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DESIGN, SYNTHESIS AND ANTIVIRAL EVALUATION OF PYRAZOLE AND PYRAZOLINE DERIVATIVES AS NOVEL INHIBITORS OF FLAVIVIRUS AND PESTIVIRUS REPLICATION

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Nothing in life is to be feared, it it only to be understood.
Now is time to understand more, so that we may fear less.
Marie Curie

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1. INTRODUCTION

The *Flaviviridae* family includes four genera: Flavivirus, Hepacivirus, Pegivirus and Pestivirus. This virus family contains human and animal pathogens of global significance, e.g. the human flaviviruses yellow fever virus (YFV), dengue virus (DENV), West-Nile virus (WNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), Zika virus (ZIKV), Murray Valley encephalitis (MVEV), St Louis encephalitis (SLEV) as well as hepacivirus hepatitis C virus (HCV). The name *Flaviviridae* refers to the jaundice occurring in course infection caused by YFV, the first identified virus of this family [1]. In humans, infection with *Flaviviridae* may lead to fulminant, hemorrhagic diseases (YFV, DENV), viral encephalitis (WNV, JEV, TBEV) or chronic hepatitis (HCV). Viruses belonging to the pestivirus genus [e.g. bovine viral diarrhea virus (BVDV)], infect only animals, leading to severe disease of the host, usually followed by death, causing significant economic losses [2, 3].

Specific antiviral therapies are currently available only for the treatment of HCV infections [4] and, to date, there is no specific treatment for any flavivirus and pestivirus infections, though commercially available vaccines for some viruses exist. The overall strategy against pestivirus mainly involves identification and removal of infected animals and vaccination. Despite therapy for pestivirus infections is not believed to be an option, current vaccines do not completely prevent the spread of infection and antiviral therapy could potentially supplement vaccination [5].

On the contrary, due to the global threat of flaviviral pandemics [6], there is an urgent need for specific antiviral drugs for the treatment of flavivirus infections since, in many countries, patients with severe cases of infections are treated only by supportive care, which includes intravenous fluids, hospitalization, respiratory support, and prevention of secondary infections.

2. PATHOGENESIS AND CLINICAL SIGNIFICANCES

2.1. Flavivirus

Most Flaviviruses are transmitted through the bite of infected mosquitoes of the species *Aedes aegypti* (YFV, DENV) and *Culex Vishnu* (JEV), or through the bite of the tick *Ixodes ricinus* (Western Europe), *Ixodes persulcatus* (Eastern Europe and Asia) or *Ixodes ovatus* (China and Japan) [6-7].

The geographical distribution of each virus is likely to be affected by its vector distribution. Therefore, climate change and development of transportation systems have allowed flaviviruses to invade new areas. Currently, many people are exposed to flaviviruses worldwide. In addition, traveling through endemic countries increases the opportunity of exposure to flaviviruses [8].

Mosquitoes are the most important vector for flaviviral diseases affecting humans, as well as being the crucial intermediate replicative vector for the normal enzootic cycle through birds and pigs, which amplify these viruses. Mosquito-borne flaviviruses and their associated diseases are found in Asia, Africa, Europe, the US and Australia, and are concentrated in wetland areas where mosquitoes abound [9]. Consequently, flavivirus-induced disease attack rates increase with rising mosquito population densities, and are further increased by human contact with amplifying vertebrate hosts, such as birds or pigs. This is associated with farming activities, such as deforestation and irrigation [10], as well as by the effects of global warming, associated with increasing temperatures and precipitation, as occurs during extended monsoon periods [11, 12].

As indicated in Table 1, an etiologic classification within the genus Flavivirus might be performed by knowing the vector.

Table 1. Classification of Flavivirus by carrier [13, 14]

FLAVIVIRUS WITH KNOWN VECTOR		
Classification	**Vector**	**Species**
Mammalian viruses	Tick	*Gadgets Gully virus, Kadam virus, Kyasanur Forest disease virus, Langat virus, Louping ill virus, Omsk hemorrhagic fever virus, Powassan virus, Royal Farm virus, Tick-borne encephalitis virus*
Aroa	Mosquito	*Aroa virus*
Dengue	Mosquito	*Dengue virus, Kedougou virus*
Japanese encephalitis	Mosquito	*Cacipacore virus, Japanese encephalitis virus, Koutango virus, Murray Valley encephalitis virus, St. Louis encephalitis virus, Usutu virus, West Nile virus, Yaounde virus*
Kokobera	Mosquito	*Kokobera virus*
Ntaya	Mosquito	*Israel turkey virus, Ntaya virus, Tembusu virus*
Spondweni	Mosquito	*Zika virus*
Yellow fever	Mosquito	*Banzi virus, Bouboui virus, Edge Hill virus, Jugra virus, Sapoya virus, Sepik virus, Uganda S virus, Wesselsbron virus, Yellow fever virus*
FLAVIVIRUS WITH UNKNOWN VECTOR		
Gender	**Species**	
Flavivirus	*Entebbe bat virus, Yokose virus, Apoi virus, Cowbone Ridge virus, Jutiapa virus, Modoc virus, Sal Vieja virus, San Perlita virus, Bukalasa bat virus, Carey Island virus, Dakar bat virus, Motana myotis leukoencephalitis virus, Phnom Penh bat virus, Rio Bravo virus*	

Due to their significant pleiotropism within the vertebrate host, Flavivirus can be broadly grouped into viruses that have the capacity to cause vascular leakage and hemorrhage (e.g. YFV and DENV), and those that cause encephalitis (e.g. WNV, JEV, TBEV) (Table 2). After entering through the skin via the bite of an infected arthropod, the virus starts to proliferate locally

and then spreads to become generalized within a short period of time, usually with a significant viremia. Despite the route of progression through the vertebrate host has not been clearly established, it seems that the virus progresses from the site of the bite to draining lymph nodes, where it replicates and is amplified before, in case of encephalitic flaviviruses, crossing the blood-brain barrier (BBB) [15]. Several hypotheses have been presented to explain the mechanism of central nervous system (CSN) penetration. These include virus entry as a result of inflammation and damage to vascular integrity [16], penetration through the olfactory bulb [17], toll-like receptor-mediated entry [18] and transcytosis across vascular endothelial cells [16]. BBB crossing is an important factor for the pathogenesis and unfavorable clinical outcome of the neurotropic viral infection [19]. Studies suggest that macrophages could serve as a reservoir for WNV, spreading the virus from the periphery to the CNS [20, 21], while other reports have shown that WNV is able to enter the CSN via anterograde axonal transport [22]. It has been shown that JEV virions bound to the endothelial surface of the CSN are internalized by endocytosis [23]. The mechanisms by which encephalitic flaviviruses induce neuronal injury in vivo are still unknown. However, in vitro studies have started to elucidate the pathways involved in WNV-induced cell death. It has been demonstrated that WNV infection triggers apoptosis in different transformed cell lines, resulting in caspase 3 activation, cytochrome c release, and exposure of phosphatidylserine on the outer leaflet of the plasma membrane [24, 25]. The cellular outcome of WNV replication depends on interactions between host and viral factors. UV-inactivated WNV failed to induce cell death, suggesting that viral replication is required to trigger apoptosis [25]. Several WNV proteins may contribute directly to this process. Ectopic expression of the WNV NS3 protein or its helicase or protease domain induced apoptosis and activation of caspase 3 and 8 [26]. Expression of

WNV capsid protein either in vitro or in the striata of mouse brain also triggered apoptosis downstream of caspase 3 and caspase 9 activation [27]. It is known that programmed cell death could have opposing functions during viral infection: it may be antiviral by inducing the death of infected cells, or it may enhance viral spread and progeny release. Cell death can also be pathological if it occurs in non-renewing cell populations, such as neurones. Thus, it has been postulated that virus-induced apoptosis may contribute to neuronal death in vivo and the pathogenesis of encephalitic flaviviruses [28, 29]. However, direct evidence for this mechanism has been lacking, and the pathways involved in the flavivirus-mediated death of neurones are not well understood.

Other pathogenic flaviviruses, principally DENV and YFV, cause a syndrome of fever and malaise, 'capillary leak' with loss of plasma volume and coagulation defects which can lead to bleeding. The 15–20 different types of viral haemorrhagic fever appear to share a similar pathogenesis, in which macrophages and dendritic cells, rather than acting as barriers to an invading pathogen, serve as the principal sites of viral replication, supporting the rapid spread of infection [30, 31, 32]. Although direct cytopathic effects can also contribute to disease severity (hepatic injury in case of YFV infection), most features of the illness are often caused by innate immune responses, as the systemic spread of virus to macrophages and dendritic cells leads to the release of mediators that modify vascular function and have procoagulant activity.

Human YFV infections exhibit three clinical stages [33]. Following a 3–6-day incubation period, illness begins with fever, headache, myalgia and viremia characterized as the "infection period", although the majority of cases are thought to result in asymptomatic infections. Following recovery from a 3–5-day "infection period", a "remission period" can occur in some patients with liver and renal failure. Finally, an "intoxication period" begins with

hemorrhagic fever and multi-organ failure, where patients also develop jaundice and thrombocytopenia. Approximately 15% of cases develop moderate/severe manifestations. The World Health Organization (WHO) estimates 200'000 cases with 30'000 deaths occur annually, mostly in Africa [34].

DENV is a flavivirus of global public health importance; an estimated 2.5 billion people in more than 100 countries are at risk of acquiring dengue viral infection with more than 50 million new infections being projected annually [35]. The economic importance of dengue in the developing world is also a major concern [36]. After the bite of an infected mosquito vector, DENV can cause a mild and self-limiting infection but may induce more severe symptoms such as dengue fever (DF) or it may progress to a potentially lethal dengue haemorrhagic fever (DHF) and in the worst cases, dengue shock syndrome (DSS). The characteristic features of DHF are increased capillary permeability without morphological damage to the capillary endothelium, thrombocytopenia, altered number and functions of leucocytes, altered haemostasis and liver damage. The pathogenesis of DHF is not fully understood, despite extensive work carried out in recent decades, due mainly to the absence of an appropriate animal model. Various mechanisms have been suggested for DHF enhancement including a role for non-neutralizing enhancing antibodies in secondary dengue infections with heterologous serotypes, memory T-cell-mediated pathogenesis, immune complex disease or complement and its products, anti-NS1 antibodies that cross-react with vascular endothelium. A cytokine Tsunami and other soluble mediators such as high concentrations of soluble IL-2 receptor, soluble CD4, soluble CD8, TNF receptors, IL-10 and macrophage migration inhibition factor are also considered important factors in dengue pathogenesis. Finally, selection of virulent virus strains (South East Asian type DENV-2) and host genetic polymorphism are others important factors largely described and certainly involved in DHF [37, 38].

Table 2. Main pathogenic flaviviruses for humans.

Virus	Abbreviation	Location of isolation	Geographic distribution	Human diseases
Dengue 1	DENV-1	Hawai	Tropics, subtropics	Fever, rash, vasculopathy, hemorrhagic fever
Dengue 2	DENV-2	New Guinea	Tropics, subtropics	Fever, rash, vasculopathy, hemorrhagic fever
Dengue 3	DENV-3	Philippines	Tropics, subtropics	Fever, rash, vasculopathy, hemorrhagic fever
Dengue 4	DENV-4	Philippines	Tropics, subtropics	Fever, rash, vasculopathy, hemorrhagic fever
Kyasanur Forest disease	KFDV	India	India	Hemorrhagic fever
Omsk hemorrhagic fever	OHFV	Russia	Western Siberia	Hemorrhagic fever/encephalitic
Yellow fever	YFV	Ghana	Sub-Saharan Africa, South America	Pantropic
Powassan virus	POWV		Western United States, Western Canada, Siberia	Encephalitis
Japanese encephalitis	JEV	Japan	Asia	Encephalitis
Langat	LGTV	Makaysia	Malaysia, Thailand, Siberia	Encephalitis
Louping ill	LIV	Scotland	Uk, Ireland	Encephalitis
Murray Valley encephalitis	MVEV	Australia	Australia, New Guinea	Encephalitis
St Louis encephalitis	SLEV	USA	South and Central	Encephalitis
Tick-borne encephalitis	TBEV	Russia	Europa, Asia	Encephalitis
West Nile	WNV	Uganda	Old World and New World	Encephalitis

2.2. Pestivirus

The genus Pestivirus includes three species: bovine viral diarrhea virus (BVDV), classical swine fever disease virus (CSFV), and ovine border disease virus (OBV). Pestiviruses infect many species of domestic and wild animals, BVDV is a prototypical representative of the pestiviruses of ruminant animals.

There are multiple methods of BVDV transmission: the virus can spread horizontally within a herd as well as transmit vertically from cow to calf. Horizontal transmission can occur via transiently infected (TI) animals that shed virus during acute infection, it can also occur due to persistently infected (PI) animals that shed virus throughout their lifespan in all bodily secretions (nasal and ocular discharges, milk/colostrum, semen, urine, and feces) [39]. Studies show that BVDV environmental survival is dependent upon temperature and moisture levels with a maximum survival in bovine farm slurry at 5°C for 3 weeks and at 20°C for 3 days [40]. There are also reports of indirect BVDV transmission from contaminated pens, rectal examination gloves, hypodermic needles, nose tongs, and ambient air [41].

Vertical transmission may occur from a PI dam *in utero* to her offspring. In vertical transmission the outcome of infection is determined by the stage of fetal maturation when exposed to the virus *in utero*. If the fetus is infected in the first trimester, it will likely abort, mummify, or show a variety of congenital defects. Infection during the second trimester results in a PI animal: in these feti, the virus is recognized as self, resulting in an immune-tolerant state and persistent viremia without seroconversion. By the third trimester of gestation (>180 days), the fetus is immune-competent and will mount an immune response that may result in abortion, or the birth of a healthy or weak and seropositive calf [42].

BVDV is known for causing a variety of disease presentations in cattle and other ungulates.There are two genotypes of the virus: BVDV-1 and BVDV-2, both of which have also been isolated from non-bovine species. The genotypes are further divided into cytopathic (CP) and non-cytopathic (NCP) subtypes. Acute infection occurs when seronegative, immunocompetent cattle are exposed to the virus. The disease is characterized by ulceration of the nose, mouth and gastrointestinal mucosa which helps the

virus to spread quickly, due to the continuous salivation, coughing, nasal discharge or diarrhea [43]. These symtoms are consequences of the damage caused by the virus to its target organs such as the lymphoid sistem, the cells of the gastrointestinal tract, glands and neurons [44, 45].

Control of pestiviral diseases is particularly difficult due to the constant viremia and viral shedding of PI animals, which must be identified and eliminated to prevent disease transmission. Existing vaccines are limited by the delay between vaccination and the onset of protection, the difficulty of differentiating serologically between vaccinated and naturally infected animals and the need for broad vaccine cross-protection against diverse virus strains. Antiviral therapy could potentially supplement vaccination by providing immediate protection in the case of an outbreak [46].

2.3. Hepacivirus

Hepatitis C virus (HCV) is the only member of the Hepacivirus genus, identified in 1989 [47]. HCV is found worldwide but the most affected regions are Africa and Central an East Asia. The distribution varies locally and can be concentrated in certain populations (drug addicts or blood products recipients) or in general population. HCV infections are largely diffused in the world and around 55-85% of infected people will develop a chronic infection; according to the World Health Organization (WHO) the number of people suffering from chronic HCV infection is between 130-150 million people globally.

HCV is a bloodborne virus, most commonly transmitted to intravenous-drug users through the sharing of syringes, transfusion of unscreened blood and blood products, reuse or inadequate sterilization of medical, tattoing or body piercing equipment [48, 49]. This virus can also be transmitted sexually and through perinatal transmission but these modes are much less common. HCV

does not spread through water, food or breast milk or by casual everyday contact such as hugging, kissing and sharing food and drinks with an infected individual. Since actually there is no available vaccine, prevention is crucial in reducing the risk of infection, especially in high-risk population and settings [50].

The incubation period is between 2 weeks and 6 months, even after this period the majority of infected people show no symptoms while those who are acutely sympthomatic may exhibit fever, fatigue, nausea, vomiting, abdominal pain, decreased appetite, dark urine, grey-coloured faeces, joint pain and jaundice (yellowing of skin and yellowing of the sclera). Since HCV infection is usually asymptomatic, just a few people are diagnosed during the acute phase, even in those who develop a chronic HCV infection this usually remains undiagnosed for decades until secondary symptoms of serious liver damage occur. To correctly decide the treatement and manage the disease, people diagnosed with chronic HCV infection should have an assessment of the degree of liver damage (fibrosis and cirrhosis) and a test to identify the genotype of the HCV strain. There are 6 different HCV genotypes which respond differently to treatement, and it is possible to be infected with more than one of them.

About 15-45% of people infected with HCV spontaneously clear the infection thanks to a strong immune reaction and some people with chronic infection do not develop liver damage, but when treatement is necessary, the goal of HCV treatment is cure. Antiviral medicines can cure 90% of persons with HCV infection, thus reducing the risk of death, but access to diagnosis and treatment is usually low [51, 52, 53]. Until recently HCV treatment was based on 48 weeks therapy with interferon and ribavin; this therapy cured approximately half of the treated patients but caused frequent and sometimes life-threatening adverse reactions. The standard care for HCV is rapidly

changing and, recently, new antiviral drugs, called direct antiviral agents (DAA) have been developed. These medicines can cure most persons with HCV infection and treatment is safer, shorter (usually 12 weeks) and better tolerated than old treatments. The production cost of DAAs is low but in many high and middle-income countries these medicines are very expensive but due to the introduction of generic versions of this drug prices have substantially dropped in some countries, primarily low income ones [54].

3. VIROLOGY

The genus Flavivirus comprises an unusually large number of taxonomically recognised species (more than 70 at the present time, of which more than 40 are human pathogens) with a global distribution. This genus also includes a large, and increasing, number of unclassified or "tentative" species that have very different characteristics from those currently recognized as members of the genus [55, 56].

Flaviviruses have historically been associated with changes in taxonomy to reflect newly-identified viruses and advances in analytical methods. Initially, the term arborviruses (later changed to arboviruses to avoid confusion with the Latin word "arbor", meaning tree) was derived as a taxonomic criterion following the discovery of several arthropod-borne viruses, later due to morphological information obtained using electron microscopy, it was possible to support the hypothesis of the existence of at least two groups of viruses:

1. A group of non-enveloped viruses, which are currently classified within the family *Reoviridae* (genera Orbivirus, Coltivirus and Seadornavirus), namely viruses with an overall diameter of 60–80 nanometers, icosahedral symmetry and several concentric capsid layers that surround a segmented double-stranded RNA (dsRNA) genome.
2. A group of enveloped viruses with a diameter of 50-60 nanometers and infectious single-stranded RNA (ssRNA) of positive polarity. The development of serological methods led to the identification of two antigenically distinct sub-groups. This division was subsequently confirmed by analysis of the genome sequences and the viruses were divided as follows:

(a) The "A group of Arbovirus" which currently includes viruses classified within the genus Alphavirus, family Togaviridae (together with the non-arboviral genus Rubivirus).

(b) The "B group of Arbovirus" which currently includes viruses classified within the genus flavivirus, family *Flaviviridae* (together with non-arboviral genera Hepacivirus and Pestivirus).

3.1. Viral genome

The flavivirus genome is a plus-sense, single stranded RNA of about 11000 nucleotides, consisting of a 5' untranslated region (UTR) carrying a canonical cap structure, a single open reading frame (ORF) and a 3' UTR which is not polyadenylated (with the exception of TBEV) but instead forms a functionally equivalent complex RNA fold [57]. The genome is packaged by the viral capsid protein (C) in a host-derived lipid bilayer containing the viral envelope protein (E) and the protein M. The first is crucial in receptor binding, membrane fusion and viral assembly while the second is processed from a larger precursor protein (Pre-M), which acts as a stability factor that protects the E protein during the replication in the infected cell (Figure 1).

The single ORF encodes a large polyprotein that is co-translationally and post-translationally processed by viral and host proteases into 10 mature viral proteins that are required for replication and assembly of new virions. The N-terminal end of the polyprotein encodes the three structural proteins C, prM/M and E, followed by seven non-structural (NS) proteins NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5 (Figure 2) [58].

NS3 (70kDa) and NS5 (104kDa) are the most fully characterized non-structural proteins with multiple enzyme activities essential for viral replication. Mutations that affect each activity impair viral replication [59]. NS3 protein has three distinct enzymatic activities: serine protease together with

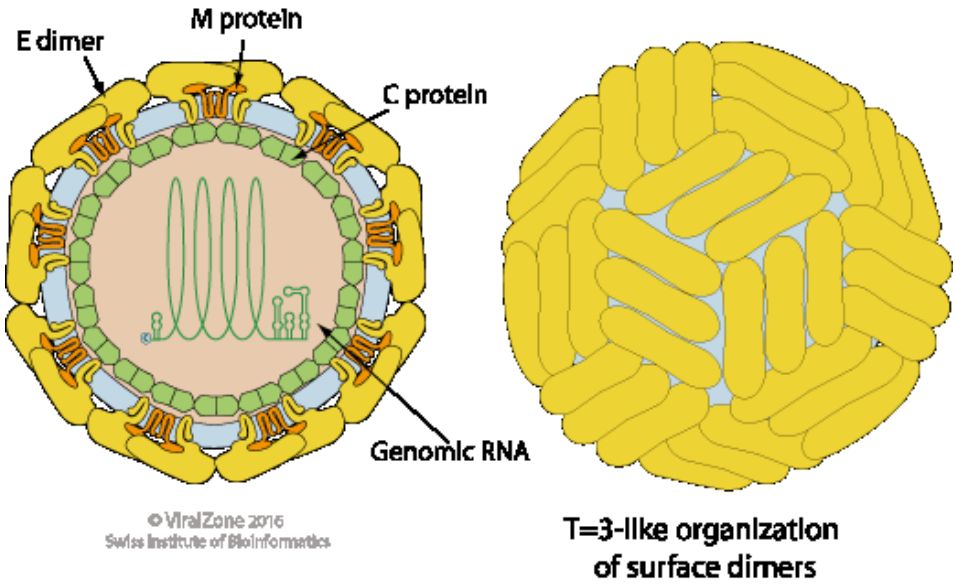


Figure 1. Flavivirus structure.

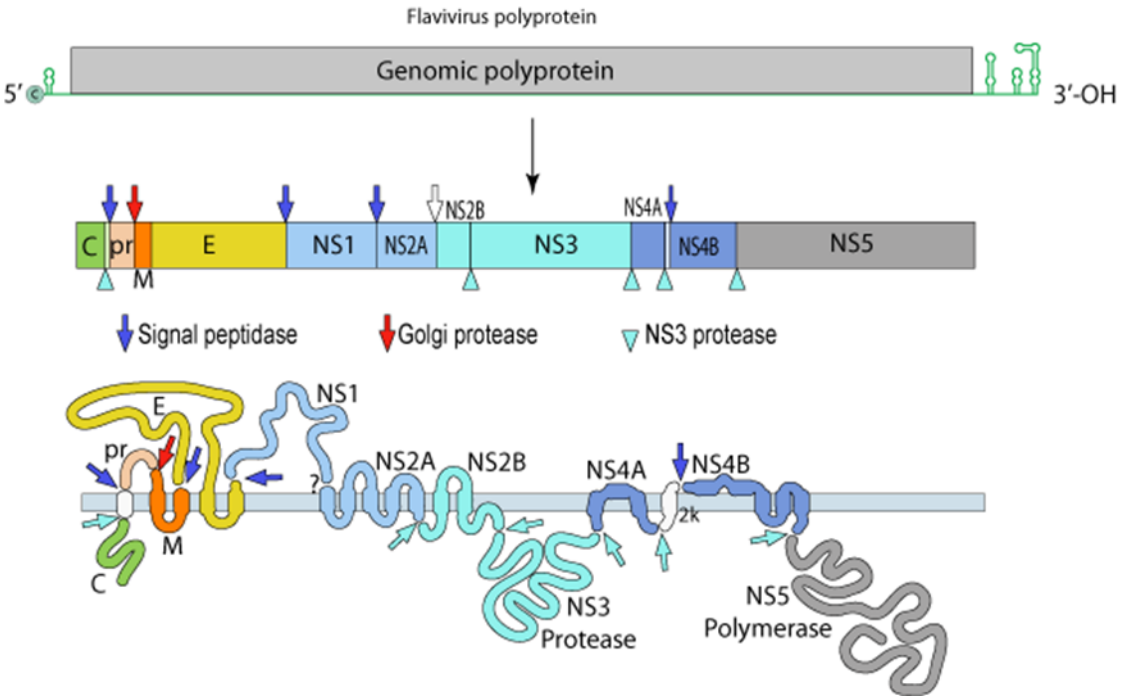


Figure 2. Schematic representation of flavivirus genome and polyprotein organization.

the cofactor NS2B, necessary for the polyprotein processing [60, 61, 62]; helicase / NTPase activity, required for unwinding the double-stranded replicative dorm of RNA; RNA triphosphatase, needed for capping nascent viral RNA [63].

NS5 is the largest and most highly conserved flaviviral protein, with sequence identity greater than 75% across all DENV serotype. It contains two distinct enzymatic activities, separated by an interdomain region: an S-adenosylmethyltransferase and an RNA-dependent RNA polymerase (RdRp) [64].

The NS1 protein (16 kDa) is required for flavivirus replication and is presumably involved in negative-strand RNA synthesis by a mechanism not yet entirely clarified. The fundamental role played by this protein in viral replication is confirmed by the fact that its deletion prevents the replication of YFV [65].

NS2A (22 kDa) is a small hydrophobic transmembrane protein that is involved in generating virus-induced membranes during virus assembly [66].

NS4A (16 kDa) is an integral membrane protein which induces membrane rearrangements to form the viral replication complex [67].

NS4B (27 kDa) is a protein which inhibits the type I interferon response of host cells and may modulate viral replication through an interaction with the NS3 protein [68, 69].

3.2. Replication cycle

Host cells for flaviviral infection include monocytes, macrophages and dendritic cells [70]. The virus attaches to the cell surface, mediated by the E protein, and enters the cell via receptor-mediated endocytosis [71, 72] (Figure 3). The low pH in the endosomal compartment triggers fusion of the viral host cell membrane mediated by the structural reorganization of E protein, which

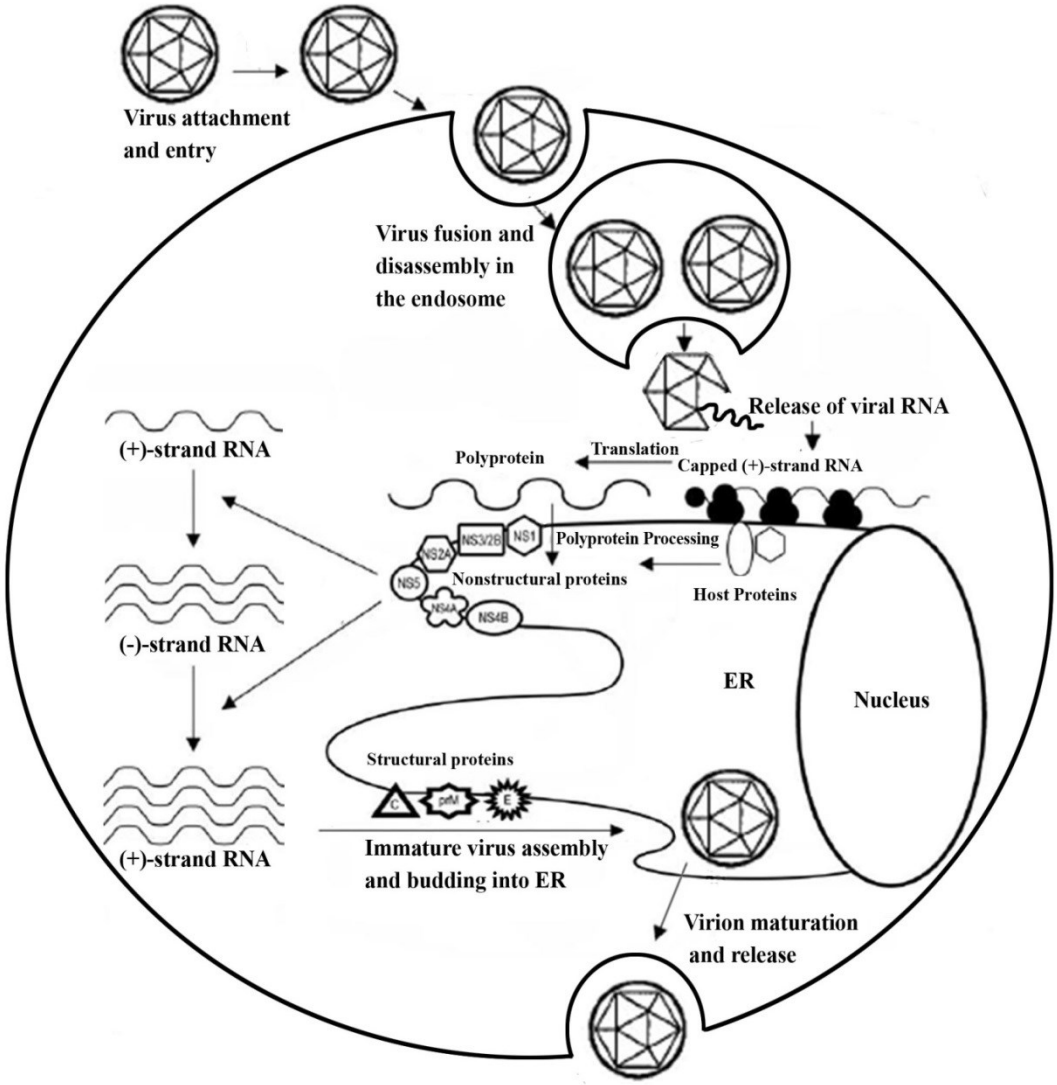


Figure 3. Flavivirus replication cycle.

promotes the release of the nucleocapsid and viral RNA into the cytoplasm (uncoating) [73]. The positive-sense RNA is translated into a single polyprotein that is co-and post-translationally cleaved by the viral protease NS2B/NS3 and by host proteases in order to produce three structural proteins and seven non-structural proteins in the following order: C - prM -E - NS1 - NS2A - NS2B - NS3 - NS4A - NS4B - NS5. The host cell peptidase, located in the lumen of the endoplasmic reticulum (ER), cleaves the sites C - PrM, PrM - E, E - NS1, NS4A - NS4B while the viral protease NS3 cleaves the sites NS2A - NS2B, NS2B - NS3, NS3 - NS4A, NS4B - NS5 [74]. The non-structural

proteins are involved in viral genome replication, which occurs in the rough endoplasmic reticulum (RER) and in the membranes from Golgi complex, called vesicular packets (VP). The RNA-dependent RNA polymerase (RpRd) NS5 generates a copy of the viral genome in the form of single-stranded negative-sense RNA devoid of cap, which serves as a template for the synthesis of new ssRNA (+) genome. The new synthesized RNA is extruded through the intermembrane space of the VP and from here out into the cytoplasm by a not yet completely clarified mechanism [75]. The assembly of virus particles occurs in the lumen of the RER. The first step in this process is the coating of the newly synthesized viral RNA with the C protein [76, 77, 78]. Next, E and PrM proteins hetero-dimerize and envelope the nucleocapsid, forming an immature virus particle that buds from the lumen of the RER into the Golgi complex [79]. However, the mechanism of interaction of the C protein within the nucleocapsid is still not clear. Maturation of virus particle occurs in the trans-Golgi network, where prM is cleaved to M by furin, along with conformational rearrangements of E protein [80, 81]. Mature virions are subsequently released by exocytosis.

4. CURRENT TARGETS AND PERSPECTIVES ON FLAVIVIRUS CHEMOTERAPY

Despite the large number of people that annually suffer from severe flavivirus infections, no clinically approved antiviral therapy is currently available to manage these diseases and only a palliative FANS therapy is provided to treat fever and pain. Vaccination is considered a reasonable method to prevent flavivirus infections; effective flavivirus vaccines against YFV, JEV and TBEV infections have been developed, but WNV and DENV vaccines have not been licensed for human use [82]. However vaccines may cause adverse effects ranging from headaches, gastrointestinal disturbances, myalgia and local reactions, to neurotropic and viscerotropic syndromes whose outcome can be fatal. Moreover, vaccination is not recommended in immunocompromised patients; unfortunately regions with the highest number of HIV infections (Africa Central and Eastern Europe) are also the most hit by YFV [83]. The aforementioned problems linked to vaccination and the absence of a specific therapy, have pushed towards the search for effective drugs in the treatment of infections by flaviviruses. For this purpose, useful targets can be the viral proteins (Tables 3 and 4) or the host proteins that play a key role in the virus replication (Tables 5 and 6).

