-61**) were evaluated by Wan *et al* for their anti-HCV activity on HCV subgenomic RNA replicon assays using Huh-5-2 Cells, Huh-9-13 Cells and Huh-6 Cells. Two compounds **57** and **61** inhibited hepatitis C virus (HCV) replication efficiently. Compound **57** showed the most potent anti-HCV activity with  $EC_{50}$  of 19.4  $\mu\text{M}$  and a greatly reduced toxicity compared to the current clinical drug in use, RBV ( $EC_{50} = 28.7 \mu\text{M}$ ). Most interestingly, the prominent selective antiviral activity was achieved for compound **57** by modulating the ribose sugar moiety into deprotected and protected forms while retaining a similar trifluoromethylphenylethynyltriazole as the nucleobase. Furthermore, the effect of the  $CF_3$  group at the *p*-position of phenyl ring in **57** was also studied using **58** and **59** containing this group at the meta and ortho position, respectively. Neither **58** nor **59** displayed favorable anti-HCV activity. Therefore, the  $CF_3$  group at *p*-position of phenyl ring seems to be critical for the observed anti-HCV activity. It was revealed by preliminary structure-activity relationship study that not only ribose moiety but also  $CF_3$  group at para position of phenyl ring and rigid triple bond functionality contributed significantly to observed antiviral activity of the compound **57** against HCV. Therefore, this compound constitutes promising lead in the search for new antiviral candidates [45].

A preliminary SAR study suggests that the appended phenyl ring as well as the rigid triple bond linker contributes significantly to the anti-HCV activity. In ongoing efforts to



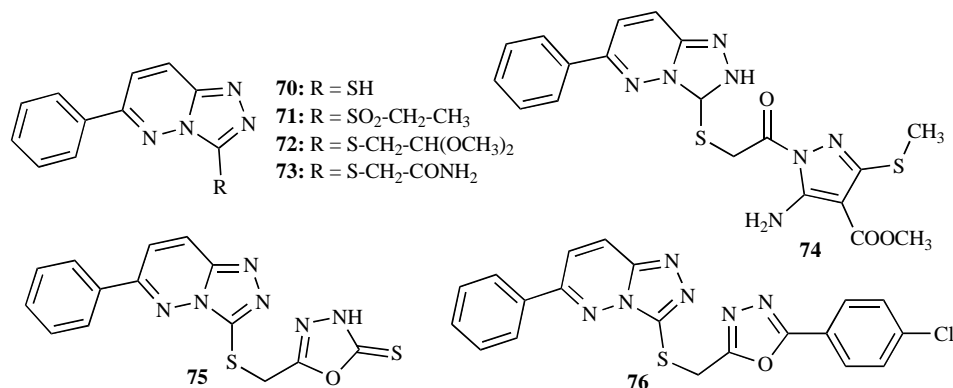
**Fig. (10).** Chemical structures of N-amino-1,2,3-triazoles.



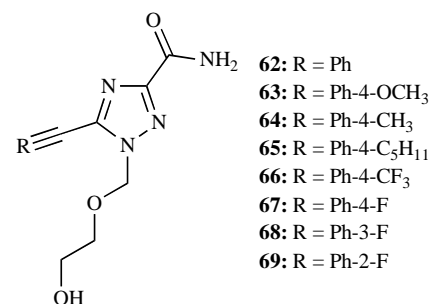
**Fig. (11).** Chemical structures of ethynyltriazole ribonucleosides.

search for potent new triazole nucleosides with antiviral activity, Zhu *et al* reported the synthesis of novel acyclic triazole nucleosides (Fig. (12): **62-69**) with various ethynyl moieties appended on the triazole nucleobase and evaluated them for their anti-HCV activity in a HCV subgenomic RNA replicon assay using Huh-5-2 cells. All the compounds with arylethynyl functionalities on the triazole ring **62-69**, except **63**, exhibited anti-HCV activity. Compound **63** exhibited no anti-HCV activity which may be due to the unfavorable electronic properties brought by the methoxyl group on the phenyl ring. This may suggest that the appended aromatic systems brought by the arylethynyl functionality on the triazole ring in **62-69** are responsible for the antiviral activity. One of the compounds, **67** was most potent and inhibited HCV subgenomic replication with a EC<sub>50</sub> of 22 µg/mL and did not inhibit proliferation of the host cell at a concentration of 50 µg/mL which is superior to standard drug RBV in selectivity, because it is much less toxic than RBV although it is slightly less potent [46].

