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Research paper

Insight into non-nucleoside triazole-based systems as viral polymerases inhibitors

Roberta Bivacqua [a], Marilia Barreca [a], Virginia Spanò [a], Maria Valeria Raimondi [a,*], Isabella Romeo [b,c], Stefano Alcaro [b,c], Graciela Andrei [d], Paola Barraja [a], Alessandra Montalbano [a]

[a] Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Via Archirafi 32, 90123, Palermo, Italy
[b] Dipartimento di Scienze della Salute, Università Magna Græcia, Viale Europa, 88100, Catanzaro, Italy
[c] Net4Science srl, Academic Spinoff, Università Magna Græcia, Viale Europa, 88100, Catanzaro, Italy
[d] Laboratory of Virology and Chemotherapy, Rega Institute for Medical Research, KU Leuven, 3000, Belgium

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ABSTRACT

Viruses have been recognized as the etiological agents responsible for many pathological conditions ranging from asymptomatic infections to serious diseases, even leading to death. For this reason, many efforts have been made to identify selective viral targets with the aim of developing efficient therapeutic strategies, devoid of drug-resistance issues. Considering their crucial role in the viral life cycle, polymerases are very attractive targets. Among the classes of compounds explored as viral polymerases inhibitors, here we present an overview of non-nucleoside triazole-based compounds identified in the last fifteen years. Furthermore, the structure-activity relationships (SAR) of the different chemical entities are described in order to highlight the key chemical features required for the development of effective antiviral agents.

1. Introduction

Viral infections still represent one of the major threats for human health as proved by the high number of emerging and re-emerging viral infections that global community has faced in the last decades. Taking into consideration the most significant outbreaks since 2002, SARS (severe acute respiratory syndrome), MERS (Middle East respiratory syndrome), EBOV (Ebola virus), ZIKV (Zika virus) and the recent COVID-19 have followed one another [1]. Despite the progress made in the discovery of new antiviral agents, many viral infections still lack a specific treatment and drug-resistance issues often make the therapeutic approach even more difficult. Therefore, the identification of efficient therapeutic strategies is still an unmet medical need that has yet to be addressed.

Knowledge of the complex mechanisms involved in the viral life cycle plays a fundamental role in the identification of promising therapeutic targets to be addressed to develop new antiviral agents. Due to their key function in viral genome replication, polymerases are very attractive targets. In particular, among the different families of polymerases identified so far, a relevant role is played by the template-directed nucleic acid polymerases (TdPPs), which include DNA-dependent DNA-polymerase (DdDP), RNA-dependent DNA-polymerase (RdDP or Reverse Transcriptase), DNA-dependent RNA-polymerase (DdRP) and RNA-dependent RNA-polymerase (RdRP) [2,3]. Based on their structure, all these polymerases are referred to as "right-hand polymerases", due to their shape that resembles a cupped right hand with "fingers," "palm," and "thumb" subdomains, each contributing to the correct arrangement of the substrate and metal ions within the catalytic site of the enzyme [2,4–6].

Small molecules based on heterocyclic core represent a valuable tool in the identification of bioactive entities, thus allowing the development of new therapeutic agents [7–11]. Among nitrogen-containing heterocycles, triazoles have received considerable attention due to their chemical versatility, coupled with the wide range of biological activities as anti-cancer [12], anti-inflammatory [13], antitubercular [14], anti-leishmanial, antitrypanosomal [15], antimalarial [16], antimicrobial [17], antibacterial [18] and antiviral [19–33]. As a matter of fact, Ribavirin (RBV) and Taribavirin (a prodrug of ribavirin) are triazole derivatives clinically used in the treatment of viral infections caused by a number of RNA viruses [34]. As other nucleoside-based compounds,

* Corresponding author.
E-mail address: mariavaleria.raimondi@unipa.it (M.V. Raimondi).

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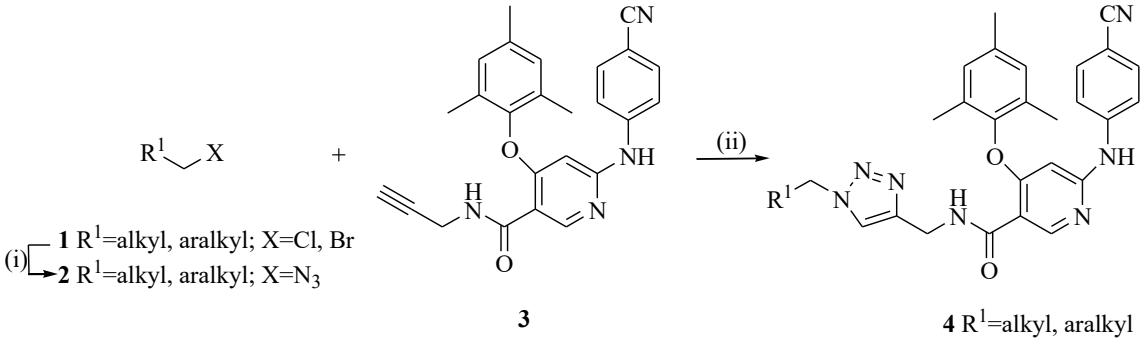
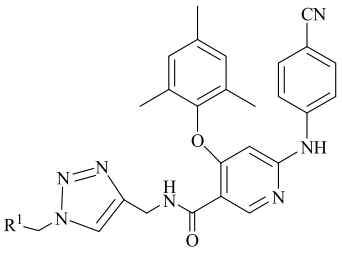
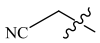
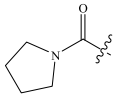
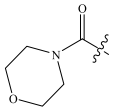


Scheme 1. Reagents and conditions: (i): NaN_3 (ii): $CuSO_4$, VcNa, THF-H_2O, 65 °C.

Table 1
Antiviral activity against IIIB and RES056 HIV-1 strains, cytotoxicity and SI of **4a-c** in MT-4 cells.



4a-c

Compd	R^1	EC_{50} (μM)		CC_{50} (μM)	SI	
		III_B	RES056		III_B	RES056
4a		0.020 ± 0.005	2.31 ± 0.57	40.15 ± 16.62	2044	17
4b		0.020 ± 0.005	1.43 ± 0.31	58.09 ± 34.60	2097	41
4c		0.020 ± 0.009	15.70 ± 7.16	180.9 ± 36.74	9279	12
NPV	–	0.26	>15.02	>15.02	>58	–
DLV	–	0.038	–	36.8	733	–

they need to be activated inside the cell through consecutive phosphorylation reactions. Unfortunately, as many other nucleoside analogues, they often have several disadvantages, such as low bioavailability, high toxicity and, most notably, the tendency to develop drug resistance [35].

Many non-nucleoside compounds have been designed in order to try to solve these problems. In this review we will focus on non-nucleoside triazole-based compounds targeting viral polymerases identified in the last fifteen years. Both 1,2,3- and 1,2,4-triazoles will be considered along with triazole-condensed systems.

2. 1,2,3-Triazole-based compounds

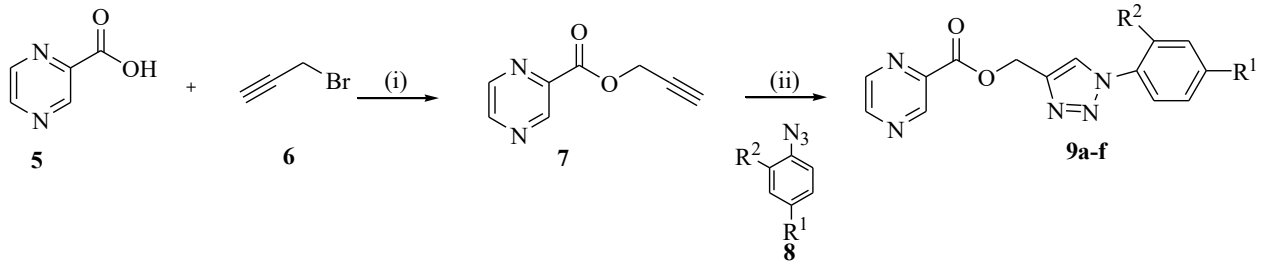
Several 1,4-disubstituted-1,2,3-triazoles with antiviral activity have been described in the literature. Among them, diarylnicotinamide 1,4-disubstituted 1,2,3-triazoles were identified as HIV-1 non-nucleoside reverse transcriptase inhibitors (NNRTI) [22]. A series of 23 compounds was initially synthesized, starting from the proper alkyl or aralkyl halide (**1**) which, upon nucleophilic substitution with sodium azide, gave the correspondent azides (**2**) (Scheme 1). Subsequent copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) "click chemistry" reaction with **3**, properly synthesized [36] led to the desired compounds **4**.

Derivatives **4** were evaluated for their antiviral activities against the wild-type (WT) HIV-1 strain IIIB, and the K103 N + Y181C double reverse transcriptase (RT) HIV-1 mutant (RES056) and the WT HIV-2 strain ROD in MT-4 cells using the tetrazolium-based colorimetric (MTT) method. Although no activity was found against the HIV-2 strain, all compounds exhibited good anti-HIV-1 (WT) activity with 50% effective concentration (EC_{50}) values ranging from 0.02 μM to 0.85 μM. Among triazole derivatives **4**, none of the substituted benzyl derivatives displayed activity against the RES056 strain (data not shown), differently from the alkyl sub-series showing EC_{50} values from 1.43 μM to 15.70 μM (Table 1). In particular, the most active compounds were **4a**, **4b** and **4c** bearing a cyanomethyl, a 2-oxo-2-(pyrrolidin-1-yl)ethyl and a 2- morpholino-2-oxoethyl moiety, respectively. They proved to be active against IIIB HIV-1 strain at 20 nM, and against RES056 strain with EC_{50} of 2.31 μM (**4a**), 1.43 μM (**4b**) and 15.70 μM (**4c**) (Table 1). Interestingly, compared to the reference non-nucleoside reverse transcriptase inhibitors (NNRTI's) Nevirapine (NPV) (EC_{50} = 0.26 μM) and Delavirdine (DLV) (EC_{50} = 0.038 μM), these derivatives proved to be more potent and less cytotoxic (CC_{50} = 40.15, 58.09 and 180.9 μM respectively), with high selectivity index (SI, ratio CC_{50}/EC_{50}) values.

Furthermore, compounds **4a-c** were tested against six different HIV-1 reverse transcriptase mutants (L100I, K103 N, E138K, Y181C, Y188L and F227L + V106A), showing good activity against most of the mutants, with high potency and low cytotoxicity (Table 2).

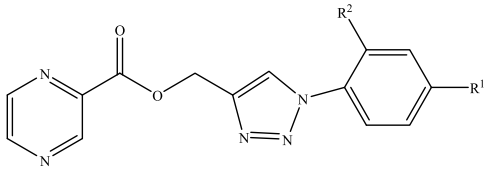
Table 2
Antiviral activity of **4a-c** against HIV-1 reverse transcriptase mutants in MT-4 cells.

Compd	EC_{50} (μM)					
	L100I	K103 N	E138K	Y181C	Y188L	F227L + V106A
4a	1.06 ± 0.22	0.25 ± 0.028	0.015 ± 0.003	0.089 ± 0.004	2.24 ± 0.070	0.33 ± 0.21
4b	0.96 ± 0.12	0.27 ± 0.006	0.014 ± 0.001	0.085 ± 0.020	1.48 ± 0.081	0.24 ± 0.008
4c	6.13 ± 0.17	0.38 ± 0.048	0.027 ± 0.001	0.24 ± 0.085	7.16 ± 0.46	0.61 ± 0.007
NPV	0.83 ± 0.32	6.78 ± 0.08	0.23 ± 0.05	6.21 ± 0.08	9.61 ± 1.86	7.17 ± 1.86
DLV	2.54 ± 0.31	3.00 ± 0	0.051 ± 0.006	2.46 ± 0.54	0.84 ± 0.01	2.27 ± 0.14



Scheme 2. Reagents and conditions: (i) K_2CO_3, DMF, rt; (ii) Sodium D-isoascorbate, $CuSO_4$. $5H_2O$, *n*-butanol/H_2O, MW, 100 °C, 2 h.

Table 3
Antiviral properties of pyrazine-triazole conjugates **9a-f** against SARS-CoV-2.



Compd	R^1	R^2	IC_{50} (μM)	CC_{50} (μM)	SI
9a	F	H	12.223	4.098	0.3
9b	Cl	H	1.150	1.961	1.7
9c	OCH_3	H	0.120	0.378	3.2
9d	NO_2	H	0.477	4.916	10.3
9e	H	Cl	0.952	3.508	3.7
9f	H	OCH_3	1.079	2.308	2.1
Favipiravir	–	–	1.382	5.262	3.8

An enzymatic assay against HIV-1 RT was performed on the most promising compounds **4b** and **4c** (according to their lowest cytotoxicity and their best SI against RES056 and HIV IIIB strains respectively), using Etravirine (ETR) as a reference drug. Both 1,2,3,triazole derivatives showed good activity, with IC_{50} values of 2.70 and 1.57 μM respectively, compared to ETR [IC_{50} (50% inhibitory concentration) value of 0.75 μM]. Molecular modeling studies performed on compounds **4b,c** and Etravirine into the NNRTIs binding pocket in WT and E138K mutated RT, revealed that the 2,4,6-trimethyl-phenoxy group and the 4-cyanoaniline moiety established key interactions with two hydrophobic pockets in the binding site. The side chain on the key residue K101 in WT RT was also involved in a hydrogen bond with the amide NH of derivatives **4b,c** in which pyrrolidine and morpholine groups respectively established additional key hydrophobic interactions, thus increasing the binding affinity to RT. All reported results confirmed that these compounds targeted HIV-1 RT, thus acting as NNRTIs [22].

Another class of 4-yl-methylen-heteroaryl decorated molecules was designed using a molecular hybridization approach. In particular, a set of pyrazine-triazole conjugates with a significant SARS-CoV-2 RdRp activity were identified [37]. The desired pyrazine-triazole conjugates **9a-f** were synthesized in a two-step reaction sequence from pyrazinoic acid **5**. Upon treatment with propargyl bromide (**6**) in presence of potassium carbonate, the pyrazine-based alkyne **7** was obtained and subsequent click chemistry reaction with substituted aromatic azides **8,** properly synthesized [38], allowed the isolation of the pyrazine-triazole conjugates **9a-f**, as shown in Scheme 2.