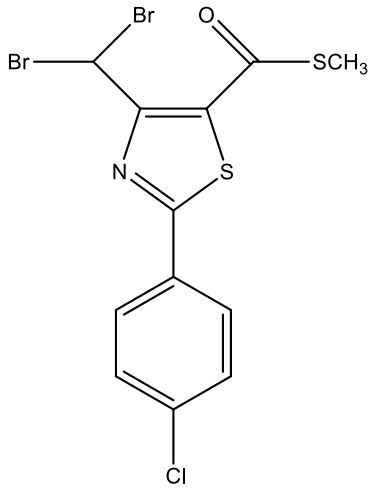
Currently, interest in developing inhibitors is limited to those viruses that cause chronic disease, viruses that have the potential to cause large-scale epidemics, or ubiquitous viruses for which the treatment of acute infection would be beneficial even if the infection was ultimately self-limiting.

4.1. Compounds targeting viral proteins

Envelope proteins. The structural proteins of the flavivirus, especially those of the envelope, are excellent targets for the development of antiviral drugs, for this purpose modeling studies have been used to identify potential inhibitors of these proteins [84].

The monoclonal antibody **mAb 2A10G6** has been shown to be effective on YFV, DENV, WNV, JEV and TBEV recognizing an epitope within the fusion loop of the E protein, thus arresting the steps of the replication cycle after binding [85].

Compounds that interact with the envelope proteins could be used to prevent entry of the virus into the host cell. Many thiazole derivatives that target the envelope proteins have shown a high activity against YFV in cell cultures, in particular ***S*-methyl-2-(4-chlorophenyl)-4-(dibromomethyl) thiazole-5-carbothioate** is moderately nontoxic (CC_{50} = 369 μM) with an EC_{50} = 1.4 μM and has a selectivity index (EC_{50}/CC_{50}) of 263 [86].



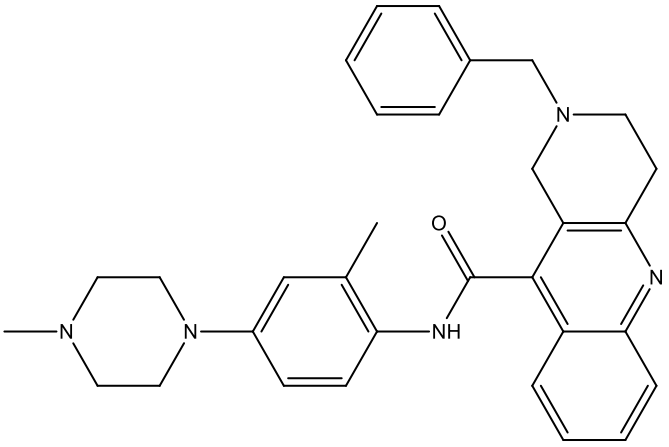
***S*-methyl-2-(4-chlorophenyl)-4-(dibromomethyl)thiazole-5-carbothioate**

NS1. The primary function of this protein remains unclear, despite the recent unveiling of its atomic structure [87] and a growing list of host molecules with which it has been found associated. It has been found that NS1 plays an

important role in the mechanisms of immune-evasion of various Flavivirus. This non-structural protein forms a complex with C1S and C4 proteins, promoting the cleavage of C4 into C4b [88]. NS1 is also a fundamental component for the synthesis of viral RNA, therefore molecules that inhibit this protein could be potential viral replication inhibitors. Further understanding of the interplay between flaviviruses and the immune system will be critical to the development of new antiviral strategies.

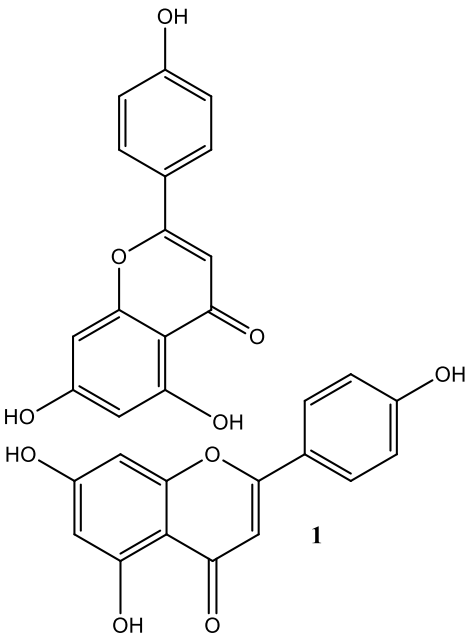
NS3. Inhibitors of this protease are currently designed by either competing with substrate binding or by disrupting the interaction between NS2B and the NS3 protease domain [89]. Despite a wealth of structural and biochemical informations available on the NS2B–NS3 protease substrate-binding pocket, no compound has progressed to the preclinical stage yet.

A competitive non peptidic inhibitor (**Compound 14**) with an EC_{50} of 5 μM and a CC_{50} greater than 300 μM has been recently identified using a computational approach that included elaboration of a pharmacophore model, the NS2B–NS3 protease X-ray crystallographic structure and a docking protocol [90].



Compound 14

In another study, flavonoids were found to be non-competitive inhibitors of DENV NS2B–NS3 protease serotypes 2 and 3 with IC_{50} (compound concentration giving a 50% inhibition in enzymatic assay) of 15–44 μM [91].



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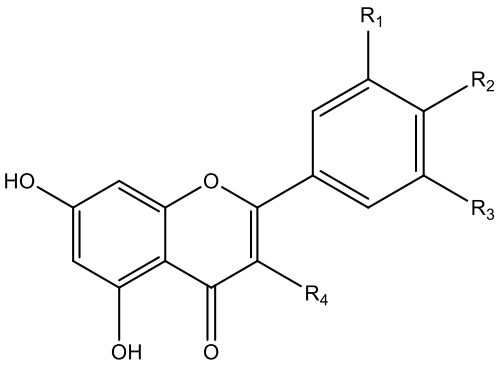
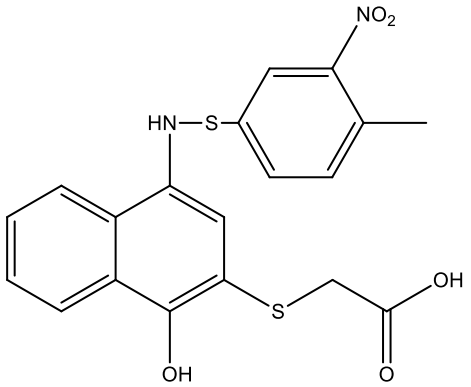


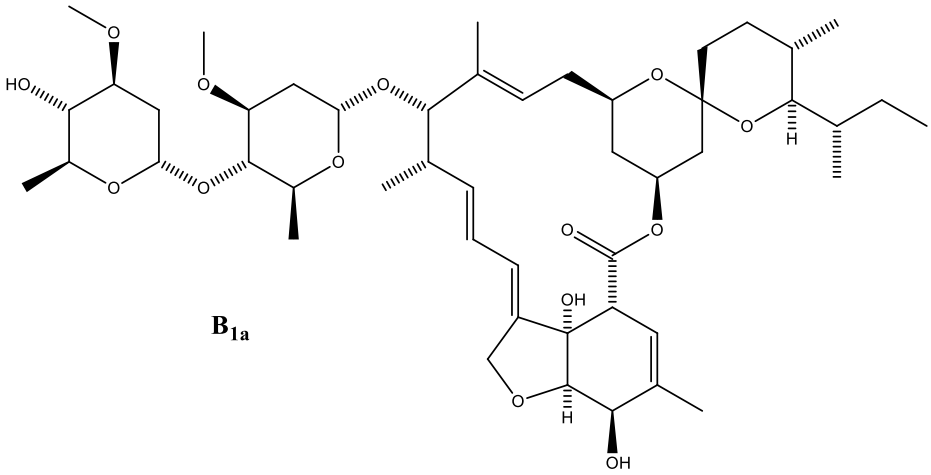
	R_1	R_2	R_3	R_4
2	OH	OH	H	o-rha α
3	OH	OH	H	glc β
4	OH	OH	OH	OH
5	OH	OH	H	OH
6	H	OH	H	OH

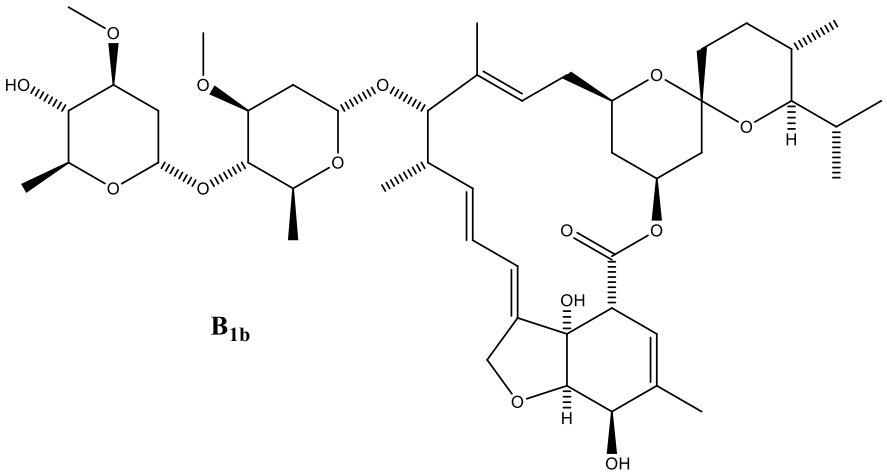
Finally, an interesting study provided evidences for the possibility of targeting the interaction between NS2B and NS3 as an effective antiviral strategy. This study reports that compound **SK-12** acts by blocking the NS2B/NS3 protease interaction, inhibiting DENV-4 replication with an EC_{50} of 3.8 μM as well as JEV with EC_{50} of 14.4 μM [92].



SK-12

By screening a library of compounds, the anthelmintic drug **ivermectin** has been shown to interact with the helicase activity of the NS3; this is surprising considering that this drug is approved for the treatment of nematodes infections. **Ivermectin** showed a high potency against YFV in cell cultures and a lower one against a wide range of other flavivirus [93].

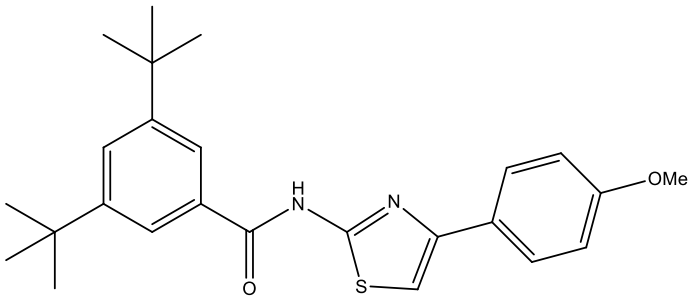




Ivermectin

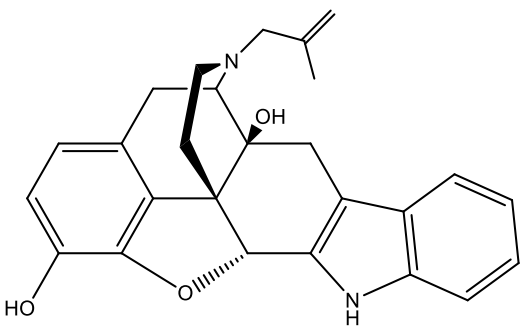
NS4B. This non-structural protein has recently emerged as a valid antiviral drug target. The aminothiazole **NITD-618** (Novartis Institute for Tropical Diseases) was identified as a potent and pan-serotype DENV inhibitor with EC_{50} ranging from 1.0 to 4.1 μM. Transient transfection studies using a luciferase DENV-2 replicon as shown that **NITD-618** acts by

suppression of viral RNA synthesis. Unfortunately the high lipophilicity of **NITD-618** resulted in poor pharmacokinetic properties which hindered testing of its in vivo efficacy in the DENV-AG129 mouse model [94].



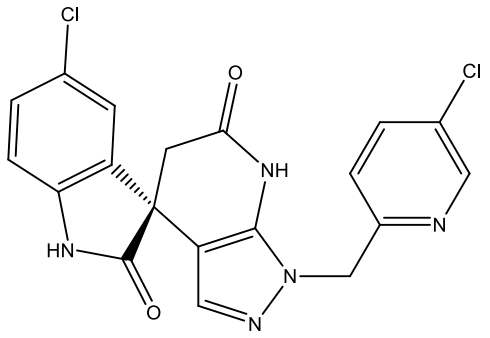
NITD-618

It has been reported the identification of a δ opioid receptor antagonist, **SDM25N**, which inhibits DENV (EC_{50} = 1.9 μM) at the step of viral RNA replication. A single amino acid substitution (F164L) in the NS4B protein that confers compound resistance was found by culturing the DENV-2 replicon cells for several passages under selection of **SDM25N**. Moreover, this compound exhibits antiviral activity only in mammalian cells, but not in the C6/36 mosquito cells, suggesting that **SDM25N** targets a function of NS4B that is only required for efficient replication in mammalian cells but not in mosquito cells, and that host environment plays a role in mediating the compound efficacy [95].



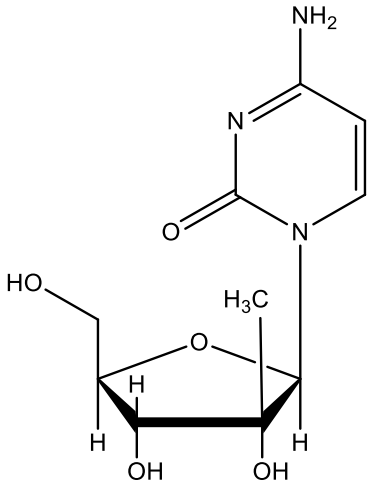
SDM25N

It was recently reported a compound class with a spiropyrazolo-pyridone core displaying a promising antiviral activity against dengue virus. In particular the **Compound 14a** has shown a good *in vivo* pharmacokinetic profile (EC_{50} = 0.042 μM) in fact treatment of DENV-2-infected AG129 mice with **Compound 14a** suppressed viremia, even when the treatment started after viral infection [96].



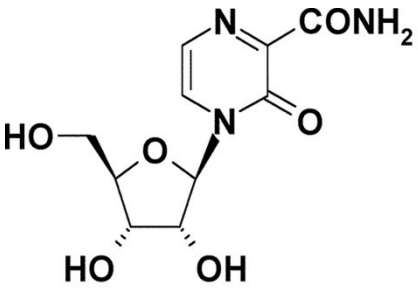
Compound 14a

NS5. This protein contains two fundamental enzymatic activities: an *S*-adenosyl methyltransferase and an RNA-dependent RNA polymerase (RdRp). **2'-C-methylcytidine** is an RNA polymerase inhibitor which reached the clinical trial fase for the treatment of HCV infections but was later abandoned due to its high toxicity in long term treatment [97]. Interestingly, this compound has also shown activity against YFV both *in vivo* and *in vitro*. In a research conducted on hamsters the treatment with **2'-C-methylcytidine** showed its effectiveness when administered two days after YFV inoculation [98]. Several other derivatives of the **2'-C–methylcytidine** showed different activities against YFV in cell cultures and hopefully a similar less toxic compound which maintains anti-YFV activity will be developed [99].

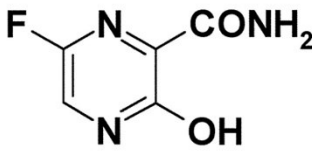


2'-C- methylcytidine

The pyrazinecarboxamide derivatives **T-705** (**favipiravir**) and **T-1106** have showed a broad spectrum of activity on RNA viruses, including some flaviviruses (WNV, YFV). In a study, **T-705** has been reported to be a potent inhibitor against influenza A, B and C viruses *in vitro*; it has been also proposed that **T-705** is converted intracellularly to the ribonucleotide T-705-ribofuranosyl-5-monophosphate (T-705 RMP) by a phosphoribosyl transferase and, upon phosphorylation, to its 5-triphosphate. This metabolite would inhibit the influenza virus RdRp in a GTP-competitive manner [100]. In addition to inhibiting YFV and WNV replication *in vitro*, improvements in survival and disease parameters were also observed after the addition of **T-705** to YFV- or WNV-infected rodents [101]. It may be assumed that the mechanism by which **T-705** inhibits viruses other than influenza is similar to the mechanism by which it is believed to inhibit influenza virus' replication, but this remains subject of further studies. Although **T-705** is slightly less effective than **T-1106** in a hamster model of yellow fever virus infection [102], it is currently under clinical trial for the treatment the influenza virus [103] and its possible approval could represent an *off label* therapeutic strategy to patients with yellow fever. In a recent study, **T-705** also showed limited *in vivo* efficacy against zika virus (ZIKV) [104].

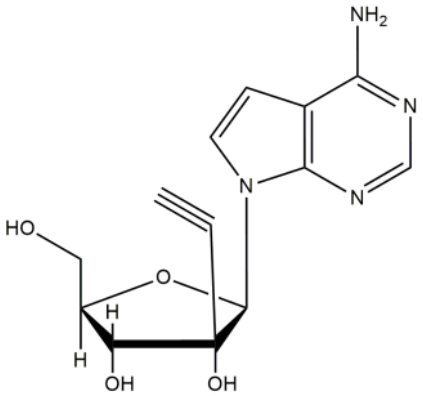


T-1106



T-705

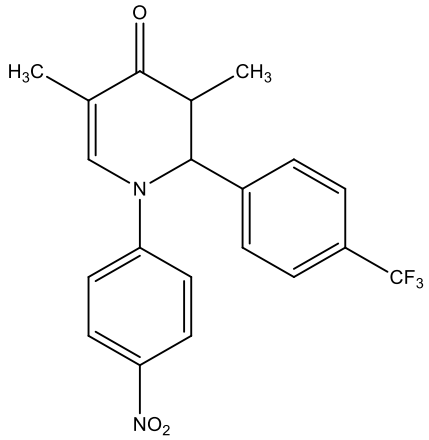
The compound **NITD008** showed a potent anti-DENV activity in mice and has also shown *in vivo* activity (rodents) against YFV [105]. **NITD008** is an adenosine nucleoside analog that contains a carbon substitution for N-7 of the purine and an acetylene at the 2′ position of ribose; the triphosphate form of **NITD008** competes with natural adenosine triphosphate substrates to incorporate into the growing RNA chain and, upon incorporation, terminates RNA elongation. It has been demonstrated that **NITD008** is also a potent inhibitor of ZIKV and can be used as reference inhibitor for future ZIKV antiviral drug screen and discovery [106].



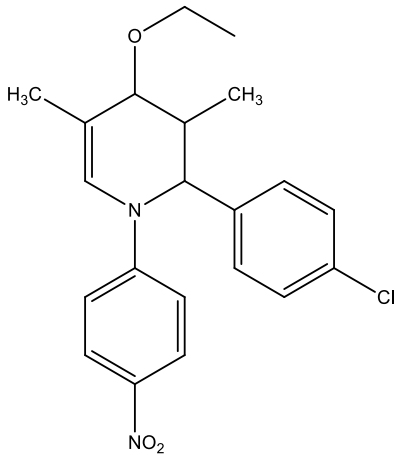
NITD008

Two 2,3-dihydro-4*H*-pyridine derivatives, **cis-3,5-dimethyl-1-(4-nitrophenyl)-2-[4-(trifluoromethyl)phenyl]-2,3-dihydropyridin-4(1*H*)-one** and **2-(4-chlorophenyl)-4-ethoxy-3,5-dimethyl-1-(4-nitrophenyl)-1,2,3,4-tetrahydropyridine**, have shown moderate activity against the YFV in cell cultures, with selectivity index of 5.6 and 10 respectively [107].

2-(4-chlorophenyl)-4-ethoxy-3,5-dimethyl-1-(4-nitrophenyl)-1,2,3,4-tetra hydropyridine was able to inhibit *in vitro* the HCV replicon as well as NS5B protein of the same virus, suggesting that the inhibition of RNA-dependent RNA polymerase could be at the basis of its mechanism of action.



Cis-3,5-dimethyl-1-(4-nitrophenyl)-2-[4-(trifluoromethyl)phenyl]-2,3-dihydropyridin-4(1*H*)-one



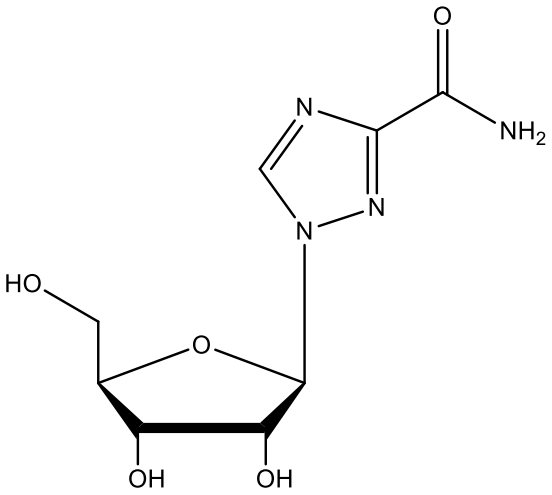
2-(4-chlorophenyl)-4-ethoxy-3,5-dimethyl-1-(4-nitrophenyl)-1,2,3,4-tetrahydropyridine

Anti-flavivirus compounds with other or unknown mechanisms of action.

Ribavirin is a broad spectrum antiviral drug which has been widely used for the treatment of HCV infections in combination with pegylated interferons [108] and in aerosol form for the treatment of pediatric respiratory syncytial virus

(RSV) infections. Almost all RNA viruses and even some DNA viruses are sensitive to the *in vitro* antiviral activity of ribavirin and some viruses are more susceptible to the action of this drug than others; flaviviruses, for example, are much less sensitive than the paramyxovirus RSV [109]. The antiviral activity of ribavirin was reported almost four decades ago, but the molecular mechanism by which the compound exerts its antiviral activity still remains a matter of debate.

Ribavirin 5-monophosphate inhibits Inosine 5-monophosphate (IMP) dehydrogenase, a cellular enzyme which converts IMP to xanthosine 5-monophosphate in the *de novo* synthesis pathway of GMP [110]. As a consequence, intracellular GTP pools are depleted, resulting in inhibition of viral (but also cellular) RNA synthesis. This compound has also shown other mechanisms of action including inhibition of viral RdRp on influenza virus [111] and inhibition of viral capping (via an effect on the viral GTase or MTase activities) on dengue virus [112].

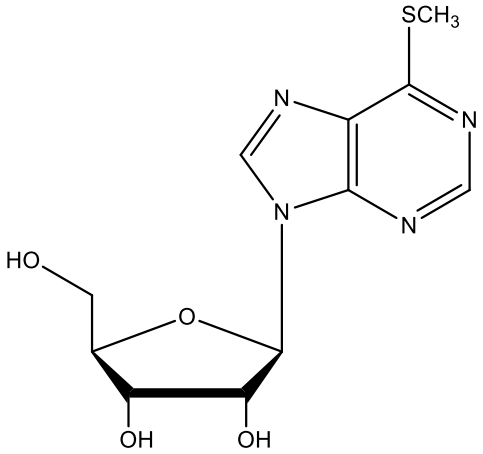


Ribavirin

Ribavirin is particularly active in animal models of hamster [113]; the effect of this drug was also studied in rhesus monkeys infected with YFV or DENV-1. Either therapeutic or prophylactic protocols were studied. Overall, no effect on viremia and survival was noted [114, 115, 116]. Since the mechanism

of anti-flavivirus activity of ribavirin is based on an aspecific mechanism, the design of safe and more potent analogues of ribavirin will likely be very difficult to achieve.

The mechanisms of action of many compounds that have shown an *in vitro* anti-flavivirus activity are still not clear. For example **6-methyl mercaptopurine riboside** (**6MMPr**) showed the ability to inhibit *in vitro* the replication of YFV and other Flaviviruses like WNV. However it has been shown that this compound is able to exacerbate WNV infection in mice [117].



6MMPr

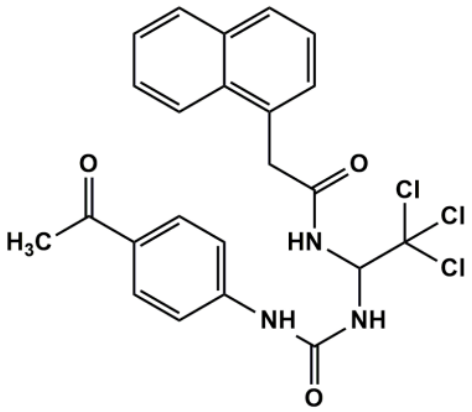
4.2. Compounds targeting host cells functions

One of the fundamental characteristics of viral diseases, including those caused by flavivirus, is the immunopathogenesis connected to the infection. Animal models inoculated with YFV have shown that pathological events which mostly threaten the animal's survival are those that occur in the late stage of the disease, suggesting that the later phases have a strong immunological component [118]. Many studies have turned their attention to the immunopathogenic mechanism of yellow fever in cell cultures, proving that there is a net reduction of cytokine induction in cells infected with the wild-

type strain of YFV, compared to cells infected with the vaccine 17-D, suggesting that decreased immune response can be a mechanism of pathogenesis [119]. Therefore the stimulation of the inflammatory response with the use of immunomodulators could represent a convenient way for the treatment of yellow fever.

Previously it has been cited the combination of ribavirin and interferons for the treatment of HCV infections and how this combination can also be used in YFV infections, the resulting reduction of the doses and duration of treatment could avoid the toxic effects; however, the therapeutic window for the use of interferon is limited. Both interferon **alfacon-1** and adenovirus-vectored interferon **DEF201** were tested on hamsters infected with YFV, demonstrating therapeutic activity when administered two/three days after the inoculation of the virus and thus before the manifestation of pathological symptoms [120, 121]. The same result is obtained by initiating treatment seven days prior to inoculation of the virus, thus suggesting the potential prophylactic use in the case of YFV epidemics.

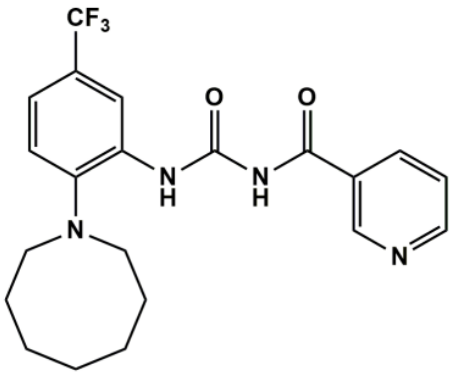
The compound **CCG-4088** recently showed anti YFV activities [122]. In fact this compound is structurally similar to a class of inhibitors of RNAase L, an antiviral protein involved in humoral immune response and in ssRNA cleavage.



CCG-4088

A further therapeutic strategy, together with the modulation of the humoral immune response, is represented by the use of antibodies. In addition to having important implications in the development of a vaccine, it was demonstrated that antibody therapy is also effective in the treatment of YFV and other viruses' infections, both in animal models and in humans.

Another useful strategy for the arrest of viral replication is to inhibit the enzymes of the host cell that are necessary for the replication of the virus. For example the inhibitor of the kinase broad spectrum **SFV785** showed activity against HCV, DENV and YFV acting on the virus assembly in the endoplasmic reticulum. This inhibitor, at a concentration of 5 μM, is able to reduce by 5 logarithmic units the viral title of YFV in cell cultures [123].



SFV785

Even some natural products showed anti-flavivirus activity. For example, phospholipases A_2 (**PLA_2**), found in the venom of the rattlesnake (*Crotalus durissis terrificus*), has shown *in vitro* activity against YFV and DENV, mainly in the pre-treatment/inactivation stages of viruses [124]. The toxin contained in the venom (crotoxin) seems to significantly inhibit inflammatory edema and cell migration when administered before or after carrageenan injection in mice, thus suggesting an anti-inflammatory activity for this compound [125].

Table 3. Compounds targeting viral proteins – activity in cell cultures.

Compound	Mechanism	Effect
mAb 2A10G6	Binding to the E protein	Neutralization
***S*-methyl-2-(4-chlorophenyl)-4-(dibromomethyl)thiazole-5-carbothioate**	Binding to the E protein	Inhibition
SK-12	Action on the NS2B-NS3 protease	Inhibition of interaction between NS2B and NS3
Ivermectin	Action on the NS3 helicase	Inhibition of replication
NITD-618	Action on the polymerase NS4B	Inhibition of replication
SDM25N	Action on the polymerase NS4B	Inhibition of replication
2'-C-methylcytidine	Action on the polymerase NS5	Inhibition of replication
NITD008	Inhibition of the polymerases	Reduces the viremia
T-705	Inhibition of the polymerases	
Cis-3,5-dimethyl-1-(4-nitrophenyl)-2-[4-(trifluoromethyl)phenyl]-2,3-dihydropyridin-4(1*H*)-one	Inhibition of the polymerases	Reduces the viremia
2-(4-chlorophenyl)-4-ethoxy-3,5-dimethyl-1-(4-nitrophenyl)-1,2,3,4-tetrahydropyridine	Inhibition of the polymerases	Reduces the viremia
Ribavirin	Inhibition of IMP dehydrogenase	Reduction of GTP and inhibition of RNA synthesis

Tabel 4. Compounds targeting viral proteins – activity in animal models.

Compound	Model	Mechanism	Effect
Compound 14a	Mouse	Action on the polymerase NS4B	Survival/protection from disease
2'-C-methylcytidine	Hamster	Action on the polymerase NS5	Survival/protection from disease
T-705	Hamster	Action on the polymerases	Survival/protection from disease
T-1106	Hamster	Action on the polymerases	Survival/protection from disease
Ribavirin	Hamster	Inhibition of IMP dehydrogenase	Survival/protection from disease

Table 5. Compounds targeting host cell functions – activity in cell culture.

Compound	Mechanism	Effect
CCG-4088	Inhibiting RNAase	Inhibition of replicon
SFV785	Inhibition of kinases	Reduction in viral power
PLA_2	Viral inactivation (?)	Reduction in viral power

Table 6. Compounds targeting host functions – activity in animal models.

Compound	Model	Mechanism	Effect
Alfacon-1	Hamster	Action on INF	Survival/protection from disease
DEF201	Hamster	Action on INF	Survival/protection from disease

References

1. Monath TP. Yellow fever: a medically neglected disease. Report on a seminar. *Rev. Infect. Dis*. (**1987**) 9, 165-175.
2. Nettelton PE & Endrican G. Ruminant pestiviruses. *Brit. Vet. J*. (**1998**) 151, 615-642.
3. Fourichon C, Beaudeau F, Bareille N, Seegers H. Quantification of economic losses consecutive to infection of a dairy herd with bovine viral diarrhoea virus. *Prev. Vet. Med*. (**2005**) 72, 177-181.
4. Carter W, Connelly S, Struble K. Reinventing HCV treatment: Past and Future perspectives. *J. Clin. Pharmacol*. (**2016)** Sep 22. doi: 10.1002/jcph.830.
5. Newcomer BW, Givens MD. Approved and experimental countermeasures against pestiviral diseases: Bovine viral diarrhoea, classical swine fever and border disease. *Antivir. Res.* (**2013**) 100, 133-150.
6. Mackaenzie JS, Gubler DJ, Petersen LR. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat. Med*. (**2004**) 10, 98-109.
7. Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet*. (**2008**) 371, 500-509.

8. Nichlas JC King, Daniel R Getts, Meghann T Getts, Sabita Rana, Bimmi Shrestha, Alison M Kesson. Immunopathology of flavivirus infections. *Immunol. Cell. Biol.* (**2007**) 85, 33–42.
9. Solomon T. Flavivirus encephalitis. *N. Engl. J. Med.* (**2004**) Vol. 351, pp. 370–378.
10. Paul WS, Moore PS, Karabatsos N, Flood SP, Yamada S, Jackson T, Tsai TF. Outbreak of Japanese encephalitis on the island of Saipan, 1990. *J. Infect. Dis.* (**1993**) 167, 1053-1058
11. Hubalek Z, Halouzka J. West Nile fever: a reemerging mosquito-borne viral disease in Europe. *Emerg. Infect. Dis*. (**1999**) 5, 643-650.
12. Lawrence J. Japanese encephalitis outbreak in India and Nepal. *Euro Surveill.* (**2005**) 10, E050922-E050924.
13. Petersen LR, Marfin AA. Shifting epidemiology of *Flaviviridae*. *J. Travel Med.* (**2005**) 12, S3-11.
14. Heinz FX, Collett MS, Purcell RH. *Flaviviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RB. Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses, (San Diego, CA). *Academic Press*. (**2000**) 7, 611-614.
15. Hayasaka D, Nagata N, Fujii Y, Hasefawa H, Sata T, Suzuki R, Gould EA, Takashima I, Koike S. Mortality following peripheral infection with tick-borne encephalitis virus results from a combination of central nervous system pathology, systemic inflammatory and stress responses. *Virology*. (**2009**) 390, 139-150.
16. Lossinsky AS, Shives RR. Structural pathways for macromolecular and cellular transport across the blood-brain barrier during inflammatory conditions. *Rev. Histol. Histopathol*. (**2004**) 19, 535-564.
17. Cook SH, Griffin DE. Luciferase imaging of a neurotropic viral infection in intact animals. *J. Virol*. (**2003**) 77, 5333-5338.
18. Wang T, Town T, Alexopoulu L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat. Med*. (**2004**) 10, pp. 1366-1373.
19. King NJ, Getts DR, Getts MT, Rana S, Sherestha B, Kesson AM. Immunopathology of flavivirus infections. *Immunol. Cell. Biol*. (**2007**) 85, 33-42.

