# Antiviral Activity of Substituted Salicylanilides – A Review

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**Abstract:** Chemotherapy of viral infections is still challenging. Salicylanilides demonstrated a wide range of biological activities including antiviral potency and the review summarizes this field. Niclosamide was described to be able to affect coronaviruses. Some salicylanilides and salicylamides could inhibit HIV virus by targeting of HIV-1 integrase or reverse transcriptase. Hepatitis C virus is another virus, which could be potentially afflicted by salicylanilides on the level of two enzymes – NS3 protease and NS5B RNA polymerase. Nitazoxanide is a nitrothiazole derivative of salicylamide useful for the treatment of protozoal and bacterial infections with an extended range of antiviral activity and innovative mechanism of action, especially against hepatitis and influenza viruses or rotaviruses. Nitazoxanide, its metabolite tizoxanide and their derivatives are a very promising stream in the development of new antiviral compounds. In this review, we summarize the antiviral activity of structures containing salicylanilide and partly salicylamide moiety.

**Keywords:** Antiviral activity, antiviral therapy, nitazoxanide, salicylamides, salicylanilides.

## 1. INTRODUCTION

Salicylanilides (2-hydroxy-*N*-phenylbenzamides) are a group of compounds having a wide range of biological activities and they have been studied for potential therapeutic or other usage for a long time. Closantel, rafoxanide, niclosamide, its sodium salt bayluscide, and salantel belong to the most known members of this group [1]. For example, they act as uncouplers on biomembranes [2-4], affect productions of interleukins and regulate an immune response [5-9], show analgesic efficacy [10], influence ion channels [11-13] *etc*. Moreover, salicylanilides influence more molecular targets with the chance to be potentially useful in cancer therapy [14-18]. Importantly, salicylanilide derivatives are known for their long time ago discovered activity against different bacteria, fungi and human and veterinarian parasites [1]. They are still investigated and modified for antimycobacterial activity against typical, atypical and resistant mycobacteria [19-26], different fungal strains [23-24, 26-29] or bacteria, especially Gram-positive [26, 28, 30-37].

Salicylanilides have been reported to cause undesirable side effect on the skin (photocontact dermatitis) due to their photosensitising properties, especially for halogenated ones like tetrachlorosalicylanilide [1, 38-40] and brominated salicylanilides [40-41]. Several studies pointed that some of salicylanilide derivatives represented by niclosamide in higher doses, may have adverse effects on genetic code and DNA due to presence of halogens and nitro group [42-46].

The salicylamide pharmacophore, which is bolded in structures in this review, was referred as an important part of antiviral compounds acting against HIV protease in 1996 [47]. Salicylamide and closely salicylanilide moieties are present in more structures developed for combat viral infections, but no systematic studies dealing with antiviral activity of salicylanilides and/or systematic screening of salicylanilide derivatives against a broad spectrum of different virus species were published.

The development of antiviral drugs and their clinical utilization ascended later than investigation of other antimicrobial agents because of several factors – in a normal host, most viral infections are self-recoverable, an existence of possible drug toxicity and the cost of the treatment. In addition, diagnostics for viral pathogens have been slow to develop and in many cases the diagnosis is based on clinical symptoms. There are challenges in the development of antiviral agents that are distinct when compared to antibacterial or antifungal agents. Antivirotics must be evaluated in a cell culture system since the viruses are obligate intracellular parasites. The affecting of virus could be harmful for cell functions due to their possible similarity [48]. One strategy to control viral infections is the vaccination, but for some of the most pressing viruses of today, no vaccine is available – for example for HIV infection or hepatitis C virus (HCV) infection [49]. Additional problems bring in many cases drug-resistance [50-51].

## 2. ANTIVIRAL ACTIVITY OF SALICYLANILIDES

### 2.1. Activity Against Coronaviruses

Coronaviruses (CoV) may cause acute and chronic respiratory, enteric and central nervous system diseases in many species including porcine, felines, bovine, avian, murine and human organisms. Until recently, the relatively low burden of human coronavirus disease hampered development of anti-coronavirus therapeutics. However, the emergence of severe acute respiratory syndrome caused by coronavirus (SARS-CoV) has accelerated the discovery of these drugs. The main targets of coronavirus therapy are SARS-CoV entry into cells, fusion and replication by several mechanisms

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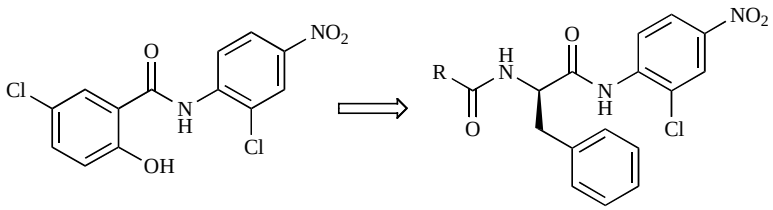


**Scheme 1.** Peptide modifications of niclosamide´s substructure.

[52]. Severe acute respiratory syndrome has recently emerged as a new human disease with considerable morbidity and mortality. It was first described in China in 2002 [53].