Their antiviral and cytotoxic properties and selectivity indexes were evaluated in VERO-E6 cell line. Among all tested derivatives, **9d** was found to be the best compound, with high potency (IC_{50} = 0.477 μM), low cytotoxicity (CC_{50} = 4.916 μM) and high selectivity index (SI = 10.3), almost three times higher than the control drug Favipiravir (IC_{50} = 1.382; CC_{50} = 5.262 μM; SI = 3.8). Even though compound **9c** exhibited the lowest IC_{50} value (0.120 μM) and therefore the greatest potency, it showed a lower selectivity index, thus confirming **9d** as the best compound (Table 3).

From a structure-activity relationship (SAR) it was possible to assess that:

- *para* methoxy group (R^1) at N-1 phenyl ring, seems to be essential for antiviral activity since its replacement with a chlorine or fluorine atom caused a reduction in activity (**9a** and **9b**).
- *ortho*-substitution (R^2) showed an opposite trend: the presence of a chlorine atom led to an increase in antiviral activity and to an improvement of SI compared to the methoxy substituted derivative (compare **9e** with **9f**).

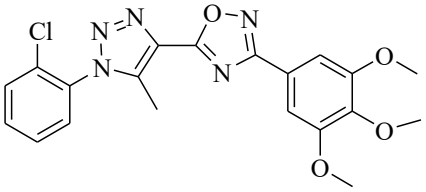


Fig. 1. Chemical structure of **Triazole-1**.

QSAR and molecular modeling studies were also performed using a co-crystalized model of RdRp with Favipiravir (PDB: 7CTT). The favorable interactions that derivative **9c** was able to establish with key amino acids residues of the enzyme active site (ARG555, CYS622, LYS798 and LYS621) were in accordance with the high activity expressed. Finally, *in silico* absorption, distribution, metabolism, and excretion (ADME) studies provided promising results, showing good water solubility and intestinal absorption for most of the tested compounds, indicating 90–95% plasma protein binding [37].

Among1,4-disubstituted-1,2,3-triazoles, **Triazole-1** (Fig. 1), emerged as an interesting hit from a high-throughput screening (HTS), performed with the aim of identifying novel respiratory syncytial virus (RSV) inhibitors [39]. Starting from a Roche internal library of about 870,000 compounds, tested at a single concentration of 10 μM, using the cytopathic effect (CPE) reduction assay on HEp-2 cells, **Triazole-1** exhibited an EC_{50} value of about 1.0 μM against both RSV A and B subtypes, without displaying any toxicity for concentrations up to 100 μM.

A time-dependent drug addition experiment was in accordance with the ability of **Triazole-1** to interfere with genome transcription and/or replication, after viral penetration in a dose-dependent manner. The viral molecular target was finally identified through deep sequence analysis of the genome a **Triazole-1**-resistant mutant virus (referred to as Triazole-1-p9) showing a single point mutation (A to G) at nucleotide 13,546, leading to an amino acid substitution (Thr to Ala) at position 1684 of the RSV L protein (RNA polymerase for both viral transcription and replication). When the effects of **Triazole-1** were evaluated on minigenome transcription using the mutant T1684A L protein, the compound proved to be significantly less potent. Furthermore, the

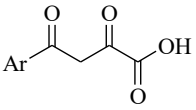
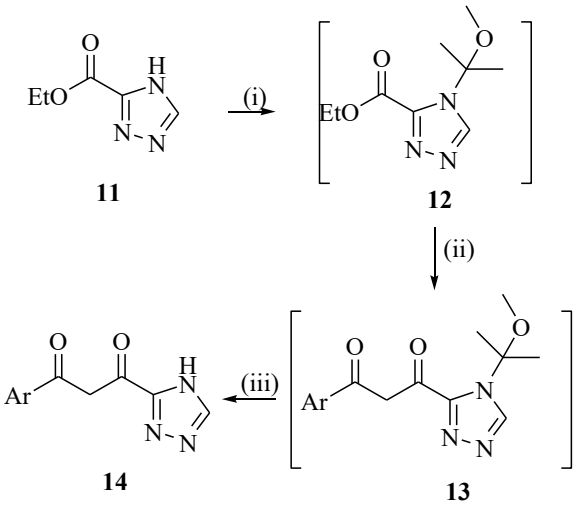


Fig. 2. Chemical structure of aryl α,γ-diketo acids **10**.



Scheme 3. Reagents: (i) 2-methoxypropene, *p*-TSA, THF; (ii) aryl methyl ketone, NaOEt; (iii) 4 N HCl (aq).

previously reported replication inhibitor (BI compound D) of the RSV RdRp remained active against the triazole-1-p9 mutant. Altogether, these data indicated that **Triazole-1** was able to bind the amino acid residue L1684 of the RSV polymerase, which is different from that targeted by other classes of inhibitors [40–42].

3. 1,2,4-Triazole-based compounds

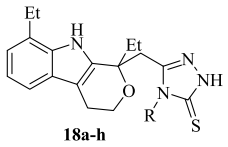
1,2,4-triazoles are structural isomers of 1,2,3-triazoles with widely documented antiviral activity [31,33,43].

With the aim of improving stability and cellular permeability of aryl α,γ-diketo acids of type **10** (Fig. 2), which are well-known inhibitors of the active site of Hepatitis C (HCV) polymerase NS5B [44], a series of diketo triazoles were synthesized as potential bioactive bioisosteres [45].

The desired compounds were obtained by reacting 1,2,4-triazole-3-carboxylate (**11**) and 2-methoxypropene in the presence of p-toluenesulfonic acid (p-TSA), thus leading to the intermediate **12** which, through a Claisen condensation with properly substituted aryl methyl ketones, afforded diketo triazoles **13**. Subsequent deprotection in acidic media allowed the isolation of the desired compounds of type **14** (Scheme 3).

All compounds were screened for their activity against HCV, using RG7128 (an oral prodrug of PSI-6130, which is a deoxycytidine analog

Table 4
Anti-HCV NS5B activity of etodolac 1,2,4-triazole derivatives **18a-h**.



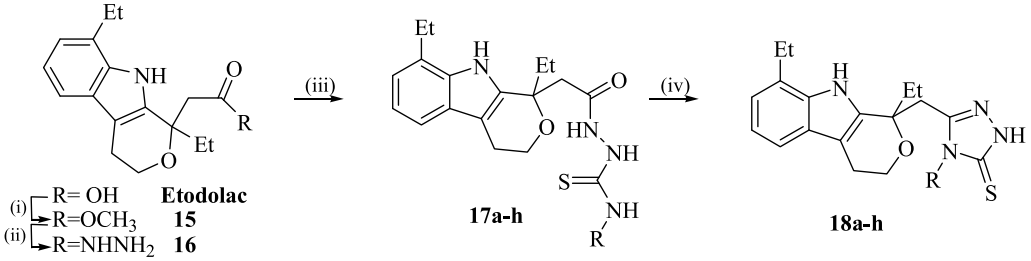
18a-h

Compd	R/Ar	Anti-NS5B activity (% inhibition, 100 μM)	IC_{50} (μM)
18a	$-CH_3$	78.9 ± 0.7	14.8 ± 1.2
18b	$-CH_2CH_3$	74.6 ± 1.1	21.6 ± 1.3
18c	$-C_3H_7$	69.0 ± 0.1	26.0 ± 1.3
18d	$-C_4H_9$	72.3 ± 0.4	25.4 ± 1.3
18e	$-CH_2{=}CHCH_2$	58.2 ± 0	40.3 ± 1.2
18f	$-CH_2C_6H_5$	3.78	–
18g	$-C_6H_5$	49.5 ± 0.1	–
18h	$-C_6H_{11}$	45.6	–

inhibiting HCV NS5B RNA polymerase) as a positive control [46] and for their cytotoxicity on the human hepatoma cell lines Huh-7. Among the tested derivatives, compound **14a** (Ar = 4-(4-fluorobenzyloxy)phenyl) emerged as the most potent, with an EC_{50} value of 3.9 μM, no cytotoxicity at 500 μM and high selectivity index (SI > 128). Western blot analysis with a specific antibody against the viral non-structural protein NS5A and quantitative RT-PCR were also performed, showing the ability of compound **14a** to reduce the viral protein and mRNA levels in a dose-dependent manner [45].

A novel series of 1,2,4-triazoles derivatives with a promising activity against HCV NS5B polymerase has also been synthesized as part of a study aimed at identifying new agents with both anti-HCV and anticancer activities [47]. In fact, viral hepatitis infection (hepatitis B or C) can often result in the onset of hepatocellular carcinoma [48]. Since cyclooxygenase (COX)-2 upregulation was also observed in HCV-induced hepatocellular carcinogenesis [49], Etodolac, a nonsteroidal anti-inflammatory drug with selective COX-2 inhibitory activity [50], exhibited anti-HCC properties at physiological doses [51–53], thus proving to be an interesting starting point for the development of new anti-cancer agents also endowed with anti-HCV NS5B polymerase activity.

The synthetic pathway used for the novel etodolac 1,2,4-triazole derivatives is reported in Scheme 4. Methyl(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-*b*]indole-1-yl)acetate **15** (R = OCH_3) was obtained from the reaction of **etodolac** (R = OH) with methanol in the presence of sulfuric acid. Subsequent condensation with hydrazine-hydrate afforded the acetohydrazide **16** (R = $NHNH_2$) [54], which was subsequently reacted with alkyl/aryl isothiocyanates in ethanol. Final cyclization of etodolac thiosemicarbazides **17a-h** in the presence of NaOH allowed the isolation of the desired 4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **18a–h**.



Scheme 4. Reagents: (i) CH_3OH/H_2SO_4; (ii) NH_2NH_2 . H_2O; (iii) R(Ar)-NCS/C_2H_5OH; (iv) NaOH (2 N), HCl.

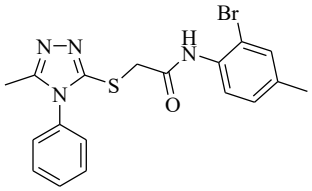


Fig. 3. Chemical structure of **VRX-387902** (**19**).

Studies on their activity against HCV NS5B polymerase indicated that five of them (**18a,b,c,d,e**) displayed ≥50% inhibition of NS5B polymerase activity. Further biological screenings established **18a** as the most potent of the series, with IC_{50} = 14.8 μM (Table 4).

Docking studies on NS5B TP-II site of both *R*- and *S*-isomers of **18a** showed that *S*-isomer forms key hydrogen bonds with specific active site residues, such as Tyr477 and Ser476, not observed in the *R*-isomer, thus suggesting that *S*-isomer could be responsible for the NS5B inhibitory activity of racemic **18a**. Considering that compound **18a** also exhibited anti-cancer properties, with EC_{50} value of 4.29 μM against Huh7 cell line, it can be considered as a promising hit for the development of new agents endowed both with anti-hepatitis C and anti-cancer activity [47].

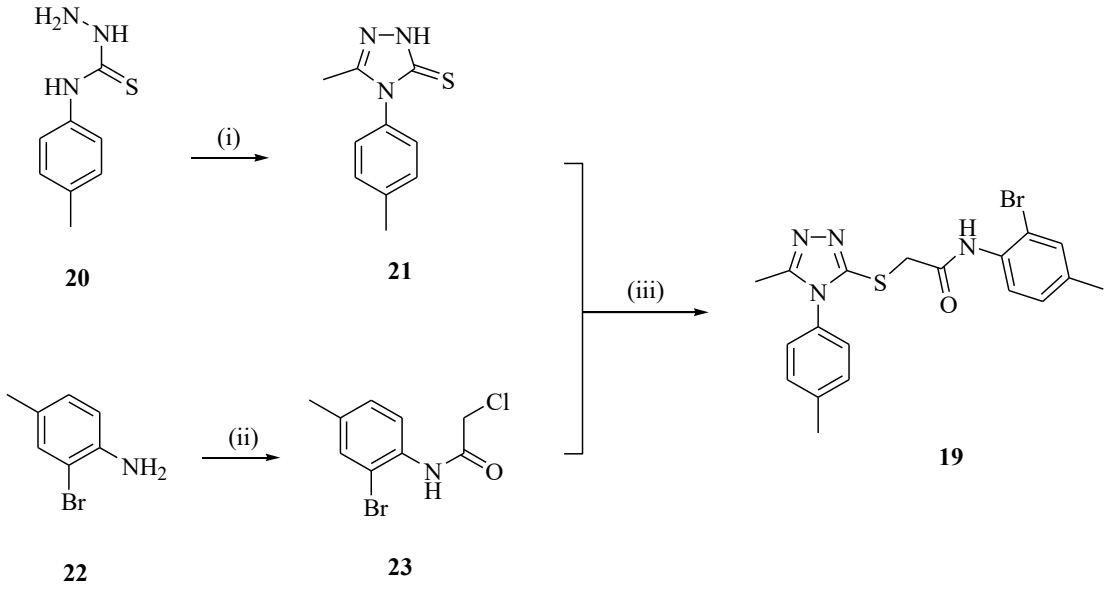
Furthermore, 1,2,4-triazoles proved to be a valuable heterocyclic ring in the development of anti-HIV agents. With the aim of identifying novel HIV-1 NNRTIs, a high-throughput screening (HTS) was performed. Starting from a library of about 87.000 compounds, using a cell-based assay, triazole **19** (**VRX-387902**) (Fig. 3) emerged as an active agent with micromolar activity in inhibiting the replication of WT (EC_{50} = 0.1 μM) and K103 N/Y181C double HIV-1 RT mutant (EC_{50} = 1.3 μM). It also showed high inhibiting activity in the purified WT HIV-1 RT enzymatic assay [55].

The synthesis of derivative **19** started from a ring cyclization of the thiosemicarbazides **20** with ethyl acetate to afford the corresponding triazoles **21**. Condensation of substituted aniline **22** with chloroacetyl chloride in dichloromethane, in the presence of diisopropylethylamine, allowed the isolation of intermediate **23**, which was then reacted with triazoles **21** in the presence of potassium carbonate to obtain the desired compound **19** (Scheme 5) [55].