Some novel 3-S-substituted-6-phenyl-[1,2,4]triazolo[4,3-b]pyridazine derivatives (Fig. (13): **70-73**) were prepared by Shamroukh *et al*. Furthermore, the preparation of 1-[2-(6-phenyl-[1,2,4]triazolo[4,3-b]pyridazin-3-ylsulfanyl)-acetyl]-1H-pyrazole derivative (Fig. (13): **74**) and 5-(6-phenyl-[1,2,4]triazolo[4,3-b]pyridazin-3-ylsulfanylmethyl)-[1,3,4]oxadiazole derivatives (Fig. (13): **75** and **76**) were also described



**Fig. (13).** Chemical structures of 3-S-substituted-6-phenyl-[1,2,4]triazolo[4,3-b]pyridazine derivatives.



**Fig. (12).** Chemical structures of 5-arylethynyltriazoleacyclonucleosides.

by them. The plaque-infectivity assay was carried out to test all the prepared compounds for antiviral activity against HAV and MBB-cell culture adapted strain. The test was performed to include the three possibilities for antiviral activity, virucidal effect, virus adsorption, and effect on virus replication for HAV. Only compounds **70-76** revealed promising anti-HAV activity. It was found obvious that at concentration of 20 µg/mL, compounds **70, 72, 74** and **75** revealed the highest antiviral activity in comparison with amantadine as control. While at a concentration of 50 µg/mL, compounds **70, 74** and **75** revealed the highest antiviral activity in comparison to standard drug amantadine as control. In general, compound **75** showed the highest effect on HAV as compared to other tested compounds and the control (amantadine), where its antiviral activity increased from 66% at concentration of 20 µg/mL to 81% at concentration of 50 µg/mL. Structure-activity correlation of the obtained results revealed that 6-phenyl-[1,2,4]triazolo[4,3-b]pyridazine-3(2H)-thione **70** showed more HAV activity than the S-alkylated compounds **71-73** and **76**. While addition of other rings e.g., a pyrazole ring in compound **74** or an oxadiazole ring in compound **75**, increased the percentage activity of HAV reduction [47].

Zhang *et al*. disclosed the finding that RBV (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) **77** (Fig. (14)) is a nontoxic antiviral agent currently licensed for the treatment of severe RSV associated with lower respiratory tract infections. RBV treatment administered in concentrations of 10 to 100 µg/mL potently inhibited RSV transcription, thereby reducing the level of RSV N transcripts to ~13% of levels in nontreated cells. Furthermore, they demonstrated that ribavirin potentiates virus-induced interferon (IFN)-stimulated

response element (ISRE) signaling to enhance the expression of antiviral IFN-stimulated response genes (ISGs), suggesting a mechanism for the efficacy of combined treatment with RBV and IFN in other chronic viral diseases [48].

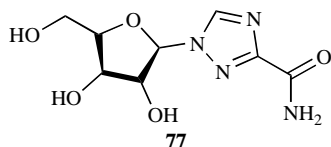


Fig. (14). Chemical structure of ribavirin.

A series of novel 1,2,3-triazole nucleosides linked to DNA nucleobases was designed and synthesized *via* the azide-alkyne 'click' chemistry reaction by Chittepu *et al.* and antiviral activity of these compounds against selected RNA viruses was reported. The 1,2,3-triazole nucleoside conjugates (Fig. (15): **78** and **79**) were incorporated into DNA using phosphoramidite building blocks and employed in solid-phase synthesis. Melting experiments demonstrated that such 1,2,3-triazole nucleosides have a negative impact on the duplex stability when they are placed opposite to the canonical bases as well as abasic sites. Nucleobases attached to the triazole ring cannot involve in the base pair formation with opposite bases because of the structural variations induced by triazole ring. The stacking of such nucleosides when positioned at end of oligonucleotides retains the stability of DNA duplexes. The duplex structures were studied by molecular modelling which supports the results of melting experiments [49].