Niclosamide (5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide; Table **1**, compound **1a**), an old antiparasitic drug, was described to be able to inhibit replication of a coronavirus causing SARS. Viral antigen synthesis was totally abolished at the concentration of 1.56 or 3.12 μM and higher. On the other side, the concentration of niclosamide that reduced cell viability to 50%, used to determine the cellular toxicity, was approximately 250 μM. It indicated that niclosamide does not interfere with the virion´s attachment to and entry into cells [53]. Additionally, niclosamide showed high inhibition activity of cythopathogenic effect of SARS-CoV at the concentration ≥ 3.3 μM and exhibited significant inhibitory effects at concentrations as low as 1 μM. Cytotoxic $IC_{50}$ was 22.1 μM, $EC_{50}$ for the inhibition of viral replication lower than 0.1 μM (therefore SI is higher than > 221). Niclosamide showed inhibitory effects on 3CL (chymotrypsin-like) protease activity with $IC_{50}$ value of 40.0 μM [54]. 3CL protease, the SARS-associated coronavirus main proteinase, is a key enzyme in proteolytic processing of the replicase polyproteins 1a and 1ab, which makes it an attractive target for development of new drugs. Niclosamide was demonstrated to bind this enzyme [55]. Various (mostly peptide) modifications of 2-chloro-4-nitroaniline, which is a niclosamide´s substructure, lead to a series of potent competitive inhibitors of the SARS 3CL protease with stepped up efficacy (Scheme **1**) [56].

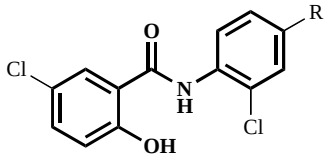
The fact that niclosamide potentially acts as a vacuolar ATPase inhibitor could be relevant to the activity against SARS-CoV. Niclosamide was found to disrupt heptamer prepore-to-pore conversion, eliminated SARS-CoV viral antigen synthesis at modest concentrations. The possibility that niclosamide does act on vacuolar ATPase is intriguing and may yield insight into its effect on viral entry [57].

Niclosamide was investigated for pharmacokinetic and toxicological properties. Its oral $LD_{50}$ is higher than 5 000 mg/kg and this drug showed no adverse effect level in the dose 2 000 mg/kg/day for four weeks (subacute toxicology study). These results indicate good safety selectivity index and profile. The oral bioavailability in rats is low, 10 % after single dose administration of 5 mg/kg compare to i. v. [58].

On the other side, niclosamide exhibited no significant inhibitory activity against human coronavirus NL 63 [59].

Two niclosamide-like salicylanilides 5-chloro-*N*-(2,4-dichlorophenyl)-2-hydroxybenzamide (Table **1**, compound **1b)** and 5-chloro-*N*-(2-chloro-4-(trifluoromethyl)phenyl)-2-hydroxybenzamide (Table **1**, compound **1c**) were designed and synthesized. These compounds and niclosamide inhibited porcine transmissible gastroenteritis virus (TGEV) replication with an $IC_{50} \geq 2$ μM [58].

**Table 1. Niclosamide-Like Salicylanilides and their Activity**



| | R | $EC_{50}$ (TGEV) |
|---|---|---|
| **1a** | $-NO_2$ | 15 μM |
| **1b** | -Cl | 3 μM |
| **1c** | $-CF_3$ | 2 μM |

## 2.2. Activity Against HIV-Viruses

Human immunodeficiency virus type-1 (HIV-1) is the etiological agent of fatal acquired immunodeficiency syndrome (AIDS), which was firstly detected in 1981. Different classes of chemotherapeutic agents are actually available to block the replication of HIV-1 – nucleoside and non-nucleoside reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, entry inhibitors and integrase inhibitors [60-61]. These drugs are mostly used in a combination therapy, named as Highly Active Anti Retroviral Therapy because of non-existence of one ideal drug. Treatment of acquired immunodeficiency syndrome continues to be one of the most challenging obstacles in chemotherapy. The compounds with anti-HIV potential use are still widely synthesized and studied [61]. Especially new mechanisms of action than having clinical used drugs are needed.

HIV-1 integrase is an essential enzyme for retroviral replication and due to the absence of any known human homologue represents an attractive and useful target for chemotherapeutic intervention. This enzyme catalyzes the insertion of the viral DNA into the genome of the host cell through a complex process [60]. This integration of viral DNA into the host cell genome is a critical step in the life cycle of HIV. The reaction is catalyzed by integrase and consists of three steps: (i) cleavage of a dinucleotide pair from the 3'-end of the viral DNA (3´-processing), (ii) insertion of the strands into the host-cell chromosome (strand transfer),

and (iii) removal of the two unpaired nucleotides at the 5'-end of the viral DNA and gap-filling process [62-63].

Among the reported HIV-1 integrase inhibitors, dioxo ("diketo") compounds represent a most promising class of compounds. The mechanism of action appears to involve the binding of the dioxoacid portion to the $Mg^{2+}$ or $Mn^{2+}$ co-factor located in the active site of the enzyme. Several such inhibitors have been tested in the clinic. There were tested three salicylanilides in a series of 17 dioxoamides for their ability to inhibit 3´-processing and strand transfer. The highest activity in both assays among all compounds performed (*Z*)-2-hydroxy-4-(3-(2-hydroxy-3,5-dinitrobenzamido)phenyl)-4-oxobut-2-enoic acid (Fig. **1**). $IC_{50}$ was 8.0 μM in the case of 3´-processing and 2.0 μM against strand transfer. The replacement of 3,5-dinitrosalicylic acid by salicylic or 3-methylsalicylic acid reduces significantly the activity. The presence of salicylic hydroxyl brings a twofold increase of activity than unsubstituted or 4-hydroxylated compounds. The results imply that another dioxo moiety is formed between the benzamide group and the C-2 phenolic group, which might bind to two divalent metal ions on the active site of enzyme [60].