In order to improve the activity against K103 N/Y181C double mutant HIV-1 strains, molecular optimization was attempted, thus leading to the identification of **VRX-480773** (**24**) (Fig. 4).

Compound **24** was synthesized as depicted in Scheme 6. Condensation of thiosemicarbazone **26** [56] with the proper substituted naphthylamine **27** allowed to obtain compound **28**, which was then reacted with intermediate **29** (synthesized as previously reported [57]) to yield the title compound **24** [55].

SAR analysis showed that switching the N-4 phenyl ring with a naphthyl group enhanced the antiviral activity, especially when a substituent at position 4 was present. An *ortho* halogen substitution on the aniline seemed crucial for the activity, as the best results were obtained



Scheme 5. Reagents and conditions: (i) CH_3COOEt, EtOH; MeONa, reflux; (ii) chloroacetyl chloride, DIPEA, CH_2Cl; (iii) K_2CO_3, DMF, rt.

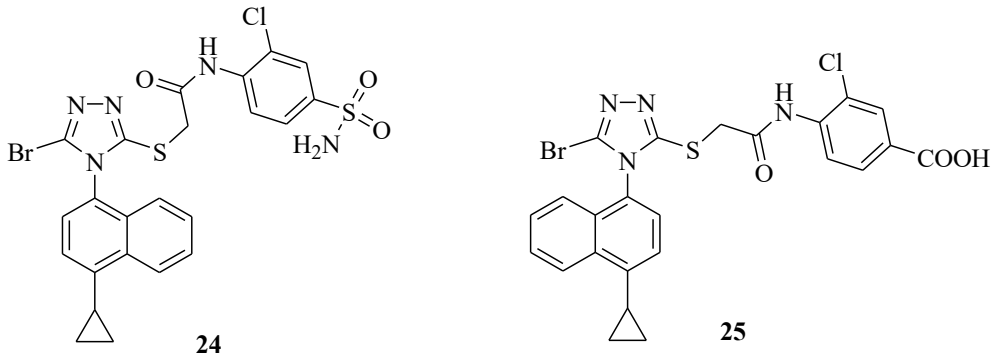
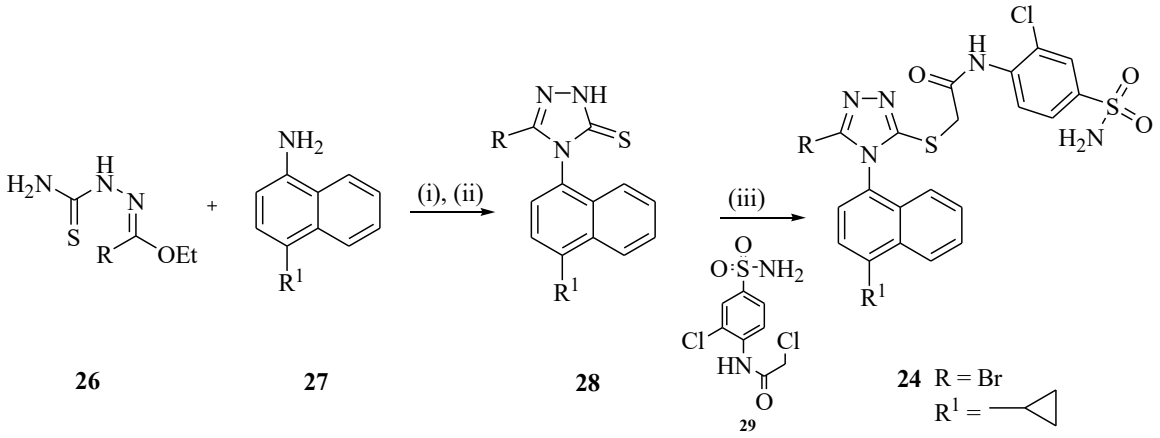


Fig. 4. Chemical structures of **VRX-480773** (**24**) and **RDEA806** (**25**).



Scheme 6. Reagents and conditions: (i) DMF, reflux; (ii) NaOH 1 N, 40 °C; (iii) K_2CO_3, DMF, rt.

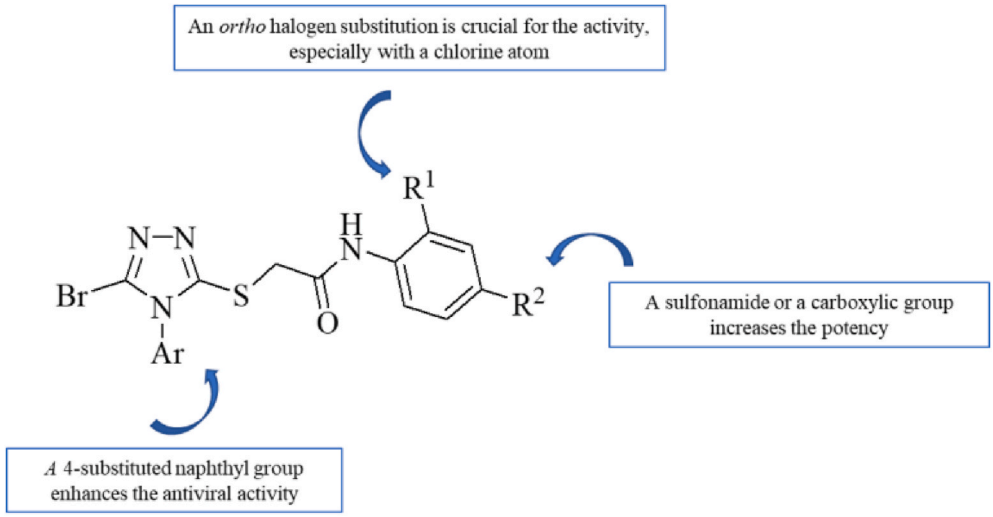


Fig. 5. Structural requirements of **VRX-480773** analogues.

when the bromine was replaced by a chlorine atom and a sulfonamide group was introduced in position 4 (R^2) of the aniline (Fig. 5).

VRX-480773 exhibited an increased *in vitro* activity, with EC_{50} values of 0.14 nM against WT HIV-1 and of 0.23 nM for K103N–Y181C RT mutant, and low cytotoxicity (CC_{50} of 3 to >100 μM). In addition, enzymatic experiments were carried out, showing that VRX-480773 inhibits WT HIV-1 RT activity with IC_{50} value of 4.0 nM, without inhibiting human DNA polymerases at up to 45 μM, thus proving to be a potent and specific HIV-1 RT inhibitor [58].

Replacement of the sulfonamide group of VRX-480773 with a carboxylic group led to **RDEA806 (25)** (Fig. 4), a novel HIV-1 NNRTI with potent *in vitro* activity against WT HIV-1 (EC_{50} = 3.05 nM) and NNRTI-resistant mutant strains. RDEA806 entered and completed the phase IIa clinical trials (https://go.drugbank.com/drugs/DB05228). Treatment with RDEA806 was well tolerated, proving it to be an interesting candidate for further clinical studies [59,60].

4. Condensed triazole systems

A commonly used strategy in drug development is the use of heterocyclic moieties to enhance the biological effect and drug-like properties of new scaffolds [61–65].

[1,2,4]Triazolo[1,5-*a*]pyrimidines have emerged as promising potent HCV polymerase NS5B inhibitors (PF-00868554 or Filibuvir) [66], targeting an allosteric site in the thumb domain of the enzyme [67].

In particular, starting from derivative **30** identified from a high-throughput screening, with potent inhibitory activity of the HCV NS5B

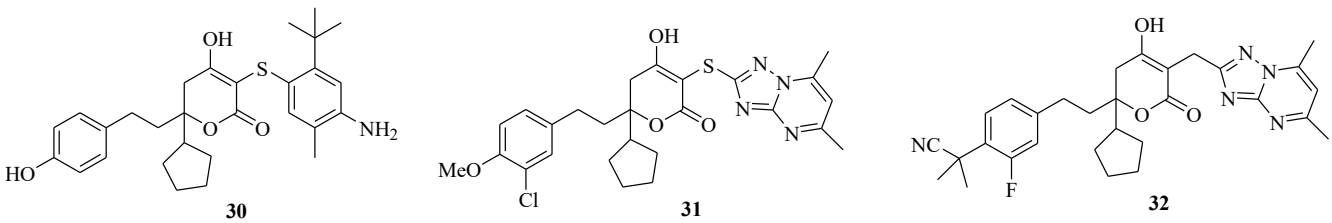
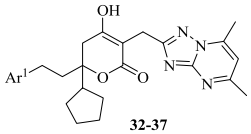
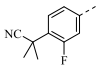
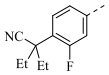
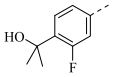


Fig. 6. Chemical structures of dihydropyrones **30-32**.

Table 5
Antiviral activities and CYP2D6 inhibitory profiles of C-linked dihydropyrones **32-37**.



32-37

Compd	Ar^1	CYP2D6 IC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
32		0.30	0.003	0.015	>32
33		>6	0.005	0.016	>32
34		>6	0.14	0.059	>32
35		–	0.016	0.067	>32
36	Enantiomer 1 of **35 (R)**	–	0.007	0.041	320
37	Enantiomer 2 of **35 (S)**	–	0.012	0.31	320

polymerase (IC_{50} value of 0.93 μM) [68], a novel class of dihydropyrones incorporating the [1,2,4]triazolo[1,5-*a*]pyrimidines system was synthesized leading to compound **31**. The latter exhibited potency at nanomolar level in the biochemical assay (IC_{50} = 36 nM), but only moderate micromolar activity in the cell-based replicon assay (EC_{50} = 3.25 μM). ADME pharmacokinetics studies also revealed its low bioavailability and poor absorption after oral administration. In the attempt of achieving compounds with good potency in both biochemical and cell-based replicon assays, with favorable pharmacokinetics profiles, a series of carbon-linked dihydropyrones was synthesized, leading to compound **32**, meeting both of these requirements (Fig. 6). Structure-activity relationship (SAR) studies highlighted that the presence of a fluorine atom at the *meta* position of the aromatic ring on the left-hand side of the molecule produced an increase in potency compared to a chlorine atom or a methyl group, as well as the *gem*-dimethyl-cyano group at the *para* position, involved in direct hydrogen bond interaction with Leu497 of the enzymatic binding site. Furthermore, both the fluorine and one of the *gem*-dimethyl groups occupied two small hydrophobic pockets formed by adjacent amino acid residues [69].

Unfortunately, compound **32** displayed strong inhibition of the cytochrome P450 isozyme 2D6 (CYP2D6), which plays an important role in the oxidation of xenobiotics [70]. Further optimization of C-linked dihydropyrones was therefore necessary. While structural modifications on the triazolopyrimidine system did not lead to any improvement in terms of CYP2D6 inhibition, the replacement of the *gem*-dimethyl-cyano group with a *gem*-diethyl-cyano or hydroxyl group (compounds **33** and **34**, Table 5) at the phenyl ring (Ar^1) caused a significant reduction in CYP2D6 inhibition. As a matter of fact, the cyano group was primarily responsible for the interaction with the CYP2D6, therefore its removal or the introduction of sterically hindered substituent, reduced the undesired inhibitory effect on the cytochrome, without compromising the potency against the HCV polymerase. Finally, the replacement of the phenyl ring with a pyridine one, allowed to obtain an optimized series of derivatives with no CYP2D6 inhibitory properties, leading to the identification of compound **35** and, more specifically, of its enantiomer 1 (R) **36** (PF-00868554), as a potent and selective NS5B polymerase inhibitor (IC_{50} = 0.007 μM, EC_{50} = 0.041 μM, CC_{50} = 320 μM) with good pharmacokinetic properties. The X-ray co-crystal structure of **36** bound to HCV polymerase (PDB code 3FRZ) was also examined, suggesting that the presence of a hydrophobic interaction between one of the pyridine ethyl groups of compound **36** and the enzymatic binding site balances the loss of the hydrogen bond between the Leu497 residue and the cyano group of compound **32** [66].

Preclinical data showed that racemic PF-00868554 was a potent and selective *in vitro* inhibitor of the 1a and 1b genotypes of HCV RdRp derived from a majority (95.8%) of HCV strains, without displaying inhibition of several human polymerases or proteases. Generation of PF-00868554-resistant replicon cells selected M423T as the predominant NS5B resistance mutation, resulting in a marked reduction in sensitivity to PF-00868554 but not to other inhibitors targeting different regions of the polymerase, supporting the use of PF-00868554 in combination therapies, due to the absence of cross-resistance. *In vivo* studies were also carried out in rodent and non-rodent species, showing good pharmacokinetic properties compatible with a potential clinical use of PF-00868554 in human patients with a certain margin of safety [71]. PF-00868554, also known as Filibuvir, passed a phase II clinical evaluation in patients with chronic Hepatitis C genotype 1 infection, although its clinical development program was then suspended [72].

[1,2,4]Triazolo[1,5-*a*]pyrimidine is a recurrent scaffold in compounds endowed with antiviral activity targeting polymerases. **Mol-5** is a novel DENV NS5-RdRp inhibitor identified [73] from an *in silico* virtual screening performed on the ChemDiv Company antiviral library (~8000 molecules, http://www.chemdiv.com/antiviral-library) using the DENV2 NS5-RdRp crystal structure (PDB code 5K5M). Among the ~8000 compounds investigated in this study, **Mol-5** (Fig. 7) emerged as an interesting compound, which was further evaluated in *in vitro* assays.

Biophysical analysis (SPRi) and cell-based assays confirmed that **Mol-5** directly inhibits DENV2 RdRp, with an IC_{50} value of 1.28 μM. CPE reduction assays were also performed on mammalian BHK-21 cells to evaluate the antiviral effects of **Mol-5**, showing an interesting EC_{50} value of 4.5 ± 0.08 μM CC_{50} value of 66.0 ± 0.04 μM was evaluated by MTT assay thus allowing to assess a selectivity index (SI) of 14.7.