20. Cardosa MJ, Gordon S, Hirsch S, Springer TA, Porterfield JS. Interaction of West Nile virus with primary murine macrophages: role of the cell activation and receptors for antibody and complement. *J. Virol*. (**1986**) 57, 952-959.
21. Rios M, Zhang MJ, Grinev A, Srinivasan K, Daniel S, Wood O, Hewlett IK, Dayton AI. Monocytes-macrophages are a potential target in human infection with West Nile virus through blood transfusion. *Transfusion*. (**2006**) 46, 659-667.
22. Hunsperger EA, Roehrig JT. Temporal analyses of the neuropathogenesis of a West Nile virus infection in mice. *J. Neurovirol*. (**2006**) 12, 129-139.
23. Liou CW, Hsu CY. Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. *Cell Tissue* Res. (**1998**) 293, 389-394.
24. Chu JJ, Ng ML. The mechanism of cell death during West Nile virus infection is dependent on initial infectious dose. *J. Gen. Virol*. (**2003**) 84, 3305-3314.
25. Parquet MC, Kumatori A, Hasebe F, Morita K, Igarashi A. West Nile virus induced bax-dependent apoptosis. *FEBS Lett*. (**2001**) 500, 17-24.
26. Ramanathan MP, Chambers JA, Pankhong P, Chattergoon M, Attatippaholkun W, Dang K, Shah N, Weiner DB. Host cell killing by the West Nile Virus NS2B-NS3 proteolytic complex: NS3 alone is sufficient to recruit caspase-8-based apoptotic pathway. *Virology*. (**2006**) 345, 56-72.
27. Yang JS, Ramanathan MP, Muthumani K, Choo AY, Jin SH, Yu QC, Hwang DS, Choo DK, Lee MD, Dang K, Tang W, Kim JJ, Weiner DB. Induction of inflammation by West Nile virus capsid through the caspase-9 apoptotic pathway. *Emerg. Infect. Dis.* (**2002**) 8, 1379-1384.
28. Raung SL, Kuo MD, Wang YM, Chen CJ. Role of reactive oxygen intermediates in Japanese encephalitis virus infection in murine neuroblastoma cells. *Neurosci. Lett.* (**2001**) 315, 9-12.
29. Weissenbock H, Bakonyi T, Chvala S, Nowotny N. Experimental Usutu virus infection of suckling mice causes neuronal and glial cell apoptosis and demyelination. *Acta Neuropathol.* (**2004**) 108, 453-460.
30. Bray M. Pathogenesis of viral haemorrhagic fever. *Curr. Opin. Immunol*. (**2005**) 17, 399-403.

31. Bray M, Geisbert TW. Ebola virus: the role of macrophages and dendritic cells in the pathogenesis of Ebola haemorrhagic fever. *Int. J. Biochem. Cell Biol*. (**2005**) 37, 1560-1566.
32. Geisbert TW, Jahrling PB. Exotic emerging viral diseases: progress and challenges. *Nat. Med*. (**2004**) 10, S110-S121.
33. Quaresma JA, Pagliari C, Medeiros DB, Duarte MI, Vasconcelos PF. Immunity and immune response, pathology and pathologic changes: progress and challenges in the immunopathology of yellow fever. *Rev Med Virol.* (**2013**) 23, 305-18.
34. World Health Organization. WHO Division of Epidemiological Surveillance and Health Situation Trend Assessment Global Health Situation and Projections Estimates. Geneva, Switzerland. (**1992**).
35. Halstead SB. Dengue. *Lancet.* (**2007**) 370,. 1644-1652.
36. Clark DV, Mammen Jr. MP, Nisalak A, Puthimethee V, Endy TP. Economic impact of dengue fever/dengue haemorrhagic fever in Thailand at the family and population levels. *Am. J. Trop. Med. Hyg*. (**2005**) 72, 786-791.
37. Noisakran S, Perng GC. Alternate hypothesis on the pathogenesis of dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS) in dengue virus infection. *Exp. Biol. Med.* (Maywood) (**2008**) 233, 401-408.
38. Martina BEE, Koraka P, Osterhaus ADME. Dengue virus pathogenesis: an integrated view. *Clin. Microbiol. Rev*. (**2009**) 22, 564-581.
39. Van Campen H, Frolich K. Pestivirus infections, in Infectious Diseases of Wild Mammals. Williams ES, Barker IK, editors. (Ames, IA: Iowa State University Press) (**2001**) 232-244.
40. Botner A, Belsham GJ. Virus survival in slurry: analysis of the stability of foot-and-mouth disease, classical swine fever, bovine viral diarrhoea and swine influenza viruses. V*et. Microbiol.* (**2012**) 157, 41-49.
41. Niskanen R, Lindberg A. Transmission of bovine viral diarrhoea virus by unhygienic vaccination procedures, ambient air, and from contaminated pens. *Vet. J.* (**2003**), 165, 125-130.
42. Thurmond M. Virus transmission, in Bovine Viral Diarrhea Virus Diagnosis, Management, and Control. Goyal S. M., Ridpath J. F., editors. (Hoboken, NJ: Blackwell Publishing), (**2005**) 91-104.

43. Thiel HJ, Plagemann PGW, Moennig V. Pestiviruses. *Fields virology*. (**1996**) 1, 1059-1073.
44. Bruschke CJ, Weerdmeester K, Van Oirschot JT, Van Rijn PA. Distribution of bovine virus diarrhoea virus in tissues and white blood cells of cattle during acute infection. *Vet. Microbiol*. (**1998**) 64, 23-32.
45. Ellis JA, West KH, Cortese VS, Myers SL, Carman S, Martin KM, Haines DM. Lesions and distribution of viral antigen following an experimental infection of young seronegative calves with virulent bovine virus diarrhea virus-type II. *Can J Vet Res*. (**1998**) 62, 161-169.
46. Newcomer BW, Givens MD. Approved and experimental countermeasures against pestiviral diseases: Bovine viral diarrhea, classical swine fever and border disease. *Antivir. Res*. (**2013**) 100, 133-150.
47. Choo QL, G Kuo, AJ Weiner, LR Overby, DW Bradley and M Houghton. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* (**1989**) 244, 359-362.
48. SC Rey and DL Thomas. "Hepatitis C" (Chapter 156). In Mandell, Douglas, and Bennett's principles and practice of infectious diseases, edited by JE Bennett, R Dolin, MJ Blaser. Philadelphia: Elsevier (**2015**).
49. Zeuzem S, G Teuber, JH Lee, B Ruster and WK Roth. Risk factors for the transmission of hepatitis C. *J. Hepatol*. (**1996**) 24, 3-10.
50. WHO. Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection. Updated version. (**2016**).
51. American Association for the Study of Liver Diseases/Infectious Diseases Society of America/International Antiviral Society–USA. HCV Guidance: Recommendations for testing, managing, and treating hepatitis C. Available at: www.hcvguidelines.org. (**2015**).
52. JM Pawlotsky, Feld JJ, Zeuzem S, Hoofnagle JH. From non-A, non-B hepatitis to hepatitis C virus cure. *J Hepatol*. (**2015**) 62, S87.
53. EASL Recommendations on treatment of Hepatitis C 2015. *J Hepatol.* (**2015**) 63, 199.
54. Polenakovik H. New Therapies for Hepatitis C Virus. *PRILOZI*. (**2015**) 36, 119-132.

55. Bollati M, Alvarez K, Asseberg R, Baronti C, Canard B, Cook S, Coutard B, Decroloy E, de Lamballerie X, Gould EA, Grard G, Grimes JM, Hilgenfeld R, Jansson AM, Malet H, Mancini EJ, Mastrangelo E, Mattevi A, Milani M, Moureau G, Neyts J, Owens RJ, Ren J, Selisko B, Speroni S, Steuber H Stuart DI, Unge T, Bolognesi M. Structure and functionality in flavivirus NS-proteins: Perspectives for drug design. *Rev. Antivir. Res*. (**2010**) 87, 125-148.

56. Moureau G, Cook S, Lemey P, Nougairede A, Forrester NL, Khasnatinov M, Charrel RN, Firth AE, Gould EA, de Lamballerie X. New Insights into Flavivirus Evolution, Taxonomy and Biogeographic History, Extended by Analysis of Canonical and Alternative Coding Sequences. *Plos One.* (**2015**) 10 (2): e0117849. doi: 10.1371/journal.pone.0117849.

57. Samsa MM, Mondotte JA, Caramelo JJ, Gamarnik AV. Uncoupling cis-acting RNA elements from coding sequences revealed a requirement of the N-terminal region of dengue virus capsid protein virus particle formation. *J. Virol*. (**2012**) 86, 1046-1058.

58. Lindenbach BD, Rice CM. Molecular biology of flaviviruses. *Adv. Virus Res*. (**2003**) 59, 23-61.

59. Matusan AE, Pryor MJ, Davidson AD, Wright PJ. Mutagenesis of the Dengue virus type 2 NS3 protein within and outside helicase motifs: effects on enzyme activity and virus replication. *J. Virol.* (**2001**) Vol. 75, pp. 9633-9643.

60. Assemberg R, Mastrangelo E, Walter TS, Verma A, Milani M, Owens RJ, Stuart DI, Grimes JM, Mancini EJ. Crystal structure of a novel conformational state of the flavivirus NS3 protein: implications for polyprotein processing and viral replication. *J. Virol*. (**2009**) 83, 12895-12906.

61. Aleshin AE, Shiryaev SA, Strongin AY, Liddington RC. Structural evidence for regulation and specificity of flaviviral proteases and evolution of the *Flaviviridae* fold. *Protein Sci*. (**2007**) 16, 795-806.

62. Bera AK, Kuhn RJ, Smith JL. Functional characterization of cis and trans activity of the Flavivirus NS2B-NS3 protease. *J. Biol. Chem*. (**2007**) 282, 12883-12892.

63. Egloff MP, Decroly E, Malet H, Selisko B, Benarroch D, Ferron F, Canard B, Structural and functional analysis of methylation and 5'-RNA sequence requirements of short capped RNAs by the methyltransferase domain of dengue virus NS5. *J. Mol. Biol*. (**2007**) 372, 723-736.

64. Davidson AD. Chapter 2. New insights into flavivirus nonstructural protein 5. *Adv. Virus Res*. (**2009**) 74, 41-101.
65. Muylaert IR, Galler R, Rice CM, Genetic analysis of the yellow fever virus NS1 protein: identification of a temperature-sensitive mutation which blocks RNA accumulation. *J. Virol*. (**1997**) 71, 291-298.
66. Leung JY, Pijlman GP, Kondratieva N, Hyde J, Mackenzie JM, Khromykh AA. Role of nonstructural protein NS2A in flavivirus assembly. *J. Virol*. (**2008**) 82, 4731-4741.
67. Miller S, Kastner S, Krijnse-Locker J, Buhler S, Bartenschlager R. The nonstructural protein 4A of dengue virus is an integral membrane protein inducing membrane alterations in a 2K-regulated manner. *J. Biol. Chem*. (**2007**) 282, 8873-8882.
68. Munoz-Jordan J, Laurent-Rolle M, Ashour J, Martinez-Sobrido L, Ashok M, Lipkin WI, Garcia Sastre A. Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. *J. Virol*. (**2005**) 79, 8004-8013.
69. Umareddy I, Chao A, Sampath A, Gu F, Vasudevan SG. Dengue virus NS4B interacts with NS3 and dissociates it from single-stranded RNA. *J. Gen. Virol*. (**2006**) 87, 2605-2614.
70. Krishnan MN, Sukumaran B, Pal U, Agaisse H, Murray JL, Hodge TW, Fikrig E. Rab 5 is required for the cellular entry of dengue and West Nile viruses. *J. Virol*. (**2007**) Vol. 81, pp. 4881-4885.
71. Barba-Spaeth G, Longman RS, Albert ML, Rice CM. Live attenuated yellow fever 17D infects human DCs and allows for presentation of endogenous and recombinant Tell epitopes. *J. Exp. Med*. (**2005**) 202, 1179-1184.
72. Lozach PY, Burleigh L, Staropoli I, Navarro-Sanchez E, Harriague J, Virelizier JL, Rey FA, Despres P, Arenzana Seisdedos F, Amara A. Dendridic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection independent of DC-SIGN internalization signals. *J. Biol. Chem*. (**2005**) 280, 23698-23708.
73. Rey FA, Heinz FX, Mandl C, Kunz C, Harrison Sc. The envelope glycoprotein from ick-borne encephalitis virus at 2A resolution. *Nature*. (**1995**) 25, 291-298.

74. Rice, C.M., Lenches, E.M., Eddy, S.R., Shin, S.J., Sheets, R.L., Strauss, J.H., Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. *Science*. (**1985**) 229, 726-733.
75. Uchil PD, Satchidanandam V. Architecture of the flaviviral replication complex protease, nuclease, and detergents reveal encasement within double layered membrane compartments. *J. Biol. Chem.* (**2003**) 278, 24388-24398.
76. Khromykh AA, Westaway EG. RNA binding properties of core protein of the flavivirus Kunjin. *Arch. Virol*. (**1996**) 141, 685-699.
77. Perera R, Kuhn RJ. Structural proteomics of dengue virus. *Curr. Opin. Microbiol*. (**2008**) 11, 369-377.
78. Perera R, Khaliq M, Kuhn RJ. Closing the door on flaviviruses: entry as a target for antiviral drug design. *Antiviral Res*. (**2008**) 80, 11-22.
79. Mackenzie JM, Westaway EG. Markers for trans-Golgi membranes and the intermediate compartment localize to induced membranes with distinct replication functions in flavivirus-infected cells. *J. Virol.* (**1999**) 73, 9555-9567.
80. Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. *Nat. Rev. Microbiol*. (**2005**) 3, 12-33.
81. Yu IM, Zhang W, Holdaway HA, Li L, Kostyuchenko VA, Chipman PR, Kuhn RJ, Rossmann MG, Chen J. Structure of the immature dengue virus at low ph primes proteolytic maturation. *Science*. (**2008**) 319, 1834-1837.
82. Tomohiro I, Atsushi Y, Eiji K. A review of successful flavivirus vaccines and the problems with those flaviviruses for which vaccines are not yet available. *Vaccine.* (**2014**) 32, 1326–1337
83. Veit O, Niedrig M, Chapuis-Taillard C, Cavassini M, Mossdorf E, Schmid P, Bae HG, Litzba N, Staub T, Hatz C, Furrer H. Immunogenicity and safety of yellow fever vaccination for 102 HIV-infected patients. Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. (**2009**) 48, 659-666.
84. Umamaheswari A, Kumar MM, Pradhan D, Marisetty H. Docking studies towards exploring antiviral compounds against envelope protein of yellow fever virus. *Interdisciplinary Sciences*. (**2011**) 3, 64–77.
85. Deng YQ, Dai JX, Ji GH, Jiang T, Wang HJ, Yang HO, Tan WL, Liu R, Yu MGe BX, Zhu QY, Qin ED, Guo YJ, Qin CF. A broadly flavivirus cross neutralizing

monoclonal antibody that recognizes a novel epitope within the fusion loop of E protein. *PloS One.* (**2011**) 6, e16059.

86. Mayhoub AS, Khaliq M, Kuhn RJ, Cushman M. Design, synthesis, and biological evaluation of thiazoles targeting flavivirus envelope proteins. *J. Med. Chem*. (**2011**) 54, 1704-1714.
87. Akey DL, Brown WC, Dutta S, Konwerski J, Jose J, Jurkiw TJ, DelProposto J,Ogata CM, Skiniotis G, Kuhn RJ, Smith JL. Flavivirus NS1 structures reveal surfaces for associations with membranes and the immune system. *Science*. (**2014**) 343, 881-885.
88. Avirutnan P, Fuchs A, Hauhart RE, Somnuke P, Youn S, Diamond MS, Atkinson JP, Antagonism of the complement component C4 by flavivirus nonstructural protein NS1. *J. Exp. Med*. (**2010**) 207, 793-806.
89. Lim SP, Wang QY, Noble CG, Chen YL, Dong H, Zou B, Yokokawa F, Nilar S, Smith P, Beer D, Lescar J, Shi PY. Ten years of dengue drug discovery: progress and prospects. *Antiviral Res*. (**2013**) 100, 500-519.
90. Li L, Basavannacharya C, Chan KW, Shang L, Vasudevan SG, Yin Z- Structure-guided discovery of a novel non-peptide inhibitor of dengue virus NS2B–NS3 protease. *Chem. Biol. Drug Des*. (**2015**) 86, 255-264.
91. De Sousa LR, Wu H, Nebo L, Fernandes JB, da Silva MF, Kiefer W, Kanitz M, Bodem J, Diederich WE, Schirmeister T, Vieira PC. Flavonoids as noncompetitive inhibitors of Dengue virus NS2B–NS3 protease: inhibition kinetics and docking studies. *Bioorg. Med. Chem*. (**2015**) 23, 466-470.
92. Pambudi S, Kawashita N, Phanthanawiboon S, Omokoko MD, Masrinoul P, Yamashita A, Limkittikul K, Yasunaga T, Takagi T, Ikuta K, Kurosu T. A small compound targeting the interaction between nonstructural proteins 2B and 3 inhibits dengue virus replication. Biochem. Biophys. *Res. Commun*. (**2013**) 440, 393-398.
93. Mastrangelo E, Pezzullo M, De Burghgraeve T, Kaptein S, Pastorino B, Dallmeier K, de Lamballerie X, Neyts J, Hanson AM, Frick DN, Bolognesi M, Milani M. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J. Antimicrob. Chemoth*. (**2012**) 67, 1884-1894.
94. Xie X, Zou J, Wang QY, Shi PY. Targeting dengue virus NS4B protein for drug discovery. *Antiviral Res*. (**2015**) 118, 39-45.

95. Van Cleef KWR, Overheul GJ, Thomassen MC, Kaptein SJF, Davidson AD, Jacobs M, Neyts J, van Kuppeveld FJM, van Rij RP. Identification of a new dengue virus inhibitor that targets the viral NS4B protein and restricts genomic RNA replication. *Antiviral Res.* (**2013**) 99, 165-171.
96. Wang Q, Dong H, Zou B, Karuna R, Wan KF, Zou J, Susila A, Yip JT, Xu H, Ding M, Chan, WL, Gu F, Seah PG, Liu W, Lakshminarayana SB, Kang C, Julien L, Blasco F, Smith PW, Shi PY. Discovery of dengue virus NS4B inhibitors. *J. Virol*. (**2015**) 89, 8233-8244.
97. Carroll SS, Olsen DB, Nucleoside analog inhibitors of hepatitis C virus replication. *Infect. Disord. Drug Targets*. (**2006**), 6, 17-29.
98. Julander JG, Jha AK, Choi JA, Jung KH, Smee DF, Morrey JD, Chu CK- Efficacy of 2'-C-methylcytidine against yellow fever virus in cell culture and in a hamster model. *Antiviral Res*. (**2010**) 86, 261-267.
99. Fogt J, Januszczyk P, Framski G, Onishi T, Izawa K, De Clercq E, Neyts J, Boryski J, Synthesis and antiviral activity of novel derivatives of 2'-beta-C-methylcytidine. *Nucleic Acids Symposium Series (Oxford)*. (**2008**) 52, 605-606.
100. Furuta Y, Takahashi K, Shiraki K, Sakamoto K, Smee DF, Barnard DL, Gowen BB, Julander JG. Morrey JD. T-705 (favipiravir) and related compounds: Novel broad-spectrum inhibitors of RNA viral infections. *Antiviral Res*. (**2009**) 82, 95-102.
101. Julander JG, Shafer K, Smee DF, Morrey JD, Furuta Y. Activity of T-705 in a hamster model of yellow fever virus infection in comparison with that of a chemically related compound, T-1106. *Antimicrob. Agents Chemother*. (**2009**) 53, 202-209.
102. Hayden F. Developing new antiviral agents for influenza treatment: what does the future hold? Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America. (**2009**) 48 (Suppl. 1), S3-S13.
103. Mumtaz N, Van Kampen JJ, Reusken CB, Boucher CA, Koopmans MP. Zika virus: Where is the treatment? *Curr. Treat. Options Infect. Dis*. (**2016**) 8, 208-211.
104. Yin Z, Chen YL, Schul W, Wang QY, Gu F, Duraiswamy J, Kondreddi RR, Niyomrattanakit P, Lakshiminaraya SB, Goh A, Xu HY, Liu W, Lim JY, Ng CY, Quiq M, Lim CC, Yip A, Wang G Chan WL, Tan HP, Lin K, Zhang B, Zou G, Bernard KA, Garrett C, Beltz K, Dong M, Weaver M, He H, Pichota A, Dartois V,

Keller TH, Shi PY. An adenosine nucleoside inhibitor of dengue virus. *Proc. Nat. Acad. Sci*. (**2009**) 106, 20435-20439.

105. Deng YQ, Zhang N, Li CF, Tian M, Hao JN, Xie XP, Shi PJ, Qin CF. Adenosine Analog NITD008 Is a Potent Inhibitor of Zika Virus. *Open Forum Infect Dis*. (**2016**) 3, ofw175.
106. Peduto A, Massa A, Di Mola A, de Caprariis P, La Colla P, Loddo R, Altamura S, Maga G, Filosa R. 2,3-Dihydro-1,2-diphenyl-substituted 4*H*-Pyridinone derivatives as new anti *Flaviviridae* inhibitors. *Chem. Biol. Drug Des*. (**2011**) 77, 441-449.
107. Ilyas JA, Vierling JM. An overview of emerging therapies for the treatment of chronic hepatitis C. *Clin. Liver Dis*. (**2011**) 15, 515-536.
108. Leyssen P, Balzarini J, De Clercq E, Neyts J. The predominant mechanism by which ribavirin exerts its antiviral activity in vitro against flaviviruses and paramyxoviruses is mediated by inhibition of IMP dehydrogenase. *J. Virol*. (**2005**) 79, 1943-1947.
109. Leyssen P, De Clercq E, Neyts J. The anti-yellow fever virus activity of ribavirin is independent of error-prone replication. *Mol. Pharmacol*. (**2006**) 69, 1461-1467.
110. Eriksson B, Helgstrand E, Johansson NG, Larsson A, Misiorny A, Noren JO, Philipson L, Stenberg K, Stening G, Stridh S, Oberg B. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. *Antimicrob. Agents Ch*. (**1977**) 11, 946-951.
111. Benarroch D, Egloff MP, Mulard L, Guerreiro C, Romette JL, Canard B. A structural basis for the inhibition of the NS5 dengue virus mRNA 2'-Omethyltransferase domain by ribavirin 5'-triphosphate. *J. Biol. Chem*. (**2004**) 279, 35638-35643.
112. Bougie I, Bisaillon M. Initial binding of the broad spectrum antiviral nucleoside ribavirin to the hepatitis C virus RNA polymerase. *J. Biol. Chem*. (**2003**) 278, 52471-52478.
113. Julander JG, Morrey JD, Blatt LM, Shafer K, Sidwell RW. Comparison of the inhibitory effects of interferon alfacon-1 and ribavirin on yellow fever virus infection in a hamster model. *Antiviral Res.* (**2007**) 73, 140-146.
114. Huggins JW, Robins RK, Canonico PG. Synergistic antiviral effects of ribavirin and the C-nucleoside analogs tiazofurin and selenazofurin against togaviruses, bunyaviruses, and arenaviruses. *Antimicrob. Agents Ch*. (**1984**) 26, 476-480.

115. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev. Infect. Dis*. (**1989**) 11, S750-761.
116. Malinoski FJ, Hasty SE, Ussery MA, Dalrymple JM. Prophylactic ribavirin treatment of dengue type 1 infection in rhesus monkeys. *Antiviral Res*. (**1990**) 13, 139-149.
117. Lim PY, Keating JA, Hoover S, Striker R, Bernard KA. A thiopurine drug inhibits West Nile virus production in cell culture, but not in mice. *PloS One*. (**2011**) 6, e26697.
118. Julander JG, Trent DW, Monath TP. Immune correlates of protection against yellow fever determined by passive immunization and challenge in the hamster model. *Vaccine*. (**2011a**) 29, 6008-6016.
119. Woodson SE, Freiberg AN, Holbrook MR. Differential cytokine responses from primary human Kupffer cells following infection with wild-type or vaccine strain yellow fever virus. *Virology*. (**2011**) 412, 188-195.
120. Julander JG, Ennis J, Turner J, Morrey JD. Treatment of yellow fever virus with an adenovirus-vectored interferon (DEF201) in a hamster model. *Antimicrob. Agents Ch*. (**2011b**) 55, 2067-2073.
121. Patkar CG, Larsen M, Owston M, Smith JL, Kuhn RJ. Identification of inhibitors of yellow fever virus replication using a replicon-based high throughput assay. *Antimicrob. Agents Ch*. (**2009**) 53, 4103-4114.
122. Low JSY, WuKX, Chen KC, Ng MM-L, Chu JJh. Narassin, a novel antiviral compound that block dengue virus expression. *Antivir. Ther*. (**2011**) 16, 1203-1218.
123. Anwar A, Hosoya T, Leong KM, Onogi H, Okuno Y, Hiramatsu T, Koyama H, Suzuki M, Hagiwara M, Garcia-Blanco MA. The kinase inhibitor SFV785 dislocates dengue virus envelope protein from the replication complex and blocks virus assembly. *PloS One*. (**2011**) 6, e23246.
124. Muller VD, Russo RR, Oliveira Cintra AC, Sartim MA, De Melo Alves-Paiva R, Figueiredo LT, Sampaio SV, Aquino VH. Crotoxin and phospholipases A2 from Crotalus durissus terrificus showed antiviral activity against dengue and yellow fever viruses. *Toxicon*. (**2012**) 59, 507-515.
125. Nunes FP, Zychar BC, Della-Casa MS, Sampaio SC, Goncalves LR, Cirillo MC. *Toxicon*. (**2010**) 55, 1100-1106.

5. SUBJECT OF PRESENT RESEARCH

Viruses belonging to *Flaviviridae* family are a major cause of infectious diseases, worldwide. The global, social and economic impact due to morbidity and even mortality associated with these infections, urgently demands effective therapeutic interventions. Success has been recently achieved with the introduction in therapy of the direct-acting antiviral agents (DAAs) for the treatment of hepatitis C. However, to date no specific drug has been approved to combat Flavivirus and Pestivirus infections, and patient care remains symptomatic.

Following the researches undertaken in our laboratory on heterocyclic compounds with antiviral activity, in the present PhD thesis the design, synthesis and antiviral evaluation of new classes of pyrazole and pyrazoline derivatives with potent and selective anti-Flavivirus or anti-BVDV activity is described.

5.1. Pyrazole-based antiviral agents

By the antiviral screening of an in house library of pyrazole compounds, (*N*-(1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)anilines (**4a-v**) were identified as a new class of potent and selective inhibitors of human respiratory syncytial virus (RSV) replication [1]. Some derivatives were also endowed with a moderate activity against BVDV, representative of *Pestivirus* genus, and against significant human pathogens belonging to the *Flavivirus* genus such as YFV, Dengue Virus (DENV) and West Nile Virus (WNV). The hit compounds exhibited activity in the micromolar range coupled with no cytotoxicity (CC_{50} > 100 μM) against the cell lines (MDBK and BHK-21) utilized for the in vitro assays [1].

References

1. Fioravanti R, Desideri N, Biava M, Droghini P, Atzori EM, Ibba C, Collu G, Sanna G, Delogu I, Loddo R. *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methyl)anilines: A novel class of anti-RSV agents. *Bioorg. Med. Chem. Lett*. (**2015**) 25, 2401-2404.

5.1.1. *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)anilines: a novel class of anti-RSV agents

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Bioorg. Med. Chem. Lett. 11 (**2015**) 2401-2404

Human respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections in infants and young children worldwide. Severe clinical manifestations due to RSV include both pneumonia and bronchiolitis that represent the most frequent causes of childhood hospitalization in developed countries [1]. Despite the incidence of RSV infection is higher during the first years of life, RSV disease can also occur in individuals of all ages. Unfortunately, due to the lack of long-lasting immunity, re-infections are common throughout life [2-4]. Prematurity, chronic conditions, such as congenital heart disease and chronic lung disease, and immunodeficiency disorder increase both the frequency and the severity of the infection associated with this pathogen, as well as mortality attributable to RSV [4-6]. In recent years, extrapulmonary manifestations associated with severe RSV infection have been frequently described. Indeed, in addition to

respiratory tract, RSV can infect heart, liver and CNS producing severe cardiopathy, hepatitis and encephalitis, respectively [7-9]. Neither vaccines nor specific antiviral drugs are currently available to prevent or to treat RSV infection. To date, current preventive strategy involves passive immunization with Palivizumab, a humanized monoclonal antibody targeting the F- protein of RSV. Despite its usefulness in preventing severe RSV disease, the use of Palivizumab is limited to high risk patients, because of its high cost and the need of monthly injection during the epidemic season [10-11]. Ribavirin, a broad spectrum antiviral agent, was the first drug licensed for the treatment of RSV infection. However, its clinical use is now limited, due to poor efficacy and elevated risk of severe side effects [12]. Therefore, there is an urgent need of identifying and developing new potent and selective antiviral drugs to treat RSV infection. Following our research on the antiviral activity of heterocyclic compounds [13-16], in this paper we report the synthesis and the anti-RSV *in vitro* activity of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline derivatives (**4a–v**). In addition, all the compounds were evaluated against a large panel of RNA viruses representative of single-stranded, negative-sense ($ssRNA^-$), and positive-sense ($ssRNA^+$), or double stranded (dsRNA) viruses, as well as against two DNA viruses.

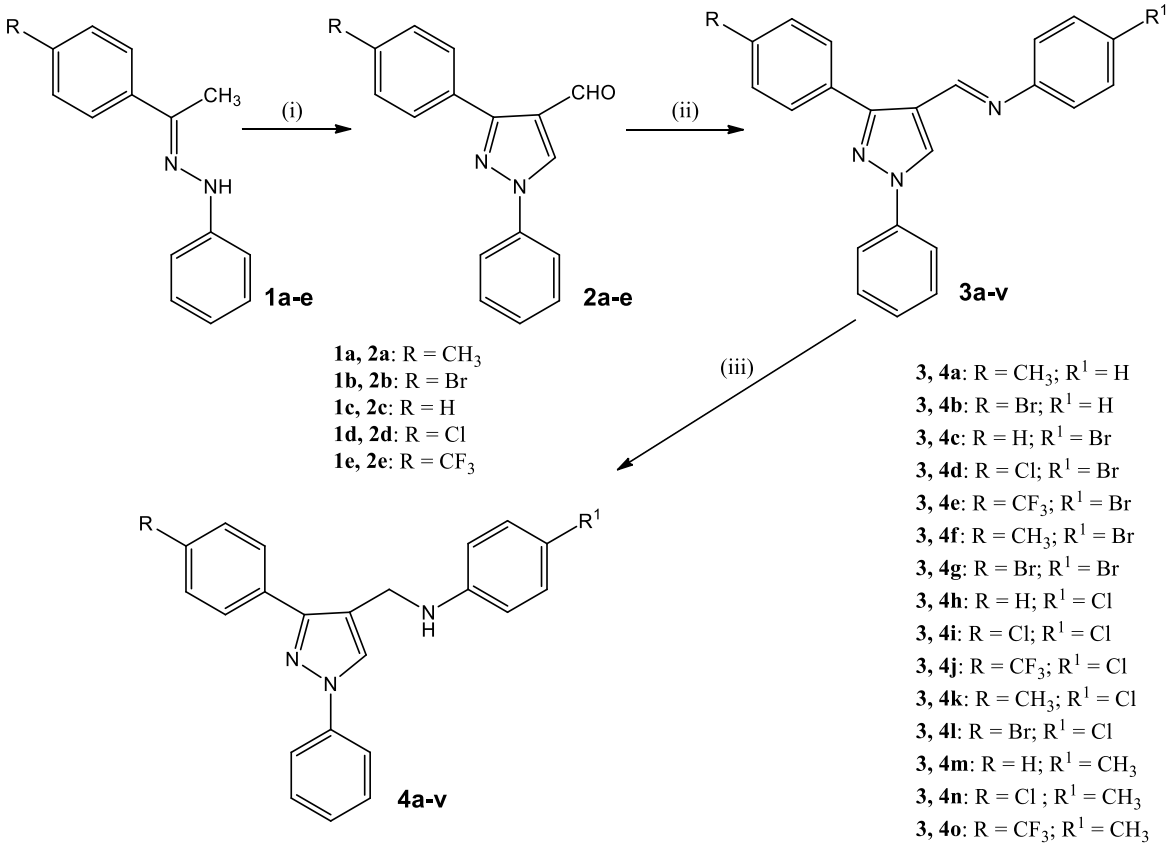
5.1.1.1. Results and discussion

5.1.1.1.1. Chemistry

As depicted in the Scheme 1, the compounds **4a–v** were prepared with some modification of the general synthetic route reported by Huang et al.[17] Initially, the required hydrazones **1a-e** were synthesized by condensation of the suitable acetophenones with phenylhydrazine hydrochloride, in the presence of anhydrous sodium acetate in ethanol. Following the procedure described by Kira et al.[18] the obtained hydrazones **1a-e** were treated with

Vilsmeier–Haack reagent (DMF–$POCl_3$), leading to the corresponding 1,3-diphenyl-1*H*-pyrazole-4-carboxaldehydes **2a-e**.