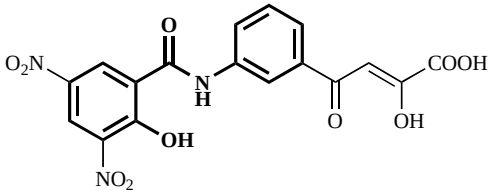



**Fig. (1).** Structure of (*Z*)-2-hydroxy-4-(3-(2-hydroxy-3,5-dinitrobenzamido)phenyl)-4-oxobut-2-enoic acid.

Niclosamide was also tested for its activity against recombinant HIV-1 integrase and showed no significant inhibitory potency at 100 μM [64]. New potential inhibitors of HIV-1 integrase have been identified primarily *via* computer-based screening. One structure containing salicylanilide core, (*E*)-3-((3-(2,4-dimethylphenylcarbamoyl)-2-hydroxynaphthalen-1-yl)diazenyl)-4-hydroxybenzenesulfonic acid (Fig. **2**), showed an integrase inhibitory activity in the presence of divalent ions with $IC_{50}$ of 13.0 μM for 3´-processing and 14.0 μM for integration ($Mn^{2+}$) and 53.0 μM and 22.0 μM for $Mg^{2+}$, respectively. It indicates that chelation of divalent metals in the active site of enzyme may be responsible

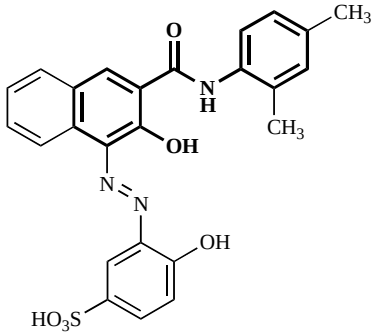



**Fig. (2).** Structure of (*E*)-3-((3-(2,4-dimethylphenylcarbamoyl)-2-hydroxynaphthalen-1-yl)diazenyl)-4-hydroxybenzenesulfonic acid.

for the potency. This compound showed cytoprotection of HIV-1 infected CEM cells and it was the most potent antiviral compound with an $EC_{50}$ of 0.8 μM. However, the compound also showed considerable cytotoxicity value $CC_{50}$ of 4.4 μM. Interestingly, salicylaldehyde Schiff base showed a lower activity and a higher cytotoxicity. The binding to HIV-1 integrase was confirmed by docking studies. The ionizable sulfonate group, amidic nitrogen a one azo-nitrogen are important for the binding to the viral enzyme [62].

Other polycyclic compounds containing "incorporated" salicylanilide substructure exhibited good anti-HIV integrase activity. The $IC_{50}$ of the most active compound (Fig. **3**) is 0.41 μM for integrase and, moreover, 2.8 for μM for RNase H domain of reverse transcriptase; both this targets share structural similarity. The molecule also exerts a 20-fold strand transfer selectivity compared to 3´-end-processing inhibition. The acetylation or carboxymethylcarbonylation of hydroxy groups led to the derivatives with abolished anti-integrase activity, but the anti-RNase H activity is conserved or improved. The presence of free hydroxy groups and the "salicylanilide´s" phenyl seem to be necessary for high activity [65].

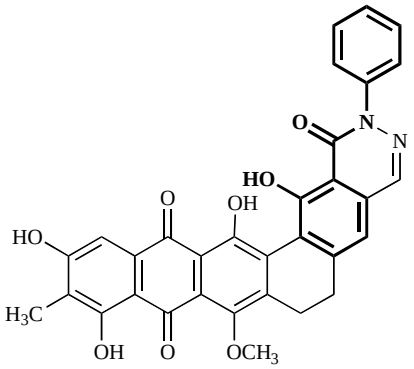



**Fig. (3).** Example of polycyclic compound containing salicylanilide moiety.

Reverse transcriptase (RT)-targeting drugs can be grouped into two classes: nucleoside analogues and non-nucleoside reverse transcriptase inhibitors. The second ones bind to a hydrophobic pocket close to, but distinct from, the RT active site and inhibit the enzyme activity by mediating allosteric changes in the RT. Despite the favorable toxicity and adherence properties, resistance develops rapidly, mostly as a consequence of single mutations [66].

*N*-Acylthiocarbamates were described being non-nucleoside inhibitors of HIV-1 reverse transcriptase inhibitors. One of these structures contains *N*-substituted *O*-acetylsalicylanilide moiety. Replacement of phenyl moiety on nitrogen by other acyls failed to improve the antiretroviral potency. Salicylanilide derivative (Fig. **4**) showed $EC_{50}$ value of 9.5 μM. This concentration provided 50% protection of MT-4 cell from the HIV-1 induced cytopathogenicity; the selectivity index was > 21 [66].

One of the potential HIV targets is the nucleocapsid protein (NCp7). HIV NCp7 is necessary for proper viral RNA-packaging and virion budding and stimulates reverse transcription *in vitro*. NCp7 is a small, basic protein that tetrahe-

drally coordinates a $Zn^{2+}$. Changes in the zinc fingers of HIV-1 NCp7 yield non-infectious particles. Therefore compounds disrupting zinc coordination to NCp7 may have an antiviral effect.

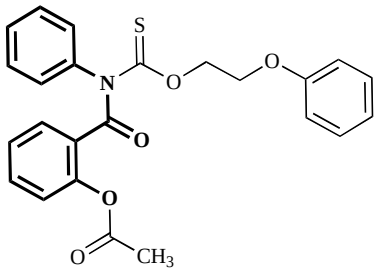


**Fig. (4).** Structure of 2-(((2-phenoxyethoxy)carbonothioyl)(phenyl) carbamoyl)phenyl acetate.

It has been described that disulfide benzamides possess a low micro molar anti-HIV activity in both acutely and chronically infected cells. The results indicate that the disulfide benzamides act after virus-cell fusion and before late-stage reverse transcription. All of the antiviral disulfide benzamides were found to eject NCp7 zinc, while some disulfide benzamides with zinc ejection activity are not antiviral. The necessity of the disulfide bond for the NCp7 zinc ejection and cellular anti-HIV activity by disulfide benzamides was examined. While the thiol compound (Fig. **5**, X = S) demonstrated no $Zn^{2+}$ ejection activity, but low micro molar antiviral activity and its oxidated form, disulphide, both antiviral and moderately ejection activity, the replacement of the free thiol with a phenolic hydroxy group abolishes both *in vitro* and cellular activities (> 100 μM) [67]. Therefore this "salicylanilide" modification (Fig. **5**, X = O) is not favorable as in the case of benzamide HIV-1 protease inhibitors.