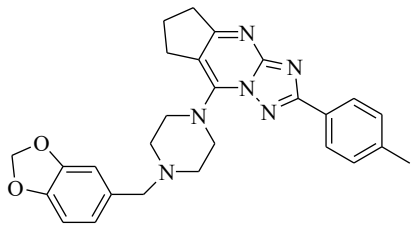


Fig. 7. Chemical structure of **Mol-5**.

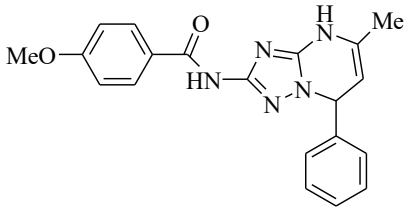


Fig. 8. Chemical structure of compound **38**.

Moreover, immunofluorescence analysis revealed that **Mol-5** reduced dsRNA production, resulting in a decrease in progenitor viral particles. To confirm that **Mol-5** inhibited DENV2 RNA replication after infection, a time of addition assay was performed. No effect was found at the pretreatment and co-incubation stages, suggesting that **Mol-5** had no virucidal activity and was not involved in viral attachment or entry processes. Conversely, a significant reduction in anti-DENV activity was found at post-treatment stage, thus supporting the hypothesis that **Mol-5** exerted its antiviral effect by inhibiting the RdRp. A plaque assay established the specific antiviral time window of **Mol-5** between 0 and 4 hpi, demonstrating the ability of compound to interfere with the early stages of viral replication. Furthermore, Western blotting experiments showed that **Mol-5** could decrease the viral structural (E) and non-structural (NS1) protein levels in a dose-dependent manner. Finally, **Mol-5** exhibited an anti-inflammatory effect mediated by the reduction of DENV2-induced STAT1 phosphorylation levels, with no effect on INF-β-induced STAT1 activation, suggesting that the anti-inflammatory activity of **Mol-5** is strictly dependent on the presence of DENV2 infection, without directly interacting with host's defenses [73].

A series of 1,2,4-triazolo[1,5-*a*]pyrimidine-2-carboxamide-based compounds were also identified as potent influenza virus (Flu) RdRp inhibitors, thanks to their ability to disrupt the interaction of its acidic protein−basic protein 1 (PA−PB1) subunits, essential for the correct assembly of the RdRp complex [74]. A structure-based drug discovery approach initially led to the identification of the dihydrotriazolopyrimidine derivative **38** (Fig. 8), which only weakly inhibited PA-PB1 interaction (IC_{50} = 170.6 μM) and showed no antiviral activity up to 100 μM [75]. Since it was devoid of cytotoxicity (CC_{50} > 250 μM in MDCK cells) [75], further investigations were performed in order to identify more potent compounds.

A first series of derivatives was rationally designed [76] starting from structural modifications on the benzamide moiety at the C-2 position of the dihydrotriazolopyrimidine core. Other structural modifications were pursued: aromatic derivatives were synthesized by oxidation of the pyridine nucleus and, based on previous computational studies [77], the effect of switching the 7-phenyl ring and 5-methyl on the central core and the presence of an inverse amide at C-2 position were also investigated. Furthermore, hybrid molecules were designed by merging the 1,2,4-triazolopyrimidine (TZP) moiety with the cycloheptathiophene-3-carboxamide (cHTC) scaffold previously identified for its ability to impart PA-PB1 inhibitory activity [75,78]. The desired derivatives were synthesized as reported in Scheme 7. 3,5-Diamino-1,2,4-triazole (**39**), was reacted with benzylideneacetone or phenyl 1-propenyl ketone thus allowing the isolation of the proper 2-amino-1,2,4-triazolo[1,5-*a*]pyrimidine synthones **40-42** which were finally reacted with substituted benzoyl chlorides, affording the desired compounds **43-45** (Scheme 7, panel A).

Derivatives bearing the inverted amide at the C-2 position (Scheme 7, panel B) were synthesized by reacting ethyl 5-amino-1,2,4-triazole-3-carboxylate (**46**) [79] with benzylideneacetone or phenyl 1-propenyl ketone thus leading to compound **47**, then oxidized with NBS to derivative **48,** and **49** respectively. Basic hydrolysis of esters **48** and **49,** followed by carbodiimide-mediated coupling reaction with 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide or aniline, in the presence of N,N-diisopropylethylamine (DIPEA), led to the desired compounds **50-53**.

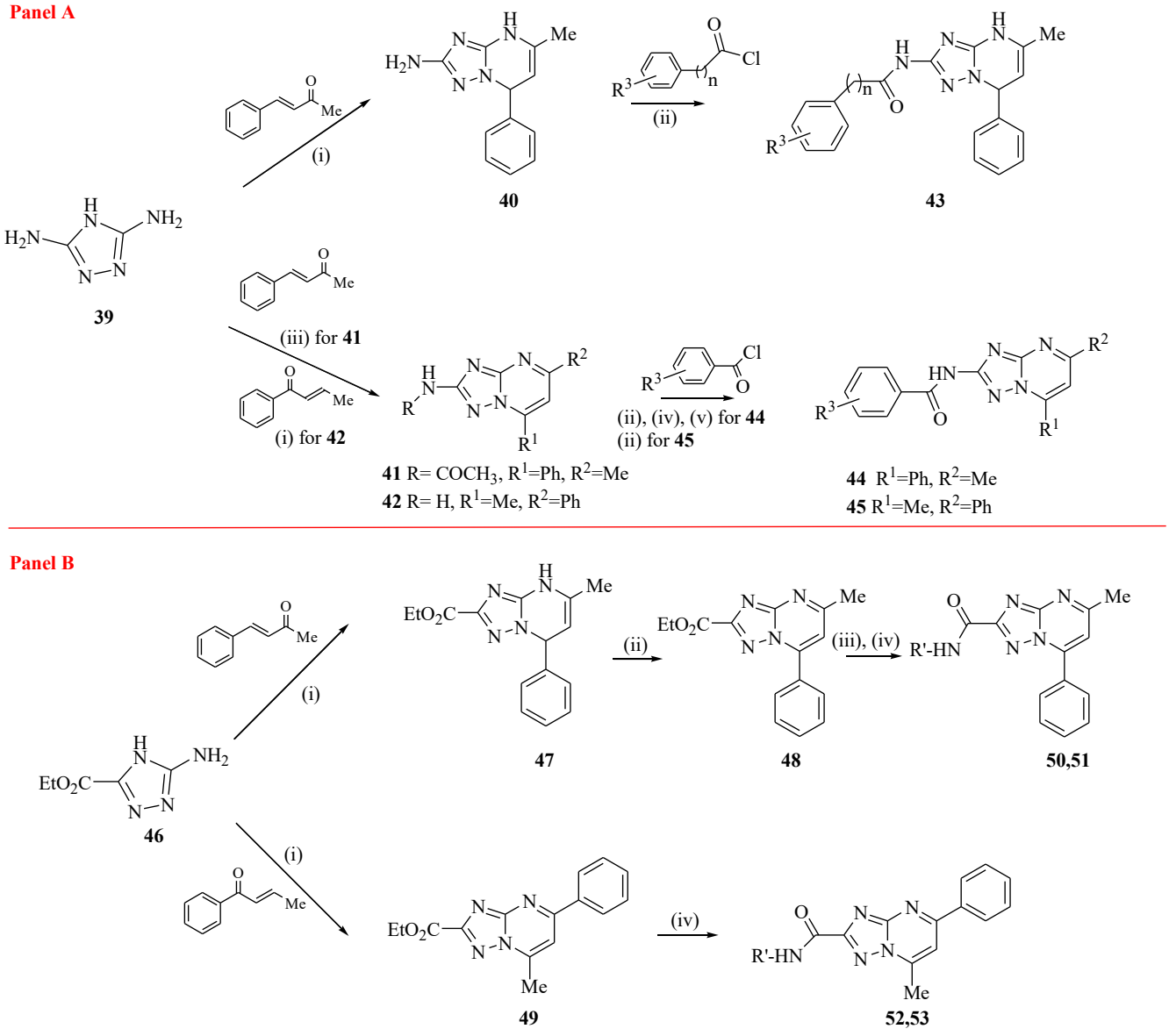
Enzyme-linked immunosorbent assay (ELISA) was performed on all derivatives to evaluate their ability to disrupt the PA-PB1 interaction along with plaque reduction assay (PRA) in MDCK cells infected with FluA virus (A/PR/8/34 strain), using RBV as a positive control (EC_{50} = 10 μM). Furthermore, all compounds were analyzed for their cytotoxicity by MTT assays in MDCK and HEK 293T cell lines.

Among compounds bearing a benzamide moiety at the C-2 position, *para*-substituted derivatives proved to be slightly more active than *ortho*- and *meta*-substituted ones. In particular, compound **43a** (R = 4-propoxyphenyl, n = 0) emerged as the most interesting, being able to inhibit both PA−PB1 interaction and viral growth with IC_{50} value of 40 μM and EC_{50} = 47 μM and CC_{50} values > 250 and 230 μM in HEK 293T and MDCK cells, respectively. In the aromatic series, the phenyl ring at C-5 and a methyl group at C-7 seem to be crucial for the antiviral activity (derivative **45** IC_{50} = 26 μM; EC_{50} = 25 μM, Table 6), since switched derivatives were devoid of biological activity.

The most interesting results were obtained with hybrid compounds **51** and **53**, with an inverted amide at C-2 position and the cycloheptathiophene-3-carboxamide substituent (Table 6). Compound **51** emerged as the most potent PA−PB1 inhibitor (IC_{50} = 1.1 μM) with modest antiviral activity (EC_{50} = 21 μM). On the other hand, compound **53** exhibited the best anti-Flu activity with an EC_{50} value of 8.0 μM, although it weakly interfered with the PA-PB1 interaction (IC_{50} = 28 μM), thus indicating a different binding mode of new triazolopyrimidine derivatives, strongly influenced by the substituents at the C-5/C-7 positions and by the group bound to the C-2 position. In fact, while compound **53** can recognize the three hydrophobic regions previously described [80] within the PB1 binding site, **51** is shifted toward the opposite side of the cavity and only interacts with the first hydrophobic region, through an efficient π−π staking interaction with W706. Compound **51**, however, is able to establish favored hydrogen bonding between its 2-carboxamide group and Q408, a key residue for PB1 binding, which might be responsible for its surprisingly high activity.

FluA minireplicon assay was also performed in transfected HEK 293T cells, showing good anti-polymerase activity for both compounds **51** and **53** both bearing a cyclohepta thiophene moiety with EC_{50} values of 12 and 16 μM respectively, comparable to that of the reference drug RBV (EC_{50} = 18 μM). Finally, the antiviral activity of these promising compounds against several strains of FluA and three strains of FluB was evaluated, thus confirming compound **53** as the most active in inhibiting the viral growth, with EC_{50} = 5 μM against both the FluA/Parma/24/09 and Flu B/Lee/40 strain. ADME studies on these new derivatives exhibited a promising profile, albeit compounds **51** and **53** showed poor solubility [76].

Further chemical manipulations of the two series of analogues were subsequently performed, maintaining the triazolopyrimidine core while widely modifying the cycloheptathiophene-3-carboxamide moiety (Table 7) [81]. Interesting results were achieved when the cHTC portion was replaced by a phenyl ring, as in compound **54** (analogue of **51**), showing only anti PA-PB1 activity (IC_{50} = 11 μM), and in compound **55** (analogue of **53**), exhibiting very good anti PA-PB1 activity (IC_{50} = 7 μM) but also interesting anti-Flu activity (EC_{50} = 31 μM in PRA assay). Replacement of the cycloheptathiophene moiety with the tricyclic cycloheptathieno-oxazinone core led to compounds **56** (analogue of **51**) and **57** (analogue of **53**) with both promising anti PA-PB1 and antiviral activity (IC_{50} = 19 μM and 15 μM respectively; EC_{50} = 26 μM and 40 μM respectively).



Scheme 7. Reagents and conditions: Panel A: (i) DMF, reflux; (ii) pyridine; (iii) DMF, Ac_2O, reflux; (iv) AcONa, NBS, ethanol, reflux; (v) 12 N HCl, ethanol, reflux; Panel B: (i) DMF, reflux; (ii) AcONa, NBS, EtOH, reflux; (iii) NaOH, MeOH, reflux; (iv) oxalyl chloride, CH_2Cl_2, DMF; then R'NH_2, CH_2Cl_2, DIPEA.

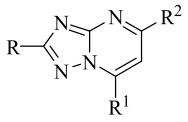
Target compounds **54** and **55**, were obtained through a multistep sequence as reported in Scheme 8. Cyclocondensation of ethyl 5-amino-1,2,4- triazole-3-carboxylate **46** [79] with 1-phenylbutane-1,3-dione in acetic acid allowed the isolation of the corresponding [1,2,4]triazolo[1,5-*a*]pyrimidine-2-carboxylates **60** and **61** [82], which were hydrolyzed to the corresponding acids and further reacted with oxalyl chloride to afford 1,2,4-triazolopyrimidine-2-carbonyl chlorides **64** and **65** (as described above [76]) (Scheme 8).

Finally, by reaction with 2-aminobenzamide in the presence of DIPEA, desired compounds **54** and **55** were obtained [82].

In order to better understand the structural requirements related to the ability to interfere with PA-PB1 interaction without showing anti-Flu activity exhibited by some [1,2,4]triazolo[1,5-*a*]pyrimidines, derivatives **54** and **55** were further investigated.

Besides the lower solubility of compound **54**, as emerged from preliminary chemical/physical assays, computational studies pointed out a different positioning in the PB1 binding site of compound **54** compared to **51,** as a cause of the reduced anti PA-PB1 activity. Similarly to **53**, compound **55** was able to interact with all the three hydrophobic regions previously described (including key residue W706), but it was additionally involved in a favorable hydrogen bonding between the 2-carboxamide NH group and the hydroxyl group of T639, an amino acid whose role has yet to be clarified. On the other hand, **54** was only able to interact with the first hydrophobic region within the PB1 binding site but, differently from **51**, the hydrogen bonding with Q408 was established with the 2-carboxamide carbonyl group of the phenyl ring. Furthermore, **54** formed a second H-bond with I621, a key residue of PA in binding of PB1, which however resulted in a different positioning and in a reduction of π-π interaction with W706, thus explaining its reduced anti PA-PB1 activity.