The subsequent condensation of **2a-e** with the appropriate anilines, performed in refluxed dry ethanol and dry benzene with traces of glacial acetic acid, provided the corresponding azomethines **3a-v**, which were finally converted into the desired N-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)anilines (**4a–v**) by reduction with sodium borohydride.

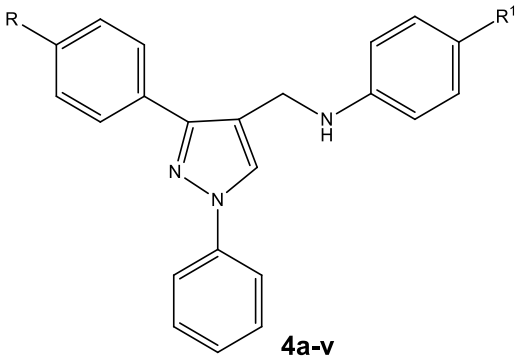


Scheme 1. Reagents and conditions: (i) $POCl_3$, DMF, 80 C, 5 h; (ii) $R^1C_6H_4$-NH_2, dry ethanol, dry C_6H_6, CH_3COOH, refluxed, 12 h; (iii) THF, $NaBH_4$, refluxed, 4 h.

5.1.1.1.2. Antiviral tests

Derivatives **4a–v** were evaluated in cell based assays for their cytotoxicity and antiviral activity against a panel of RNA and DNA viruses. Among single-stranded, positive RNA viruses ($ssRNA^+$), we considered a retrovirus (Human Immunodeficiency Virus type 1, HIV-1), two Picornaviruses (Coxsackie Virus type-5, CVB-5, and Poliovirus type-1, Sabin strain, Sb-1), and viruses representative of two of the three genera of the *Flaviviridae* family, that is, a Flavivirus (Yellow Fever Virus, YFV), and a Pestivirus (Bovine Viral Diarrhea Virus, BVDV). Among single-stranded, negative RNA viruses ($ssRNA^-$) a *Paramyxoviridae* (Respiratory Syncytial Virus, RSV) and a *Rhabdoviridae* (Vesicular Stomatitis Virus, VSV) were selected as representatives. Among double-stranded RNA (dsRNA) viruses, a *Reoviridae* family member (Reo-1) was included. Finally, two representatives of DNA virus families were also included: Herpes Simplex Virus type-1, HSV-1 (*Herpesviridae*) and Vaccinia Virus, VV (*Poxviridae*). Efavirenz (EFV), pleconaril, ribavirin, 6-azauridine (NM 299), 20-C-methylcytidine (NM 107), ACG (acyclovir), and mycophenolic acid (M 5255) were used as reference inhibitors of ssRNA+, ssRNA-, dsRNA and DNA viruses respectively. Cytotoxicity and antiviral activity of the compounds **4a–v** and reference inhibitors are reported in Tables 1 and 2.

Table 1. Cytotoxicity and antiviral activity of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl) anilines **4a–v** against ssRNA$^+$ (BVDV, YFV) and ssRNA$^-$ (RSV) viruses.



4a-v

Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	BHK-21 [d]CC_{50} (µM)	YFV [e]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	Vero76 [h]CC_{50} (µM)	RSV [i]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}
4a	CH_3	H	>100	>100	-	>100	38.0±6.0	>2.6	52	23.0±2.5	2.3
4b	Br	H	>100	>100	-	>100	20.0±4.0	>5.0	52	9.0±1.5	5.8
4c	H	Br	>100	31.0±0.5	>3.2	>100	32.0±4.0	>3.1	52	9.0±2.0	5.8
4d	Cl	Br	>100	>100	-	>100	28.0±5.0	>3.6	43	7.0±1.5	6.1
4e	CF_3	Br	64	>64	-	28	>28	-	17	5.0±0.5	3.4
4f	CH_3	Br	>100	>100	-	>100	>100	-	92	11.0±4.5	8.4
4g	Br	Br	>100	>100	-	>100	54.0±6.0	>1.8	88	8.0±2.5	11.0
4h	H	Cl	>100	29.0±1.5	>3.4	>100	29.0±3.5	>3.4	65	6.0±1.0	10.8
4i	Cl	Cl	>100	31.0±2.0	>3.2	73	>73	-	90	13.0±2.5	6.9
4j	CF_3	Cl	44	>44	-	31	>31	-	10	> 10	-
4k	CH_3	Cl	>100	>100	-	>100	34.0±0.5	>2.9	>100	14.0±1.5	>7.1
4l	Br	Cl	>100	≥100	-	>100	43.0±0.5	>2.3	>100	9.0±0.5	>11.1
4m	H	CH_3	>100	53.0±3.0	>1.9	>100	65.0±5.0	>1.5	>100	28.0±2.5	>3.6
4n	Cl	CH_3	>100	55.0±5.0	>1.8	>100	>100	-	>100	13.0±2.5	>7.7
4o	CF_3	CH_3	>100	54.0±4.0	>1.8	88	>88	-	82	25.0±3.0	>3.3
4p	CH_3	CH_3	>100	>100	-	>100	>100	-	>100	28.0±2.5	>3.6
4q	Br	CH_3	>100	>100	-	>100	>100	-	>100	11.0±1.0	>9.1
4r	H	CF_3	34	>34	-	37	>37	-	17	>17	-
4s	Cl	CF_3	31	>31	-	17	>17	-	18	>18	-
4t	CF_3	CF_3	26	>26	-	27	>37	-	26	>26	-
4u	CH_3	CF_3	>100	>100	-	88	>100	-	45	>45	-
4v	Br	CF_3	46	>46	-	20	>28	-	28	>28	-
Ref. compounds											
NM 299						>100	46.0±3.3	>2.2	10.0±1.6	0.9±0.4	11.1
Ribavirin			57.0±6.2	19.0±5.3	3.0				>100	35.0±5.0	>2.8
NM 108						60					

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC50 and EC50. [d] Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTTmethod. [e]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [i]Compound concentration required to reduce the plaque number of RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

Data listed in Table 1 showed that most of the tested compounds exhibited antiviral activity against RSV in the micromolar range (EC_{50}s ranging from 5 μM to 28 μM). Only the analogues bearing a trifluoromethyl group in R^1 (**4r–4v**) and **4j**, (where R = CF_3), were inactive against this virus up to the CC_{50} for Vero76 cells, a nonhuman cell line suitable for the replication of RSV. Moreover, the presence of this group whether in position R^1 (**4r–1v**) or R (**4e**, **4j**, **4o**) seems to increase the compound cytotoxicity not only on Vero76 cells but also on the other nonhuman cell lines (MDBK and BHK-21) utilized in this study. When tested against these last two cell lines, the removal of the trifluoromethyl group or its replacement with a methyl group or with a halogen completely abolished the compound cytotoxicity up to the highest concentration tested (100 μM). The only exception to this generalization was represented by **4i**, (where R = R^1 = Cl), that exhibited a low cytotoxicity against BHK-21 cells (CC_{50} = 73 μM). Moreover, several *N*-((1,3-diphenyl)-1*H*-pyrazol-4-yl)methyl)aniline derivatives have already been reported to have remarkable antiproliferative effects against MCF-7 and B16-F10 cancer cell lines and potent cyclin dependent kinase 2 (CDK2) inhibitory activities [1]. The antiproliferative effects seem to be associated to the presence of a strong electron-withdrawing substituent in R^1, however, trifluoromethyl derivatives had not been tested. Interestingly, as reported in Table 2, all the compounds **4a–v**, included the trifluoromethyl analogues, are devoid of toxicity for the human MT-4 cell line up to the highest concentration tested (100 μM).

Concerning the anti-RSV activity (Table 1), all the compounds able to interfere with RSV replication exhibited an interesting potency, comparable or better than ribavirin (EC_{50} = 35 μM). Unfortunately, all derivatives were found less potent than 6-Azauridine, used as reference drug. SAR studies showed that the replacement of the trifluoromethyl group in R^1 with the

methyl group (**4m–q**) strongly reduced the cytotoxicity and led to the appearance of anti-RSV activity in the micromolar concentrations (EC_{50}s ranging from 11 μM to 28 μM). More potent compounds were obtained with the introduction in R^1 of an electron-withdrawing group such as a chlorine (**4h–1l**) or a bromine (**4c–1g**) atom (EC_{50}s ranging from 5 μM to 14 μM). However, both these substitutions generally resulted in compounds with increased cytotoxicity against Vero76 cells with respect to the corresponding 4-methylanilines (**4m–1q**). In particular, **4e**, where R = CF_3 and R^1 = Br, exhibited the most potent anti-RSV activity (EC_{50} = 5 μM) of the entire series of compounds, however, its selectivity was modest (SI = 3.4) due to its significant cytotoxicity against Vero76 cells (CC_{50} = 17 μM). Among analogues showing anti-RSV activity, only **4a** and **4o** exhibited a lower selectivity index (SI = 2.26 and 3.28, respectively) than **1e**. Although less potent than **4e**, the halo substituted anilines **4g**, **4h** and **4l** (EC_{50} = 8 μM, 6 μM, and 9 μM, respectively) presented comparable or higher selectivity (SI = 11.00, 10.83, and >11.1, respectively) than the reference drug 6-Azauridine (SI = 11.1).

When tested against HIV-1, Reo-1, CVB-5, Sb-1, VV, HSV-1 and VSV, all the compounds were devoid of antiviral activity up to the highest concentration tested (100 μM) (Table 2), while some analogues showed a moderate protection against BVDV and YFV, representative of Pestivirus and Flavivirus genera, respectively, of the *Flaviviridae* family (Table 1). In particular, six compounds (**4c**, **4h**, **4i**, **4m–o**) inhibited BVDV with EC_{50}s ranging from 29 μM to 55 μM and nine compounds (**4a–d**, **4g**, **4h**, **4k–m**) inhibited YFV with EC_{50}s ranging from 20 μM to 65 μM. All the active compounds were non cytotoxic against the proper cell line (MDBK and BHK-21, respectively) at concentration up to 100 μM. Although no evident relationship can be observed between the structure of compounds and the

Table 2. Cytotoxicity and antiviral activity of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl) anilines **4a–v** against $ssRNA^+$ (HIV-1, CVB-5, Sb-1), $ssRNA^-$ (VDV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

Compds	R	R^1	MT-4 $^aCC_{50}$ (µM)	HIV-1 $^bEC_{50}$ (µM)	BHK-21 $^cCC_{50}$ (µM)	Reo-1 $^dEC_{50}$ (µM)	Vero76 $^eCC_{50}$ (µM)	CVB-5	Sb-1	VV	HSV-1	VSV
								$^fEC_{50}$ (µM)				
4a	CH_3	H	>100	>100	>100	>100	52	>52	>52	>52	>52	>52
4b	Br	H	>100	>100	>100	>100	52	>52	>52	>52	>52	>52
4c	H	Br	>100	>100	>100	>100	52	>52	>52	>52	>52	>52
4d	Cl	Br	>100	>100	>100	>100	43	>43	>43	>43	>43	>43
4e	CF_3	Br	>100	>100	28	>28	17	>17	>17	>17	>17	>17
4f	CH_3	Br	>100	>100	>100	>100	92	>92	>92	>92	>92	>92
4g	Br	Br	>100	>100	>100	>100	88	>88	>88	>88	>88	>88
4h	H	Cl	>100	>100	>100	>100	65	>65	>65	>65	>65	>65
4i	Cl	Cl	>100	>100	73	>73	90	>90	>90	>90	>90	>90
4j	CF_3	Cl	>100	>100	31	>31	80	>80	>80	>80	>80	>80
4k	CH_3	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4l	Br	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4m	H	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4n	Cl	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4o	CF_3	CH_3	>100	>100	88	>88	82	>82	>82	>82	>82	>82
4p	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4q	Br	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4r	H	CF_3	>100	>100	37	>37	75	>75	>75	>75	>75	>75
4s	Cl	CF_3	>100	>100	17	>17	18	>18	>18	>18	>18	>18
4t	CF_3	CF_3	>100	>100	27	>27	26	>26	>26	>26	>26	>26
4u	CH_3	CF_3	>100	>100	88	>88	45	>45	>45	>45	>45	>45
4v	Br	CF_3	>100	>100	20	>20	28	>28	>28	>28	>28	>28
Ref. compounds												
EFV			37.0±0.01	0.002±0.0001								
NM 107					>100	6.0±2.9						
Pleconaril							80.0±6.0	0.005±0.002	2.2±0.6			
ACG							>100				2.8±0.2	
M5255							19.0±3.0			1.5±0.2		

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c] Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kideney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock.infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1) and VSV (Vescicular Stomatitis Virus) by 50% in VERO76 monolayers.

appearance of the activity against BVDV and YFV, it should be noted that only derivatives **4c**, **4h**, **4m,** unsubstituted in R, were found to be active against BVDV, YFV, and RSV. Whereas these compounds exhibited a modest and similar potency against the two viruses representative of two of

the three genera of the *Flaviviridae* family, the activity against RSV was always higher.

In conclusion, a series of *N*-((1,3-diphenyl-1*H*-pyrazol-4- yl)methyl) anilines were synthesized and tested in cell-based assays against a large panel of RNA and DNA viruses. Results of cytotoxicity and antiviral activity showed that most of the analogues were endowed with promising and selective activity against RSV. Initial SAR studies indicated that the presence of trifluoromethyl group in R^1 negatively impact both potency and cytotoxicity, whereas the halo-substituted analogues can be considered good candidates for the development of new potent and selective anti-RSV agents.

5.1.1.2. Experimental

5.1.1.2.1. Chemistry

Chemicals were purchased from Sigma-Aldrich and used without further purification. Melting points were determined on a Stuar Scientific SMP1 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AM-400 spectrometer (400MHz) in $CDCl_3$ or DMSO-d_6, and chemical shifts were reported in ppm (δ). All compounds were routinely checked by thin-layer chromatography (TLC). TLC was performed on silica gel or aluminium oxide fluorescent coated plates (Fluka, DC-Alufolien Kieselgel or aluminum oxide F254). Compound purity was determined by elemental analysis and was confirmed to be > 95% for all the tested compounds. Analytical results are within ±0.40% of the theoretical values.

1-phenyl-2-(1-phenylethylidene)hydrazones **1a-e** [19] and 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes **2a-e** [17] were synthesized according to literature procedures.

5.1.1.2.2. General procedure for the synthesis of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)anilines (4a-v).

The appropriate aniline (17 mmol) was added to a solution of the appropriate 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **2a-e** (17 mmol) in dry ethanol (100 ml), dry benzene (50 ml) and 2 drops of glacial acetic acid. The mixture was refluxed for 12 h and the water formed during the reaction was eliminated using a Dean-Stark apparatus. After cooling, the solvent was evaporated under reduced pressure to give the crude (*E*)-*N*-[(1,3-diphenyl-1*H*-pyrazol-4-yl)methylene]aniline **3a-v** which was used without further purification. To a solution of compound **3a-v** (4 mmol) in tetrahydrofuran (100 ml) sodium borohydride was added (40 mmol) and the mixture was refluxed for 4 h. The solvent was then evaporated under reduced pressure and the residue was distributed between water and chloroform. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was crystallized with suitable solvent.

5.1.1.2.2.1. *N*-((1-Phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)methyl)aniline (4a). Yield 48%; m.p. = 103 – 106°C from EtOH. Anal. Calc. for $C_{23}H_{21}N_3$: C 81.38, H 6.24, N 12.38; found C 81.36, H 6.25, N 12.39. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.02 (s, 1H), 7.75 (d, 2H, *J* = 7.6 Hz), 7.70 (d, 2H, *J* = 7.6 Hz), 7.22 – 7.48 (m, 7 H), 6.81 (t, 1H, *J* = 7.6 Hz), 6.73 (d, 2H, *J* = 6.8 Hz), 4.40 (s, 2H), 2.42 (s, 3H). ^{13}C NMR ($CDCl_3$): δ (ppm) 150.51, 148.63, 139.51, 137.23, 130.11, 129.52, 129.17, 128.87, 128.73, 127.27, 126.01, 118.88, 117.97, 116.12, 112.34, 38.24, 20.80.

5.1.1.2.2.2. *N*-((3-(4-Bromophenyl)-1-phenyl-*1H*-pyrazol-4-yl)methyl) aniline (4b). Yield 43%; m.p. = 124 – 127 °C from CH_3CN. Anal. Calc. for $C_{22}H_{18}BrN_3$: C 65.36, H 4.49, Br 19.76; N 10.39; found C 65.36, H 4.50, Br

19.76; N 10.38. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.06 (s, 1H), 7.74 (d, 2H, *J* = 7.6 Hz), 7.68 (d, 2H, *J* = 6.8 Hz), 7.48 – 7.58 (m, 4H), 7.24 – 7.34 (m, 3H), 6.85 (t, 1H, *J* = 6.8 Hz), 6.75 (d, 2H, *J* = 6.8 Hz), 4.38(s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 149.25, 148.57, 139.34, 132.11, 131.55, 129.57, 129.26, 129.19, 128.86, 126.28, 121.27, 119.10, 118.10, 116.21, 112.40, 38.15.

5.1.1.2.2.3. 4-Bromo-*N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline (4c) Yield 59%; m.p. = 107 – 110 °C from CH_3CN. Anal. Calc. for $C_{22}H_{18}BrN_3$: C 65.36, H 4.49, Br 19.76; N 10.39; found C 65.35, H 4.50, Br 19.76; N 10.39. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.01 (s, 1H), 7.74 – 7.76 (m, 4H), 7.28 – 7.47 (m, 8H), 6.58 (d, 2H, *J* = 7.6 Hz), 4.37 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 150.45, 147.79, 139.43, 132.82, 131.37, 129.55, 128.93, 128.60, 127.94, 127.33, 126.17, 118.56, 118.06, 114.22, 106.78, 38.12.

5.1.1.2.2.4. *N*-((3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-chlorobenzenamine (4d) [17]. Yield 83%; m.p. = 125 – 128 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{22}H_{17}BrClN_3$; C 60.22, H 3.91, Br 18.21, Cl 8.08, N 9.58; found C 60.21, H 3.90, Br 18.22, Cl 8.08, N 9.59.

5.1.1.2.2.5. 4-Bromo-*N*-((3-(4-(trifluoromethyl)phenyl)1-phenyl-1*H*-pyrazol-4-yl)methyl)aniline (4e). Yield 42%; m.p. = 126 – 129 °C from C_6H_{12}. Anal. Calc. for $C_{23}H_{17}BrF_3N_3$: C 58.49, H 3.63, Br 16.92, F 12.07, N 8.90; found C 58.50, H 3.62, Br 16.92, F 12.08, N 8.89. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.05 (s, 1H), 7.91(d, 2H, *J* = 6.8 Hz), 7.75 (d, 2H, *J* = 8.0 Hz), 7.70 (d, 2H, *J* = 7.2 Hz), 7.29 – 7.49 (m, 5H), 6.60 (d, 2H, *J* = 6.4 Hz), 4.38 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 148.84, 147.72, 139.26, 136.80,

131.36, 129.59, 129.43, 128.14 (q, $J_{C\text{-}F}$ = 31 Hz), 127.78, 126.50, 125.50 (q, $J_{C\text{-}F}$ = 3 Hz), 124.91 (q, $J_{C\text{-}F}$ = 270 Hz), 119.15, 118.24, 114.30, 106.93, 38.05.

5.1.1.2.2.6. 4-Bromo-*N*-((1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)methyl) aniline (4f) [1]. Yield 48%; m.p. = 110 – 111 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [1]. Anal. Calc. for $C_{23}H_{20}BrN_3$: C 66.04, H 4.82, Br 19.10, N 10.04; found C 66.02, H 4.83, Br 19.10, N 10.05.

5.1.1.2.2.7. 4-Bromo-*N*-((3-(4-bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl) methyl)aniline (4g) [17]. Yield 74%; m.p. = 124 – 128°C from EtOH. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{22}H_{17}Br_2N_3$: C 54.68, H 3.55, Br 33.07, N 8.70; found C 54.68, H 3.54, Br 33.07, N 8.71.

5.1.1.2.2.8. 4-Chloro-*N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)aniline (4h). Yield 46%; m.p. = 105 – 108 °C from CH_3CN. Anal. Calc. for $C_{22}H_{18}ClN_3$: C 73.43, H 5.04, Cl 9.85, N 11.68; found C 73.45, H 5.03, Cl 9.85, N 11.67. ^{1}H-NMR δ (ppm) ($CDCl_3$): 4.38 (s, 2H), 6.62 (d, 2H, *J* = 8.0 Hz), 7.16 (d, 2H, *J* = 7.6 Hz), 7.28-7.47 (m, 6H), 7.74-7.76 (m, 4H), 8.01 (s, 1H). ^{13}C NMR (DMSO-d_6): δ (ppm) 150.45, 147.46, 139.44, 132.84, 129.55, 128.95, 128.60, 128.57, 127.94, 127.33, 126.17, 119.38, 118.60, 118.05, 113.64, 38.21.

5.1.1.2.2.9. 4-Chloro-*N*-((3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl) methy)aniline (4i) [17]. Yield 78%; m.p. = 110 – 113 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{22}H_{17}Cl_2N_3$: C 67.01, H 4.35, Cl 17.98, N 10.66; found C 67.00, H 4.36, Cl 17.98, N 10.66.

5.1.1.2.2.10. 4-Chloro-*N*-((3-(4-trifluoromethyl)phenyl)1-phenyl-1*H*-pyrazol-4-yl)methyl)aniline (4j). Yield 50%; m.p. = 111 – 115 °C from C_6H_{12}. Anal. Calc. for $C_{23}H_{17}ClF_3N_3$: C 64.57, H 4.00, Cl 8.29, F 13.32, N 9.82; found C 64.56, H 4.02, Cl 8.28, F 13.32, N 9.82. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.05 (s, 1H), 7.92 (d, 2H, *J* = 8.0 Hz), 7.76 (d, 2H, *J* = 8.4 Hz), 7.71 (d, 2H, *J* = 8.4 Hz), 7.50 (t, 2H, *J* = 8.0 Hz), 7.35 (t, 1H, *J* = 7.6 Hz), 7.18 (d, 2H, *J* = 8.8 Hz), 6.65 (d, 2H, *J* = 8.8 Hz), 4.38 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 148.84, 147.39, 139.26, 136.81, 129.59, 129.43, 128.55, 128.13 (q, $J_{C\text{-}F}$ = 31 Hz), 127.78, 126.49, 125.49 (q, $J_{C\text{-}F}$ = 3 Hz), 124.26 (q, $J_{C\text{-}F}$ = 270 Hz), 119.53, 119.19, 118.23, 113.73, 38.15.

5.1.1.2.2.11. 4-Chloro-*N*-((1-phenyl-3-(*p*-tolyl)-1*H*-pyrazol-4-yl)methyl) aniline (4k) [17]. Yield 77%; m.p. = 106 – 108 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{23}H_{20}ClN_3$: C 73.89, H 5.39, Cl 9.48, N 11.24; found C 73.88, H 5.40, Cl 9.48, N 11.24.

5.1.1.2.2.12. *N*-((3-(4-Bromophenyl)-1-phenyl-*1H*-pyrazol-4-yl)methyl)-4-chloroaniline (4l) [17]. Yield 62%; m.p. = 125 – 127 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{22}H_{17}BrClN_3$: C 60.22, H 3.91, Br 18.21, Cl 8.08, N 9.58; found C 60.22, H 3.90, Br 18.22, Cl 8.08, N 9.58.

5.1.1.2.2.13. *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (4m). Yield 35%; m.p. = 95 – 97 °C from EtOH. Anal. Calc. for $C_{23}H_{21}N_3$: C 81.38, H 6.24, N 12.38; found C 81.39, H 6.25, N 12.36. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.01 (s, 1H), 7.85 (d, 2H, *J* = 7.6 Hz), 7.77 (d, 2H, *J* = 7.6 Hz), 7.29

– 7.48 (m, 6H), 7.06 (d, 2H, *J* = 7.6 Hz), 6.65 (d, 2H, *J* = 7.6 Hz), 4.39 (s, 2H), 2.30 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ (ppm) 150.43, 146.40, 139.49, 132.96, 129.54, 129.32, 128.82, 128.57, 127.88, 127.36, 126.10, 124.54, 119.23, 118.02, 112.52, 38.49, 20.09.

5.1.1.2.2.14. *N*-((3-(4-Chlorophenyl)-1-phenyl-*1H*-pyrazol-4-yl)methyl)-4-methylaniline (4n) [17]. Yield 76%; m.p. = 89 – 91 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{23}H_{20}ClN_3$: C 73.89, H 5.39, Cl 9.48, N 11.24; found C 73.90, H 5.40, Cl 9.48, N 11.22.

5.1.1.2.2.15. *N*-((3-(4-(Trifluoromethyl)phenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (4o). Yield 69%; m.p. = 98 – 100 °C from C_6H_{12}. Anal. Calc. for $C_{24}H_{20}F_3N_3$: C 70.75, H 4.95, F 13.99, N 10.31; found C 70.74, H 4.96, F 14.00, N 10.30. ^{1}H-NMR δ (ppm) ($CDCl_3$): 7.99 – 8.02 (m, 3H), 7.77 (d, 2H, *J* = 7.6 Hz), 7.71 (d, 2H, *J* = 8.4 Hz), 7.50 (t, 2H, *J* = 7.6 Hz), 7.35 (t, 1H, *J* = 7.6 Hz), 7.08 (d, 2H, *J* = 8.4 Hz), 6.66 (d, 2H, *J* = 8.4 Hz), 4.38 (s, 2H), 2.31 (s, 3H). ^{13}C NMR (DMSO-d_6): δ (ppm) 149.9, 146.3, 139.7, 136.3, 131.0, 129.8, 129.6, 129.3 (q, J_{C-F} = 31 Hz), 126.2, 126.2, 126.1, 125.6, 124.1(q, J_{C-F} = 3 Hz), 123.0 (q, J_{C-F} = 270 Hz), 119.9, 117.2, 113.4, 36.4, 21.3.

5.1.1.2.2.16. 4-methyl-*N*-((1-phenyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl) aniline (4p) [17]. Yield 68%; m.p. = 89 – 91 °C from CH_3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{24}H_{23}N_3$: C 81.55, H 6.56, N 11.89; found C 81.52, H 6.58, N 11.89.

5.1.1.2.2.17. *N*-((3-(4-Bromophenyl)-1-phenyl-*1H*-pyrazol-4-yl)methyl)-4-methylaniline (4q) [17]. Yield 54%; m.p. = 89 – 92 °C from CH3CN. The compound exhibited spectroscopic data identical to those previously reported [17]. Anal. Calc. for $C_{23}H_{20}BrN_3$: C 66.04, H 4.82, Br 19.10, N 10.04; found C 66.03, H 4.83, Br 19.10, N 10.04.

5.1.1.2.2.18. 4-(trifluoromethyl)-*N*-((1,3-diphenyl-1*H*-pyrazol-4-yl) methyl)aniline (4r). Yield 67%; m.p.= 110 – 112 °C Crystallized EtOH. Anal. Calc. for $C_{23}H_{18}F_3N_3$: C 70.22, H 4.61, F 14.49, N 10.68; found C 70.23, H 4.62, F 14.49, N 10.66. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.01 (s, 1H), 7.75 – 7.78 (m, 4H), 7.39 – 7.50 (m, 7H), 7.33 (d, 1H, J = 7.2 Hz), 6.71 (d, 2H, J = 8.4 Hz), 4.44 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 151.38, 150.48, 139.42, 132.78, 129.54, 129.01, 128.61, 127.98, 127.32, 126.19 (q, J_{C-F} = 3 Hz), 125.34 (q, J_{C-F} = 268 Hz), 118.18, 118.09, 115.67 (q, J_{C-F} = 32 Hz), 111.60, 37.73, 15.05.

5.1.1.2.2.19. *N*-((3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-(trifluoromethyl)aniline (4s). Yield 65%; m.p. = 100 – 103 °C from MeOH; Anal. Calc. for $C_{23}H_{17}ClF_3N_3$: C 64.57, H 4.00, Cl 8.29, F 13.32, N 9.82; found C 64.59, H 4.01, Cl 8.27, F 13.32, N 9.80. ^{1}H-NMR δ (ppm) ($CDCl_3$): 7.97(s, 1H), 7.72 – 7.76 (m, 4H), 7.41 – 7.55 (m, 6H), 7.34 (t, 1H, J = 7.6 Hz), 6.69 (d, 2H, J = 8.4 Hz), 4.39 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 151.35, 149.24, 139.31, 132.70, 131.62, 129.57, 129.28, 128.94, 128.68, 126.35, 126.18 (q, J_{C-F} = 4 Hz), 126.53 (q, J_{C-F} = 268 Hz), 118.27, 118.16, 115.72 (q, J_{C-F} = 32 Hz), 111.65, 37.66.

5.1.1.2.2.20. 4-(Trifluoromethyl)-*N*-((3-(4-(trifluoromethyl)phenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)aniline (4t). Yield 68%; m.p. = 97 – 100 °C from C_6H_{12}. Anal. Calc. for $C_{24}H_{17}F_6N_3$: C 62.47, H 3.71, F 24.71, N 9.11; found C 62.49, H 3.70, F 24.70, N 9.11. ^{1}H-NMR δ (ppm) ($CDCl_3$): 8.02 (s, 1H), 7.93 (d, 2H, *J* = 8.0 Hz), 7.76 (d, 2H, *J* = 8.4 Hz), 7.71 (d, 2H, *J* = 8.0 Hz), 7.46 – 7.53 (m, 4H), 7.36 (t, 1H, *J* = 7.6 Hz), 6.72 (d, 2H, *J* = 8.4 Hz), 4.44 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 151.33, 148.87, 139.25, 136.75, 129.57, 129.49, 128.19 (q, J_{C-F} = 32 Hz), 127.77, 126.51, 126.16, (q, J_{C-F} = 4.7 Hz), 125.50 (q, J_{C-F} = 4.7 Hz), 125.30 (q, J_{C-F} = 267 Hz), 124.23 (q, J_{C-F} = 270 Hz), 118.76, 118.26, 115.83 (q, J_{C-F} = 31 Hz), 111.68, 37.66.

5.1.1.2.2.21. 4-(Trifluoromethyl)-*N*-((1-phenyl-3(-*p*-tolyl)-1H-pyrazol-4-yl)methyl)aniline (4u). Yield 73%; m.p. = 128 – 130 °C from C_6H_{12}. Anal. Calc. for $C_{24}H_{20}F_3N_3$: C 70.75, H 4.95, F 13.99, N 10.31; found C 70.75, H 4.96, F 13.98, N 10.31. ^{1}H-NMR δ (ppm) ($CDCl_3$): 7.97(s, 1H), 7.75 (d, 2H, *J*= 7.6 Hz), 7.67 (d, 2H, *J*= 8.0 Hz), 7.44 – 7.50 (m, 4H), 7.31 (t, 1H, *J* = 7.6 Hz), 7.27 (d, 2H, *J* = 8.4 Hz), 6.69 (d, 2H, *J* = 8.4 Hz), 4.42 (s, 2H), 2.42 (s, 3H). ^{13}C NMR (DMSO-d_6): δ (ppm) 151.38, 150.52, 139.45, 137.32, 129.96, 129.53, 129.17, 128.88, 127.22, 126.18 (q, J_{C-F} = 3 Hz), 126.11, 125.34 (q, J_{C-F} = 270 Hz), 118.03, 118.00?, 115.62 (q, J_{C-F} = 31 Hz), 111.57, 37.74, 20.76.