There are no food and drug administration (FDA) approved drugs for the treatment of hemorrhagic fever with renal syndrome (HFRS), a serious human illnesses caused by hantaviruses. Clinical studies using ribavirin (RBV) to treat HFRS patients suggest that it provides an improved prognosis when given early in the course of disease. Given the unique antiviral activity of RBV and the lack of other lead scaffolds, Chung *et al.* prepared diverse series of 3-substituted 1,2,4-triazole- $\beta$ -ribosides and identified one with antiviral activity, 1- $\beta$ -D-ribofuranosyl-3-ethynyl-[1,2,4]triazole **80** (Fig. (16)) (ETAR). ETAR showed an  $EC_{50}$  value of 10 and 4.4  $\mu$ M for Hantaan virus (HTNV) and Andes virus, respectively. ETAR had weak activity against Crimean Congo hemorrhagic fever virus, but had no activity against Rift Valley fever virus. Intraperitoneally delivered ETAR

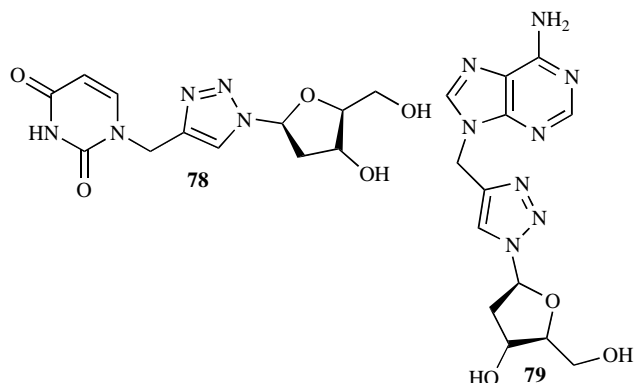


Fig. (15). Chemical structures of 1,2,3-triazole nucleosides.

offered protection to suckling mice challenged with HTNV with a ~25% survival at 12.5 and 25 mg/kg ETAR, and a MTD of  $17.1 \pm 0.7$  days. ETAR was phosphorylated in Vero E6 cells to its 5'-triphosphate and reduced cellular GTP levels. In contrast to RBV, ETAR did not increase mutation frequency of the HTNV genome, which suggests it has a different mechanism of action than RBV. ETAR is an exciting and promising lead compound that will be elaborated in further synthetic investigations as a framework for the rational design of new antivirals for treatment of HFRS [50].

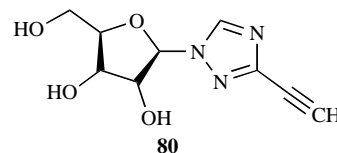


Fig. 16. 1- $\beta$ -D-ribofuranosyl-3-ethynyl-[1,2,4]triazole.

Li *et al.* designed and synthesized sixteen novel 4-triazole modified zanamivir analogues (Fig. (17): **81-86**) by using click chemistry reactions as specific neuraminidase (NA) inhibitors to develop more potent, long-lasting therapeutic drugs for anti-AIV infection. The synthesized compounds were tested against avian influenza virus (AIV, H5N1) for their preliminary anti-AIV activities. Compounds **81** and **83-85** exerted moderate inhibition against AIV (H5N1), whereas compound **82** showed promising inhibitory activity with  $EC_{50}$  of 6.4  $\mu$ M, which is very close to that of standard drug zanamivir ( $EC_{50} = 2.8 \mu$ M). The structure-activity relationship (SAR) studies suggested that comparing compound **81** with compounds **83-86**, the substitutions on phenyl ring substantially play an important role in potent anti-AIV activity of compounds. Generally, alkyl (**83** and **84**) and alkoxy (**85**) substituents are favorable to the activities of these compounds, however, introduction of halogen (**86**) substituent results in loss of activity. Among non-aromatic substituents of  $R^1$ , propyl alcohol of compound **82** is the most favorable to increase activity. Molecular modelling result provides information about the binding model between inhibitors and neuraminidase, and is helpful for future design of neuraminidase inhibitors as anti-AIV agents [51].