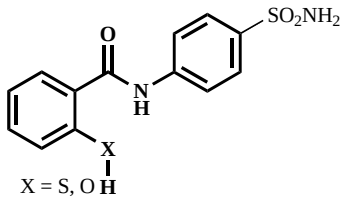


**Fig. (5).** Structure of *N*-(4-sulfamoylphenyl)benzamides.

### 2.3. Activity Against Hepatitis Viruses

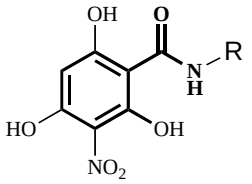
Hepatitis C virus (HCV) is a common pathogen with an estimated 170 million people infected worldwide. HCV is a RNA virus classified as a separate genus of the *Flaviviridae* family and it is the major etiological agent of non-A, non-B hepatitis [68]. Hepatitis C is a blood-borne, infectious, viral disease caused by HCV. The chronic infection can result in cirrhosis of the liver, liver cancer and failure [69].

As it is the case with HIV, efforts to develop anti-HCV agents have focused on the inhibition of key viral enzymes for virus replication cycle including NS3-NS4A protease, where we distinguish several different approaches of inhibition mechanisms. In addition to the HCV serine protease, NS3 possesses an RNA helicase and a nucleotide triphosphatase domain. HCV helicase represents a relatively new and unproven antiviral target. Although it is not yet known whether helicase inhibition would be effective in suppressing HCV replication, it has nonetheless been targeted in the search for novel therapies for HCV infection. Potential anti-HCV compounds cause inhibition of the unwinding through intercalation of polynucleotide chain or affecting internal ribosomal entry [68, 70].

Unsubstituted salicylanilide was tested in high-throughput screening for its activity on hepatitis C virus replication with the result to be proviral agent increasing HCV replication [71].

One study evaluated twelve 2,4,6-trihydroxy-3-nitrobenzamide derivatives (Table **2**; compounds **2a** – **2l**) as small inhibitors of HCV NS3-NS4A protease and other serine proteases. In this series were seven salicylanilide derivatives. *N*-tridecyl derivative **2b** exhibited the lowest $IC_{50}$ of 2.3 μg/mL (5.8 μM) and 1.2 μg/mL in the presence and absence of NS4A, respectively. All salicylanilides were active and the most potent was derivative **2k** with $IC_{50}$ 8.5 μg/mL (22.2 μM) and 3.1 μg/mL in the presence and absence of NS4A, respectively. The results suggest that these derivatives inhibit NS3 protease activity without affecting NS4A. The presence of second phenyl moiety or long chain substituent increased activity. The kinetic analyses revealed that the mode of action is a combination of non-competitive and uncompetitive inhibition. Unfortunately, the majority of these compounds displayed a low specificity, exhibiting strong inhibitory effects on other human serine proteases, chymotrypsin and elastase, several compounds more effective than HCV protease. Derivative **2d** was found to be the most selective inhibitor [72].

**Table 2. Anti-HCV Protease NS3 Activity of Salicylamides**



| | R | % inhibition at 100 μg/mL | $IC_{50}$ |
|---|---|---|---|
| **2a** | *n*-$C_7H_{15}$ | 86.8 | 101.5 μM |
| **2b** | *n*-$C_{13}H_{27}$ | 100 | 5.8 μM |
| **2c** | cyclohexyl | 81.1 | 101.9 μM |
| **2d** | 2-phenylethyl | 69.8 | 145.2 μM |
| **2d** | 2-(4-phenoxy-phenoxy)ethyl | 71.0 | 76.9 μM |
| **2f** | *p*-tolyl | 84.8 | 80.5 μM |
| **2g** | *m*-tolyl | 90.4 | 147.6 μM |
| **2h** | *o*-tolyl | 77.8 | 155.5 μM |
| **2i** | 3,4-dimethoxyphenyl | 64.9 | 149.0 μM |
| **2j** | 2,3-dichlorophenyl | 96.1 | 28.1 μM |
| **2k** | 4-phenoxyphenyl | 100 | 22.2 μM |
| **2l** | 4-bromophenyl | 94.1 | 52.6 μM |

It was screened a library of 2 000 compounds for inhibitors of HCV serine proteinase NS3. Only three compounds were found to be active with $IC_{50} < 10^{-5}$ M and one of them was salicylanilide with no inhibition acting on common serine proteinases. $IC_{50}$ of 3-bromo-5-chloro-*N*-(5-chloro-2-(1-chloronaphthalen-2-yloxy)phenyl)-2-hydroxybenzamide (Fig. **6**) was 6.2 μM and in the concentration of $10^{-5}$ M, where it reduced the HCV serine protease activity by more than 50%, did not significantly affect the activity of chymotrypsin, factor Xa, kallikrein, plasmin, α-thrombin and trypsin. Interestingly, the replacement of phenolic hydroxyl group by 4-methylphenylsulfonamido moiety resulted in the compounds with similar bioactivity. The salicylanilide was found being a non-competitive inhibitor and its inhibition constant value was estimated to be 3.6 μM.