5.1.1.2.2.22. *N*-((3-(4-Bromophenyl)-1-phenyl-1*H*-pyrazol-4-yl)methyl)-4-(trifluoromethyl)aniline (4v). Yield 56%; m.p. = 101 – 105 °C from EtOH. Anal. Calc. for $C_{23}H_{17}BrF_3N_3$: C 58.49, H 3.63, Br 16.92, F 12.07, N 8.90; found C 58.49, H 3.62, Br 16.92, F 12.08, N 8.90. 1H-NMR δ (ppm) ($CDCl_3$): 8.02 (s, 1H), 7.74 (d, 2H, *J* = 7.6 Hz), 7.65 (d, 2H, *J* = 8.4 Hz), 7.58 (d, 2H, *J* = 8.4 Hz), 7.45 – 7.51 (m, 4H), 7.34 (t, 1H, *J* = 7.2 Hz), 6.72 (d, 2H, *J* = 8.4 Hz), 4.41 (s, 2H). ^{13}C NMR (DMSO-d_6): δ (ppm) 151.33, 149.27,

139.31, 131.98, 131.57, 129.55, 129.21, 126.34, 126.18 (q, J_{C-F} = 3 Hz), 125.32 (q, J_{C-F} = 268 Hz), 121.33, 118.28, 118.15, 115.78 (q, J_{C-F} = 32 Hz), 111.65, 37.67.

5.1.1.3. Biology

5.1.1.3.1. Test compounds

Compounds were dissolved in DMSO at 100 µM, and then diluted in culture medium.

5.1.1.3.2. Cells and Viruses

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of RNA and DNA viruses were the following: $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4); Madin Darby Bovine Kidney (MDBK) [ATCC CCL22 (NBL-1) *Bos Taurus*], Baby Hamster Kidney (BHK-21) [ATCC CCL10 (C-13) *Mesocricetus auratus*] and Monkey kidney (Vero 76) [ATCCCRL 1587 *Cercopithecus Aethiops*]. Viruses were purchased from American Type Culture Collection (ATCC), with the exception of Yellow Fever Virus (YFV) and Human Immunodeficiency Virus type-1 (HIV-1). Viruses representative of positive-sense single stranded RNAs ($ssRNA^+$) were: (i) *Retroviridae*: the III_B laboratory strain of HIV-1, obtained from the supernatant of the persistently infected $H9/III_B$ cells (NIH 1983); (ii) *Flaviviridae*: yellow fever virus (YFV) [strain 17-D vaccine (Stamaril Pasteur J07B01)] and bovine viral diarrhoea virus (BVDV) [strain NADL (ATCC VR-534)]; (iii) *Picornaviridae*: human enterovirus B [coxsackie type B5 (CV-B5), strain Faulkner, (ATCC VR-185)], and human

enterovirus C [poliovirus type-1 (Sb-1), Sabin strain Chat (ATCC VR-1562)]. Viruses representative of negative-sense, single-stranded RNAs (ssRNA$^-$) were: (iv) *Paramyxoviridae*: human respiratory syncytial virus (RSV) [strain A2 (ATCC VR-1540)]; (v) *Rhabdoviridae*: vesicular stomatitis virus (VSV) [lab strain Indiana (ATCC VR 158)]. The virus representative of double-stranded RNAs (dsRNA) *Reoviridae* was reovirus type-1 (Reo-1) [simian virus 12, strain 3651 (ATCC VR- 214)]. DNA virus representatives were: (vi) *Poxviridae*: vaccinia virus (VV) [strain Elstree (Lister Vaccine) (ATCC VR-1549)]; and (vii) *Herpesviridae*: human herpesvirus 1 (HSV-1) [strain KOS (ATCC VR- 1493)].

5.1.1.3.3. Cytotoxicity Assays

Cytotoxicity assays were run in parallel with antiviral assays. Exponentially growing MT-4 cells were seeded at an initial density of $1x10^5$ cells/mL in 96-well plates in RPMI-1640 medium, supplemented with 10% foetal bovine serum (FBS), 100 units/mL penicillin G and 100 mg/mL streptomycin. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere, in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37 °C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [4]. MDBK and BHK cells were seeded in 96-well plates at an initial density of $6x10^5$ and $1x10^6$ cells/mL, respectively, in Minimum Essential Medium with Earle's salts (MEM-E), L glutamine, 1 mM sodium pyruvate and 25 mg/L kanamycin, supplemented with 10% horse serum (MDBK) or 10% foetal bovine serum (FBS) (BHK). Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 72 hrs at 37 °C by the

MTT method. Vero76 cells were seeded in 96-well plates at an initial density of $5x10^5$ cells/mL, in Dulbecco's Modified Eagle Medium (D-MEM) with L-glutamine and 25 mg/L kanamycin, supplemented with 10% FBS. Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 48-96 h at 37 °C by the by the MTT method.

5.1.1.3.4. Antiviral assays

Antiviral activity against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m.o.i.) of 0.01. Briefly, 50 µL of RPMI containing $1x10^4$ MT-4 cells were added to each well of flat bottom microtitre trays, containing 50 µL of RPMI without or with serial dilutions of test compounds. Then, 20 µL of a HIV-1 suspension containing 100 $CCID_{50}$ were added. After 4-day of incubation at 37 °C, cell viability was determined by the MTT method. Antiviral activity against YFV and Reo-1 was based on inhibition of virus-induced cytopathogenicity in BHK-21 cells acutely infected with a m.o.i. of 0.01. Activity of compounds activity against BVDV was based on inhibition of virus-induced cytopathogenicity in MDBK cells acutely infected with a m.o.i. of 0.01. Briefly, BHK and MDBK cells were seeded in 96-well plates at a density of $5x10^4$ and $3x10^4$ cells/well, respectively, and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 µL of a proper virus dilution in maintenance medium [MEM-Earl with L-glutamine, 1 mM sodium pyruvate and 0.025 g/L kanamycin, supplemented with 0.5% inactivated FBS] to give an m.o.i of 0.01. After 2 hrs, 50 µL of maintenance medium, without or with serial dilutions of test

compounds, were added. After a 3-/4-day incubation at 37 °C, cell viability was determined by the MTT method [20].

Antiviral activity against CV-B5, Sb-1, VV, HSV-1, VSV and RSV was determined by plaque reduction assays in infected cell monolayers. To this end, Vero 76-cells were seeded in 24-well plates at a density of $2x10^5$ cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium [Dulbecco's Modified Eagle Medium (D-MEM) with L-glutamine and 4500 mg/L D-glucose and 0.025 g/L kanamycin, supplemented with 10% FBS] at 37 °C in a humidified CO_2 (5%) atmosphere. Then, monolayers were infected for 2 h with 250 µL of proper virus dilutions to give 50 to 100 PFU/well. Following removal of unadsorbed virus, 500 µL of maintenance medium [D-MEM with L-glutamine and 4500 mg/L Dglucose, supplemented with 1% inactivated FBS] containing 0.75% methylecellulose, without or with serial dilutions of test compounds, were added. Cultures were incubated at 37 °C for 2 (Sb-1 and VSV), 3 (CVB-5, VV and HSV-1) or 5 days (RSV) and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted.

5.1.1.3.5. Linear regression analysis

The extent of cell growth/viability and viral multiplication, at each drug concentration tested, were expressed as percentage of untreated controls. Concentrations resulting in 50% inhibition (CC_{50} or EC_{50}) were determined by linear regression analysis.

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References

1. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Singleton RJ, O'Brien KL, Roca A, Wright PF, Bruce N, Chandran A, Theodoratou E, Sutanto A, Sedyaningsih ER, Ngama M, Munywoki PK, Kartasasmita C, Simões EAF, Rudan I, Weber MW, Campbel H. *Lancet*. (**2010**) 375, 1545.
2. Gonzalez PA, Bueno SM, Carreno LJ, Riedel CA, Kalergis AM. *Rev. Med. Virol*. (**2012**) 22, 230.
3. Falsey AR, Singh HK, Walsh EE. *J. Med. Virol*. (**2006**) 78, 1493.
4. Falsey AR, Hennessey P A, Formica MA, Cox C, Walsh EE. *N. Engl. J. Med*. (**2005**) 352, 1749.
5. Aujard Y, Fauroux B. *Respir. Med.* (**2002**) 96 (Suppl. B), S9–14.
6. Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, Fukuda *K. J.A.M.A.* (**2003**) 289, 179.
7. Eisenhut M. *Lancet*. (**2006**) 368, 988.
8. Eisenhut M. *Crit. Care*. (**2006**) 10, R107.
9. Morichi S, Kawashima H, Ioi H, Yamanaka G, Kashiwagi Y, Hoshika A, Nakayama T, Watanabe Y. *J Infect. Chemother*. (**2011**) 17, 776.
10. Palivizumab (Synagis) for prevention of RSV infection. *Med. Lett. Drugs. Ther.* (**1999**) 41, 3–4.
11. Harkensee C, Brodlie M, Embleton ND, McKean *M. J. Infect*. (**2006**) 52, 2.
12. Prince GA. *Expert Opin. Investig. Drugs.* (**2001**) 10, 297.
13. Tonelli M, Vazzana I, Tasso B, Boido V, Sparatore F, Fermeglia M, Paneni MS, Posocco P, Pricl S, La Colla P, Ibba C, Secci B, Collu G, Loddo R. B*ioorg. Med. Chem.* (**2009**) 17, 4425.

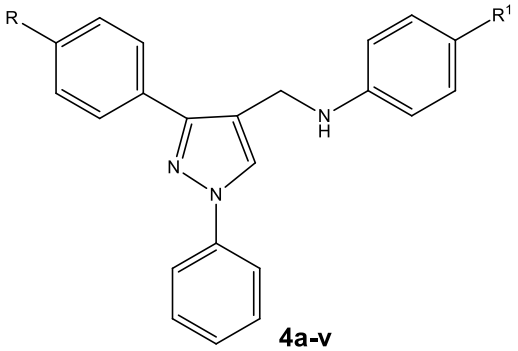
14. Tonelli M, Simone M, Tasso B, Novelli F, Boido V, Sparatore F, Paglietti G, Pricl S, Giliberti G, Blois S, Ibba C, Sanna G, Loddo R, La Colla P. *Bioorg. Med. Chem*. (**2010**) 18, 2937.
15. Conti C, Proietti Monaco L, Desideri N. *Bioorg. Med. Chem*. (**2011**) 19, 7357.
16. Conti C, Proietti Monaco L, Desideri N. *Bioorg. Med. Chem.* (**2014**) 22, 1201.
17. Huang X F, Lu X, Zhang Y, Song G, He Q, Li Q, Yang X, Wei Y, Zhu H. *Bioorg. Med. Chem*. (**2012**) 20, 4895.
18. Kira M A, Abdel Raeman M O, Gadalla K Z *Tetrahedron Lett*. (**1969**) 2, 109.
19. Bekhit AA, Ashour HMA, Bekhit AA, Abdel-Rahman HM, Bekhit SA. *J. Enz. Inhib. Med. Chem*. (**2009**) *24,* 296.
20. Pauwels R, Balzarini J, Baba M, Snoeck R, Schols D, Herdewijn P, Desmyter J, De Clercq E. *J. Virol. Methods*. (**1988**) *20*, 309.

5.1.2. Anti DENV-2 and WNV studies of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)anilines

N-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl)anilines (**4a**, **4b**, **4c**, **4d**, **4g**, **4h**, **4i**, **4k**, **4l**, **4m**, **4n**, **4o**) able to interfere with YFV and/or BVDV replication were successively tested against two additional significant human pathogens such as DENV-2 and WNV, both belonging to the Flavivirus genus of the *Flaviviridae* family. Cytotoxicity and antiviral activity against DENV-2 and YFV of the selected inhibitors were reported in Table 3. For comparison, Table 3 includes also previously obtained data on YFV and BVDV replication.

Results showed that the antiviral activity of tested compounds extended to DENV-2 replication, while WNV replication was only marginally affected by few compounds (**4g**, **4i**, **4n**, **4o**). When compared the antiviral activity against the selected viruses belonging to the *Flaviviridae* family, all the tested compounds exhibited the higher potency against DENV-2.

Table 3. Cytotoxicity and antiviral activity of *N*-((1,3-diphenyl-1*H*-pyrazol-4-yl)methyl) anilines **4a–v** against ssRNA$^+$ (BVDV, YFV, DENV-2, WNV) viruses.



4a-v

Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	[c]SI CC_{50}/ EC_{50}	BHK-21 [d]CC_{50} (µM)	YFV [e]EC_{50} (µM)	[c]SI CC_{50}/ EC_{50}	[f]DENV-2 EC_{50} (µM)	[c]SI CC_{50}/ EC_{50}	[g]WNV EC_{50} (µM)
4a	CH_3	H	>100	>100	-	>100	38.0±6.0	>2.6	30.0±4.0	>3.3	>100
4b	Br	H	>100	>100	-	>100	20.0±4.0	>5.0	22.0±6.0	>4.5	>100
4c	H	Br	>100	31.0±0.5	>3.2	>100	32.0±4.0	>3.1	>100	-	>100
4d	Cl	Br	>100	>100	-	>100	28.0±5.0	>3.6	11.0±0.4	>9.1	>100
4g	Br	Br	>100	>100	-	>100	54.0±6.0	>1.8	23.0±6.0	>4.3	80.0±8.5
4h	H	Cl	>100	29.0±1.5	>3.4	>100	29.0±3.5	>3.4	13.0±5.0	>7.7	>100
4i	Cl	Cl	>100	31.0±2.0	>3.2	73	>73	-	14.5±3.5	5.0	57.5±0.5
4k	CH_3	Cl	>100	>100	-	>100	34.0±0.5	>2.9	12.1±3.0	>8.3	>100
4l	Br	Cl	>100	≥100	-	>100	43.0±0.5	>2.3	12.9±2.3	>7.7	>100
4m	H	CH_3	>100	53.0±3.0	>1.9	>100	65.0±5.0	>1.5	11.2±3.6	>8.9	>100
4n	Cl	CH_3	>100	55.0±5.0	>1.8	>100	>100	-	11.1±1.6	>9.0	39.0±1.0
4o	CF_3	CH_3	>100	54.0±4.0	>1.8	88	>88	-	14.3±0.7	6.1	59.0±1.1
Ref. compounds											
NM 299						>100	46.0±3.3	>2.2			
Ribavirin			57.0±6.2	19.0±5.3	3.0						
NM 108						60			1.2	50	0.65

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC50 and EC50. [d] Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method.

5.1.2.1. Experimental

5.1.2.1.1. Biology

The cytotoxicity and antiviral assays were performed by Prof. Roberta Loddo, Department of Biomedical Sciences, Microbiology and Virology Section, University of Cagliari.

5.1.2.1.1.1. Test compounds

Compounds were dissolved in DMSO at 100 μM, and then diluted in culture medium.

5.1.2.1.1.2. Cells and Viruses

Baby Hamster Kidney (BHK-21) [ATCC CCL10 (C-13) *Mesocricetus auratus*] was utilized to support the multiplication of Dengue virus type 2 (DENV-2) and West Nile virus (WNV). Cell line was purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. DENV-2 and WNV utilized were clinical isolate.

5.1.2.1.1.3. Cytotoxicity Assays

BHK cells were seeded in 96-well plates at an initial density of $1x10^6$ cells/mL in Minimum Essential Medium with Earle's salts (MEM-E), L glutamine, 1 mM sodium pyruvate and 25 mg/L kanamycin, supplemented with 10% horse serum (MDBK) or 10% foetal bovine serum (FBS) (BHK). Cell cultures were then incubated at 37 °C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 72 hrs at 37 °C by the MTT method.

5.1.2.1.1.4. Antiviral assays

Antiviral activity against DENV-2 and WNV was based on inhibition of virus-induced cytopathogenicity in BHK-21 cells acutely infected with a m.o.i. of 0.01. Briefly, BHK cells were seeded in 96-well plates at a density of

$5x10^4$ cells/well and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in a humidified CO_2 (5%) atmosphere. Cell monolayers were then infected with 50 µL of a proper virus dilution in maintenance medium [MEM-Earl with L-glutamine, 1 mM sodium pyruvate and 0.025 g/L kanamycin, supplemented with 0.5% inactivated FBS] to give an m.o.i of 0.01. After 2 hrs, 50 µL of maintenance medium, without or with serial dilutions of test compounds, were added. After a 3-/4-day incubation at 37 °C, cell viability was determined by the MTT method [20].

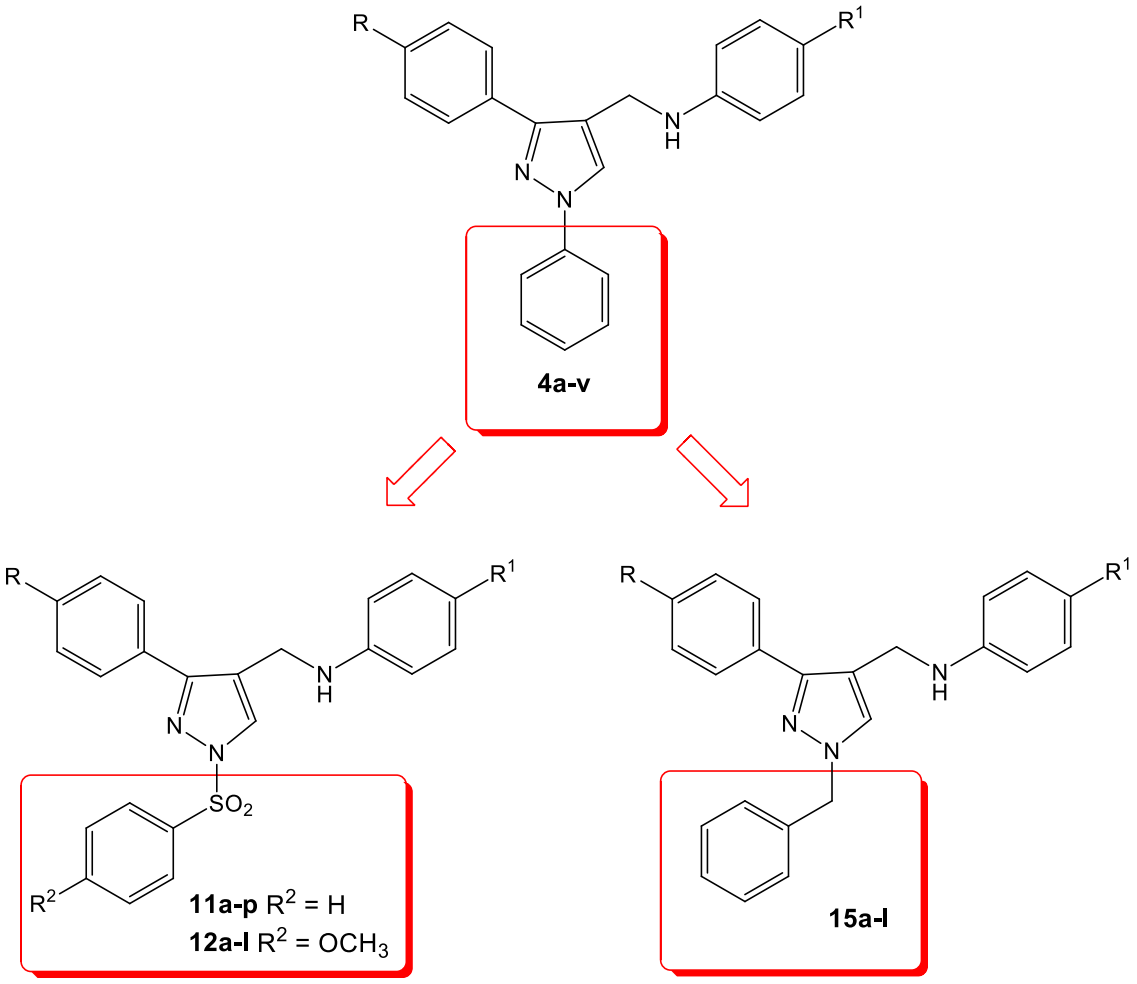
5.1.2.1.1.5. Linear regression analysis

The extent of cell growth/viability and viral multiplication, at each drug concentration tested, were expressed as percentage of untreated controls. Concentrations resulting in 50% inhibition (CC_{50} or EC_{50}) were determined by linear regression analysis.

5.1.3. Design, synthesis, antiviral evaluation and SAR studies of new 1-(phenylsulfonyl) or 1-benzyl-substituted 1*H*-pyrazol -4-yl-methylanilines and (*E*)-1,3-diphenyl-1-styryl-1*H*-pyrazoles

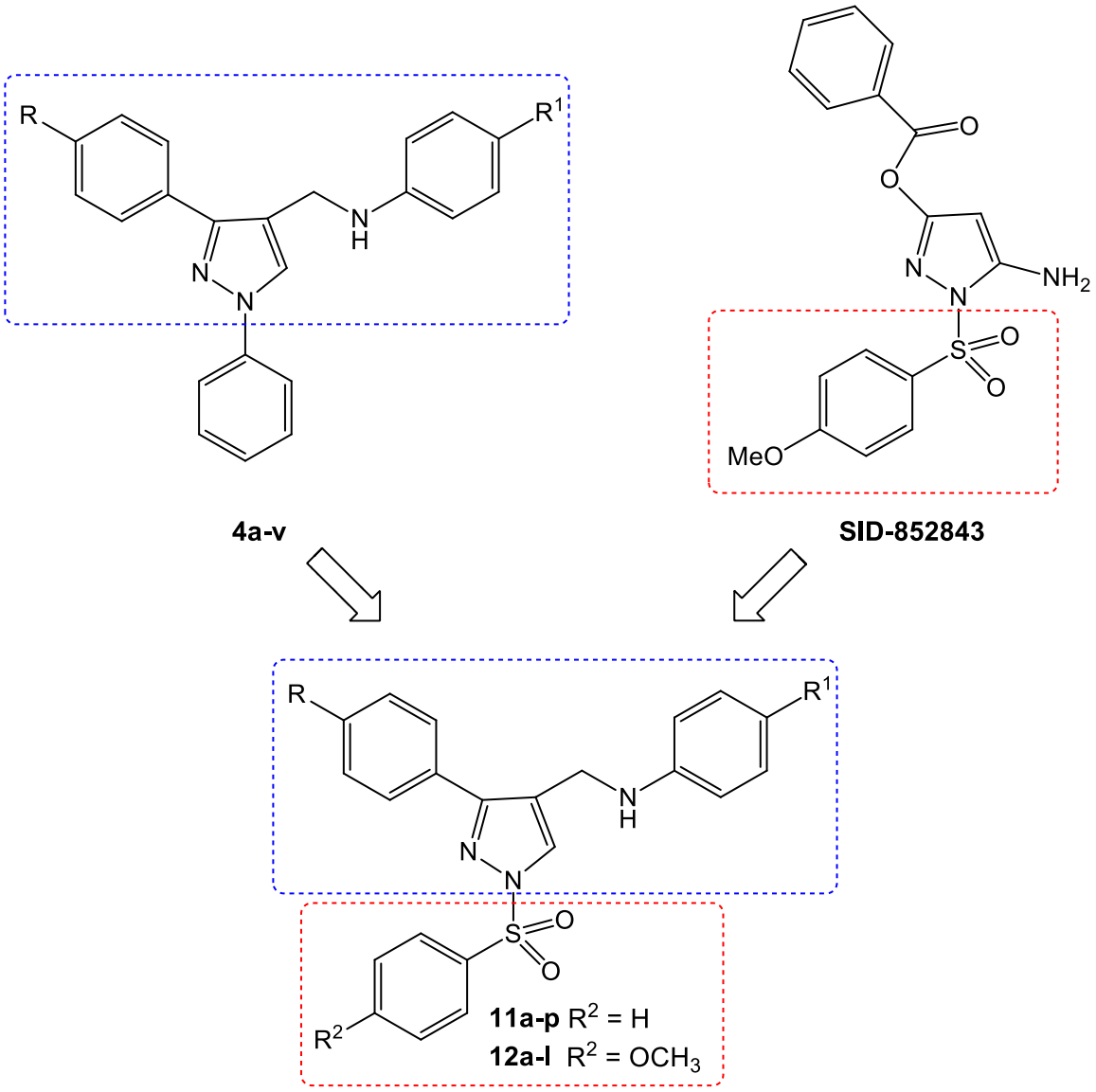
In an effort to identify compounds more active against RSV and/or against viruses belonging to *Flaviviridae* family and to maintain the low cytotoxicity of the hit compounds, we initially planned to modify the substituent at position N1 of pyrazole ring. Two new series of compounds containing phenylsulfonyl group (**11a-p**, **12a-l**) or a benzyl moiety (**15a-l**) were designed and synthesized (Figure 1).

FIGURE 1



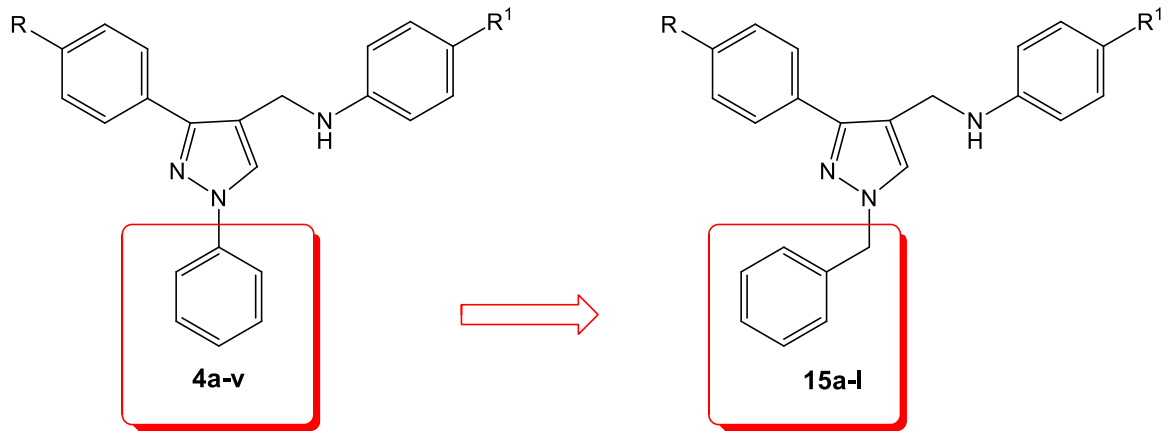
The identification of 5-amino-1-(phenylsulfonyl)-1*H*-pyrazol-3-yl benzoate derivatives (SID) as allosteric inhibitors of West Nile Virus (WNV) NS2B-NS3 proteinase [1] suggested to combine the 1-phenylsulfonyl fragment of SID with the *N*-((3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline core of compounds **4a-v** in order to identify new promising *Flaviviridae* inhibitors (Figure 2).

FIGURE 2



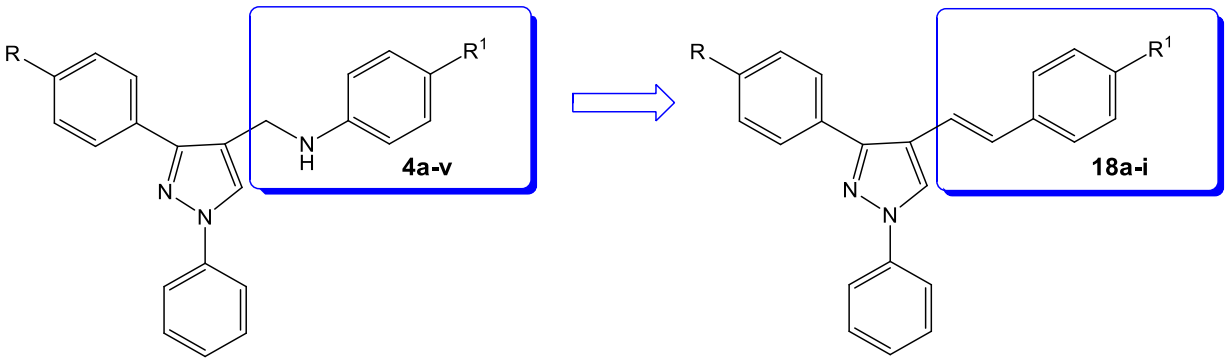
To further investigate the impact on antiviral activity of the substituent at position N1 of the pyrazole ring, the phenyl ring was also replaced by a benzyl moiety (Figure 3).

FIGURE 3



In order to verify the importance for the antiviral activity of the basic substituent at position 4 of pyrazole ring, we also prepared a new series of 4-styryl analogues **18a-i** of compounds **4a-v** (Figure 4).

FIGURE 4



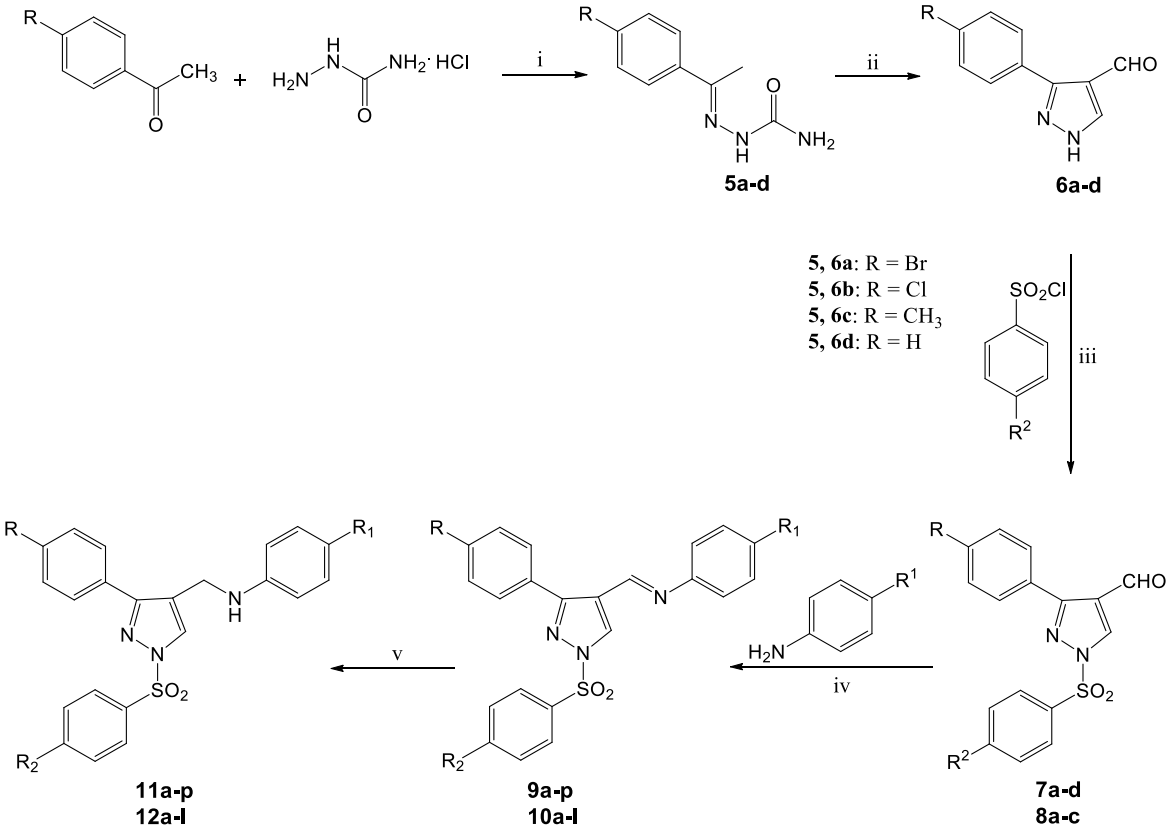
5.1.3.1. Results and discussion

5.1.3.1.1. Chemistry

As shown in Scheme 2, the compounds **11a-p** and **12a-l** were synthesized in five steps starting from the condensation of the suitable acetophenone with semicarbazide hydrochloride, in the presence of sodium acetate. The obtained semicarbazones **5a-d** were treated with Vilsmeier-Haack reagent (DMF-$POCl_3$) to give the corresponding 3-phenyl-1*H*-pyrazole-4-carbaldehydes (**6a-d**) which were converted into the respective 3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazole-4-carbaldehydes (**7a-d** and **8a-c**) by treatment with the appropriate benzenesulfonyl chloride under basic condition. The subsequent condensation with the appropriate anilines provided the corresponding Schiff bases **9a-p** and **10a-l** which were finally converted into the desired *N*-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)anilines (**11a-p** and **12a-l**) by reduction with sodium borohydride.