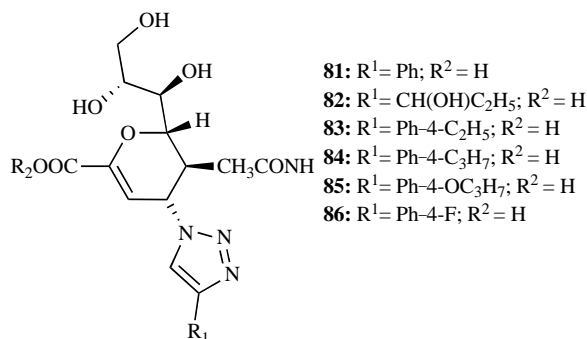
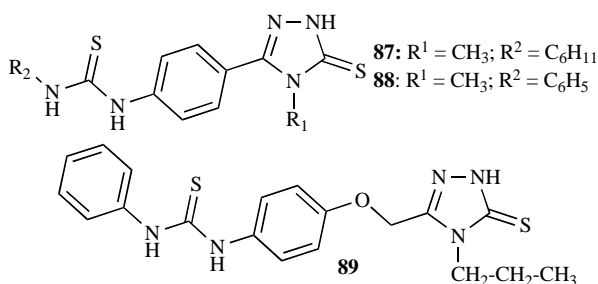


Fig. (17). Chemical structures of triazole-modified zanamivir analogues.

A series of some novel thiourea derivatives obtained from 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones (Fig. (18): **87-89**) were synthe-



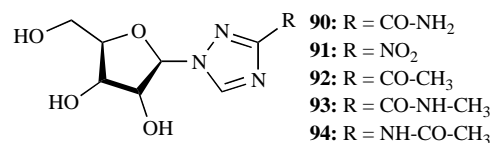
sized by Kucukguzel *et al* for evaluation of their antiviral potency against HIV-1 (IIIB) and HIV-2 (ROD) strains in MT-4 cells, as well as other selected viruses such as HSV-1, HSV-2, Coxsackie virus B4, Sindbis virus and Varicella-zoster virus using HeLa, Vero, HEL and E6SM cell cultures. Among the derivatives screened, compound **87** which had a MCC value of 400 mg/mL, showed weak activity against the herpes simplex family [HSV-1 (KOS), HSV-2 (G) and HSV-1 TK<sup>-</sup> (ACVR)] with the MIC value of 48 mg/mL. Compound **88** also demonstrated a weak activity against HSV-1 TK<sup>-</sup> (ACVR) with the MIC value of 48 mg/mL. The most active derivative in this series was compound **89** which showed moderate protection against Coxsackie virus B4 with an MIC value of 16 µg/mL and a selectivity index of 5. This compound was also active against thymidine kinase positive Varicella-zoster virus (TK<sup>+</sup> VZV, OKA strain) with an EC<sub>50</sub> value of 9.9 µg/mL and also possessed low toxicity on human embryonic lung (HEL) cells (MCC of >100 mg/mL) as compared to standard drug trovirdine [52].



**Fig. (18).** Chemical structures of 5-[(4-aminophenoxy)methyl]-4-alkyl/aryl-2,4-dihydro-3H-1,2,4-triazole-3-thiones.

The conversion of RBV to the monophosphate by adenosine kinase is the rate-limiting step in activation of this broad spectrum antiviral drug. The effect of variation of the 3-substituents in a series of bioisosteric and homologated 1-β-D-ribofuranosyl-1,2,4-triazoles (Fig. (19): **90-94**) on activity with human adenosine kinase (hADK) was studied by Kumarapperuma *et al* to develop new antiviral drugs for RNA viruses. The potential for inhibition of hADK activity was evaluated by monitoring the formation of RBV monophosphate in the presence of compounds **91-94** by using hamster ADK. Enzyme activity was only detected with three of RBV analogs (**91**, **92** and **94**). However, these analogs were less effective substrates than **90**. Computational meth-