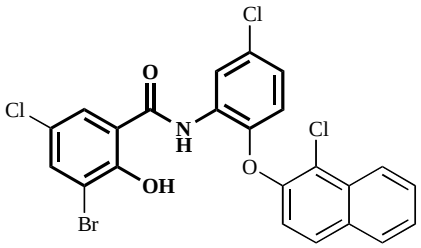



**Fig. (6).** Structure of 3-bromo-5-chloro-*N*-(5-chloro-2-(1-chloronaphthalen-2-yloxy)phenyl)-2-hydroxybenzamide.

Some structure modification of this structure was made to examine the effect of substituents. The replacement of chlorine by bromine on naphthalene ring in combination with replacement of bromine on salicylic ring and the changes in position of substituents on anilide ring resulted in the almost comparable activity. The presence of the large hydrophobic group (halogenated naphthoxy moiety) in the aniline ring is necessary for a high inhibitory activity, and its replacement with chlorine resulted in a large reduction of the activity [73].

The HCV NS5B gene encodes an RNA-dependent RNA polymerase which enzymatic activity is critical to the replication of viral RNA genome. Various compounds were reported to affect this target including halogenated salicylanilides, which were identified in a high throughput screening. The structure-activity relationship (Fig. **7**) suggested that 4,6-diiodo substitution on the salicylic ring is preferable to other halogen substitutions. The replacement of phenolic hydroxyl by amino group diminished the activity. On the other side, the substitutions on the anilide ring did not change the activity significantly, only quantitative – bulky hydrophobic moieties on the anilide part of molecule have the best activity. The amide linkage can be replaced by the imine without loss of activity. Methylation of either the hydroxyl group or the amide group of the salicylamide moiety abolished the activity, as well as *O*-acetylation. The most efficacious was 2-hydroxy-3,5-diiodo-*N*-(4-(phenylamino) phenyl)benzamide (Fig. **8**) with $IC_{50}$ value of 1.9 μM for 1b polymerase. The derivatives were shown to act non-competitively. More importantly, this class of compounds demonstrated broad genotype activity against genotype 1 – 3 HCV NS5B polymerases and a replication cell culture activity. Therefore, halogenated salicylanilides represent a novel class of allosteric inhibitors. However, one disadvantage is their poor solubility [74].

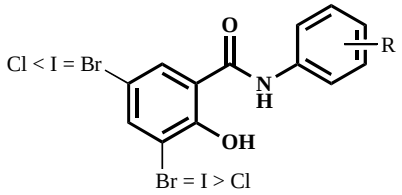



**Fig. (7).** Halogenated salicylanilides.

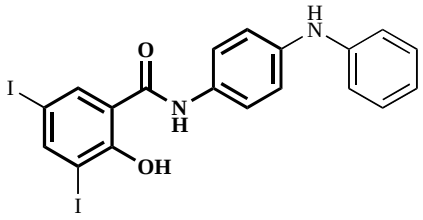
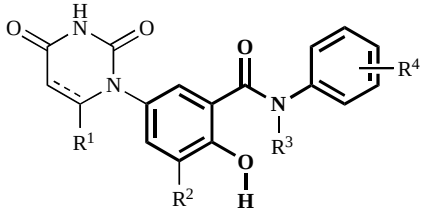



**Fig. (8).** Structure of 2-hydroxy-3,5-diiodo-*N*-(4-phenylamino) phenyl)benzamide.

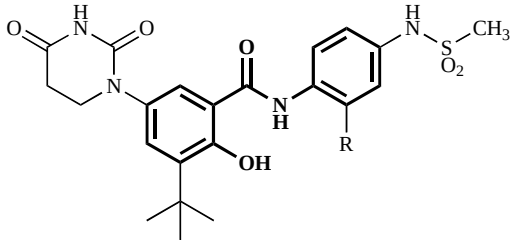
Abbot Laboratories patented a group of uracil and thymine derivatives for treatment of HCV infection. One subgroup of these derivates contain salicylanilide moiety (Fig. **9**). Hydroxy group could be replaced by methoxy group or by hydrogen and the anilide ring by some heteroaryls (thiophene, thiazole or *ortho*-condensed phenyl rings), but these modifications did not prove a better activity, they have a comparable or a little worse efficacy.

These compounds were tested in the HCV polymerase (NS5B) inhibition assay for their activity to inhibit HCV polymerase from two strains (1a and 1b). The most active were 4 compounds (Fig. **10a**, **10b**, **11**, **12**) with $IC_{50} < 0.01$ μM against 1b and $IC_{50}$ higher than 0.01 and lower than 0.1 μM against 1a polymerase. In the HCV RNA replication assay showed the best activity only compounds with partly hydrogenated pyrimidine ring or fully hydrogenated in the combination with 4-methylsulfonylamido moiety on anilide ring with the result having $EC_{50}$ in the interval from 0.01 to 1.0 μM [69].




$R^1$ = H, $CH_3$; $R^2$ = H, $CH_2CH_3$, *tert*-butyl; $R^3$ = H, $CH_3$
$R^4$ = 4-: H, $NR´SO_2R´´$, $SO_2NH_2$, $CH_2NHSO_2CH_3$,
2-: H, $OCH_3$, $SO_2R$
dashed line means a possible presence of the double bond

**Fig. (9).** Uracil and thymine derivatives having salicylanilide moiety.



R = H (**10a**), $OCH_3$ (**10b**)

**Fig. (10).** Structure of 3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-hydroxy-*N*-(4-(methylsulfonamido)phenyl)benzamide and its methoxy analogue.