Table 6
Structure and biological activity of selected triazolopyrimidines.



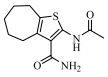
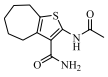
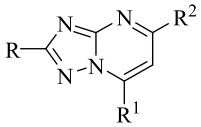
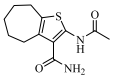
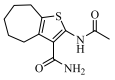
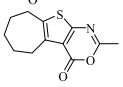
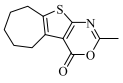
Compd	R	R^1	R^2	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM) HEK 293T cells	CC_{50} (μM) MDCK cells
45		Me	Ph	26 ± 5	25 ± 1	196 ± 11	>250
50		Ph	Me	160 ± 11	42 ± 5	110 ± 15	>250
51		Ph	Me	1.1 ± 0.3	21 ± 4	>250	>250
52		Me	Ph	128 ± 15	49 ± 2	>250	>250
53		Me	Ph	28 ± 1	8 ± 2	>250	>250
RBV	–	–	–	10 ± 2	>250	>100	10 ± 2

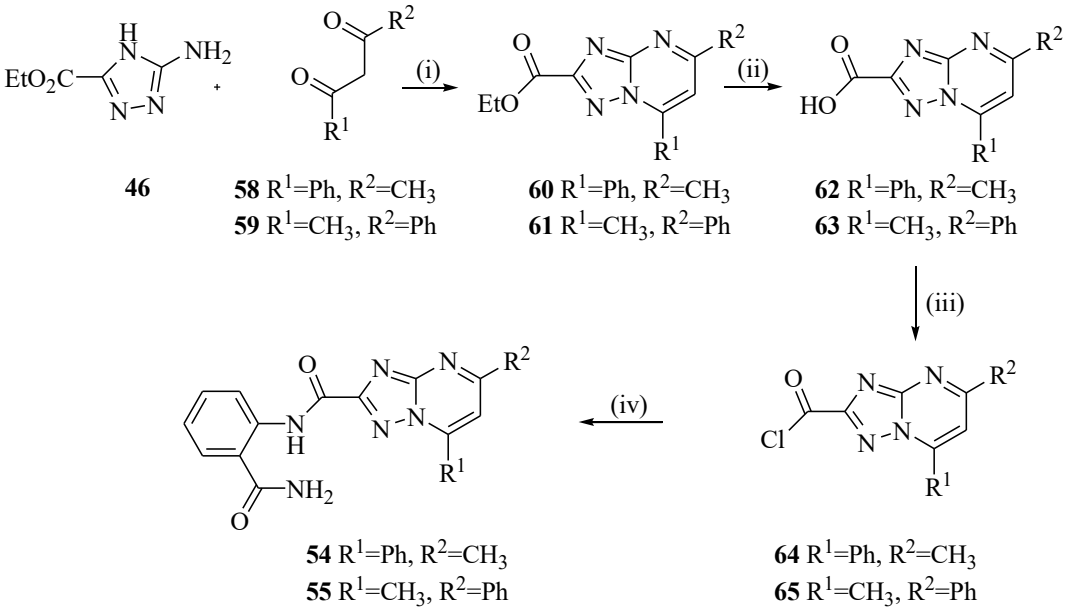
Table 7
Structure and biological activity of selected analogues of compounds **51 and 53**.



Compd	R	R^1	R^2	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM) MDCK cells
51		Ph	Me	1.1 ± 0.3	21 ± 4	>250
53		Me	Ph	28 ± 1	8 ± 2	>250
54		Ph	Me	11 ± 3	>100	>250
55		Me	Ph	7 ± 1	31 ± 10	>250
56		Ph	Me	19 ± 1	26 ± 1	101 ± 2
57		Me	Ph	15 ± 5	40 ± 6	>50
RBV	–	–	–	–	10 ± 2	>250

A minireplicon assay on HEK 293T cells was performed on the most promising compound **55** to verify if the anti-PA-PB1 activity observed *in vitro* produced a similar inhibitory activity of FluA RdRp in a cellular assay. Compound **55** emerged as a potent inhibitor of FluA polymerase activity with an EC_{50} value of 5.8 ± 2.0 μM, even higher than that of RBV (EC_{50} value of 24 ± 4 μM), thus confirming its ability to inhibit RdRp activity by interfering with PA-PB1 interaction [81].

To further investigate the effect of substitutions on the triazolopyrimidine core, another series of analogues was designed and synthesized by the same research group, focusing on the role of the amide bond on C-2 position, on the replacement of the carbamoyl moiety and on the effects of the presence of a sole phenyl ring in C-5, C-6 and C-7 positions [81]. Among 5-methyl-7-phenyl-TZP derivatives, compound **66**, with a benzo[*d*] [1,3]oxazin-4-one ring at the C-2 position of the TZP nucleus, exhibited the best anti-PA-PB1 activity (IC_{50} = 19.5 μM), also showing a good anti-Flu activity (EC_{50} = 16 μM). A weak inhibition of PA-PB1 interaction but no antiviral activity was observed in derivatives in which the 2-carbamoyl moiety was replaced by a carboxylic acid (**69**) or a methyl ester (**71**), thus confirming the 2-carbamoylphenyl ring as the best C-2 substituent within the 7-methyl-5-phenyl-TZP series. Compounds with only a phenyl ring at the C-5, C-6 or C-7 positions of the triazolopyrimidine nucleus were devoid of both anti-PA-PB1 and



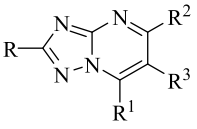
Scheme 8. Reagents and conditions: (i) glacial acetic acid, reflux; (ii) NaOH, MeOH, reflux; (iii) oxalyl chloride, CH_2Cl_2, DMF, r.t.; (iv) 2-aminobenzamide, DIPEA, CH_2Cl_2, rt.

anti-Flu activity when decorated with an unsubstituted benzoyl moiety at the C-2 position (compounds **72-74**), while the presence of an inverted amide functionalized with 2-carbamoylphenyl (**75** and **76**) or cycloheptathiophene-3- carboxamide moiety (**77** and **78**) at the C-2 position of the same derivatives conferred a good anti-PA-PB1 activity without showing anti-Flu activity (Table 8).

The synthesis of TZP derivatives is reported in Scheme 9. 5-Methyl-7-phenyl- [1,2,4]triazolo[1,5-*a*] pyrimidine-2-carbonyl chloride **64** and 7-methyl-5-phenyl- [1,2,4]triazolo[1,5-*a*]pyrimidine-2-carbonyl chloride **65** [76]were reacted with methyl 2-aminobenzoate in presence of DIPEA to obtain methyl esters **70** and **71**, then hydrolyzed with LiOH in H_2O/THF mixture at 50 °C to give acid derivatives **68** and **69**. The latter was then cyclized in acetic anhydride at 100 °C to afford benzo-oxazinone derivative **67**, while compound **66** was obtained by reaction of **64** with 2-aminobenzoic acid in CH_2Cl_2 in presence of DIPEA.

A minireplicon assay on HEK 293T cells showed an EC_{50} value of 21 ± 3 μM for the most promising compound **66** thus confirming its ability to interfere with RdRP catalytic activity by inhibiting PA-PB1 interaction. Additionally, over the course of this study an interesting derivative (**79**, Fig. 9) emerged as a good inhibitor of PA-PB1 interaction (IC_{50} =

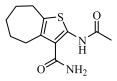
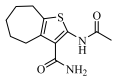
Table 8
Structure and anti-Flu activity of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives.

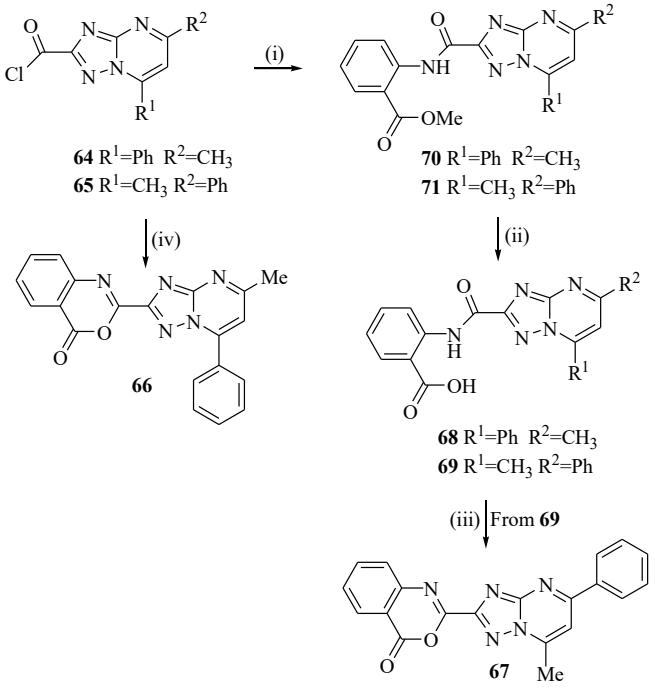


Compd	R	R^1	R^2	R^3	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM) MDCK cells
66		Ph	CH_3	H	19.5 ± 4.9	16 ± 5.7	>250
67		CH_3	Ph	H	123 ± 45	>100	>200
68		Ph	CH_3	H	116 ± 43	>100	>250
69		CH_3	Ph	H	32 ± 14	>100	>250
70		Ph	CH_3	H	26.5 ± 2.1	>80	>250
71		CH_3	Ph	H	22.3 ± 6.4	>100	>250
72		Ph	H	H	>200	>100	>250

(*continued on next page*)

Table 8 (*continued*)

Compd	R	R^1	R^2	R^3	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM) MDCK cells
73		H	Ph	H	165	>100	>250
74		H	H	Ph	>200	>100	>250
75		Ph	H	H	21 ± 4.2	>100	>250
76		H	H	Ph	21 ± 8	>100	>250
77		Ph	H	H	>200	>60	>200
78		H	H	Ph	22 ± 3	>100	100 ± 9
RBV	–	–	–	–	–	10 ± 2	>250



Scheme 9. Reagents and conditions: (i) methyl 2-aminobenzoate, DIPEA, CH_2Cl_2, rt; (ii) LiOH, H_2O/THF (1:1), 50 °C; (iii) Ac_2O, 100 °C; (iv) 2-aminobenzoic acid, DIPEA, CH_2Cl_2, rt.

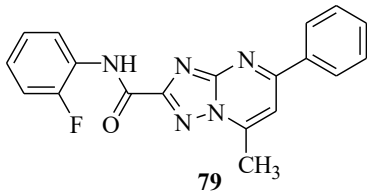


Fig. 9. Chemical structure of compound **79**.

17.5 μM). It also showed an interesting anti-SARS-CoV-2 activity (EC_{50} = 34.47 ± 2.99 μM) and no cytotoxic effect up to 100 μM on Vero E6 cells, thus suggesting that triazolopyrimidine scaffold may also be promising in the identification of anti-CoV agents [83].

PA-PB1 interaction has also been suggested as potential target of a [1,2,4]triazolo[4,3-*a*]pyrimidine derivative **ANA-1** (Fig. 10) with promising anti-Flu activity [84]. A high throughput screening performed

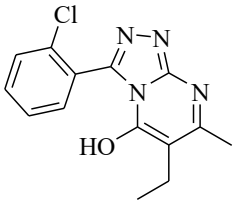


Fig. 10. Chemical structure of **ANA-1**.

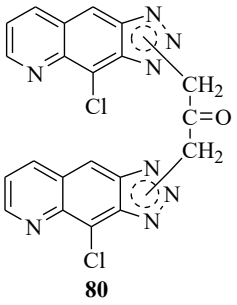
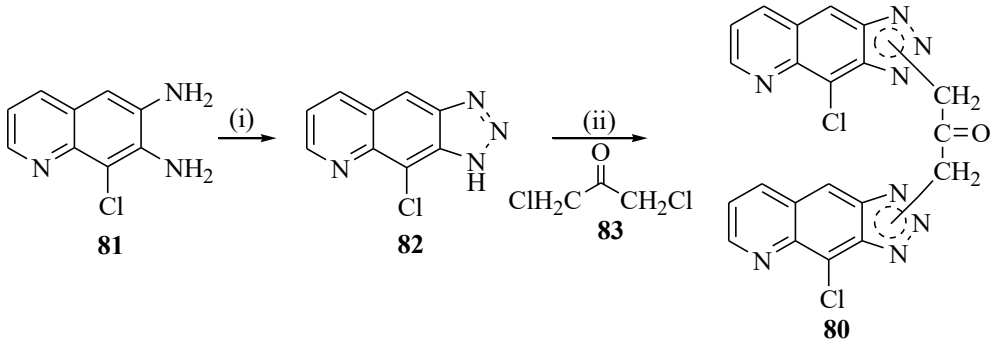


Fig. 11. Chemical structure of triazolo [4,5-*g*]quinoline **80**.

on a chemical library of 950 candidates [85] led to the identification of 3-(2-chlorophenyl)-6-ethyl-7-methyl [1,2,4]triazolo[4,3-*a*]pyrimidin-5-ol, named **ANA-1** capable of inhibiting virus polymerase activity with an IC_{50} value of 30 μM. A plaque reduction assay on influenza H1N1 virus showed an EC_{50} value of 0.55 ± 0.10 μM, while low cytotoxicity (CC_{50} = 125 ± 18 μM) was found by MTT assay on MDCK cells, with an interesting selectivity index (SI = 227).

In vitro antiviral activity of **ANA-1** was determined by multi-cycle virus growth assays, showing inhibition of different subtypes of influenza virus in a dose-dependent manner, with most promising antiviral effect against H9N2 virus infection (EC_{50} = 0.09 ± 0.03 μM). *In vivo* studies were also performed, proving that **ANA-1** could inhibit viral replication on mice. Mechanism of action studies proved that **ANA-1** suppressed viral replication by interfering with polymerase activity. Molecular docking studies pointed out that **ANA-1** interacted with an allosteric site of the C terminus of PA (PA_C), probably inducing conformational changes that hindered interaction with PB1 [84].