A similar synthetic procedure was utilized for the synthesis of the 1-benzyl analogues **15a-l** (Scheme 3). For this purpose 3-phenyl-1*H*-pyrazol-4-carbaldehydes **6a-c** were alkylated with benzyl bromide in the presence of sodium hydride to give the corresponding 1-benzyl-3-phenyl-1*H*-pyrazole-4-carbaldehydes **13a-c**.

The subsequent condensation with the appropriate anilines, followed by reduction of intermediate Schiff bases (**14a-l**) were conducted in a similar manner as described for the 1-(phenylsulfonyl) analogues **11a-p** and **12a-l** and resulted in the designed *N*-((1-benzyl-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline **15a-l**.

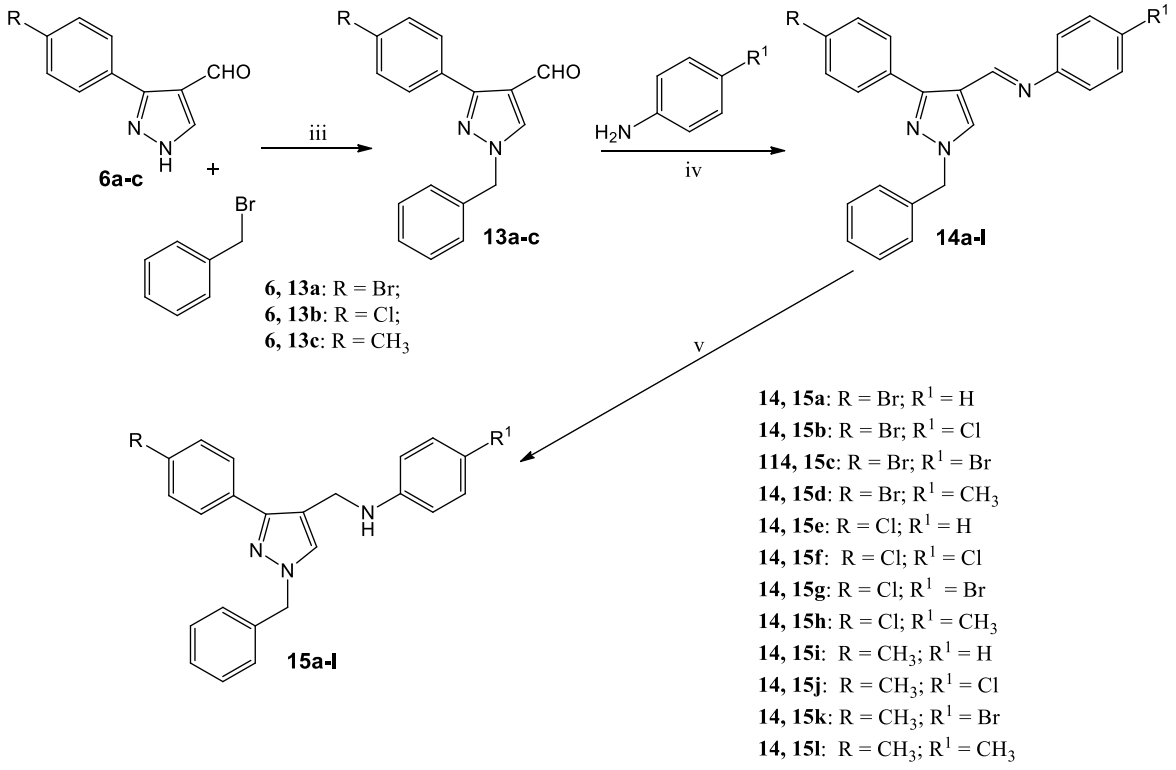


9, 11a R = Br; R^1 = H; R^2 = H;
9, 11b R = Br; R^1 = Cl; R^2 = H;
9, 11c R = Br; R^1 = Br; R^2 = H;
9, 11d R = Br; R^1 = CH_3; R^2 = H;
9, 11e R = Cl; R^1 = H; R^2 = H;
9, 11f R = Cl; R^1 = Cl; R^2 = H;
9, 11g R = Cl; R^1 = Br; R^2 = H;
9, 11h R = Cl; R^1 = CH_3; R^2 = H;
9, 11i R = CH_3; R^1 = H; R^2 = H;
9, 11j R = CH_3; R^1 = Cl; R^2 = H;
9, 11k R = CH_3; R^1 = Br; R^2 = H;
9, 11l R = CH_3; R^1 = CH_3; R^2 = H;
9, 11m R = H; R^1 = H; R^2 = H;
9, 11n R = H; R^1 = Cl; R^2 = H;
9, 11o R = H; R^1 = Br; R^2 = H;
9, 11p R = H; R^1 = CH_3; R^2 = H;

10, 12a R = Br; R^1 = H; R^2 = OCH_3
10, 12b R = Br; R^1 = Cl; R^2 = OCH_3
10, 12c R = Br; R^1 = Br; R^2 = OCH_3
10, 12d R = Br; R^1 = CH_3; R^2 = OCH_3
10, 12e R = Cl; R^1 = H; R^2 = OCH_3
10, 12f R = Cl; R^1 = Cl; R^2 = OCH_3
10, 12g R = Cl; R^1 = Br; R^2 = OCH_3
10, 12h R = Cl; R^1 = CH_3; R^2 = OCH_3
10, 12i R = CH_3; R^1 = H; R^2 = OCH_3
10, 12j R = Cl; R^1 = Cl; R^2 = OCH_3
10, 12k R = Cl; R^1 = Br; R^2 = OCH_3
10, 12l R = Cl; R^1 = CH_3; R^2 = OCH_3

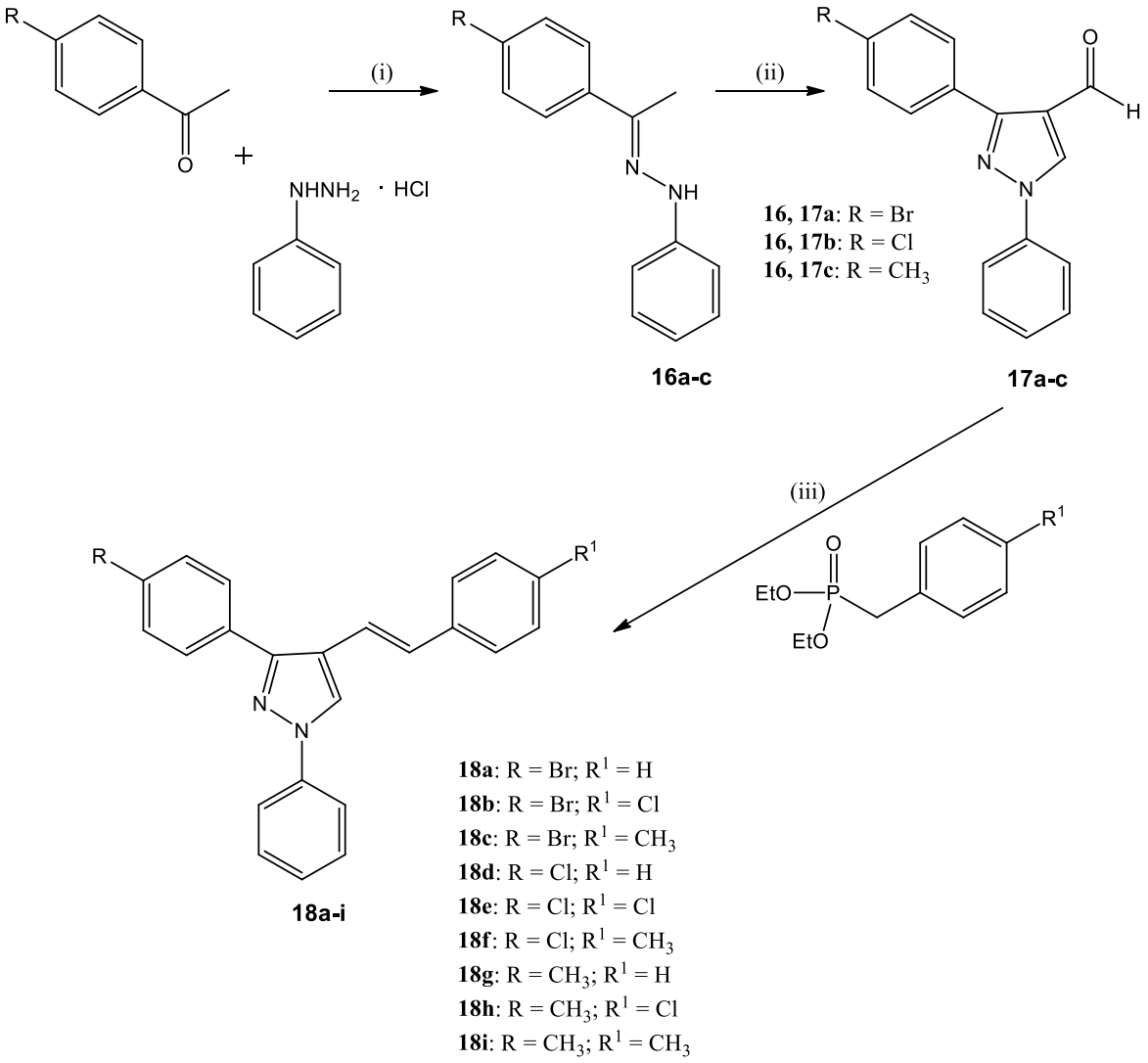
7a: R = Br; R^2 = H
7b: R = Cl; R^2 = H
7c: R = CH_3; R^2 = H
7d: R = H; R^2 = H
8a: R = Br; R^2 = OCH_3
8b: R = Cl; R^2 = OCH_3
8c: R = CH_3; R^2 = OCH_3

Scheme 2. Reagents and conditions: (i) 1) EtOH, AcONa, r.t. 2) Semicarbazide hydrochloride, water, refluxed, 6h 3) r.t., 18h; (ii) 1) dry DMF, $POCl_3$, 0 °C, 30' 2) 65 °C, 6h 3) r.t., 18h; (iii) dry THF, NaH, r.t., 24h; (iv) dry EtOH, glacial AcOH, 80-90 °C, 6h; (v) dry THF, $NaBH_4$, r.t., 24h.



Scheme 3. Reagents and contitions: (iii) dry THF, NaH, r.t., 24h; (iv) dry EtOH, glacial AcOH, 80-90 °C, 6h; (v) dry THF, $NaBH_4$, r.t., 24h.

(*E*)-1,3-Diphenyl-1-stiril-1*H*-pyrazoles **18a-i** were synthetized according to the three steps procedure shown in Scheme 4. Initially 1-phenyl-2-(1-phenylethylidene)hydrazones **16a-c** and the corresponding 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes **17a-c** were prepared following the procedure previously described [2]. The subsequent Horner-Wadsworth-Emmons reaction of aldehydes **17a-c** with commercially available diethyl benzylphosphonates, carried out using sodium hydride as base in dry THF, provided only the (*E*) isomers **18a-i**. The trans configuration of the double bound was confirmed on the basis of the coupling constant values of the vinylic protons ($J_{\alpha\text{-}\beta}$ = 16.4 Hz).



Scheme 4. Reagents and conditions: (i) 1) EtOH, AcONa, r.t. 2) Phenylhydrazine hydrochloride, water, refluxed, 3h; (ii) 1) dry DMF, POCl3, 0 °C, 30' 2) 65 °C, 6h; (iii) dry THF, NaH, r.t., 24h.

5.1.3.1.2. Antiviral tests

All the synthesized pyrazole derivatives (**11a-p**, **12a-l**, **15a-l** and **18a-i**) were assayed for antiviral activity against a large panel of RNA and DNA viruses. Cytotoxicity and antiviral activity of the new compounds (**11a-p**, **12a-l**, **15a-l**, and **18a-i**) and reference inhibitors are reported in Tables 3 – 10.

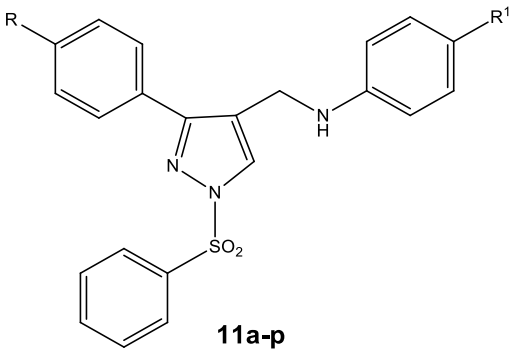
The majority of new *N*-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)anilines **11a-p** exhibited a better activity than the parent

compounds **4a-v** against YFV (EC_{50} ranging from 2.7 μM to 12.2 μM), coupled with high selectivity (SI ranging from >8.20 to >37.04) due to the absence of cytotoxicity for BHK-21 cell line up to the highest concentration tested (100 μM). All these derivatives showed better activity and selectivity than the reference drug 6-Azauridine (EC_{50} = 46.0 μM, SI > 2.2). Despite the presence of a p-methoxy substituent on the phenylsulfonyl group was required for a potent antiviral activity in SID compounds [1], the p-methoxy phenylsulfonyl analogues **12a-l** were totally inactive or less potent YFV inhibitors with respect to corresponding **11a-p**. On the contrary, the introduction of a p-methoxy substituent converted inactive or poor effective unsubstituted compounds **11a-p** in the more potent anti-BVDV agents **12a-l**. In particular, **12a** showed the best anti-BVDV potency and selectivity (EC_{50} = 5.0 μM, SI >20). The 1-(phenylsulfonyl)analogues able to interfered with YFV and/or BVDV replication were also tested against DENV-2 and WNV. Surprisingly, the replacement of the phenyl ring at N1 position with a phenylsulfonyl group completely abolished the activity against these two Flavivirus.

Similarly to the N-phenyl derivatives **4a-v**, several N-phenylsulfonyl analogues **11a-p** exhibited anti-RSV activity in the micromolar concentration (EC_{50} ranging from 9.0 μM to 23.0 μM) generally coupled with high selectivity (SI ranging from 5.3 to >11.1). SAR studies indicated that the *para* substitution at the 3-phenyl ring is necessary for RSV inhibitory activity, in contrast the introduction of the p-methoxy substituent on the phenylsulfonyl moiety abolished the activity (Table 4 and 6).

When tested against Reo-1, CVB-5, Sb-1, VV, HSV-1 and HIV-1, the compounds were devoid of antiviral activity up to the highest concentration tested, with the exceptions of analogues **11n** and **11o** which showed moderate activity against CBV-5 and **11a** that affected the HIV-1 replication (Table 5).

Table 4. Cytotoxicity and antiviral activity of *N*-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline **11a–p** against ssRNA$^+$ (BVDV, YFV, DENV-2, WNV) and ssRNA$^-$ (RSV) viruses.



11a-p

Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	BHK-21 [c]CC_{50} (µM)	YFV [d]EC_{50} (µM)	[e]SI CC_{50}/ EC_{50}	DENV-2 [f]EC_{50} (µM)	WNV [g]EC_{50} (µM)	Vero76 [h]CC_{50} (µM)	RSV [i]EC_{50} (µM)	[e]SI CC_{50}/ EC_{50}
11a	Br	H	>100	18.0±4.0	>100	3.0±1.0	>33.3	>100	>100	87.0	16.5±1.5	5.3
11b	Br	Cl	>100	>100	>100	3.5±1.5	>28.6	>100	>100	90.0	13.5±1.5	6.7
11c	Br	Br	>100	>100	>100	6.5±3.5	>15.4	>100	>100	90.0	15.0±3.0	6.0
11d	Br	CH_3	>100	>53.0	>100	5.5±2.5	>18.2	>100	>100	93.0	11.0±1.0	8.5
11e	Cl	H	>100	>100	>100	7.8±0.4	>12.8	>100	>100	>100	10.0±2.0	>10.0
11f	Cl	Cl	>100	42.5±2.5	>100	4.6±0.3	>21.7	>100	>100	>100	9.0±1.0	>11.1
11g	Cl	Br	>100	>100	>100	6.0±1.4	>16.7	>100	>100	>100	13.5±1.5	>7.4
11h	Cl	CH_3	>100	>100	>100	7.0±1.6	>14.3	>100	>100	>100	23.0±2.5	>4.4
11i	CH_3	H	>100	>100	>100	12.2±2.8	>8.2	>100	>100	>100	11.0±1.0	>9.1
11j	CH_3	Cl	>100	>100	>100	4.7±0.9	>21.3	>100	>100	>100	11.0±1.0	>9.1
11k	CH_3	Br	>100	>100	>100	2.7±1.3	>37.0	>100	>100	>100	13.0±1.0	>7.7
11l	CH_3	CH_3	>100	>100	>100	3.7±0.65	>27.0	>100	>100	>100	11.0±1.0	>9.1
11m	H	H	>100	>100	>100	>100	-	-	-	>100	>100	-
11n	H	Cl	>100	>100	>100	7.1±0.35	>14.1	>100	>100	45.0	>45.0	-
11o	H	Br	>100	>100	>100	6.1±1.7	>16.4	>100	>100	44.0	>44.0	-
11p	H	CH_3	>100	>100	>100	>100	-	-	-	≥100	>100	-
Ref. Compds.												
Ribavirin			55.0	16.0±2.0						55.0	37.5±2.5	1.5
6-Azauridine					>100	46.0±1.5	>2.2			≥100	1.8±0.25	≥55.5
NM108					60			1.2	0.65			

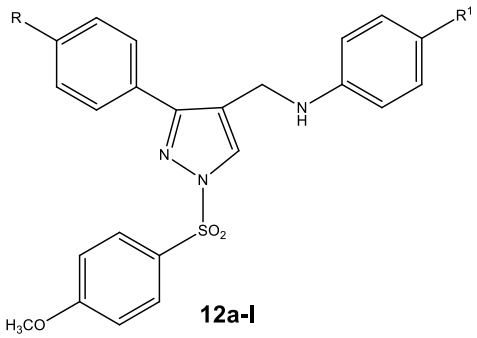
Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [e] Selectivity index (SI) was the ratio between CC_{50} and EC_{50}. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [i]Compound concentration required to reduce the plaque number of RSV (Respiratory Syncytial Virus) by 50% in VERO 76 monolayers.

Table 5. Cytotoxicity and antiviral activity of *N*-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline **11a–p** against ssRNA$^+$ (HIV-1, CVB-5, Sb-1), ssRNA$^-$ (VDV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

Compds	R	R^1	MT-4 $^aCC_{50}$ (μM)	HIV-1 $^bEC_{50}$ (μM)	BHK-21 $^cCC_{50}$ (μM)	Reo-1 $^dEC_{50}$ (μM)	Vero76 $^eCC_{50}$ (μM)	CVB-5	Sb-1	VV	HSV-1	VSV
								EC_{50} ^{f}(μM)				
11a	Br	H	>100	20	>100	>100	87.0	>87.0	>87.0	>87.0	>87.0	>87.0
11b	Br	Cl	>100	>100	>100	>100	90.0	>90.0	>90.0	>90.0	>90.0	>90.0
11c	Br	Br	>100	>100	>100	>100	90.0	>90.0	>90.0	>90.0	>90.0	>90.0
11d	Br	CH_3	>100	>100	>100	>100	93.0	>93.0	>93.0	>93.0	>93.0	>93.0
11e	Cl	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11f	Cl	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11g	Cl	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11h	Cl	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11i	CH_3	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11j	CH_3	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11k	CH_3	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11l	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11m	H	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
11n	H	Cl	>100	>100	>100	>100	45.0	20.0±5.0	>45.0	>45.0	>45.0	>45 .0
11o	H	Br	20	>20	>100	>100	44.0	24.0±11.0	>44.0	>44.0	>44.0	>44.0
11p	H	CH_3	>100	>100	>100	>100	≥100	>100	>100	>100	>100	>100
Ref. Compds.												
EFV			39	0.002								
ACG							>100				2.9±0.1	
Pleconaril							70.0	0.0025±0.0005	2.0±0.1			
M 5255							20.0			2.0±0.1		
NM107					>100	7.5±1.5						

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kideney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1) and VSV (Vescicular Stomatitis Virus) by 50% in VERO76 monolayers.

Table 6. Cytotoxicity and antiviral activity of *N*-((1-((4-methoxyphenyl)sulfonyl)-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline **12a–l** against ssRNA$^+$ (BVDV, YFV, DENV-2, WNV) and ssRNA$^-$ (RSV) viruses.



12a-l

Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	[c]SI CC_{50}/ EC_{50}	BHK-21 [d]CC_{50} (µM)	YFV [e]EC_{50} (µM)	[c]SI CC_{50}/ EC_{50}	DENV-2 [f]EC_{50} (µM)	WNV [g]EC_{50} (µM)	Vero76 [h]CC_{50} (µM)	RSV [i]EC_{50} (µM)
12a	Br	H	>100	5.0±1.4	>20.0	>100	>100	-	>100	>100	>100	>100
12b	Br	Cl	>100	21.0±4.2	>4.8	>100	21.0±4.0	>4.8	>100	>100	>100	>100
12c	Br	Br	>100	25.0±2.8	>4.0	>100	>100	-	>100	>100	>100	>100
12d	Br	CH_3	>100	>100	-	>100	17.5±6.4	>5.7	>100	100	>100	>100
12e	Cl	H	>100	>100	-	>100	>100	-	-	-	>100	>100
12f	Cl	Cl	>100	8.5±3.5	>11.8	>100	16.5±9.6	>6.1	>100	>100	>100	>100
12g	Cl	Br	>100	13.0±2.8	>7.7	>100	18.0±2.8	>5.5	>100	>100	>100	>100
12h	Cl	CH_3	>100	>100	-	>100	>100	-	-	-	>100	>100
12i	CH_3	H	>100	>100	-	>100	>100	-	-	-	>100	>100
12j	CH_3	Cl	>100	>100	-	>100	>100	-	-	-	>100	>100
12k	CH_3	Br	>100	18.5±7.8	>5.4	>100	>100	-	>100	100	>100	>100
12l	CH_3	CH_3	>100	>100	-	>100	>100	-	-	-	>100	>100
Ref. Compds.												
Ribavirin			>100	16.0±2.0	>6.2							
6-Azauridine						>100	46.0±2.5	>2.2			9.3±1.6	1.1±0.4
NM 108						60			1.2	0.65		

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC_{50} and EC_{50}.
[d]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [i]Compound concentration required to reduce the plaque number of RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

Table 7. Cytotoxicity and antiviral activity of *N*-((1-((4-methoxyphenyl)sulfonyl)-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline **12a–l** against $ssRNA^+$ (HIV-1, CVB-5, Sb-1), $ssRNA^-$ (VDV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

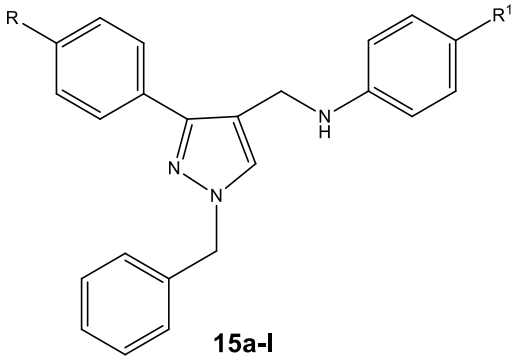
Compds	R	R^1	MT-4 $^aCC_{50}$ (μM)	HIV-1 $^bEC_{50}$ (μM)	BHK-21 $^cCC_{50}$ (μM)	Reo-1 $^dEC_{50}$ (μM)	Vero76 $^cCC_{50}$ (μM)	CVB-5	Sb-1	VV	HSV-1	VSV
								$^fEC_{50}$ (μM)				
12a	Br	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12b	Br	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12c	Br	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12d	Br	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12e	Cl	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12f	Cl	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12g	Cl	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12h	Cl	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12i	CH_3	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12j	CH_3	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12k	CH_3	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
12l	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ref. Compds.												
EFV			37	0.002								
ACG							>100				2.4±0.6	
Pleconaril							77.0±6.8	0.005±0.002	2.0±0.6			
M 5255							19.3±2.5			1.4±0.2		
NM 107					>100	6.0±2.1						

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kidney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock.infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1) and VSV (Vescicular Stomatitis Virus) by 50% in VERO76 monolayers.

The substitution of the phenyl with a benzyl moiety at position N1 of the pyrazole ring resulted in *N*-((1-benzyl-3-phenyl-1*H*-pyrazol-4-yl)methyl)anilines **15a-l**. Similarly to related N-phenylsulfonyl derivatives **11a-p**, most of the N-benzyl compounds exhibited a potent inhibition of YFV replication (EC_{50} ranging from 9.8 μM to 13.9 μM) and no toxicity against BHK-21 cells up to the higher concentration tested (100 μM), thus comparing favorably with the reference compound 6-Azauridine (EC_{50} = 46.0 μM, SI > 2.2) (Table 8). With the exception of **15g**, the benzyl derivatives **15a-l** also affected the BVDV replication, however their potency was generally modest

(EC_{50} ranging from 15.6 μM to 57.0 μM). Differently to related N-phenylsulfonyl derivatives **11a-p**, the N-benzyl analogues **15a-l** showed additional activity against DENV-2, although with a lesser degree of potency than the parent N-phenyl derivatives **4a-v**.

Table 8. Cytotoxicity and antiviral activity of *N*-((1-benzyl-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline **15a–l** against $ssRNA^+$ (BVDV, YFV, DENV-2, WNV) and $ssRNA^-$ (RSV) viruses.



15a-l

Compds	R	R^1	MDBK [a]CC_{50} (μM)	BVDV [b]EC_{50} (μM)	[c]SI CC_{50}/ EC_{50}	BHK-21 [d]CC_{50} (μM)	YFV [e]EC_{50} (μM)	[c]SI CC_{50}/ EC_{50}	DENV-2 [f]EC_{50} (μM)	[c]SI CC_{50}/ EC_{50}	WNV [g]EC_{50} (μM)	Vero76 [h]CC_{50} (μM)	RSV [i]EC_{50} (μM)
15a	Br	H	>100	29.1±2.9	>3.4	>100	11.6±0.5	>8.6	>100	-	>100	17	>17
15b	Br	Cl	>100	19.2±3.2	>5.2	>100	9.8±0.5	>10.2	18.5±1.5	>5.4	>100	9.1	>9.1
15c	Br	Br	>100	46.3±1.3	>2.2	>100	10.6±1.4	>9.4	>100	-	>100	25	11.1±1.0
15d	Br	CH_3	>100	27.5±1.5	>3.6	>100	10.0±1.6	10	28.0±1.0	>3.6	>100	16.4	>16.4
15e	Cl	H	>100	27.5±0.5	3.6	>100	13.9±1.7	>7.2	33.5±1.5	3.0	>100	34	>34
15f	Cl	Cl	>100	31.0±6.0	>3.2	>100	10.5±2.5	>9.5	18.0±1.0	>5.5	>100	8.2	>8.2
15g	Cl	Br	>100	>100	-	>100	>100	-	21.0±2.0	>4.8	>100	>100	>100
15h	Cl	CH_3	>100	44.3±3.8	>2.3	>100	13.1±1.4	>7.6	22.5± 4.5	>4.4	>100	76	>76
15i	CH_3	H	>100	30.0±6.0	>3.3	>100	>100	-	37.0±0.0	>2.7	>100	15.1	>15.1
15j	CH_3	Cl	>100	15.6±1.4	>6.4	>100	10.4±1.4	>9.6	21.0±2.0	>4.8	>100	15	>15
15k	CH_3	Br	>100	57.0±5.0	>1.7	>100	12.4±1.6	>8.1	>100	-	>100	>100	13.1±1.0
15l	CH_3	CH_3	>100	57.0±4.0	>6.4	>100	>100	-	>100	-	>100	>100	>100
Ref. Compds.													
Ribavirin			>100	13.6±1.7	>7.3								
6-Azauridine						>100	46±2.5	>2.2				9.3±1.6	1.1±0.4
NM 108			>100	1.3±0.3	>76.9	68±6.0	2.0±0.2	34	1.9±0.1	>35.8	0.8±0.2		

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC_{50} and EC_{50}. [d]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [i]Compound concentration required to reduce the plaque number of RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

Only the 4-bromoaniline derivatives **15b** and **15k** showed anti-RSV activity and selectivity (EC_{50} = 11 μM and 13 μM; SI =2.3 and 7.7, respectively) while differently substituted N-benzyl derivatives are not able to inhibit the replication of RSV up to the corresponding EC_{50} for Vero76 cells (Table 8). It is interesting to note that the lack of cytotoxicity against Vero76 cells was observed up to the highest concentration tested (100 μM), when the cultures were incubated with serial dilution of N-benzyl derivatives **15a-l** at 37 °C for 2 days and 3 days, whereas a remarkable increase of cytotoxicity was generally observed after incubation of Vero76 cells for 5 days, time necessary to observe the RSV-induced cytopathogenicity (Tables 8).

N-benzyl derivatives were also generally inactive against WNV (Table 8) and representative members of other virus families, only **15d**, **15f** and **15i** were able to affect the CVB-5 replication at high concentrations and **15e**, **15i** and **15k** exhibited a moderate activity against HIV-1 (Table 9).

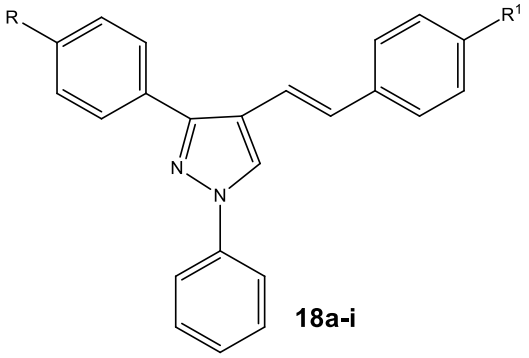
The new synthesized (*E*)-1,3-diphenyl-4-styryl-1*H*-pyrazoles (**18a-i**) were generally no toxic against host cell lines used to support the replication of selected viruses (Tables 10 and 11). On the other hand, this substitution completely abolished the anti-RSV activity. Concerning the activity against viruses belonging to *Flaviviridae* family, several styryl derivatives retained the ability to interfere with BVDV, YFV and DENV-2 replication, however, their potency were generally moderate. In contrast, only analogs **18d** and **18i** showed a modest activity and selectivity against WNV (EC_{50} = 30.5 μM and 60.0 μM, SI = 3.3 and 1.7, respectively). SAR studies indicate that the presence of a methyl group in R (**18g-i**) led to analogues active against BVDV, YFV and DENV-2, while the BVDV replication was inhibited also by analogues bearing a methyl group in R^1 (**18c** and **18f**). Exception to this rule was the 4-chlorophenyl analogue **18d** that was endowed with activity against BVDV, YFV and WNV (Table 10).

Table 9. Cytotoxicity and antiviral activity of *N*-((1-benzyl-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline **15a–l** against $ssRNA^+$ (HIV-1, CVB-5, Sb-1), $ssRNA^-$ (VDV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

Compds	R	R[1]	MT-4 [a]CC_{50} (µM)	HIV-1 [b]EC_{50} (µM)	BHK-21 [c]CC_{50} (µM)	Reo-1 [d]EC_{50} (µM)	Vero76 [e]CC_{50} (µM)	CVB-5	Sb-1	VV	HSV-1	VSV
								[f]EC_{50} (µM)				
15a	Br	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
15b	Br	Cl	97	>97	>100	>100	>100	>100	>100	>100	>100	>100
15c	Br	Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
15d	Br	CH_3	>100	>100	>100	>100	>100	23.2±3.8	>100	>100	>100	>100
15e	Cl	H	69	9.1±2.1	>100	>100	>100	>100	>100	>100	>100	>100
15f	Cl	Cl	59	> 59	>100	>100	>100	50.7±1.3	>100	>100	>100	>100
15g	Cl	Br	79	> 79	>100	>100	>100	>100	>100	>100	>100	>100
15h	Cl	CH_3	70	>70	>100	>100	>100	>100	>100	>100	>100	>100
15i	CH_3	H	>100	12.7±1.3	>100	>100	>100	32.3±3.7	>100	>100	>100	>100
15j	CH_3	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
15k	CH_3	Br	>100	60.5±7.8	>100	>100	>100	>100	>100	>100	>100	>100
15l	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ref. Compds.												
EFV			38.0±2.0	0.002±0.0								
ACG							> 100				2.4±0.6	
Pleconaril							77.0±6.8	0.005±0.002				
M 5255							19.3±2.5			1.4±0.2		
NM 107					>100	6.0±2.1						

[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kideney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1) and VSV (Vescicular Stomatitis Virus) by 50% in VERO76 monolayers.