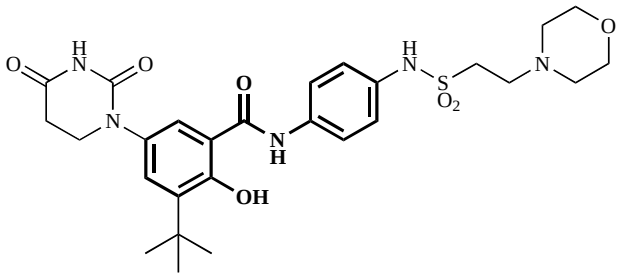


**Fig. (11).** Structure of 3-*tert*-butyl-5-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-2-hydroxy-*N*-(4-(2-morpholinoethylsulfonamido) phenyl)benzamide.

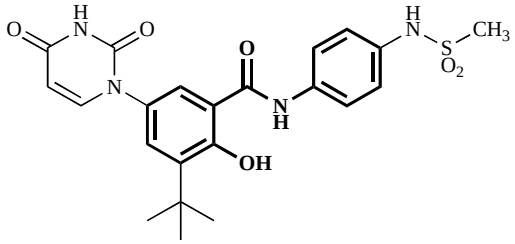


**Fig. (12).** Dihydropyrimidine derivative of hydroxybenzamide.

## 2.4. Activity Against Herpes Viruses

Different severe human infections are caused by herpes simplex viruses, varicella-zoster virus (VZV), and human cytomegalovirus (HCMV) from the family *Herpesviridae*. The seroprevalence of human herpes viruses is high and reactivations occur commonly [75].

Two salicylanilide structures with thiourea moieties (Fig. **13**, **14**) were synthesized and evaluated against human HCMV, herpes simplex virus type 1 (HSV-1) and VZV. *N*-(4-(3-(5-chloro-2,4-dimethoxyphenyl)thioureido)phenyl)-2-hydroxybenzamide (Fig. **13**) inhibited these viral strains with $IC_{50}$ of 6.0 μg/mL for HMCV and HSV and 4.0 μg/mL for VZV with 90% inhibition in the concentration of 10.0 μg/mL. The acetylation of phenolic group did not dramatically change the IC values [76]. The level of 1-hydroxy-2-naphtamide (Fig. **14**) activity was determined with $IC_{50}$ being 8.0 μg/mL for HMCV, 4.0 μg/mL for HSV and virus varicella-zoster was affected from 41% by the concentration of 10.0 μg/mL [77]. Thus, these compounds inhibit effectively the growth and replication of herpes viruses.

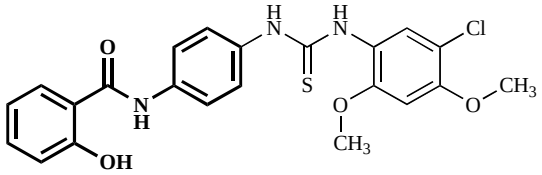


**Fig. (13).** Structure of *N*-(4-(3-(5-chloro-2,4-dimethoxyphenyl) thioureido)phenyl)-2-hydroxybenzamide.

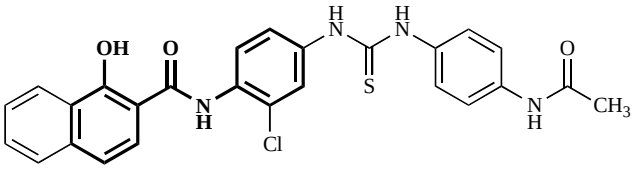


**Fig. (14).** Structure of *N*-(4-(3-(4-acetamidophenyl)thioureido)-2-chlorophenyl)-1-hydroxy-2-naphtamide.

## 2.5. Salicylamide Derivatives

A wide range of antiviral compounds could be notionally derived from salicylamide (2-hydroxybenzamide), which is a structural part of the salicylanilide molecule.

2-Hydroxy-*N'*-(2-hydroxybenzoyl)benzohydrazide (Fig. **15**) was identified as a lead compound against recombinant HIV-1 integrase, but showing limiting cytotoxicity. Based on this compound, a series of hydrazides was derived and tested against HIV-1-infected CEM cells, some with perspective findings [64]. It was shown that the length of the linker and the presence of the 2-hydroxyl group are critical for the inhibitory potency. Although one 2-hydroxyl group could be replaced by a sulphanyl group, the removal of one or both the 2-hydroxyls, eventually their replacement with amino, carboxyl groups or halogens yielded inactive compounds [78].

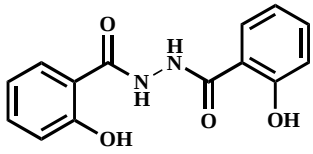


**Fig. (15).** 2-Hydroxy-*N'*-(2-hydroxybenzoyl)benzohydrazide.

More compounds containing *N*-substituted salicylamide or salicylhydrazide pharmacophore were described to affect HIV integrase, but some were inactive. It implicates that the activity is not only the function of pharmacophore presence [65, 78-81]. For example, unsubstituted salicylamide is inactive, whereas salicylhydrazide presented a moderate activity [80]. Similarly, in the case of 3-amino-2-hydroxy-4-phenylbutanoic acid derivatives the 2-hydroxyphenylcarbamoyl fragment diminishes the activity whereas 3-hydroxyphenylacarbamoyl exhibited a very good increasing potency [82]. Their hydrazide thioanalogues were similarly effective against the integrase, but less cytotoxic [83].

*N*-benzylsalicylamide fragment could be notionally found in some molecules of 8-hydroxyquinoline integrase inhibitors (Fig. **16**) [84]. Combination of salicylamide and rho-

danine fragments (Fig. **17**) led to compounds with a moderate anti-HIV-1 integrase activity [85].