Condensation of 1,2,3-triazole scaffold with the quinoline nucleus led to a novel series of triazolo[4,5-*g*]quinolones, which were evaluated for their antiviral activity against different RNA viruses [86]. Among them, the bis-triazoloquinoline **80** (Fig. 11) emerged as an attractive compound.



Scheme 10. Reagents: (i) $NaNO_2$, HCl; (ii) 1,3-dichloroacetone **83** (2:1 M ratio), KOH, DMF.

The synthesis of compound **80** is described in Scheme 10. Diazotization of the 6,7-diaminoquinolines **81** [87] and subsequent cyclization of the corresponding diazonium salt afforded compound **82** [88]which, upon reaction with 1,3-dichloroacetone **83** (2:1 M ratio), led to the desired compound **80**.

Compound **80** exhibited a promising antiviral activity against Bovine Viral Diarrhoea virus (BVDV) in a cell-based assay, with an EC_{50} value of 1 ± 0.09 μM, no cytotoxicity for MDBK, MT-4, BHK and Vero-76 cells ($CC_{50} > 100$ μM) and a good Selectivity Index (SI > 100). In order to investigate the target of the novel compound, an enzyme assay was performed, showing that **80** was a potent inhibitor of the BVDV RNA-dependent RNA polymerase, with an IC_{50} value of 0.4 ± 0.05 μM. However, a deeper insight into the mechanism of action and the structure-activity relationship of these compounds is necessary to obtain more active compounds.

5. Conclusions

The increased spread of viral infections all over the world, also due to the outbreak of the current COVID-19 pandemic, represents an important focus in pharmaceutical research, especially since many viral infections still lack a specific treatment. Among all the potential targets for the development of new antiviral agents, polymerases seem to be promising, due to the crucial role they play in the replication of the viral genome. Furthermore, their structure is highly conserved among various viral strains and their mechanism of action profoundly differs from that of human polymerases [89]. To this date, many novel non-nucleoside compounds have been designed to obtain more effective antiviral agents. Among the most promising scaffolds, triazoles emerged as one of the most important pharmacophore systems, also due to the wide range of biological and pharmacological activities they are endowed with. For this reason, in this review we have focused our attention on non-nucleoside 1,2,3-triazole and 1,2,4-triazole-based systems which are able to inhibit the polymerase activity of different viruses (Influenza virus, HCV, BVDV, RSV, SARS-CoV-2, DENV and HIV-1) in the micromolar/nanomolar range.

Overall, from the herein reported overview, it seems that 1,2,4-triazoles, both fused and not, are endowed with better biological properties with respect to 1,2,3-isomers. In particular, some of the 1,2,4-triazole derivatives showed high inhibiting activity against HIV-1 Reverse Transcriptase, thus proving that the 1,2,4-triazole nucleus represents a valuable scaffold worthy of being further explored in the development of potent RNA-dependent DNA-polymerase inhibitors. On the other hand, 1,2,4-condensed triazoles act as important pharmacophores in inhibiting RNA-dependent RNA polymerases both of HCV and Flu viruses with EC_{50} up to nanomolar values.

Synthesis, biological properties, and structure-activity relationships (SAR) of the most relevant compounds herein discussed, might provide a helpful contribution in the identification of new effective antiviral agents.

List of abbreviations

SARS = severe acute respiratory syndrome
SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2
MERS = Middle East respiratory syndrome
EBOV = Ebola virus
ZIKV = Zika virus
TdPPs = template-directed nucleic acid polymerases
DdDP = DNA-dependent DNA-polymerase
RdDP = RNA-dependent DNA-polymerase (or Reverse Transcriptase)
DdRP = DNA-dependent RNA-polymerase
RdRP = RNA-dependent RNA-polymerase
RBV = Ribavirin
HIV-1 = Human Immunodeficiency Virus 1
NNRTI = non-nucleoside reverse transcriptase inhibitors
VcNa = Vitamin C sodium salt
THF = Tetrahydrofuran
WT = wild type
RT = reverse transcriptase
MTT = tetrazolium-based colorimetric method
NPV = Nevirapine
DLV = Delavirdine
SI = selectivity index
ETR = Etravirine
DMF = N,N-Dimethylformamide
MW = microwave
HTS = high-throughput screening
RSV = respiratory syncytial virus
CPE = cytopathic effect reduction assay
HCV = Hepatitis C virus
p-TSA = p-Toluenesulfonic acid
HCC = hepatocellular carcinoma
DIPEA = N,N-Diisopropylethylamine
TMS = trimethylsilyl group
LDA = Lithium diisopropylamide
DENV = Dengue virus
hpi = hours post-infection
STAT1 = Signal transducer and activator of transcription 1
Flu = Influenza virus
PA-PB1 = acidic protein−basic protein 1
TZP = 1,2,4-triazolopyrimidine
cHTC = cycloheptathiophene-3-carboxamide
NBS = N-bromosuccinimide
PRA = plaque reduction assay
EDC = N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide
HOBt = 1-hydroxybenzotriazole
BVDV = Bovine Viral Diarrhoea virus

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Isabella Romeo reports financial support was provided by Calabria Region.

Data availability

Data will be made available on request.

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References

[1] M. Trovato, R. Sartorius, L. D'Apice, R. Manco, P. De Berardinis, Viral emerging diseases: challenges in developing vaccination strategies, Front. Immunol. 11 (2020) 1–20, https://doi.org/10.3389/fimmu.2020.02130.

[2] P. Tavares, J.R. Harris, D. Bhella, Virus protein and nucleoprotein complexes. http://link.springer.com/10.1007/978-981-10-8456-0, 2018.

[3] H. Ago, T. Adachi, A. Yoshida, M. Yamamoto, N. Habuka, K. Yatsunami, M. Miyano, Crystal structure of the RNA-dependent RNA polymerase of hepatitis C virus, Structure 7 (1999) 1417–1426, https://doi.org/10.1016/S0969-2126(00)80031-3.

[4] E. Johansson, N. Dixon, Replicative DNA polymerases, Cold Spring Harbor Perspect. Biol. 5 (2013) 1–14, https://doi.org/10.1101/cshperspect.a012799.

[5] C. Ferrer-Orta, A. Arias, C. Escarmís, N. Verdaguer, A comparison of viral RNA-dependent RNA polymerases, Curr. Opin. Struct. Biol. 16 (2006) 27–34, https://doi.org/10.1016/j.sbi.2005.12.002.

[6] J. Černý, B. Černá Bolfíková, P.M. Paolo, L. Grubhoffer, D. Růžek, A deep phylogeny of viral and cellular right-hand polymerases, Infect. Genet. Evol. 36 (2015) 275–286, https://doi.org/10.1016/j.meegid.2015.09.026.

[7] M. Barreca, V. Spanò, M.V. Raimondi, R. Bivacqua, S. Giuffrida, A. Montalbano, A. Cavalli, F. Bertoni, P. Barraja, GPCR inhibition in treating lymphoma, ACS Med. Chem. Lett. 13 (2022) 358–364, https://doi.org/10.1021/acsmedchemlett.1c00600.

[8] M. Barreca, A.M. Ingarra, M.V. Raimondi, V. Spanò, A.P. Piccionello, M. De Franco, L. Menilli, V. Gandin, G. Miolo, P. Barraja, A. Montalbano, New tricyclic systems as photosensitizers towards triple negative breast cancer cells, Arch Pharm. Res. (Seoul) 45 (2022) 806–821, https://doi.org/10.1007/s12272-022-01414-1.

[9] V. Spanò, M. Barreca, V. Cilibrasi, M. Genovese, M. Renda, A. Montalbano, L.J. V. Galietta, P. Barraja, Evaluation of fused pyrrolothiazole systems as correctors of mutant CFTR protein, Molecules 26 (2021) 1–25, https://doi.org/10.3390/molecules26051275.

[10] M. Barreca, V. Spanò, M.V. Raimondi, C. Tarantelli, F. Spriano, F. Bertoni, P. Barraja, A. Montalbano, Recurrence of the oxazole motif in tubulin colchicine site inhibitors with anti-tumor activity, Eur. J. Med. Chem. Reports. 1 (2021), 100004, https://doi.org/10.1016/j.ejmcr.2021.100004.

[11] I. Romeo, F. Mesiti, A. Lupia, S. Alcaro, Current updates on naturally occurring compounds recognizing sars-cov-2 druggable targets, Molecules 26 (2021) 1–27, https://doi.org/10.3390/molecules26030632.

[12] K. Arul, A. Anton Smith, In-Silico design, synthesis and in vitro antitubercular activity of novel 1, 2, 4 - triazole derivatives, Int. J. Pharm. Pharmaceut. Sci. 6 (2014) 213–217.

[13] D. Sarigol, A. Uzgoren-Baran, B.C. Tel, E.I. Somuncuoglu, I. Kazkayasi, K. Ozadali-Sari, O. Unsal-Tan, G. Okay, M. Ertan, B. Tozkoparan, Novel thiazolo[3,2-b]-1,2,4-triazoles derived from naproxen with analgesic/anti-inflammatory properties: synthesis, biological evaluation and molecular modeling studies, Bioorg. Med. Chem. 23 (2015) 2518–2528, https://doi.org/10.1016/j.bmc.2015.03.049.

[14] M.H. Shaikh, D.D. Subhedar, L. Nawale, D. Sarkar, F.A. Kalam Khan, J. N. Sangshetti, B.B. Shingate, 1,2,3-Triazole derivatives as antitubercular agents: synthesis, biological evaluation and molecular docking study, Medchemcomm 6 (2015) 1104–1116, https://doi.org/10.1039/c5md00057b.

[15] R. Adam, P. Bilbao-Ramos, B. Abarca, R. Ballesteros, M.E. González-Rosende, M. A. Dea-Ayuela, F. Estevan, G. Alzuet-Piña, Triazolopyridopyrimidines: an emerging family of effective DNA photocleavers. DNA binding. Antileishmanial activity, Org. Biomol. Chem. 13 (2015) 4903–4917, https://doi.org/10.1039/c5ob00280j.

[16] R. Chopra, K. Chibale, K. Singh, Pyrimidine-chloroquinoline hybrids: synthesis and antiplasmodial activity, Eur. J. Med. Chem. 148 (2018) 39–53, https://doi.org/10.1016/j.ejmech.2018.02.021.

[17] P. Nagender, G. Malla Reddy, R. Naresh Kumar, Y. Poornachandra, C. Ganesh Kumar, B. Narsaiah, Synthesis, cytotoxicity, antimicrobial and anti-biofilm activities of novel pyrazolo[3,4-b]pyridine and pyrimidine functionalized 1,2,3-triazole derivatives, Bioorg. Med. Chem. Lett 24 (2014) 2905–2908, https://doi.org/10.1016/j.bmcl.2014.04.084.

[18] S. Nagarajan, P. Shanmugavelan, M. Sathishkumar, R. Selvi, A. Ponnuswamy, H. Harikrishnan, V. Shanmugaiah, An eco-friendly water mediated synthesis of 1,2,3-triazolyl-2- aminopyrimidine hybrids as highly potent anti-bacterial agents, Chin. Chem. Lett. 25 (2014) 419–422, https://doi.org/10.1016/j.cclet.2013.12.017.

[19] M. Liu, Q. Xu, S. Guo, R. Zuo, Y. Hong, Y. Luo, Y. Li, P. Gong, Y. Liu, Design, synthesis, and structure-activity relationships of novel imidazo[4,5-c]pyridine derivatives as potent non-nucleoside inhibitors of hepatitis C virus NS5B, Bioorg. Med. Chem. 26 (2018) 2621–2631, https://doi.org/10.1016/j.bmc.2018.04.029.

[20] H. Kaoukabi, Y. Kabri, C. Curti, M. Taourirte, J.C. Rodriguez-Ubis, R. Snoeck, G. Andrei, P. Vanelle, H.B. Lazrek, Dihydropyrimidinone/1,2,3-triazole hybrid molecules: synthesis and anti-varicella-zoster virus (VZV) evaluation, Eur. J. Med. Chem. 155 (2018) 772–781, https://doi.org/10.1016/j.ejmech.2018.06.028.

[21] G. Wu, W.A. Zalloum, M.E. Meuser, L. Jing, D. Kang, C.H. Chen, Y. Tian, F. Zhang, S. Cocklin, K.H. Lee, X. Liu, P. Zhan, Discovery of phenylalanine derivatives as potent HIV-1 capsid inhibitors from click chemistry-based compound library, Eur. J. Med. Chem. 158 (2018) 478–492, https://doi.org/10.1016/j.ejmech.2018.09.029.

[22] Y. Tian, Z. Liu, J. Liu, B. Huang, D. Kang, H. Zhang, E. De Clercq, D. Daelemans, C. Pannecouque, K.H. Lee, C.H. Chen, P. Zhan, X. Liu, Targeting the entrance channel of NNIBP: discovery of diarylnicotinamide 1,4-disubstituted 1,2,3-triazoles as novel HIV-1 NNRTIs with high potency against wild-type and E138K mutant virus, Eur. J. Med. Chem. 151 (2018) 339–350, https://doi.org/10.1016/j.ejmech.2018.03.059.

[23] I.A. Seliem, S.S. Panda, A.S. Girgis, Y. Moatasim, A. Kandeil, A. Mostafa, M.A. Ali, E.S. Nossier, F. Rasslan, A.M. Srour, R. Sakhuja, T.S. Ibrahim, Z.K.M. Abdel-samii, A.M.M. Al-Mahmoudy, New quinoline-triazole conjugates: synthesis, and antiviral properties against SARS-CoV-2, Bioorg. Chem. 114 (2021), 105117, https://doi.org/10.1016/j.bioorg.2021.105117.

[24] V.N. Holanda, E.M. de A. Lima, W.V. da Silva, R.T. Maia, R. de L. Medeiros, A. Ghosh, V.L. de M. Lima, R.C.B.Q. de Figueiredo, Identification of 1,2,3-triazole-phthalimide derivatives as potential drugs against COVID-19: a virtual screening, docking and molecular dynamic study, J. Biomol. Struct. Dyn. 40 (2022) 5462–5480, https://doi.org/10.1080/07391102.2020.1871073.