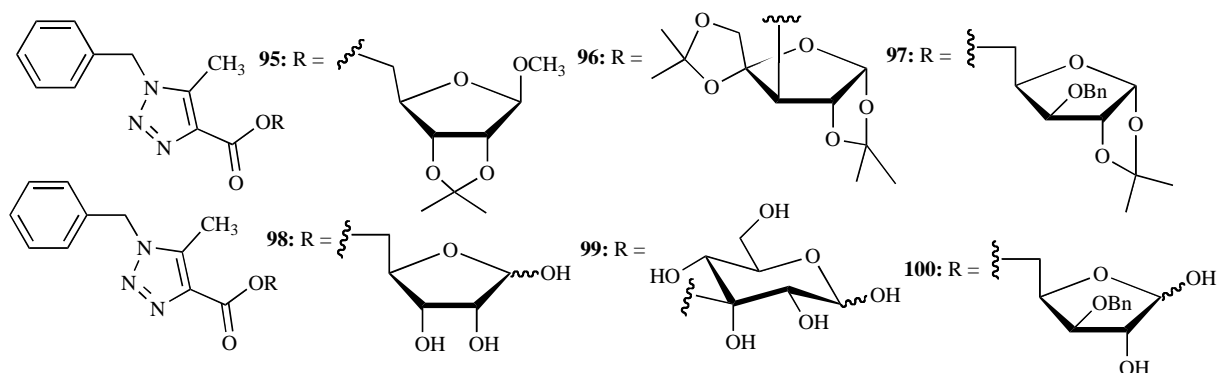
ods were employed to characterize possible structural contributions to hADK activity within this series. The compounds evaluated fall within two general classes, isosteres possessing overall steric volume similar to **90** but altered functionality that affects H-bonding (**91** and **92**), and homologated analogs possessing increased steric profiles and altered hydrophobicity (**93** and **94**). The 3-nitro derivative **91** was the most active synthetic analog. This compound possesses a molecular electrostatic potential map that is similar to **90** (RBV), the smallest volume, highest lipophilicity and dipole moment/volume. Nitro group was noted to be isostructural to the carboxamide but typically exhibited weaker intermolecular H-bond accepting capabilities. It was observed to undergo phosphorylation with 18 % enzyme activity as compared to standard drug RBV (100 %) [53].



**Fig. (19).** Chemical structures of 1-β-D-ribofuranosyl-1,2,4-triazoles.

Da Silva *et al* described the synthesis of two new classes of triazoles (Fig. (20): **95-100**) including several 1-benzyl-1H-1,2,3-triazoles attached to different carbohydrate templates and reported their *in vitro* inhibitory profile at different concentrations (0.01-50 µM) against HIV-1 RT by using recombinant HIV-1 enzyme and AZT as antiviral standard. Initially all compounds were tested at 50 µM. All compounds inhibited HIV RT to some degree and derivatives with the protected carbohydrate (**95-97**) presented an inhibitory profile higher than compounds **98-100** containing unprotected carbohydrate groups. Importantly, **95-97** produced the highest inhibitory values (63-65%) at 50 µM. The determination of the IC<sub>50</sub> of the most active compounds against HIV-RT (**95** = 2.2 ± 0.8 µM, **96** = 5.0 ± 0.5 µM and **97** = 1.98 ± 0.4 µM) revealed values for these compounds higher than AZT, but similar to DDC (zalcitabine) and lamivudine and lower than DDI (didanosine). Thus these data suggest that triazoles **95-97** may be considered as promising lead molecules for further synthetic and biological exploration for generating new anti-HIV-RT compounds [54].

Treatment with HIV-1 protease inhibitors (PIs), a component of HAART, often results in viral resistance. Structural and biochemical characterization of a 6X protease mu-