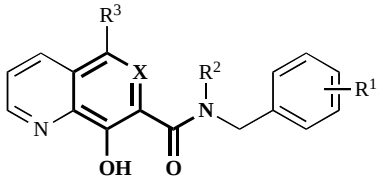


**Fig. (16).** 8-Hydroxyquinoline derivatives.

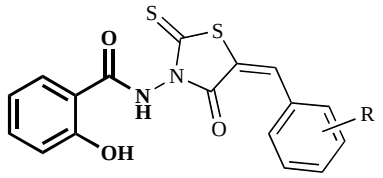


**Fig. (17).** *N*-(5-Benzylidene-4-oxo-2-thioxothiazolidin-3-yl)-2-hydroxybenzamide derivatives.

Benzamides containing carboxylic acid motifs were described being non-nucleoside inhibitors of hepatitis virus NS5B polymerase including one salicylamide (Fig. **18**). The compound exhibited almost best anti-polymerase activity with $IC_{50}$ of 0.08 μM [86].

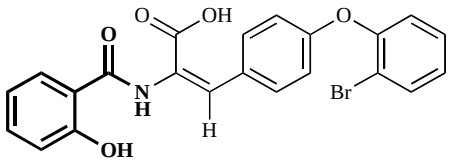


**Fig. (18).** Structure of (*E*)-3-(4-(2-bromophenoxy)phenyl)-2-(2-hydroxybenzamido)acrylic acid.

Salicylamides have a potential to be anti-influenza agents. For example, compound BMY-27709 (Fig. **19**) affects effectively influenza A H1 and H2 strains, whereas H3 strains are insusceptible [87-88]. It blocks fusion of viral particles and host cell membrane and thus viral entry; the mechanism embodies in the prevention of the low pH induced conformation rearrangement of hemagglutinin and the compound by specifically docking in a hydrophobic pocket around the fusion peptide. The salicylic hydroxyl seems to be necessary for the activity, the chlorine could be replaced by small lipophilic moieties like methyl. The removal of primary amino group is possible and the polar substituents at the position 3 diminish the activity. Corresponding carbothioamides are also advantageous. Some effectual derivatives [89-92] and antagonists [93] were synthesized.

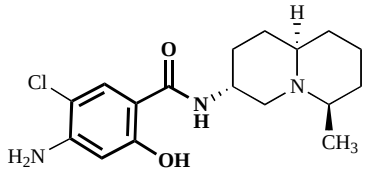


**Fig. (19).** Compound BMY-27709.

Two derivatives NSC125044 (Fig. **20**) and JJ3297 (Fig. **21**) were showed to inhibit influenza protein NS1A, which is an important factor of virulence enabling evading of the immune response and perspective target [87, 94-95].

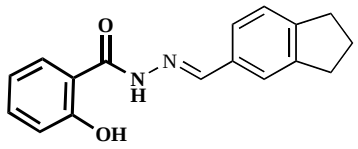


**Fig. (20).** Compound NSC125044.

*N*-benzylsalicylamide Ro 09-0881 (Fig. **22**) has been determined to be highly active ($IC_{50} \geq 0.01$ μM) inhibitor that binds to the capside of human rhinoviruses [96].

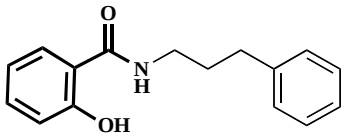


**Fig. (21).** Structure of 2-hydroxy-*N*-(3-phenylpropyl)benzamide, JJ3297.

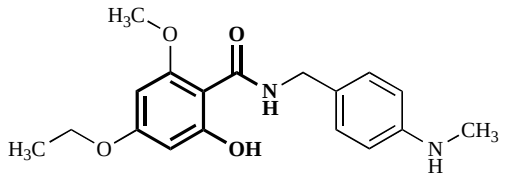


**Fig. (22).** *N*-Benzylsalicylamide Ro 09-0881.

### *2.5.1. Thiazolide Derivatives*

Some 4-thiazolidinones and carbothioamides derived from diflunisal (Fig. **23**) were evaluated for the activity against different viruses. None of the compounds inhibited vesicular stomatitis virus, Coxsackie virus, RSV, parainfluenza-3 virus, reovirus, Sindbis virus, Punto Toro virus, HSV-1, HSV-2 and vaccinia virus-induced cytopathicity at subtoxic concentrations. *N*-methyl and *N*-allyl hydrazinecarbothioamide derivatives showed activity against Vaccinia virus and HSV-1, *N*-phenyl and *N*-(4-methyl)phenyl ones against HSV-1 and Punto Toro virus. Diflunisal thiosemicarbazides presented higher efficacy than corresponding 4-thiazolidinones [97].

Nitazoxanide (2-(5-nitrothiazol-2-ylcarbamoyl)phenyl acetate; NTZ; Fig. **24a**) is a new anti-infective thiazolide used originally for treating protozoal infections. NTZ and its active deacetylated metabolite tizoxanide (2-hydroxy-*N*-(5-nitrothiazol-2-yl)benzamide; TIZ; Fig. **24b**) have presented a broad-spectrum of antiviral activity against DNA and RNA viruses [98-99]. Thiazolides exhibited good pharmacokinetic properties and a very favourable safety profile [99].