[25] C.Y. Wu, K.Y. King, C.J. Kuo, J.M. Fang, Y.T. Wu, M.Y. Ho, C.L. Liao, J.J. Shie, P. H. Liang, C.H. Wong, Stable benzotriazole esters as mechanism-based inactivators of the severe acute respiratory syndrome 3CL protease, Chem. Biol. 13 (2006) 261–268, https://doi.org/10.1016/j.chembiol.2005.12.008.

[26] K. Karypidou, S.R. Ribone, M.A. Quevedo, L. Persoons, C. Pannecouque, C. Helsen, F. Claessens, W. Dehaen, Synthesis, biological evaluation and molecular modeling of a novel series of fused 1,2,3-triazoles as potential anti-coronavirus agents, Bioorg. Med. Chem. Lett 28 (2018) 3472–3476, https://doi.org/10.1016/j.bmcl.2018.09.019.

[27] K. Anand, J. Ziebuhr, P. Wadhwani, J.R. Mesters, R. Hilgenfeld, (3CL pro) structure : basis for design of anti-SARS drugs, Science 300 (2014) 1763–1767, https://doi.org/10.1126/science.1085658, 80.

[28] M. Turlington, A. Chun, S. Tomar, A. Eggler, V. Grum-Tokars, J. Jacobs, J. S. Daniels, E. Dawson, A. Saldanha, P. Chase, Y.M. Baez-Santos, C.W. Lindsley, P. Hodder, A.D. Mesecar, S.R. Stauffer, Discovery of N-(benzo[1,2,3]triazol-1-yl)-N-(benzyl)acetamido)phenyl) carboxamides as severe acute respiratory syndrome coronavirus (SARS-CoV) 3CLpro inhibitors: identification of ML300 and noncovalent nanomolar inhibitors with an induced-fit binding, Bioorg. Med. Chem. Lett 23 (2013) 6172–6177, https://doi.org/10.1016/j.bmcl.2013.08.112.

[29] J. Jacobs, V. Grum-Tokars, Y. Zhou, M. Turlington, S.A. Saldanha, P. Chase, A. Eggler, E.S. Dawson, Y.M. Baez-Santos, S. Tomar, A.M. Mielech, S.C. Baker, C. W. Lindsley, P. Hodder, A. Mesecar, S.R. Stauffer, Discovery, synthesis, and structure-based optimization of a series of N -(tert -Butyl)-2-(N -arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent noncovalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL, J. Med. Chem. 56 (2013) 534–546, https://doi.org/10.1021/jm301580n.

[30] A.O. Adedeji, K. Singh, N.E. Calcaterra, M.L. DeDiego, L. Enjuanes, S. Weiss, S. G. Sarafianos, Severe acute respiratory syndrome coronavirus replication inhibitor that interferes with the nucleic acid unwinding of the viral helicase, Antimicrob. Agents Chemother. 56 (2012) 4718–4728, https://doi.org/10.1128/AAC.00957-12.

[31] N.H. Zaher, M.I. Mostafa, A.Y. Altaher, Design, synthesis and molecular docking of novel triazole derivatives as potential CoV helicase inhibitors, Acta Pharm. 70 (2020) 145–159, https://doi.org/10.2478/acph-2020-0024.

[32] L.J. Stockman, R. Bellamy, P. Garner, SARS: systematic review of treatment effects, PLoS Med. 3 (2006) 1525–1531, https://doi.org/10.1371/journal.pmed.0030343.

[33] M. Berry, B.C. Fielding, J. Gamieldien, Potential broad spectrum inhibitors of the Coronavirus 3CLpro: a virtual screening and structure-based drug design study, Viruses 7 (2015) 6642–6660, https://doi.org/10.3390/v7122963.

[34] R. Kharb, M. Shahar Yar, P. Chander Sharma, Recent advances and future perspectives of triazole analogs as promising antiviral agents, Mini-Rev. Med. Chem. 11 (2010) 84–96, https://doi.org/10.2174/138955711793564051.

[35] S.L. Deev, M.V. Yasko, I.L. Karpenko, A.N. Korovina, A.L. Khandazhinskaya, V. L. Andronova, G.A. Galegov, T.S. Shestakova, E.N. Ulomskii, V.L. Rusinov, O. N. Chupakhin, M.K. Kukhanova, 1,2,4-Triazoloazine derivatives as a new type of herpes simplex virus inhibitors, Bioorg. Chem. 38 (2010) 265–270, https://doi.org/10.1016/j.bioorg.2010.09.002.

[36] Z. Liu, W. Chen, P. Zhan, E. De Clercq, C. Pannecouque, X. Liu, Design, synthesis and anti-HIV evaluation of novel diarylnicotinamide derivatives (DANAs) targeting

the entrance channel of the NNRTI binding pocket through structure-guided molecular hybridization, Eur. J. Med. Chem. 87 (2014) 52–62, https://doi.org/10.1016/j.ejmech.2014.09.054.
[37] I.A. Seliem, A.S. Girgis, Y. Moatasim, A. Kandeil, A. Mostafa, M.A. Ali, M. S. Bekheit, S.S. Panda, New pyrazine conjugates: synthesis, computational studies, and antiviral properties against SARS-CoV-2, ChemMedChem 16 (2021) 3418–3427, https://doi.org/10.1002/cmdc.202100476.
[38] H.M. Faidallah, S.S. Panda, J.C. Serrano, A.S. Girgis, K.A. Khan, K.A. Alamry, T. Therathanakorn, M.J. Meyers, F.M. Sverdrup, C.S. Eickhoff, S.G. Getchell, A. R. Katritzky, Synthesis, antimalarial properties and 2D-QSAR studies of novel triazole-quinine conjugates, Bioorg. Med. Chem. 24 (2016) 3527–3539, https://doi.org/10.1016/j.bmc.2016.05.060.
[39] L. Wang, Q. Zhu, K. Xiang, Y. Zhang, B. Li, X. Yu, G. Yang, C. Liang, H. Yun, M. Zhang, N. Qin, L. Gao, Discovery of a novel respiratory syncytial virus replication inhibitor, Antimicrob. Agents Chemother. 65 (2021), https://doi.org/10.1128/AAC.02576-20.
[40] K. Sudo, Y. Miyazaki, N. Kojima, M. Kobayashi, H. Suzuki, M. Shintani, Y. Shimizu, YM-53403, a unique anti-respiratory syncytial virus agent with a novel mechanism of action, Antivir. Res. 65 (2005) 125–131, https://doi.org/10.1016/j.antiviral.2004.12.002.
[41] S.W. Mason, C. Lawetz, Y. Gaudette, F. Dô, E. Scouten, L. Lagacé, B. Simoneau, M. Liuzzi, Polyadenylation-dependent screening assay for respiratory syncytial virus RNA transcriptase activity and identification of an inhibitor, Nucleic Acids Res. 32 (2004) 4758–4767, https://doi.org/10.1093/nar/gkh809.
[42] M. Liuzzi, S.W. Mason, M. Cartier, C. Lawetz, R.S. McCollum, N. Dansereau, G. Bolger, N. Lapeyre, Y. Gaudette, L. Lagacé, M.-J. Massariol, F. Dô, P. Whitehead, L. Lamarre, E. Scouten, J. Bordeleau, S. Landry, J. Rancourt, G. Fazal, B. Simoneau, Inhibitors of respiratory syncytial virus replication target cotranscriptional mRNA guanylylation by viral RNA-dependent RNA polymerase, J. Virol. 79 (2005) 13105–13115, https://doi.org/10.1128/jvi.79.20.13105-13115.2005.
[43] J. Zhao, H.A. Aisa, Synthesis and anti-influenza activity of aminoalkyl rupestonates, Bioorg. Med. Chem. Lett 22 (2012) 2321–2325, https://doi.org/10.1016/j.bmcl.2012.01.056.
[44] V. Summa, A. Petrocchi, P. Pace, V.G. Matassa, R. De Francesco, S. Altamura, L. Tomei, U. Koch, P. Neuner, Discovery of α,γ-diketo acids as potent selective and reversible inhibitors of hepatitis C virus NS5b RNA-dependent RNA polymerase, J. Med. Chem. 47 (2004) 14–17, https://doi.org/10.1021/jm0342109.
[45] W.H. Song, M.M. Liu, D.W. Zhong, Y.L. Zhu, M. Bosscher, L. Zhou, D.Y. Ye, Z. H. Yuan, Tetrazole and triazole as bioisosteres of carboxylic acid: discovery of diketo tetrazoles and diketo triazoles as anti-HCV agents, Bioorg. Med. Chem. Lett 23 (2013) 4528–4531, https://doi.org/10.1016/j.bmcl.2013.06.045.
[46] P.G. Reddy, D. Bao, W. Chang, B.K. Chun, J. Du, D. Nagarathnam, S. Rachakonda, B.S. Ross, H.R. Zhang, S. Bansal, C.L. Espiritu, M. Keilman, A.M. Lam, C. Niu, H. M. Steuer, P.A. Furman, M.J. Otto, M.J. Sofia, 2′-Deoxy-2′-α-fluoro-2′-β-C-methyl 3′,5′-cyclic phosphate nucleotide prodrug analogs as inhibitors of HCV NS5B polymerase: discovery of PSI-352938, Bioorg. Med. Chem. Lett 20 (2010) 7376–7380, https://doi.org/10.1016/j.bmcl.2010.10.035.
[47] P. Çikla-Süzgün, N. Kaushik-Basu, A. Basu, P. Arora, T.T. Talele, I. Durmaz, R. Çetin-Atalay, S.G. Küçükgüzel, Anti-cancer and anti-hepatitis C virus NS5B polymerase activity of etodolac 1,2,4-triazoles, J. Enzym. Inhib. Med. Chem. 30 (2015) 778–785, https://doi.org/10.3109/14756366.2014.971780.
[48] B.I. Carr, Hepatocellular carcinoma: current management and future trends, Gastroenterology 127 (2004) 218–224, https://doi.org/10.1053/j.gastro.2004.09.036.
[49] V. Koga, S. Sakisaka, M. Ohishi, T. Kawaguchi, E. Taniguchi, K. Sasatomi, M. Harada, T. Kusaba, M. Tanaka, R. Kimura, O. Nakashima, S. Nakashima, M. Kojiro, T. Kurohiji, M. Sata, Expression of Cyclooxygenase-2 in human hepatocellular carcinoma: relevance to tumor dedifferentiation, Hepatology 29 (1999) 688–696, https://doi.org/10.1002/hep.510290355.
[50] C.A. Demerson, L.G. Humber, N.A. Abraham, G. Schilling, R.R. Martel, C. Pace-Asciak, Resolution of etodolac and antiinflammatory and prostaglandin synthetase inhibiting properties of the enantiomers, J. Med. Chem. 26 (1983) 1778–1780, https://doi.org/10.1021/jm00366a025.
[51] J. Behari, G. Zeng, W. Otruba, M.D. Thompson, P. Muller, A. Micsenyi, S.S. Sekhon, L. Leoni, S.P.S. Monga, R-Etodolac decreases β-catenin levels along with survival and proliferation of hepatoma cells, J. Hepatol. 46 (2007) 849–857, https://doi.org/10.1016/j.jhep.2006.11.017.
[52] W. Liu, H. Nakamura, T. Tsujimura, J. Cheng, T. Yamamoto, Y. Iwamoto, H. Imanishi, S. Shimomura, T. Yamamoto, T. Hirasawa, S. Inagaki, S. Nishiguchi, T. Hada, Chemoprevention of spontaneous development of hepatocellular carcinomas in fatty liver Shionogi mice by a cyclooxygenase-2 inhibitor, Cancer Sci. 97 (2006) 768–773, https://doi.org/10.1111/j.1349-7006.2006.00237.x.
[53] J. Cheng, H. Imanishi, W. Liu, H. Nakamura, T. Morisaki, K. Higashino, T. Hada, Involvement of cell cycle regulatory proteins and MAP kinase signaling pathway in growth inhibition and cell cycle arrest by a selective cyclooxygenase 2 inhibitor, etodolac, in human hepatocellular carcinoma cell lines, Cancer Sci. 95 (2004) 666–673, https://doi.org/10.1111/j.1349-7006.2004.tb03327.x.
[54] P. Çikla, D. Özsavci, Ö. Bingöl-Özakpinar, A. Şener, Ö. Çevik, S. Özbaş-Turan, J. Akbuğa, F. Şahin, Ş.G. Küçükgüzel, Synthesis, cytotoxicity, and pro-apoptosis activity of etodolac hydrazide derivatives as anticancer agents, Arch. Pharm. (Weinheim) 346 (2013) 367–379, https://doi.org/10.1002/ardp.201200449.
[55] M. De La Rosa, H.W. Kim, E. Gunic, C. Jenket, U. Boyle, Y. hyo Koh, I. Korboukh, M. Allan, W. Zhang, H. Chen, W. Xu, S. Nilar, N. Yao, R. Hamatake, S.A. Lang, Z. Hong, Z. Zhang, J.L. Girardet, Tri-substituted triazoles as potent non-nucleoside inhibitors of the HIV-1 reverse transcriptase, Bioorg. Med. Chem. Lett 16 (2006) 4444–4449, https://doi.org/10.1016/j.bmcl.2006.06.048.
[56] F. Malbec, R. Milcent, G. Barbier, Derives de la dihydro-2,4-triazole-1,2,4 thione-3, J. Heterocycl. Chem. 21 (1984) 1689–1698, https://doi.org/10.1002/jhet.5570210624.
[57] C.W. Andrews, J.H. Chan, G.A. Freeman, K.R. Romines, H.J. Tidwell, Benzophenones as inhibitors of reverse transcriptase, WO 01/17982 A1, https://doi.org/10.1517/13543776.11.10.1637, 2001.
[58] Z. Zhang, W. Xu, Y.H. Koh, H.S. Jae, J.L. Girardet, L.T. Yeh, R.K. Hamatake, Z. Hong, A novel nonnucleoside analogue that inhibits human immunodeficiency virus type 1 isolates resistant to current nonnucleoside reverse transcriptase inhibitors, Antimicrob. Agents Chemother. 51 (2007) 429–437, https://doi.org/10.1128/AAC.01032-06.
[59] G. Moyle, M. Boffito, A. Stoehr, A. Rieger, Z. Shen, K. Manhard, B. Sheedy, V. Hingorani, A. Raney, M. Nguyen, T. Nguyen, V. Ong, L.T. Yeh, B. Quart, Phase 2a randomized controlled trial of short-term activity, safety, and pharmacokinetics of a novel nonnucleoside reverse transcriptase inhibitor, RDEA806, in HIV-1-positive, antiretroviral-naïve subjects, Antimicrob. Agents Chemother. 54 (2010) 3170–3178, https://doi.org/10.1128/AAC.00268-10.
[60] W. Li, X. Li, E. De Clercq, P. Zhan, X. Liu, Discovery of potent HIV-1 non-nucleoside reverse transcriptase inhibitors from arylthioacetanilide structural motif, Eur. J. Med. Chem. 102 (2015) 167–179, https://doi.org/10.1016/j.ejmech.2015.07.043.
[61] M. Barreca, V. Spanò, R. Rocca, R. Bivacqua, A.C. Abel, A. Maruca, A. Montalbano, M.V. Raimondi, C. Tarantelli, E. Gaudio, L. Cascione, A. Rinaldi, R. Bai, M. O. Steinmetz, A.E. Prota, S. Alcaro, E. Hamel, F. Bertoni, P. Barraja, Development of [1,2]oxazoloisoindoles tubulin polymerization inhibitors: further chemical modifications and potential therapeutic effects against lymphomas, Eur. J. Med. Chem. 243 (2022), 114744, https://doi.org/10.1016/j.ejmech.2022.114744.
[62] M. Barreca, A.M. Ingarra, M.V. Raimondi, V. Spanò, M. De Franco, L. Menilli, V. Gandin, G. Miolo, P. Barraja, A. Montalbano, Insight on pyrimido[5,4-g] indolizine and pyrimido[4,5-c]pyrrolo[1,2-a]azepine systems as promising photosensitizers on malignant cells, Eur. J. Med. Chem. 237 (2022), 114399, https://doi.org/10.1016/j.ejmech.2022.114399.
[63] V. Cilibrasi, V. Spanò, R. Bortolozzi, M. Barreca, M.V. Raimondi, R. Rocca, A. Maruca, A. Montalbano, S. Alcaro, R. Ronca, G. Viola, P. Barraja, Synthesis of 2H-Imidazo[2′,1′:2,3] [1,3]thiazolo[4,5-e]isoindol-8-yl-phenylureas with promising therapeutic features for the treatment of acute myeloid leukemia (AML) with FLT3/ITD mutations, Eur. J. Med. Chem. 235 (2022), 114292, https://doi.org/10.1016/j.ejmech.2022.114292.
[64] M. Labbozzetta, M. Barreca, V. Spanò, M.V. Raimondi, P. Poma, M. Notarbartolo, P. Barraja, A. Montalbano, Novel insights on [1,2]oxazolo[5,4-e]isoindoles on multidrug resistant acute myeloid leukemia cell line, Drug Dev. Res. 83 (2022) 1331–1341, https://doi.org/10.1002/ddr.21962.
[65] K. Grillone, C. Riillo, R. Rocca, S. Ascrizzi, V. Spanò, F. Scionti, N. Polerà, A. Maruca, M. Barreca, G. Juli, M. Arbitrio, M.T. Di Martino, D. Caracciolo, P. Tagliaferri, S. Alcaro, A. Montalbano, P. Barraja, P. Tassone, The new microtubule-targeting agent SIX2G induces immunogenic cell death in multiple myeloma, Int. J. Mol. Sci. 23 (2022) 1–16, https://doi.org/10.3390/ijms231810222.
[66] H. Li, J. Tatlock, A. Linton, J. Gonzalez, T. Jewell, L. Patel, S. Ludlum, M. Drowns, S.V. Rahavendran, H. Skor, R. Hunter, S.T. Shi, K.J. Herlihy, H. Parge, M. Hickey, X. Yu, F. Chau, J. Nonomiya, C. Lewis, Discovery of (R)-6-cyclopentyl-6-(2-(2,6-diethylpyridin-4-yl)ethyl)-3-((5, 7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl) methyl)-4-hydroxy-5, 6-dihydropyran-2-one (PF-00868554) as a potent and orally available hepatitis C virus polymerase inhibitor, J. Med. Chem. 52 (2009) 1255–1258, https://doi.org/10.1021/jm8014537.
[67] R.A. Love, H.E. Parge, X. Yu, M.J. Hickey, W. Diehl, J. Gao, H. Wriggers, A. Ekker, L. Wang, J.A. Thomson, P.S. Dragovich, S.A. Fuhrman, Crystallographic identification of a noncompetitive inhibitor binding site on the hepatitis C virus NS5B RNA polymerase enzyme, J. Virol. 77 (2003) 7575–7581, https://doi.org/10.1128/JVI.77.13.7575–7581.2003.
[68] H. Li, J. Tatlock, A. Linton, J. Gonzalez, A. Borchardt, P. Dragovich, T. Jewell, T. Prins, R. Zhou, J. Blazel, H. Parge, R. Love, M. Hickey, C. Doan, S. Shi, R. Duggal, C. Lewis, S. Fuhrman, Identification and structure-based optimization of novel dihydropyrones as potent HCV RNA polymerase inhibitors, Bioorg. Med. Chem. Lett 16 (2006) 4834–4838, https://doi.org/10.1016/j.bmcl.2006.06.065.
[69] H. Li, A. Linton, J. Tatlock, J. Gonzalez, A. Borchardt, M. Abreo, T. Jewell, L. Patel, M. Drowns, S. Ludlum, M. Goble, M. Yang, J. Blazel, R. Rahavendran, H. Skor, S. Shi, C. Lewis, S. Fuhrman, Allosteric inhibitors of hepatitis C polymerase: discovery of potent and orally bioavailable carbon-linked dihydropyrones, J. Med. Chem. 50 (2007) 3969–3972, https://doi.org/10.1021/jm0704447.
[70] S.J. Gardiner, E.J. Begg, Pharmacogenetics, drug-metabolizing enzymes, and clinical practice, Pharmacol. Rev. 58 (2006) 521–590, https://doi.org/10.1124/pr.58.3.6.521.
[71] S.T. Shi, K.J. Herlihy, J.P. Graham, J. Nonomiya, S.V. Rahavendran, H. Skor, R. Irvine, S. Binford, J. Tatlock, H. Li, J. Gonzalez, A. Linton, A.K. Patick, C. Lewis, Preclinical characterization of PF-00868554, a potent nonnucleoside inhibitor of the hepatitis C virus RNA-dependent RNA polymerase, Antimicrob. Agents Chemother. 53 (2009) 2544–2552, https://doi.org/10.1128/AAC.01599-08.
[72] M. Rodriguez-Torres, E.M. Yoshida, P. Marcellin, S. Srinivasan, V.S. Purohit, C. Wang, J.L. Hammond, A phase 2 study of filibuvir in combination with pegylated IFN alfa and ribavirin for chronic HCV, Ann. Hepatol. 13 (2014) 364–375, https://doi.org/10.1016/s1665-2681(19)30843-9.
[73] Y. hong Wan, W. yu Wu, S. xin Guo, S. jun He, X. dong Tang, X. yun Wu, K. S. Nandakumar, M. Zou, L. Li, X. guang Chen, S. wen Liu, X. gang Yao, [1,2,4] Triazolo[1,5-a]pyrimidine derivative (Mol-5) is a new NS5-RdRp inhibitor of DENV2 proliferation and DENV2-induced inflammation, Acta Pharmacol. Sin. 41 (2020) 706–718, https://doi.org/10.1038/s41401-019-0316-7.