Table 10. Cytotoxicity and antiviral activity of (*E*)-1,3-diphenyl-4-styryl-1*H*-pyrazoles **18a–i** against ssRNA$^+$ (BVDV, YFV, DENV-2, WNV) and ssRNA$^-$ (RSV) viruses.



18a-i

Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	BHK-21 [d]CC_{50} (µM)	YFV [e]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	DENV-2 [f]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	WNV [g]EC_{50} (µM	Vero76 [h]CC_{50} (µM)	RSV [i]EC_{50} (µM)
18a	Br	H	>100	>100	-	>100	>100	-	>100	-	>100	36	>36
18b	Br	Cl	>100	>100	-	>100	>100	-	>100	-	>100	>100	>100
18c	Br	CH_3	>100	59.0±3.0	>1.7	>100	>100	-	>100	-	>100	>100	>100
18d	Cl	H	>100	42.1±4.1	>2.4	>100	28.0±1.5	>3.6	>100	-	30.5±1.5	39	>39
18e	Cl	Cl	>100	>100	-	>100	>100	-	>100	-	>100	60	>60
18f	Cl	CH_3	>100	50.5±2.5	>1.9	>100	>100	-	>100	-	>100	>100	>100
18g	CH_3	H	>100	61.0±7.0	>1.6	>100	38.0±1.0	>2.6	13.5±1.3	>7.4	>100	82	>100
18h	CH_3	Cl	>100	40.5±4.5	>2.5	>100	63.0±5.0	>1.6	50.0±3.0	>2.0	>100	>100	>100
18i	CH_3	CH_3	>100	32.5±0.5	>3.1	>100	48.0±5.0	>2.1	18.5±0.5	>5.4	60.0±2.0	>100	>100
Ref. Compds													
Ribavirin			>100	13.6±1.7	>7.3								
6-Azauridine						>100	46.0±2.5	>2.2				9.3±1.6	1.1±0.
NM108			>100	1.3±0.3	>76.9	68.0±6.0	2.0±0.2	34	1.9±0.1	>35.8	0.8±0.2		

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC_{50} and EC_{50}. [d]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [h]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [i]Compound concentration required to reduce the plaque number of RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

Table 11. Cytotoxicity and antiviral activity of (*E*)-1,3-diphenyl-4-styryl-1*H*-pyrazoles **18a–i** against ssRNA$^+$ (HIV-1, CVB-5, Sb-1), ssRNA$^-$ (VDV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

Compds	R	R[1]	MT-4 [a]CC_{50} (µM)	HIV-1 [b]EC_{50} (µM)	BHK-21 [c]CC_{50} (µM)	Reo-1 [d]EC_{50} (µM)	Vero76 [e]CC_{50} (µM)	CVB-5	Sb-1	VV	HSV-1	VSV
								[f]EC_{50} (µM)				
18a	Br	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18b	Br	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18c	Br	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18d	Cl	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18e	Cl	Cl	>100	>100	>100	>100	60	>60	>60	>60	>60	>60
18f	Cl	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18g	CH_3	H	>100	43.0±3.2	>100	>100	>100	>100	>100	>100	>100	>100
18h	CH_3	Cl	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
18i	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ref. Compds.												
EFV			38.0±2.0	0.002±0.0								
ACG							>100				2.4±0.6	
Pleconaril							77.0±6.8	0.005±0.002	2.0±0.6			
M 5255							19.3±2.5			1.4±0.2		
NM107					>100	6.0±2.1						

[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kidney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1) and VSV (Vescicular Stomatitis Virus) by 50% in VERO76 monolayers.

5.1.3.2. Experimental

5.1.3.2.1. Chemistry

Chemicals were purchased from Sigma-Aldrich and used without further purification. Melting points were determined on a Stuar Scientific SMP1 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer in $CDCl_3$ or DMSO-d_6, and chemical shifts were reported in ppm (δ). All compounds were routinely checked by thin-layer chromatography (TLC). TLC was performed on silica gel or aluminium oxide fluorescent coated plates (Fluka, DC-Alufolien Kieselgel or aluminum oxide F254). Compound purity was determined by elemental analysis and was confirmed to be > 95% for all the tested compounds. Analytical results are within ±0.40% of the theoretical values.

5.1.3.2.1.1. General procedure for the synthesis of the (*E*)-2-(1-phenylethylidene)hydrazinecarboxamides (5a-d).

A solution of semicarbazide hydrochloride (2.3 mmol) in water (43 ml) was added dropwise to a stirred solution of the appropriate acetophenone (2.2 mmol) and sodium acetate (3 mmol) in ethanol (43 ml) at room temperature. After the addition, the mixture was refluxed for 6 h under magnetic stirring, cooled at room temperature and stirred 18 h. Afterwards the ethanol was removed under reduced pressure and the precipitate was collected by filtration and used for the next reaction without further purification.

5.1.3.2.1.1.1. (*E*)-2-(1-(4-Bromophenyl)ethylidene)hydrazinecarboxamide (5a). Yield: 77%, m.p. = 198 – 201 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 9.47 (s, 1H, NH), 7.81 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.54 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz), 6.58 (s, 2H, NH_2), 2.18 (s, 3H, CH_3). ^{13}C NMR (DMSO-d6, 100 MHz): δ (ppm) 157.23, 142.84, 137.44, 130.99, 127.99, 121.74, 13.05.

5.1.3.2.1.1.2. (*E*)- 2-(1(4-Chlorophenyl)ethylidene)hydrazinecarboxamide (5b). Yield: 74%, m.p. = 198 – 199 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): *δ* (ppm) 9.45 (s, 1H, NH), 7.88 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.4 Hz), 7.40 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.4 Hz), 6.57 (s, 2H, NH_2), 2.18 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): *δ* (ppm) 157.22, 142.73, 137.06, 133.01, 128.07, 127.70, 13.09.

5.1.3.2.1.1.3. (*E*)-2(1(p-Tolyl)ethylidene)hydrazinecarboxamide (5c). Yield: 87%, m.p. = 199 – 200°C (lit. = 200 °C) [3]. The compound exhibited spectroscopic data identical to those previously reported [3].

5.1.3.2.1.1.4. (*E*)-2-(1-Phenylethylidene)hydrazinecarboxamide (5d). Yield: 83%, m.p. = 196 – 197 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 9.40 (s, 1H, NH), 7.84 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 6.4 Hz), 7.37 – 7.35 (m, 3H, H3, H4, H5), 6.53 (s, 2H, NH_2), 2.19 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 157.29, 143.93, 138.17, 128.32, 128.08, 125.84, 13.21.

5.1.3.2.1.2. General procedure for the synthesis of the 3-phenyl-1*H*-pyrazol-4-carbaldeydes (6a-d).

To a solution of the suitable (*E*)-2-(1-phenylethylidene) hydrazinecarboxamides **5a-d** (12 mmol) in dry DMF (9 ml) cooled in ice bath, $POCl_3$ (2 ml) was added dropwise. The mixture was stirred in ice bath for 30 min and then heated at 65 °C for 6 hours. After cooling and stirring at room temperature for 18 hours, the mixture was diluted with water and ice, neutralized with NaOH 2N and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue was purified by crystallization from $CHCl_3$/n-Hexane or from $CHCl_3$/Pet. Intermediate **6c** was purified by column chromatography on silica gel eluting with a mixture of AcOEt and n-Hexane 1:1.

5.1.3.2.1.2.1. 3-(4-Bromophenyl)-1*H*-pyrazole-4-carbaldehyde (6a). Yield 97%, m.p. = 127 – 128 °C (lit. = 163 – 165 °C) [4] from $CHCl_3$/n-Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 13.80 (s, 1H, NH), 9.90 (s, 1H, CHO), 8.49 (s, 1H, pyrazole-H5), 7.83 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.69 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 184.57, 152.81, 138.86, 138.56, 131.41, 130.39, 122.36, 119.85.

5.1.3.2.1.2.2. 3-(4-Chlorophenyl)-1*H*-pyrazole-4-carbaldehyde (6b). Yield: 99%, m.p. = 130 – 131 °C (lit. = 142 – 144 °C) [4] from AcOEt/n-

Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 13.70 (s, 1H, NH), 9.91 (s, 1H, CHO), 8.49 (s, 1H, pyrazole-H5), 7.90 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.56 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 184.64, 140.60, 133.71, 130.18, 128.49, 128.54, 125.39, 119.88.

5.1.3.2.1.2.3. 3-(p-Tolyl)-1*H*-pyrazole-4-carbaldehyde (6c). Yield: 60%, m.p. = 123 – 124 °C (lit. = 123 – 125 °C) [4] from AcOEt/n-Hexane. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 12.58 (s, 1H, NH), 9.94 (s, 1H, CHO), 8.05 (s, 1H, pyrazole-H5), 7.53 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 7.29 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 7.6 Hz), 2.43 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 185.22, 150.08, 140.5, 138.80, 129.87, 128.69, 125.33, 120.03, 21.41.

5.1.3.2.1.2.4. 3-Phenyl-1*H*-pyrazole-4-carbaldehyde (6d). Yield: 93%, m.p. = 129–130 °C (lit. = 145 °C) [4] from $CHCl_3$/n-Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 13.72 (s, 1H, NH), 9.91 (s, 1H, CHO), 8.60 (s, 1H, pyrazole-H5), 7.83 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.56 – 7.50 (m, 3H, H3, H4, H5). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 184.71, 140.01, 133.08, 130.10, 128.76, 127.08, 126.49, 110.48.

5.1.3.2.1.3. General procedure for the synthesis of the 3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-carbaldehydes (7a-d) and the 1-((4-methoxyphenyl)sulfonyl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde (8a-c).

NaH (6.8 mmol) was added to a stirred solution of the appropriate 3-phenyl-1*H*-pyrazole-4-carbaldehyde **6a-d** (6.8 mmol) in dry THF (100 ml). The mixture was stirred 30 min at room temperature, than the suitable benzenesulfonyl chloride (9.6 mmol) was added. After stirring for 24 h at room temperature, water was added to the mixture and THF was removed under reduced pressure. The obtained suspension was extracted with ethyl

acetate and the organic phase was washed with brine and dried over Na_2SO_4 anhydrous, filtered and evaporated to dryness. The residue was purified by crystallization from suitable solvent.

5.1.3.2.1.3.1. 3-(4-Bromophenyl)-1-(phenylsulfonyl)-1*H*-pyrazole-4-carbaldehyde (7a). Yield: 85%, m.p. = 121 – 122 °C from AcOEt/Pet.Et. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.97 (s, 1H, CHO), 8.71 (s, 1H, pyrazole-H5), 8.11 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 7.25 (t, 1H, H4, $J_{3\text{-}4}$ = 7.6 Hz), 7.66 – 7.56 (m, 6H, H3, H5, H2', H6', H3', H5'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 183.63, 155.33, 137.24, 135.84, 135.45, 131.84, 130.58, 129.73, 129.02, 128.73, 124.56, 122.61.

5.1.3.2.1.3.2. 3-(4-Clorophenyl)-1-(phenylsulphonyl)-1*H*-pyrazole-4-carbaldehyde (7b). Yield: 60%, m.p. = 121 – 122 °C from EtOH/n-Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 9.95 (s, 1H, CHO), 9.41 (s, 1H, pyrazole-H5), 8.15 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 7.87 (t, 1H, H4, $J_{3\text{-}4}$ = 7.6 Hz), 7.83 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.4 Hz), 7.74 (t, 2H, H3, H5, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ = 7.6 Hz), 7.54 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 8.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 184.65, 153.70, 140.92, 136.02, 135.10, 134.75, 130.52, 130.27, 128.74, 128.55, 128.26, 122.62.

5.1.3.2.1.3.3. 1-(Phenylsulfonyl)-3-(p-tolyl)-1*H*-pyrazole-4-carbaldehyde (7c). Yield: 90%, m.p. = 126 °C da AcOEt/Pet. Et. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.98 (s, 1H, CHO), 8.69 (s, 1H, pyrazole-H5), 8.10 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.69 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.61 – 7.56 (m, 4H, H2', H6', H3, H5), 7.24 (d, 2H, H3', H5', $J_{2\text{-}3}$ = 8.0 Hz), 2,38 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 184.47, 156.87, 140.18, 136.14, 136.07, 135.27, 129.65, 129.41, 128.95, 128.67, 127.22, 122.65, 21.36.

5.1.3.2.1.3.4. 3-Phenyl-1-(phenylsulphonyl)-1*H*-pyrazole-4-carbaldehyde (7d). Yield: 50%, m.p. = 110 – 111 °C from EtOH/n-Hexane. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.92 (s, 1H, CHO), 8.71 (s, 1H, pyrazole-H5), 8.11 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.71 – 7.69 (m, 3H, H2', H6', H4), 7.59 (t, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz), 7.46 – 7.44 (m, 3H, H3', H4', H5'). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 184.38, 156.81, 140.93, 136.16, 135.99, 135.34, 130.07, 129.98, 129.68, 129.07, 128.70, 122.66.

5.1.3.2.1.3.5. 3-(4-Bromophenyl)-1-((4-methoxyphenyl)sulfonyl)-1*H*-pyrazole-4-carbaldehyde (8a). Yield: 62%, m.p. = 110 – 112 °C from AcOEt/n-Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 9.94 (s, 1H, CHO), 9.33 (s, 1H, pyrazole-H5), 8.07 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.75 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.67 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.23 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz), 3.87 (s, 1H, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 184.84, 164.93, 153.43, 140.27, 131.46, 130.96, 130.70, 129.24, 125.86, 123.43, 122.32, 115.50, 56.09.

5.1.3.2.1.3.6. 3-(4Chlorophenyl)-1-((4-methoxyphenyl)sulfonyl)-1*H*-pyrazole-4-carbaldehyde (8b). Yield: 35%, m.p. = 114 – 115 °C from AcOEt/n-Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 9.93 (s, 1H, CHO), 9.34 (s, 1H, pyrazole-H5), 8.07 (d, 2H, H2, H6, $J_{2\text{-}3}$= 8.0 Hz), 7.82 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.52 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.23 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz), 3.87 (s, 1H, OCH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 185.15, 165.42, 153.85, 140.94, 135.17, 131.46, 130.98, 129.35, 129.01, 126.33, 122.81, 115.98, 56.58.

5.1.3.2.1.3.7. 1-((4-Methoxyphenyl)sulfonyl)-3-(p-tolyl)-1*H*-pyrazole-4-carbaldehyde (8c). Yield: 68%, m.p. = 107 – 108 °C from AcOEt/n-Hexane. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 9.94 (s, 1H, CHO), 9.27 (s, 1H, pyrazole-H5), 8.07 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.68 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.27 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.22 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz), 3.86 (s, 1H, OCH_3), 2.35 (s, 1H, -CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 185.24, 165.34, 155.25, 140.26, 140.04, 131.40, 129.52, 129.11, 127.65, 126.52, 122.79, 115.94, 56.55, 21.36.

5.1.3.2.1.4. General procedure for the synthesis of (*E*)-*N*-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methylene)aniline (9a-p) and (*E*)-*N*-((1-((4-methoxyphenyl)sulfonyl)-3-phenyl-1*H*-pyrazol-4-yl)methylene)aniline (10a-l).

The suitable aniline (1.09 mmol) was added to a solution of the appropriate 1-phenylsulfonyl-1*H*-pyrazol-4-carbaldehyde **7a-d** or **8a-c** (1.2 mmol) in dry ethanol (30 ml) and glacial acetic acid (0.1 ml). The mixture was refluxed for 6 h under magnetic stirring. After cooling, water was added and ethanol was removed under reduced pressure. The obtained suspension was extracted with diethyl ether and the organic layer was washed with brine, dried on Na_2SO_4 anhydrous, filtered and evaporated to dryness. The residual oil was used for the next reaction without further purification.

5.1.3.2.1.5. General procedure for the synthesis of *N*-[(3-phenil-1-(phenylsulfonil)-1*H*-pyrazol-4-il)methyl]aniline (11a-p) and *N*-((1-((4-methoxyphenyl)sulfonyl)-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline (12a-l).

To a stirred solution of the crude 1-phenylsulphonyl-1*H*-pirazol-4-yl-methyleneaniline **9a-p** or **10a-l** (1 mmol) in dry THF (17 ml), $NaBH_4$ (10 mmol) was added and the mixture was stirred at room temperature for 24 h.

After this period, water was added and THF was removed under reduced pressure. The suspension was extracted with ethyl acetate and the organic phase was washed with brine, dried under Na_2SO_4 anhydrous, filtered and evaporated to dryness. The residue obtained was purified by crystallization from suitable solvent.

5.1.3.2.1.5.1. *N*-((3-(4-Bromophenyl)-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (11a). Yield: 36%; m.p. = 110 – 11 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{22}H_{18}BrN_3O_2S$: C 56.42, H 3.87, Br 17.06, N 8.97, S 6.85; found C 56.61, H 3.88, Br 17.04, N 8.98, S 6.84. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.10 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.2 Hz), 7.64 (t, 1H, H4, $J_{3\text{-}4}$ = 7.2 Hz), 7.57 – 7.48 (m, 6H, H3, H5, H2', H6', H3', H5'), 7.18 (t, 2H, H3'', H5'', $J_{2''\text{-}3''} = J_{3''\text{-}4''}$ = 7.6 Hz), 6.77 (t, 1H, H4'', $J_{3''\text{-}4''}$ = 7.6 Hz), 6.59 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 7.6 Hz), 4.23 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.67, 147.24, 137.08, 134.57, 131.90, 131.86, 130.41, 129.56, 129.42, 129.40, 128.19, 123.53, 120.83, 118.48, 113.19, 39.12.

5.1.3.2.1.5.2. *N*-((3-(4-Bromophenyl)-1-(phenysulfonyl)-1*H*-pyrazol-4-yl)methyl)-4-chloroaniline (11b). Yield: 52%; m.p. = 137 – 138 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{22}H_{17}BrClN_3O_2S$: C 52.55, H 3.41, Br 15.89, Cl 7.05, N 8.36, S 6.38; found C 52.66, H 3.40, Br 15.95, Cl 7.06, N 8.33, S 6.40. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.65 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.55 – 7.49 (m, 6H, H3, H5, H2', H6', H3', H5'), 7.11 (d, 2H, H3'', H5'', $J_{2''\text{-}3''}$ = 8.4 Hz), 6.49 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 8.4 Hz), 4.20 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.61, 145.70, 136.99, 134.64, 131.94, 131.80, 130.31, 129.50, 129.45, 129.23, 128.18, 123.61, 123.10, 120.40, 114.24, 39.16.

5.1.3.2.1.5.3. 4-Bromo-*N*-((3-(4-bromophenyl)-1-(phenylsulfonyl)-1*H*-przl-4-yl)methyl)aniline (11c). Yield: 42%; m.p. = 150 –151 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{22}H_{17}Br_2N_3O_2S$: C 48.28, H 3.13, Br 29.20, N 7.68, S 5.86; found C 48.39, H 3.14, Br 29.29, N 7.66, S 5.87. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.65 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.56 – 7.49 (m, 6H, H3, H5, H2', H6', H3', H5'), 7.24 (d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 8.4 Hz), 6.44 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 8.4 Hz), 4.20 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.59, 146.10, 136.98, 134.64, 132.10, 131.94, 131.79, 130.28, 129.48, 129.44, 128.17, 123.60, 120.32, 114.71, 110.14, 39.04

5.1.3.2.1.5.4. *N*-((3-(4-Bromophenyl)-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (11d). Yield: 37%; m.p. = 112 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{23}H_{20}BrN_3O_2S$: C 57.27, H 4.18, Br 16.56, N 8.71, S 6.65; found C 57.08, H 4.19, Br 16.53, N 8.73, S 6.63. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.09 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.63 (t, 1H, 4H, $J_{3\text{-}4}$ = 8.0 Hz), 7.57 – 7.48 (m, 6H, H3, H5, H2', H6', H3', H5'), 6.98 (d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 7.6 Hz), 6.51 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.6 Hz), 4.19 (s, 2H, CH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.69, 144.99, 137.09, 134.54, 131.87, 131.84, 130.44, 129.87, 129.57, 129.40, 128.17, 127.73, 123.48, 121.02, 113.36, 39.41, 20.41.

5.1.3.2.1.5.5. *N*-((3-(4-Chlorophenyl)-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (11e). Yield: 21%, m.p. = 117 – 118 °C from n-Hexane; Anal. Calc. for $C_{22}H_{18}ClN_3O_2S$: C 62.33, H 4.28, Cl 8.36, N 9.91, S 7.56; found C 62.14, H 4.29, Cl 8.39, N 9.94, S 7.53. IR: 3316, 1375, 1886 cm-1. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.12 (s, 1H, pyrazole-H5), 8.02 (d, 2H,

H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 7.64 (t, 1H, H4, $J_{3\text{-}4}$ = 7.6 Hz), 7.60 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 7.6 Hz), 7.53 (t, 2H, H3, H5, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ = 7.6 Hz), 7.33 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 7.6 Hz), 7.18 (t, 2H, H3", H5", $J_{2"\text{-}3"}$ = $J_{3"\text{-}4"}$ = 7.6 Hz), 6.78 (t, 1H, H4", $J_{3"\text{-}4"}$ = 7.6 Hz), 6.60 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.6 Hz), 4.24 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.72, 16.96, 137.09, 135.22, 134.58, 131.98, 129.87, 129.42, 129.40, 129.31, 128.92, 128.17, 120.50, 118.84, 113.54, 39.30.

5.1.3.2.1.5.6. 4-Cloro-*N*-((3-(4-chlorophenyl)-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (11f). Yield: 21%, m.p. = 142 – 143 °C from n-Hexane; Anal. Calc. for $C_{22}H_{17}Cl_2N_3O_2S$: C 57.65, H 3.74, Cl 15.47, N 9.17, S 7.00; found C 57.83, H 3.75, Cl 15.50, N 9.19, S 6.88. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.12 (s, 1H, pyrazole-H5), 8.02 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.66 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.58 – 7.53 (m, 4H, H2', H6', H3, H5), 7.35 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 6.8 Hz), 7.10 (d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 7.2 Hz), 6.52 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.2 Hz), 4.20 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.67, 144.97, 136.93, 135.32, 134.66, 131.98, 129.74, 129.46, 129.43, 129.25, 128.96, 128.18, 123.73, 119.92, 114.78, 39.44.

5.1.3.2.1.5.7. 4-Bromo-*N*-((3-(4-chlorophenyl)-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (11g). Yield: 28%, m.p. = 142–143 °C from n-Hexane; Anal. Calc. for $C_{22}H_{17}BrClN_3O_2S$: C 52.55, H 3.41, Br 15.89, Cl 7.05, N 8.36, S 6.38; found C 52.48, H 3.42, Br 15.96, Cl 7.04, N 8.38, S 6.37. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.14 (s, 1H, pyrazole-H5), 8.02 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.67 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.57 – 7.54 (m, 4H, H2', H6', H3, H5), 7.35 (d, 2H, H3", H5", J2"-3" = 7.6 Hz), 7.24 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 8.4 Hz), 6.48 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.6 Hz), 4.24 (s, 2H,

CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.66, 145.40, 136.95, 135.33, 134.66, 132.12, 132.01, 129.74, 129.46, 129.25, 128.96, 128.17, 119.89, 115.28, 110.83, 39.35.

5.1.3.2.1.5.8. *N*-[(3-(4-Chlorophenyl)-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl]-4-methylanyline (11h). Yield 29%, m.p. = 100 – 101 °C from n-Hexane; Anal. Calc. for $C_{23}H_{20}ClN_3O_2S$: C 63.08, H 4.60, Cl 8.10, N 9.59, S 7.32; found C 63.30, H 4.62, Cl 8.06, N 9.62, S 7.30. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.13 (s, 1H, pyrazole-H5), 8.02 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.66 – 7.60 (m, 3H, H4, H2, H6), 7.53 (t, 2H, H3, H5, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ = 8.0 Hz), 7.33 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 8.0 Hz), 6.98 (d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 7.6 Hz), 6.53 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.6 Hz), 4.21 (s, 3H, CH_3), 2.25 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.72, 144.38, 137.04, 135.22, 134.55, 131.98, 129.88, 129.41, 129.33, 128.90, 128.27, 128.17, 120.61, 113.79, 39.65, 20.44.

5.1.3.2.1.5.9. *N*-[(1-(Phenylsulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl] aniline (11i). Yield: 20%, m.p. = 128 – 129 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{23}H_{21}N_3O_2S$: C 68.46, H 5.25, N 10.41, S 7.95; found C 68.66, H 5.26, N 10.38, S 7.97. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.09 (s, 1H, pyrazole-H5), 7.99 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.60 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.54 – 7.47 (m, 4H, H3, H5, H2', H6'), 7.17 – 7.14 (m, 4H, H3', H5', H3", H5"), 6.76 (t, 1H, H4", $J_{3"\text{-}4"}$ = 8.0 Hz), 6.58 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 8.0 Hz), 4.25 (s, 2H, CH_2), 2.34 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.89, 146.92, 139.12, 137.25, 134.37, 131.72, 129.38, 129.33, 128.55, 128.08, 127.90, 120.76, 118.57, 114.96, 113.49, 39.39, 21.29.

5.1.3.2.1.5.10. 4-Chloro-*N*-[(1-(phenylsulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl]aniline (11j). Yield: 22%, m.p. = 115 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{23}H_{20}ClN_3O_2S$: C 63.08, H 4.60, Cl 8.10, N 9.59, S 7.32; found C 62.89, H 4.61, Cl 8.07, N 9.57, S 7.29. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.05 (s, 1H, pyrazole-H5), 7.98 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.2 Hz), 7.61 (t, 1H, H4, $J_{3\text{-}4}$ = 7.2 Hz), 7.54 – 7.48 (m, 4H, H3, H5, H2', H6'), 7.17 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 7.2 Hz), 7.07 (d, 2H, H3'', H5'', $J_{2''\text{-}3''}$ = 8.0 Hz), 6.47 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 8.0 Hz), 4.25 (s, 2H, CH_2) 2.34 (s, 3H, CH_3) ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.85, 145.52, 139.22, 137.15, 134.44, 131.66, 129.41, 129.35, 129.13, 128.45, 128.06, 127.84, 123.05, 120.43, 114.47, 39.36, 21.29.

5.1.3.2.1.5.11. 4-Bromo-*N*-[(1-(phenylsulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl]aniline (11k). Yield: 24%, m.p. = 110°C from AcOEt/n-Hexane; Anal. Calc. for $C_{23}H_{20}BrN_3O_2S$: C 57.27, H 4.18, Br 16.56, N 8.71, S 6.65; found C 57.41, H 4.19, Br 16.52, N 8.69, S 6.64. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm 8.03 (s, 1H, pyrazole-H5), 7.98 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.61 (t, 1H, H4, $J_{3\text{-}4}$ = 8.0 Hz), 7.51 – 7.48 (m, 4H, H3, H5, H2', H6'), 7.21 – 7.15 (m, 4H, H3', H5', H3'', H5''), 6.42 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 7.6 Hz), 4.20 (s, 2H, CH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.84, 146.00, 139.22, 137.14, 134.44, 131.99, 131.66, 129.41, 129.35, 128.45, 128.05, 127.84, 120.41, 114.91, 110.05, 39.23, 21.29

5.1.3.2.1.5.12. 4-Methyl-*N*-[(1-(phenysulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl]aniline (11l). Yield: 41%, m.p. = 123 – 124 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{24}H_{23}N_3O_2S$: C 69.04, H 5.55, N 10.06, S 7.68; found C 68.81, H 5.57, N 10.25, S 7.65. ^{1}H NMR ($CDCl_3$, 400 MHz): δ

(ppm) 8.08 (s, 1H, pyrazole-H5), 8.00 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.2 Hz), 7.61 (t, 1H, 4H, $J_{3\text{-}4}$ = 7.2 Hz), 7.55 – 7.47 (m, 4H, H3, H5, H2', H6'), 7.17 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ = 7.6 Hz), 6.97 (d, 2H, H3'', H5'', $J_{2''\text{-}3''}$ = 8.0 Hz), 6.51 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 8.0 Hz), 4.2 (s, 2H, CH_2), 2.34 (s, 3H, CH_3-Ar), 2.24 (s, 3H, CH_3-Ar''). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.90, 144.75, 139.09, 137.29, 134.34, 131.68, 129.81, 129.36, 129.31, 128.61, 128.08, 127.91, 127.78, 121.00, 113.61, 39.69, 21.29, 20.42.

5.1.3.2.1.5.13. *N*-[(3-Phenyl-1-(phenysulfonyl)-1*H*-pyrazol-4-yl)methyl] aniline (11m). Yield: 64%, m.p. = 139°C, from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{22}H_{19}N_3O_2S$: C 67.84, H 4.92, N 10.79, S 8.23; found C 68.02, H 4.93, N 10.76, S 8.20. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.1 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.66 – 7.61 (m, 3H, H4, H2', H6'), 7.52 (t, 2H, H3, H5, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ =8.0 Hz), 7.39 – 7.37 (m, 3H, H3', H4', H5'), 7.17 (t, 2H, H3'', H5'', $J_{2''\text{-}3''}$ = $J_{3''\text{-}4''}$ = 7.6 Hz), 6.76 (t, 1H, H4'', $J_{3''\text{-}4''}$ = 7.6 Hz), 6.58 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 7.6 Hz), 4.27 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.77, 147.26, 137.17, 134.45, 131.66, 131.45, 129.36, 129.34, 129.11, 128.70, 128.12, 128.01, 121.04, 118.32, 113.20, 39.20.

5.1.3.2.1.5.14. 4-Chloro-*N*-[(3-phenyl-1-(phenylsulphonyl)-1*H*-pyrazol-4-yl)methyl]aniline (11n). Yield: 14%, m.p. = 136 – 137 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{22}H_{18}ClN_3O_2S$: C 62.33, H 4.28, Cl 8.36, N 9.91, S 7.56; found C 62.48, H 4.29, Cl 8.35, N 9.88, S 7.54. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.09 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.65 – 7.60 (m, 3H, H4, H2', H6'), 7.52 (t, 2H, H3, H5, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ = 8.0 Hz), 7.38 – 7.37 (m, 3H, H3', H4', H5'), 7.08 (d, 2H, H3'', H5'', $J_{2''\text{-}3''}$ = 8.4 Hz), 6.49 (d, 2H, H2'', H6'', $J_{2''\text{-}3''}$ = 8.4 Hz), 4.25 (s, 1H, CH_2). ^{13}C NMR ($CDCl_3$, 100

MHz): δ (ppm) 155.79, 145.29, 137.11, 134.52, 131.74, 131.33, 129.39, 129.18, 129.16, 128.72, 128.12, 127.98, 123.33, 120.35, 114.62, 39.42.

5.1.3.2.1.5.15. 4-Bromo-*N*-[(3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl]aniline (11o). Yield: 14%, m.p. = 122 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{22}H_{18}BrN_3O_2S$: C 56.42, H 3.87, Br 17.06, N 8.97, S 6.85; found C 56.28, H 3.89, Br 17.08, N 8.95, S 6.84. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.09 (s, 1H, pyrazole-H5), 8.01 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 7.66 – 7.59 (m, 3H, H4, H2', H6'), 7.53 (t, 2H, H3, H5, $J_{2\text{-}3} = J_{3\text{-}5}$ = 7.6 Hz), 7.37 – 7.35 (m, 3H, H3', H4', H5'), 7.22 (d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 7.6 Hz), 6.45 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.6 Hz), 4.25 (s, 2H, CH2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.79, 145.67, 137.10, 134.53, 132.06, 131.75, 131.31, 129.40, 129.20, 128.73, 128.13, 127.98, 120.25, 115.13, 110.46, 39.34.

5.1.3.2.1.5.16. 4-Methyl-*N*-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (11p). Yield: 62% m.p. = 199°C, from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{23}H_{21}N_3O_2S$: C 68.46, H 5.25, N 10.41, S 7.95; found C 68.65, H 5.27, N 10.44, S 7.92. IR: 3396, 1376, 1185 cm-1. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.12 (s, 1H, pyrazole-H5), 8.02 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.64 – 7.61 (m, 3H, H4, H2', H6'), 7.52 (t, 2H, H3, H5, $J_{2\text{-}3} = J_{3\text{-}4}$ = 8.0 Hz), 7.38 – 7.36 (m, 3H, H3', H4', H5'), 6.97 (d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 7.2 Hz), 6.52 (d, 2H, H2", H6", $J_{2"\text{-}3"}$ = 7.2 Hz), 4.25 (s, 2H, CH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.86, 144.51, 137.25, 134.41, 131.77, 131.47, 129.84, 129.34, 129.08, 128.67, 128.14, 128.05, 128.03, 120.92, 113.77, 39.74, 20.42.