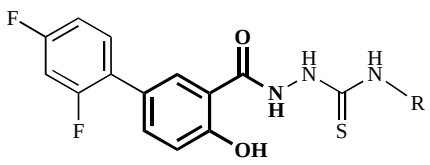
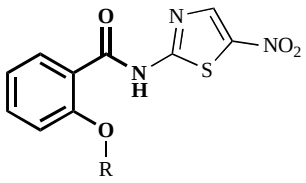


**Fig. (23).** Structure of 4-thiazolidinones.



R = $COCH_3$ (**24a**), H (**24b**)

**Fig. (24).** Nitazoxanide (**24a**) and tizoxanide (**24b**).

Antiviral properties of NTZ were discovered when patients with AIDS co infected with hepatitis B and C were treated for cryptosporidiosis [100]. The investigation of NTZ and TIZ antiviral activity was focused into treatment of hepatitis viruses (HBV and HCV) and these drugs and some of their derivatives were described to be very promising with low nano- and micro molar $EC_{50}$ (0.15 μM) and good SI *in vitro* and in clinical studies (now undergoing Phase II) alone or in the combinations with other antiviral compounds like interferon, ribavirine, lamivudine, adefovir, telaprevir, and 2′-C-methylcytidine [99, 101-110]. A range of NTZ derivatives have been tested for antiviral activity, from which some were found to be more effective than the parent compound [98, 110].

The antiviral mechanism of action of nitazoxanide is different from the mechanism of action against protozoa and anaerobic bacteria *via* direct inhibition against the pyruvate-ferrodoxin oxidoreductase reaction. A pilot clinical study suggested that NTZ can augment the antiviral effect of interferon. NTZ mechanism of action also involves increased activation (autophosphorylation) of protein kinase activated by double-stranded RNA (PKR), an interferon-induced mediator of the cellular antiviral response. By this way NTZ promoted PKR, a key step in activating PKR´s kinase activity for eIF2 alpha. This eukaryotic initiation factor alpha phosphorylation, a modification known to mediate host cell antiviral defenses, lead to inhibition of translation initiation. NTZ-induced eIF2 alpha phosphorylation was reduced in the presence of specific inhibitors of PKR autophosphorylation. Due to described processes targeting host functions NTZ enhances natural cellular antiviral mechanisms and the development of antiviral resistance is significantly more difficult than for drugs directly targeting a viral function or enzymes [101, 111].

After serial passage in increasing concentrations of NTZ or TIZ replicon cell exhibited lower phenotypic sensitivities to the cytotoxic and antiviral effects. The susceptibilities of HCV resistant replicons to other antivirotics did not change; moreover the sensitivity to interferon was increased. However, it was not possible to transfer the TIZ-resistant phenotype to naive cells by transfection of HCV RNA from these lines indicating that primary resistance was conferred by changes in the host, not the virus [112-113].

NTZ has been described *in vitro* ($EC_{50}$ 0.5 μg/mL) and in clinics to be efficacious in the treatment and cytoprotection of rotaviruses or noroviruses diarrhoea and gastroenteritis [114-116].

NTZ, TIZ and second generation thiazolides were described being effective against influenza A [98, 117] virus (H1N1 PR8, WSN and A/FI human strains and avian A/Ck strain). Thiazolides produced inhibition of the replication and they act at post-translational level by selectively blocking the maturation of the viral hemagglutinin, thus impairing hemagglutinin intracellular trafficking and insertion into the host plasma membrane, a step necessary for correct assembly. Also the viral exit from the cell is prevented. NTZ showed $EC_{50}$ from 0.5 to 1.0 μg/mL, TIZ was similarly very active, additionally a very effective inhibited replication of influenza B strain B/Parma/3/04. 5-desnitro-5-methylsulfonyl derivative of NTZ was found to be 10 times active than parent compound or TIZ [117].

About activity against other viruses, thiazolides were referred to affect at micro molar concentrations, which are non-toxic to uninfected cells, many viruses including simian SA11 rotavirus, Sendai virus, respiratory syncytial virus, coronavirus, vesicular stomatitis virus, adenovirus (Ad5), paramyxovirus and herpes simplex virus type 1 (HSV-1). $IC_{50}$ and SI varied between 0.5 and 2.0 μg/mL and 25 and > 100, respectively [98]. On the other side, they did not significantly affect the replication of human rhinovirus and picornavirus [117].

NTZ and some of its modifications are effective in the treatment of a wide range of human parasitic and protozoal infections [99, 110, 118-123], especial gastrointestinal, and infections caused by anaerobic bacteria [99, 119-120, 124-129]. NTZ (and its metabolite TIZ) could be also useful as antiviral and antiprotozoal agent in the veterinary medicine [130-134].

The wide publicity and presentation of very good properties of NTZ and TIZ evoke same questions and equivocalness which is necessary to answer before routinely usage [100, 135-137].

## 3. CONCLUSION

Therapy of infections caused by different viruses is still challenging and there exists a strong objective need for a development of novel agents. No sufficient pharmacotherapeutic arsenal for the treatment of many serious infection diseases is available. The review deals with the antiviral activity of structures containing salicylamide (2-hydroxybenzamide) and salicylanilide (2-hydroxy-*N*-phenylbenzamide) moieties. In general, salicylanilides have been studied for many years showing a wide spectrum of interesting biological activities with a perspective potential usage.

Salicylamides (including salicylanilides) have shown a suppression inhibitory activity against different virus species – coronaviruses, HCV virus (targeted on NS3-NS4A protease and NS5B polymerase), herpes viruses, influenza viruses, or HIV virus (where could affect HIV-1 integrase and reverse transcriptase). We are presenting a survey of findings in the area of their antiviral activity in the last fifteen years.

The most promising compounds with some completed clinical trials and now undergoing next clinical testing are nitazoxanide and its metabolite tizoxanide as a broad-spectrum of thiazolide antivirotics with a unique mechanism of action. They induce of IF2 phosphorylation *via* activation of PKR, key mediators of the intracellular host antiviral response.

Thus the field of design, synthesis and evaluation of new perspective antiviral agents bring many opportunities. Based on presented knowledge, the investigation of salicylanilide and salicylamide derivatives may reveal another derivatives with a better activity (special need exists for hepatitis and HIV viruses) or compounds that affect new viral species.

## ACKNOWLEDGEMENTS

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