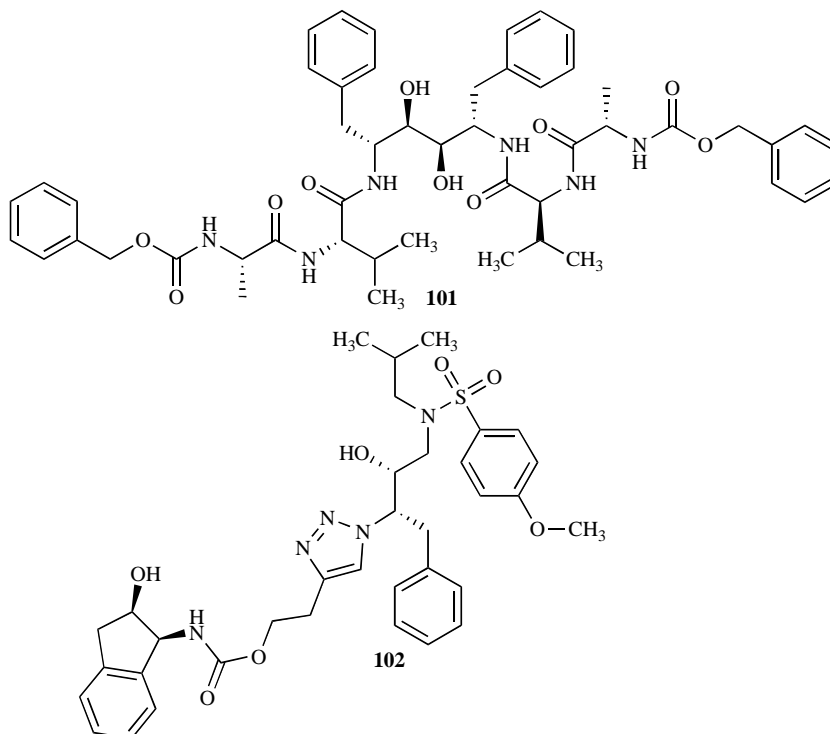


**Fig. (20).** Chemical structures of 1-benzyl-1H-1,2,3-triazoles having different carbohydrate templates.

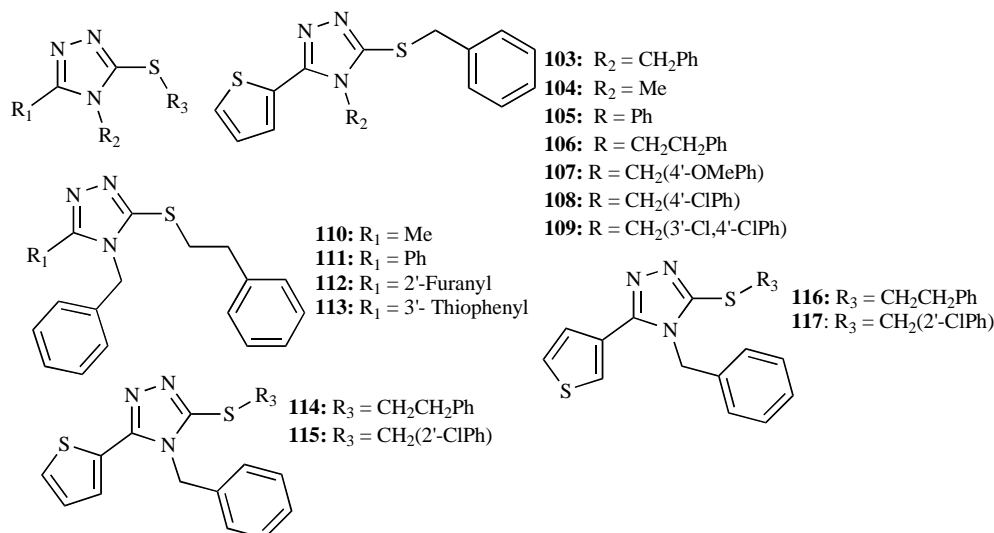
tant arising from *in vitro* selection with compound **101**, a C2-symmetric diol PI, has been previously described [55]. Giffin *et al* reported that compound **102**, a copper (I)-catalyzed 1,2,3-triazole derived compound (Fig. **21**) previously shown to be potentially effective against WT protease ( $IC_{50} = 6.0$  nM), has low nM activity ( $IC_{50} = 15.7$  nM) against the multidrug-resistant 6X protease mutant. Compound **102** displayed similar efficacy against WT and 6X HIV-1 in viral replication assays. While structural studies of compound **101** bound to WT and mutant proteases revealed a progressive change in binding mode in the mutants, the compound **102** crystal structure showed nearly identical in-

teractions in the WT protease complex which provide the basis for new inhibitors to target WT and multidrug resistant viruses [56].

A novel series of 3,4,5-trisubstituted 1,2,4-*H* triazole derivatives (Fig. (**22**): **103-117**) was synthesized by Kirschberg *et al* and investigated for HIV-1 RT inhibition. The results obtained for seven simple derivatives (**103-109**) sharing a thiophenyl substituent as  $R^1$  and a benzyl group as  $R^3$  showed that 4-benzyl derivate (**103**) was the only one that displayed anti-HIV activity with  $EC_{50}$  value of  $3.7 \mu\text{m}$ , albeit with a moderate selectivity index. Shortening or lengthening the distance of the phenyl ring from the core or removing it



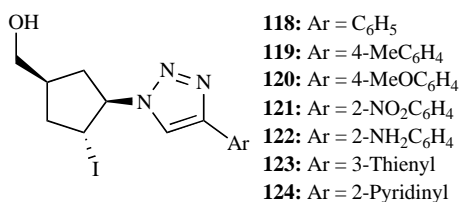
**Fig. (21).** Chemical structure of (1-(3-hydroxy-4-(Nisobutyl-4-methoxyphenylsulfonamido)-1-phenylbutan-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl-2-hydroxy-2,3-dihydro-1*H*-inden-1-ylcarbamate.