[74] P. Resa-Infante, N. Jorba, R. Coloma, J. Ortin, The influenza virus RNA synthesis machine, RNA Biol. 8 (2011) 207–215, https://doi.org/10.4161/rna.8.2.14513.
[75] G. Muratore, L. Goracci, B. Mercorelli, A. Foeglein, P. Digard, G. Cruciani, G. Palù, A. Loregian, Small molecule inhibitors of influenza A and B viruses that act by disrupting subunit interactions of the viral polymerase, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 6247–6252, https://doi.org/10.1073/pnas.1119817109.
[76] S. Massari, G. Nannetti, J. Desantis, G. Muratore, S. Sabatini, G. Manfroni, B. Mercorelli, V. Cecchetti, G. Palù, G. Cruciani, A. Loregian, L. Goracci, O. Tabarrini, A Broad anti-influenza hybrid small molecule that potently disrupts the interaction of polymerase acidic protein-basic protein 1 (PA-PB1) subunits, J. Med. Chem. 58 (2015) 3830–3842, https://doi.org/10.1021/acs.jmedchem.5b00012.
[77] S. Lepri, G. Nannetti, G. Muratore, G. Cruciani, R. Ruzziconi, B. Mercorelli, G. Palu, A. Loregian, L. Goracci, Optimization of small-molecule inhibitors of in fl uenza virus polymerase: from thiophene-3-carboxamide to polyamido sca ff olds, J. Med. Chem. 57 (2014) 4337–4350, https://doi.org/10.1021/jm500300r.
[78] S. Massari, G. Nannetti, L. Goracci, L. Sancineto, G. Muratore, S. Sabatini, G. Manfroni, B. Mercorelli, V. Cecchetti, M. Facchini, G. Palù, G. Cruciani, A. Loregian, O. Tabarrini, Structural investigation of cycloheptathiophene-3-carboxamide derivatives targeting influenza virus polymerase assembly, J. Med. Chem. 56 (2013) 10118–10131, https://doi.org/10.1021/jm401560v.
[79] V.M. Chernyshev, A.V. Chernysheva, V.A. Taranushich, Synthesis of esters and amides of 5-amino-1,2,4-triazole-3-carboxylic and 5-amino-1,2,4-triazol-3-ylacetic acids, Russ. J. Appl. Chem. 79 (2006) 783–786, https://doi.org/10.1134/S1070427206050168.
[80] H. Liu, X. Yao, Molecular basis of the interaction for an essential subunit PA-PB1 in influenza virus RNA polymerase: insights from molecular dynamics simulation and free energy calculation, Mol. Pharm. 7 (2010) 75–85, https://doi.org/10.1021/mp900131p.
[81] S. Massari, C. Bertagnin, M.C. Pismataro, A. Donnadio, G. Nannetti, T. Felicetti, S. Di Bona, M.G. Nizi, L. Tensi, G. Manfroni, M.I. Loza, S. Sabatini, V. Cecchetti, J. Brea, L. Goracci, A. Loregian, O. Tabarrini, Synthesis and characterization of 1,2,4-triazolo[1,5-a]pyrimidine-2-carboxamide-based compounds targeting the PA-PB1 interface of influenza A virus polymerase, Eur. J. Med. Chem. 209 (2021), 112944, https://doi.org/10.1016/j.ejmech.2020.112944.
[82] S. Massari, J. Desantis, G. Nannetti, S. Sabatini, S. Tortorella, L. Goracci, V. Cecchetti, A. Loregian, O. Tabarrini, Efficient and regioselective one-step synthesis of 7-aryl-5-methyl- and 5-aryl-7-methyl-2-amino-[1,2,4]triazolo[1,5-a] pyrimidine derivatives, Org. Biomol. Chem. 15 (2017) 7944–7955, https://doi.org/10.1039/c7ob02085f.
[83] M.C. Pismataro, T. Felicetti, C. Bertagnin, M.G. Nizi, A. Bonomini, M.L. Barreca, V. Cecchetti, D. Jochmans, S. De Jonghe, J. Neyts, A. Loregian, O. Tabarrini, S. Massari, 1,2,4-Triazolo[1,5-a]pyrimidines: efficient one-step synthesis and functionalization as influenza polymerase PA-PB1 interaction disruptors, Eur. J. Med. Chem. 221 (2021), 113494, https://doi.org/10.1016/j.ejmech.2021.113494.
[84] S. Yuan, H. Chu, H. Zhao, K. Zhang, K. Singh, B.K.C. Chow, R.Y.T. Kao, J. Zhou, B. J. Zheng, Identification of a small-molecule inhibitor of influenza virus via disrupting the subunits interaction of the viral polymerase, Antivir. Res. 125 (2016) 34–42, https://doi.org/10.1016/j.antiviral.2015.11.005.
[85] R.Y. Kao, D. Yang, L.S. Lau, W.H.W. Tsui, L. Hu, J. Dai, M.P. Chan, C.M. Chan, P. Wang, B.J. Zheng, J. Sun, J.D. Huang, J. Madar, G. Chen, H. Chen, Y. Guan, K. Y. Yuen, Identification of influenza A nucleoprotein as an antiviral target, Nat. Biotechnol. 28 (2010) 600–605, https://doi.org/10.1038/nbt.1638.
[86] A. Carta, I. Briguglio, S. Piras, P. Corona, G. Boatto, M. Nieddu, P. Giunchedi, M. E. Marongiu, G. Giliberti, F. Iuliano, S. Blois, C. Ibba, B. Busonera, P. La Colla, Quinoline tricyclic derivatives. Design, synthesis and evaluation of the antiviral activity of three new classes of RNA-dependent RNA polymerase inhibitors, Bioorg. Med. Chem. 19 (2011) 7070–7084, https://doi.org/10.1016/j.bmc.2011.10.009.
[87] A. Carta, M. Palomba, P. Corona, Synthesis of substituted aminoquinolines as useful intermediates for preparation of aromatic n-tricyclic systems, Heterocycles 68 (2006) 1715–1722, https://doi.org/10.3987/COM-06-10782.
[88] P. Sanna, A. Carta, G. Paglietti, Synthesis of two novel tricyclic rings: triazolo[4,5-g]- quinolines and pyrido[2,3-g]quinoxalines derived from 6,7-diaminoquinolines, Heterocycles 53 (2000) 423–432, https://doi.org/10.3987/COM-99-8766.
[89] D.S. Stec, M.G. Hill, P.L. Collins, Sequence analysis of the polymerase L gene of human respiratory syncytial virus and predicted phylogeny of nonsegmented negative-strand viruses, Virology 183 (1991) 273–287, https://doi.org/10.1016/0042-6822(91)90140-7.