**Fig. (22).** Chemical structures of 3,4,5-trisubstituted 1,2,4-*H* triazole derivatives.

altogether led to dramatic loss of activity, as did the introduction of chlorine or methoxy substituents. The replacement of the aromatic ring with alkyl substituents led to loss of antiviral activity, while extension of the linker by one methylene unit (**114** and **116**) reduced the activity when compared to the parent (**103**). Compounds **115** and **117** with EC<sub>50</sub> values of 0.44 and 0.131 μm respectively represented the most active NNRTIs in this report when compared with standard antiviral drugs like EFV and NVP [57].

A series of 4-aryl-1,2,3-triazolo-2',3'-dideoxy-2'-iodocarbanucleosides (Fig. (23):**118-124**) was synthesized by Perez-Castro *et al* and evaluated for their inhibitory activities against cytomegalovirus (CMV Davis strain) and varicella-zoster virus (TK<sup>+</sup>VZV, thymidine kinase positive strain, and TK<sup>-</sup>VZV, thymidine kinase deficient strain) in HEL cells. Most of the compounds did not show any specific antiviral effects (i.e. minimal antiviral effective concentration 5-fold lower than minimal cytotoxic concentration) against any of the viruses in the assay systems used, except for compound **118** which exhibited specific inhibitory potential against TK<sup>+</sup>VZV (EC<sub>50</sub> = 4.5 μgmL<sup>-1</sup>) [58].



**Fig. (23).** Chemical structures of 4-aryl-1,2,3-triazolo-2',3'-dideoxy-2'-iodocarbanucleosides.

## CONCLUSION

This review focuses on diverse and potent antiviral activity profile of the triazole derivatives reported in the past ten years and is an attempt to provide an outlook on the latest research developments on scope of triazole derivatives as promising antiviral agents. Information provided in this manuscript can be useful to prospective researchers and medicinal chemists working in this arena for further study of this scaffold in order to exploit its biological potential appropriately and to develop the pharmacologically significant antiviral agents of future for treatment of various viral ailments.

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## ABBREVIATIONS

AIDS	=	Acquired immune deficiency syndrome
AIV	=	Avian influenza virus
AZT	=	Azidothymidine
CC <sub>50</sub>	=	50% cytotoxic concentration
DDC	=	Zalcitabine
DDI	=	Didanosine

DNA	=	Deoxyribonucleic acid
EC <sub>50</sub>	=	50% effective concentration
EFV	=	Efavirenz
ETAR	=	1-β-D-ribofuranosyl-3-ethynyl-[1,2,4]triazole
FDA	=	Food and drug administration
HAART	=	Highly active antiretroviral therapy
hADK	=	Human adenosine kinase
HAV	=	Hepatitis-A virus
HCV	=	Hepatitis C virus
HEL	=	Human embryonic lung
HFRS	=	Hemorrhagic fever with renal syndrome
HIV	=	Human immune deficiency virus
HSV	=	Herpes simplex virus
HTNV	=	Hantaan virus
HTS	=	High throughput screening
IC <sub>50</sub>	=	50% inhibitory concentration
IFN	=	Interferon
ISGs	=	Interferon-stimulated response genes
ISRE	=	Interferon stimulated response element
MIC	=	Minimum inhibitory concentration
MOI	=	Multiplicity of infection
NNRTI	=	Non-nucleoside reverse transcriptase inhibitors
NRTI	=	Nucleoside and nucleotide reverse transcriptase inhibitors
NVP	=	Nevirapine
PHIV	=	Pseudo-type HIV
PI	=	Protease inhibitor
RBV	=	Ribavirin
RNA	=	Ribonucleic acid
RSV	=	Respiratory syncytial virus
SAR	=	Structure activity relationship
VZV	=	Varicella-zoster virus
WT	=	Wild type

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