5.1.3.2.1.5.17. *N*-((3-Bromophenyl)-1-((4-methoxphenyl)sulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (12a). Yield: 41%, m.p. = 131 – 134 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{23}H_{20}BrN_3O_3S$: C 55.43, H 4.04, Br 16.03, N 8.43, S 6.43; found C 55.58, H 4.05, Br 16.08, N 8.46, S 6.44. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.96 (s, 1H, pyrazole-H5), 7.58 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.8 Hz), 7,57 (d, 2H, H2, H6, J_{2-3} = 8.4 Hz), 7.52(d, 2H, H3', H5', $J_{2'-3'}$ = 8.8 Hz), 7.19 (t, 2H, H3", H5", $J_{2"-3"} = J_{3"-4"}$ = 8.0 Hz), 6.98 (d, 2H, H3, H5, J_{2-3} = 8.4 Hz), 6.78 (t, 1H, H4", $J_{3"-4"}$ = 8.0 Hz), 6.60 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 4.24 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.04, 146.70, 144.43, 131.37, 131.09, 130.15, 130.02, 129.05, 128.89, 127.65, 122.89, 119.80, 117.97, 116.32, 114.15, 112.26, 55.28, 38.59.

5.1.3.2.1.5.18. *N*-((3-(4-bromophenyl)-1-((4-methoxphenyl)sulfonyl)-1*H*-pyrazol-4-yl)methyl)-4-chloroaniline (12b). Yield: 50%, m.p. = 153 – 154 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{23}H_{19}BrClN_3O_3S$: C 51.84, H 3.59, Br 15.00, Cl 6.65, N 7.89, S 6.02; found C 52.05, H 3.58, Br 15.03, Cl 6.64, N 7.92, S 5.99. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07 (s, 1H, pyrazole-H5), 7.95 (d, 2H, H2, H6, J_{2-3} = 8.8 Hz), 7.52 (m, 4H, H2', H6', H3', H5'), 7.12 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.8 Hz), 6.98 (d, 2H, H3, H5, J_{2-3} = 8.8 Hz), 6.50 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.8 Hz), 4.21 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.05, 153.77, 144.81, 132.55, 131.39, 131.14, 130.14, 129.88, 128.99, 128.73, 127.52, 122.96, 119.17, 114.17, 114.01, 55.30, 38.79.

5.1.3.2.1.5.19. 4-Bromo-*N*-((3-(4-bromophenyl)-1-((4-methoxphenyl) sulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (12c). Yield: 63%, m.p. = 162 – 164 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{23}H_{19}Br_2N_3O_3S$: C 47.85, H 3.32, Br 27.68, N 7.28, S 5.55; found C 47.86, H 3.33, Br 27.67, N 7.28, S

5.55. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.06 (s, 1H, pyrazole-H5), 7.94 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.52 (m, 4H, H2, H6, H3', H5'), 7.25 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.8 Hz), 6.98 (d, 2H, H3, H5, J_{2-3} = 8.8 Hz), 6.46 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.8 Hz), 4.20 (s, 2H, CH_2), 3.86 (3H, -OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.03, 153.02, 145.57, 132.55, 131.58, 131.39, 131.05, 130.13, 129.89, 128.97, 127.51, 122.96, 119.32, 114.22, 114.17, 55.31, 38.53.

5.1.3.2.1.5.20. *N*-((3-(4-Bromophenyl)-1-((4-methoxphenyl)sulfonyl)-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (12d). Yield: 50%, m.p. = 164 – 166 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{24}H_{22}BrN_3O_3S$: C 56.25, H 4.33, Br 15.59, N 8.20, S 6.26; found C 56.39, H 4.34, Br 15.65, N 8.17, S 6.27. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm.06 (s, 1H, pyrazole-H5), 7.99 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.8 Hz), 7.61 (d, 2H, H2, H6, J_{2-6} = 8.8 Hz), 7.53 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.8 Hz), 7.03 - 6.99 (m, 4H, H3, H5, H3", H5"), 6.55 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 4.23 (s, 2H, CH_2), 3.89 (s, 3H, OCH_3), 2.28 (s, 1H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 163.97, 153.85, 144.43, 131.34, 131.08, 130.14, 129.36, 129.07, 127.67, 127.27, 122.86, 120.22, 119.98, 114.13, 112.87, 55.28, 38.91, 30.43.

5.1.3.2.1.5.21. *N*-((3-(4-Chlorophenyl)-1-(4-methoxphenyl)sulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (12e). Yield: 42%, m.p. = 143 – 145 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{23}H_{20}ClN_3O_3S$: C 60.86, H 4.44, Cl 7.81, N 9.26, S 7.06; found C 61.04, H 4.45, Cl 7.78, N 9.29, S 7.04. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.09 (s, 1H, pyrazole-H5), 7.95 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.64 (d, 2H, H2, H6, J_{2-3} = 8.0 Hz), 7.35 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 7.19 (t, 2H, H3", H5" $J_{2"-3"}$ = $J_{3"-4"}$ = 8.0 Hz), 6.98 (d, 2H, H3, H5, J_{2-3} = 8.0 Hz), 6.77 (t, 1H, H4", $J_{3"-4"}$ = 8.0 Hz), 6.60 (d, 2H, H2", H6", $J_{2"-3"}$ =

8.0 Hz), 4.24 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 163.98, 153.78, 146.73, 134.60, 131.09, 130.13, 129.56, 128.88, 128.79, 128.40, 127.66, 119.83, 117.94, 114.15, 112.67, 55.28, 39.60.

5.1.3.2.1.5.22. 4-Chloro-*N*-((3-(4-chlorophenyl)-1-((4-methoxyphenyl) sulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (12f). Yield: 36%, m.p. = 158 – 159 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{23}H_{19}Cl_2N_3O_3S$: C 56.65, H 3.92, Cl 14.52, N 8.60, S 6.57; found C 56.72, H 3.91, Cl 14.55, N 8.57, S 6.56. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.08 (s, 1H, pyrazole-H5), 7.94 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.59 (d, 2H, H2, H6 J_{2-3} = 7.2 Hz), 7.35 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 7.12 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.0 Hz), 6.98 (d, 2H, H3, H5 J_{2-3} = 7.2 Hz), 6.51 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 4.21 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.53, 154.25, 145.47, 135.18, 133.05, 131.61, 130.64, 129.92, 128.95, 128.01, 123.21, 119.77, 114.40, 114.39, 55.82, 39.22.

5.1.3.2.1.5.23. 4-Bromo-*N*-((3-(4-chlorophenyl)-1-((4-methoxyphenyl) sulfonyl)-1*H*-pyrazol-4-yl)methyl)aniline (12g). Yield: 35%, m.p. = 169 – 171 °C from AcOEt/n-Hexane; Anal. Calc. $C_{23}H_{19}BrClN_3O_3S$: C 51.84, H 3.59, Br 15.00, Cl 6.65, N 7.89, S 6.02; found C 51.64, H 3.60, Br 14.96, Cl 6.63, N 7.92, S 6.04. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07 (s, 1H, pyrazole-H5), 7.95(d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.59 (d, 2H, H2, H6 J_{2-3} = 7.2 Hz), 7.35 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 7.26 (m, 2H, H3", H5"), 6.99 (d, 2H, H3, H5, J_{2-3} = 7.2 Hz), 6.46 (d, 2H, H2", H6", $J_{2"-3"}$= 8.0 Hz), 4.22 (s, 2H, CH_2), 3.87 (s,3H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.55, 154.22, 146.01, 135.19, 132.09, 131.59, 130.69, 129.92, 128.94, 129.23, 128.94, 128.04, 119.80, 114.82, 114.68, 110.18, 55.82, 39.09.

5.1.3.2.1.5.24. *N*-((3-(4-Chlorophenyl)-1-((4-methoxyphenyl)sulfonyl)-1H -pyrazol-4-yl)methyl)-4-methylaniline (12h). Yield: 35%, m.p. = 169 – 171 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{24}H_{22}ClN_3O_3S$: C 61.60, H 4.74, Cl 7.58, N 8.98, S 6.85; found C 61.75, H 4.75, Cl 7.61, N 9.01, S 6.83. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.12 (s, 1H, pyrazole-H5), 7.99 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.64 (d, 2H, H2, H6, J_{2-3} = 7.2 Hz), 7.37 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 7.01 (m, 4H, H3, H5, H3", H5"), 6.46 (d, 2H, H2", H6", $J_{2"-3"}$= 8.0 Hz), 4.22 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 2.28 (s, 3H, -CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.47, 154.33, 144.82, 135.07, 131.61, 130.63, 130.10, 129.87, 129.32, 128.88, 128.94, 128.23, 127.89, 120.42, 114.63, 113.50, 55.78, 39.49, 20.42.

5.1.3.2.1.5.25. *N*-((1-((4-Methoxyphenyl)sulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)aniline (12i). Yield: 40%, m.p. = 154 – 155 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{24}H_{23}N_3O_3S$: C 66.49, H 5.35, N 9.69, S 7.40; found C 66.68, H 5.37, N 9.66, S 7.32. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.97 (s, 1H, pyrazole-H5), 7.95 (d, 2H, H2, H6, J_{2-3} = 8.0 Hz), 7.54 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.19 – 7.15 (m, 4H, H3', H5', H3", H5"), 6.96 (d, 2H, H3, H5, J_{2-3} = 8.0), 6.77 (t, 1H, H4" $J_{3"-4"}$= 7.6 Hz), 6.60 (d, 2H, H2", H6", $J_{2"-3"}$ = 7.6 Hz), 4.27 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 163.81, 154.99, 146.88, 138.52, 130.83, 130.05, 128.87, 128.83, 128.21, 127.93, 127.39, 120.04, 117.70, 114.06, 112.62, 55.25, 38.70, 20.80.

5.1.3.2.1.5.26. 4-Chloro-*N*-((1-(4-methoxyphenyl)sulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)aniline (12j). Yield: 56%, m.p. = 157 – 158 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{24}H_{22}ClN_3O_3S$: C 61.60, H 4.74, Cl 7.58, N 8.98, S 6.85; found C 61.78, H 4.75, Cl 7.56, N 8.95, S 6.87. ^{1}H NMR

($CDCl_3$, 400 MHz): δ (ppm) 8.03 (s, 1H, pyrazole-H5), 7.93 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.2 Hz), 7.52 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.18 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.0 Hz), 7.09 (d, 2H, H3", H5", $J_{2''\text{-}3''}$ = 8.0 Hz), 6.95 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 7.2 Hz), 6.48 (d, 2H, H2", H6", $J_{2''\text{-}3''}$ = 8.0 Hz), 4.22 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3) . ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.37, 155.44, 145.82, 139.10, 131.31, 130.60, 129.39, 129.13, 128.63, 128.33, 127.84, 122.77, 120.11, 114.58, 114.24, 55.77, 39.25, 21.31.

5.1.3.2.1.5.27. 4-Bromo-*N*-((1-(4-methoxyphenyl)sulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)aniline (12k). Yield: 33%, m.p. = 151 – 153 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{24}H_{22}BrN_3O_3S$: C 56.25, H 4.33, Br 15.59, N 8.20, S 6.26; found C 56.08, H 4.34, Br 15.63, N 8.23, S 6.23. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07 (s, 1H, pyrazole-H5), 7.96 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.8 Hz), 7.54 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.28 – 7.20 (m, 4H, H3', H5', H3", H5"), 7.01 (d, 2H, H3, H5, $J_{2\text{-}3}$ = 8.8 Hz), 6.47(d, 2H, H2", H6", $J_{2''\text{-}3''}$ = 8.8 Hz), 4.26 (s, 2H, CH_2), 3.88 (s, 3H, OCH_3), 2.38 (s, 3H, -CH_3) . ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 164.36, 155.45, 146.26, 139.13, 132.01, 131.34, 130.54, 129.41, 128.57, 128.24, 127.83, 120.06, 114.68, 114.59, 109.76, 55.80, 39.11, 21.35.

5.1.3.2.1.5.28. *N*-((1-((4-Methoxyphenyl)sulfonyl)-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (12l). Yield: 25%, m.p. = 145 – 146 °C from $CHCl_3$/n-Hexane; Anal. Calc. for $C_{25}H_{25}N_3O_3S$: C 67.09, H 5.63, N 9.39, S 7.16; found C 66.90, H 5.64, N 9.40, S 7.15. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.07 (s, 1H, pyrazole-H5), 7.95 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.8 Hz), 7.56 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.18 (d, 2H, H3', H5', $J_{2'\text{-}3'}$ 8.0 Hz), 6.99 – 6.95 (m, 4H, H3, H5, H3", H5"), 6.53(d, 2H, H2", H6", $J_{2''\text{-}3''}$ = 8.8 Hz), 4.24 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3), 2.25 (s, 3H, CH_3). ^{13}C NMR

($CDCl_3$, 100 MHz): δ (ppm) 163.80, 155.02, 144.41, 138.46, 130.85, 130.04, 129.30, 128.84, 128.26, 128.01, 127.40, 127.38, 120.09, 114.04, 113.00, 55.23, 39.13, 20.80, 19.91.

5.1.3.2.1.6. General procedure for the synthesis of the 1-benzyl-3-phenyl-1*H*-pyrazole-4-carbaldehydes (13a-c).

NaH (4 mmol) was added to a stirred solution of the appropriate 3-phenyl-1*H*-pyrazole-4-carbaldehyde **6a-c** (4 mmol) in dry THF (90 ml). The mixture was stirred for 30 min at room temperature, than benzyl bromide (5 mmol) was added. After stirring for 24 h at room temperature, water was added to the mixture and THF was removed under reduced pressure. The suspension obtained was extracted with ethyl acetate and the organic phase was washed with brine and dried on Na_2SO_4 anhydrous, filtered and evaporated to dryness. The residue was purified by column chromatography on silica gel eluting with a mixture of AcOEt and n-Hexane (1:3).

5.1.3.2.1.6.1. 1-Benzyl-3-(4-bromophenyl)-1*H*-pyrazole-4-carbaldehyde (13a). Yield: 78%, m.p. = 83 – 84 °C from n-Hexane. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.87 (s, 1H, CHO), 7.92 (s, 1H, pyrazole-H5), 7.67 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.4 Hz), 7.57 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.4 Hz), 7.38 – 7.36 (m, 3H, H3, H4, H5), 7.30 (d, 2H, H2, H6, J_{2-3} = 7.6 Hz), 5.32 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 183.78, 151.94, 134.38, 134.01, 131.28, 130.06, 129.90, 128.65, 128.34, 127.85, 122.91, 120.81, 56.25.

5.1.3.2.1.6.2. 1-Benzyl-3-(4-chlorophenyl)-1*H*-pyrazole-4-carbaldehyde (13b). Yield: 52%, m.p. = 95 – 96 °C from n-Hexane. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 9.77 (s, 1H, CHO), 7.82 (s, 1H, pyrazole-H5), 7.63 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.4 Hz), 7.32 – 26 (m, 5H, H3, H4, H5, H2', H6'), 7.19 (d, 2H,

H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 5.22 (s, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 183.77, 151.90, 134.60, 134.37, 134.05, 130.86, 129.63, 128.64, 128.32, 127.84, 126.59, 120.82, 56.23.

5.1.3.2.1.6.3. 1-Benzyl-3-(p-tolyl)-1*H*-pyrazole-4-carbaldehyde (13c). Yield: 64%, m.p. = 100 – 101 °C from n-Hexane. ^1H NMR (DMSO, 400 MHz): δ (ppm) 9.86 (s, 1H, CHO), 8.65 (s, 1H, pyrazole-H5), 7.71 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.0 Hz), 7.38 – 7.36 (m, 6H, H3, H4, H5, H3', H5'), 7.25 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 5.35 (s, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 184.45, 152.00, 138.21, 137.21, 136.25, 128.99, 128.78, 128.65, 128.35, 127.97, 127.92, 120.93, 55.26, 20.81.

5.1.3.2.1.7. General procedure for the synthesis of *N*-((1-benzyl-3-phenyl-1*H*-pyrazol-4-yl)methylene)aniline (14a-l).

The suitable aniline (2.4 mmol) was added to a solution of the appropriate 1-benzyl-3-phenyl-1*H*-pyrazole-4-carbaldehyde **13a-c** (2.3 mmol) in dry ethanol (40 ml) and glacial acetic acid (0.1 ml). The mixture was refluxed for 6 h under magnetic stirring. After cooling, water was added and the ethanol was removed under reduced pressure. The obtained suspension was extracted with ethyl acetate and the organic layer was washed with brine, dried on Na_2SO_4 anhydrous, filtered and evaporated to dryness. The residual oil was used for the next reaction without further purification.

5.1.3.2.1.8. General procedure for the synthesis of the *N*-((1-benzyl-3-phenyl-1*H*-pyrazol-4-yl)methyl)aniline (15a-l).

To a stirred solution of the crude 1-benzyl-3-phenyl-1*H*-pyrazol-4-yl-methyleneaniline **14a-l** (2.4 mmol) in dry THF (67 ml) at room temperature, $NaBH_4$ (2.4 mmol) was added and the mixture was stirred at room

temperature for 24 h. After this period, water was added and THF was removed under reduced pressure. The suspension was extract with ethyl acetate and the organic phase was washed with brine, dried under Na_2SO_4 anhydrous, filtered and evaporated to dryness. The residue obtained was purified by column chromatography on silica gel eluting with a mixture of AcOEt and n-Hexane (1:3).

5.1.3.2.1.8.1. *N*-((1-Benzyl-3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methyl) aniline (15a). Yield: 23%, m.p. = 102 – 103 °C from n-Hexane; Anal. Calc. for $C_{23}H_{20}BrN_3$: C 66.04, H 4.82, Br 19.10, N 10.04; found C 66.35, H 4.82, Br 19.04, N 10.08. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.60 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.6 Hz), 7.49 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.6 Hz), 7.38 – 7.31 (m, 4H, pyrazole-H5, H3, H4, H5), 7.26 – 7.23 (m, 2H, H2, H6), 7.18 (t, 2H, H3", H5", $J_{2"-3"}$ = $J_{3"-4"}$ = 8.0 Hz), 6.74 (t, 1H, H4", $J_{3"-4"}$ = 8.0 Hz), 6.68 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 5.29 (s, 2H, CH_2Ar), 4.21 (s, 2H, CH_2NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.82, 147.62, 136.20, 132.30, 131.77, 130.66, 129.33, 129.13, 128.91, 128.24, 127.89, 121.83, 118.09, 116.90, 113.16, 56.28, 39.23.

5.1.3.2.1.8.2. *N*-((1-Benzyl-3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methyl)-4-chloroaniline (15b). Yield: 34%, m.p. = 131 – 132 °C from n-Hexane; Anal. Calc. for $C_{23}H_{19}BrClN_3$: C 61.01, H 4.23, Br 17.65, Cl 7.83, N 9.28; found C 60.85, H 4.24, Br 17.69, Cl 7.85, N 9.30. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.57 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.5 Hz), 7.50 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.5 Hz), 7,36 – 7.32 (m, 4H, pyrazole-H5, H3, H4, H5), 7.26 – 7.25 (m, 2H, H2, H6), 7.11 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.8 Hz), 6.51 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.8 Hz), 5.29 (s, 2H, CH_2Ar), 4.18 (s, 2H, CH_2NH). $_{13}$C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.79, 146.08, 136.11, 132.19, 131.80, 130.59, 129.14, 129.07, 128.93, 128.29, 127.90, 122.65, 121.90, 116.47, 114.18, 56.31, 39.28.

5.1.3.2.1.8.3. *N*-((1-Benzyl-3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methyl)-4-bromoaniline (15c). Yield: 16%, m.p. = 143 – 144 °C from n-Hexane; Anal. Calc. for $C_{23}H_{19}Br_2N_3$: C 55.56, H 3.83, Br 32.14, N 8.45; found C 55.77, H 3.82, Br 32.01, N 8.47. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.55 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.50 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 7.39 – 7.32 (m 4H, pyrazole-H5, H3, H4, H5), 7.27 – 7.23 (m, 4H, H2, H6, H3", H5"), 6.49 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 5.30 (s, 2H, CH_2Ar), 4.19 (s, 2H, CH_2NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.78, 146.46, 136.11, 132.19, 132.01, 131.81, 130.61, 129.07, 128.93, 128.29, 127.90, 121.91, 116.40, 114.70, 109.74, 56.31, 39.19.

5.1.3.2.1.8.4. *N*-((1-Benzyl-3-(4-bromophenyl)-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (15d). Yield: 10%, m.p. = 109 – 110 °C from n-Hexane; Anal. Calc. for $C_{24}H_{22}BrN_3$: C 66.67, H 5.13, Br 18.48, N 9.72; found C 66.89, H 5.13, Br 18.41, N 9.75. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.59 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.4 Hz), 7,49 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.4 Hz), 7.41 (s, 1H, pyrazole-H5), 7.37 – 7.31 (m, 3H, H3, H4, H5), 7.26 (d, 2H, H2, H6, J_{2-3} = 7.2 Hz), 6.99 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.0 Hz), 6.56 (d, 2H, H2", H6", $J_{2"-3"}$ =8.0 Hz), 5.29 (s, 2H, CH_2Ar), 4.20 (s, 2H, CH_2NH), 2.25 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.85, 14.16, 136.22, 132.31, 131.75, 130.69, 129.81, 129.15, 128.89, 128.22, 127.87, 127.54, 121.79, 116.93, 113.49, 56.26, 39.64, 20.46.

5.1.3.2.1.8.5. *N*-((1-Benzyl-3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methyl) aniline (15e). Yield: 16%, m.p. = 115 – 116 °C from n-Hexane; Anal. Calc. for $C_{23}H_{20}ClN_3$: C 73.89, H 5.39, Cl 9.48, N 11.24; found C 74.10, H 5.41, Cl 9.45, N 11.20. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.67 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.4 Hz), 7.38 – 7.31 (m, 6H, pyrazole-H5, H3, H4, H5, H3', H5'), 7.26 – 7.24 (m, 2H, H2, H6), 7.18 (t, 2H, H3", H5", $J_{2"-3"} = J_{3"-4"}$ = 8.0 Hz), 6.74 (t,

H, H4", $J_{3"-4"}$ = 8.0 Hz), 6.62 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 5.29 (s, 2H, CH_2Ar), 4.21 (s, 2H, CH_2NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.82, 147.65, 136.22, 133.60, 131.84, 130.65, 129.34, 128.91, 128.83, 128.40, 128.24, 127.89, 118.06, 116.87, 113.14, 56.27, 39.22.

5.1.3.2.1.8.6. *N*-((1-Benzyl-3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methyl)-4-chloroaniline (15f). Yield: 59%, m.p. = 140 – 141 °C from n-Hexane; Anal. Calc. for $C_{23}H_{19}Cl_2N_3$: C 67.65, H 4.69, Cl 17.37, N 10.29; found C 67.86, H 4.70, Cl 17.43, N 10.33. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.61 (d, 2H, , H3', H5', $J_{2'-3'}$ = 8.44 Hz), 7,40 – 732 (m 6H, pyrazole-H5, H3, H4, H5, H2', H6'), 7.27 – 725 (m, 2H, H2, H6), 7.11 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.7 Hz), 6.54 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.7 Hz), 5.30 (s, 2H, CH_2Ar), 4.20 (s, 2H, CH_2NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.78, 146.09, 136.14, 133.68, 131.76, 130.58, 129.14, 128.93, 128.86, 128.78, 128.29, 127.89, 122.66, 116.45, 114.20, 56.29, 39.30.

5.1.3.2.1.8.7. *N*-((1-Benzyl-3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methyl)-4-bromoaniline (15g). Yield: 63%, m.p. = 133 – 134 °C from n-Hexane; Anal. Calc. for $C_{23}H_{19}BrClN_3$: C 61.01, H 4.23, Br 17.65, Cl 7.83, N 9.28; found C 61.20, H 4.22, Br 17.59, Cl 7.86, N 9.31. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.62 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.3 Hz), 7.37 – 7.34 (m 6H, pyrazole-H5, H3, H4, H5, H2', H6'), 7.26 – 7.23 (m, 4H, H2, H6, H3", H5"), 6.48 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.7 Hz), 5.29 (s, 2H, CH_2Ar), 4.18 (s, 2H, CH_2NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.78, 146.30, 136.08, 133.55, 131.98, 130.56, 128.88, 128.82, 128.74, 128.24, 127.85, 116.25, 114.76, 109.82, 56.25, 39.22

5.1.3.2.1.8.8. *N*-((1-Benzyl-3-(4-chlorophenyl)-1*H*-pyrazol-4-yl)methyl)-4-methylaniline (15h). Yield: 27%, m.p. = 106 – 107 °C from n-Hexane; Anal. Calc. for $C_{24}H_{22}ClN_3$: C 74.31, H 5.72, Cl 9.14, N 10.83; found C 74.02, H 5.74, Cl 9.17, N 10.80. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.66 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.4 Hz), 7.38 – 7.31 (m, 6H, pyrazole-H5, H3, H4, H5, H2', H6'), 7.25 (d, 2H, H2, H6, J_{2-3} = 7.8 Hz), 7.01 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.2 Hz), 6.55 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.2 Hz), 5.29 (s, 2H, CH_2Ar), 4.19 (s, 2H, CH_2NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 148.78, 145.25, 136.21, 133.52, 131.83, 130.61, 129.76, 128.85, 128.79, 128.77, 128.18, 127.83, 127.39, 116.95, 113.37, 56.20, 39.55, 20.42.

5.1.3.2.1.8.9. *N*-((1-Benzyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)aniline (15i). Yield: 14%, m.p. = 106 – 107 °C from n-Hexane; Anal. Calc. for $C_{24}H_{23}N_3$: C 81.55, H 6.56, N 11.89; found C 81.36, H 6.55, N 11.87. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.60 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.35 – 7.29 (m, 4H, pyrazole-H5, H3, H4, H5), 7.26 – 7.22 (m, 2H, H2, H6), 7.20 – 7.14 (m, 4H, H3', H5', H3", H5"), 6.71 (t, 1H, H4", $J_{3"-4"}$ = 8.0 Hz), 6.60 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 5.28 (s, 2H, CH_2Ar), 4.23 (s, 2H, CH_2NH), 2.35 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 149.92, 147.94, 137.46, 136.50, 130.51, 130.31, 129.38, 129.28, 128.85, 128.12, 128.87, 127.49, 117.74, 116.81, 113.06, 56.15, 39.26, 21.29.

5.1.3.2.1.8.10. *N*-((1-Benzyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)-4-chloro aniline (15j). Yield: 17%, m.p. = 91 – 92 °C from n-Hexane; Anal. Calc. for $C_{24}H_{22}ClN_3$: C 74.31, H 5.72, Cl 9.14, N 10.83; found C 74.59, H 5.73, Cl 9.16, N 10.86. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.54 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.37 – 7.31 (m, 4H, pyrazole-H5, H3, H4, H5), 7.26 - 725 (m, 2H, H2, H6), 7.19 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 7.09 (d, 2H, H3", H5", $J_{2"-3"}$

= 8.8 Hz), 6.53 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.8 Hz), 5.30 (s, 2H, CH_2Ar), 4.22 (s, 2H, CH_2NH), 2.36 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 149.96, 146.12, 137.55, 136.39, 130.37, 130.26, 129.39, 129.07, 128.86, 128.16, 127.87, 127.45, 122.55, 116.22, 114.29, 56.18, 39.44, 21.27.

5.1.3.2.1.8.11. *N*-((1-Benzyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)-4-bromo aniline (15k). Yield: 26%, m.p. = 94–95 °C from n-Hexane; Anal. Calc. for $C_{24}H_{22}BrN_3$: C 66.67, H 5.13, Br 18.48, N 9.72; found C 66.51, H 5.1, Br 18.42, N 9.69 ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.55 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.34 – 7.30 (m, 4H, pyrazole-H5, H3, H4, H5), 7.26 – 7.18 (m, 6H, H2, H6, H3', H5', H3", H5"), 6.48 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.8 Hz), 5.29 (s, 2H, CH_2Ar), 4.20 (s, 2H, CH_2NH), 2.35 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 149.93, 146.60, 137.56, 136.39, 131.94, 130.36, 130.25, 129.39, 128.86, 128.16, 127.87, 127.43, 116.20, 114.72, 109.51, 56.18, 39.30, 21.27.

5.1.3.2.1.8.12. *N*-((1-Benzyl-3-(p-tolyl)-1*H*-pyrazol-4-yl)methyl)-4-methyl aniline (15l). Yield: 15%, m.p. = 104 – 105 °C from n-Hexane; Anal. Calc. for $C_{25}H_{25}N_3$: C 81.71, H 6.86, N 11.43; found C 81.95, H 6.84, N 11.47. ^1H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.60 (d, 2H, , H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.35 – 7.29 (m, 4H, pyrazole-H5, H3, H4, H5), 7.25 – 7.22 (m, 2H, H2, H6), 7.18 (d, 2H, H3', H5', $J_{2'-3'}$ = 8.0 Hz), 6.97 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.4 Hz), 6.53 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.4 Hz), 5.28 (s, 2H, CH_2Ar), 4.21 (s, 2H, CH_2NH), 2.35 (s, 1H, CH_3), 2.23 (s, 1H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 149.91, 145.64, 137.41, 136.54, 130.55, 130.30, 129.76, 129.37, 128.83, 128.09, 128.86, 127.49, 127.05, 116.96, 113.30, 56.14, 39.64, 21.29, 20.46.

5.1.3.2.1.9. General procedure for the synthesis of the (*E*)-1,3-diphenyl-4-styryl-1*H*-pyrazole (18a-i).

A solution of the appropriate diethyl benzyphosphonate (1.9 mmol) in dry THF (10 ml) was added dropwise to a stirred suspension of sodium hydride (1.9 mmol) in dry THF (5 ml). The mixture was stirred for 10 min at room temperature, then a solution of the appropriate 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **17a-c** (1.8 mmol) in dry THF (5 ml) was added dropwise. After stirring for 24 hours at room temperature, water was added to the mixture and THF was removed under reduced pressure. The solid collected by filtration was washed with water, and purified by column chromatography on silica gel eluting with a mixture of ethyl acetate and n-Hexane 1:3.

5.1.3.2.1.9.1. (*E*)-3-(4-Bromophenyl)-1-phenyl-4-styryl-1*H*-pyrazole (18a). Yield: 13%, m.p. = 139 – 140 °C from n-Hexane; Anal. Calc. for $C_{23}H_{17}BrN_2$: C 68.84, H 4.27, Br 19.91, N 6.98; found C 68.99, H 4.28, Br 19.84, N 7.00. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.17 (s, 1H, pyrazole-H5), 7.77 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.64 – 7.59 (m, 4H, H3, H5, H3', H5'), 7.49 – 7.43 (m, 4H, H2, H6, H2'', H6''), 7.37 – 7.29 (m, 3H, H4, H3'', H5''), 7.27 – 7.24 (m, 1H, H4''), 7.04 (d, 1H, Hα, $J_{\alpha-\beta}$ = 16.4 Hz), 6.95 (d, 1H, Hβ, $J_{\alpha-\beta}$ = 16.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.63, 139.76, 137.31, 132.14, 131.81, 130.07, 129.53, 129.44, 128.77, 127.59, 126.77, 126.23, 124.68, 122.39, 120.27, 119.11, 118.28.

5.1.3.2.1.9.2. (*E*)-3-(4-Bromophenyl)-4-(4-chlorostyryl)-1-phenyl-1*H*-pyrazole (18b). Yield: 15%, m.p. = 175 – 176 °C from n-Hexane; Anal. Calc. for $C_{23}H_{16}BrClN_2$: C 63.40, H 3.70, Br 18.34, Cl 8.14, N 6.43; found C 63.21,

H 3.71, Br 18.41, Cl 8.11, N 6.44. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.15 (s, 1H, pyrazole-H5), 7.77 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 7.6 Hz), 7.60 (s, 4H, H2', H6', H2'', H6''), 7.47 (t, 2H, H3, H5, $J_{2\text{-}3}$ = 7.6 Hz), 7.35 – 7.29 (m, 5H, H4, H3', H5', H3'', H5''), 6.99 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 6.88 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.66, 139.68, 135.80, 133.09, 132.02, 131.80, 130.04, 129.50, 128.88, 128.02, 127.32, 126.82, 124.69, 122.44, 119.94, 119.09, 118.70.

5.1.3.2.1.9.3. (*E*)-3-(4-Bromophenyl)-4-(4-methylstyryl)-1-phenyl-1*H*-pyrazole (18c). Yield: 10%, m.p. = 149 – 150 °C from n-Hexane; Anal. Calc. for $C_{24}H_{19}BrN_2$: C 69.41, H 4.61, Br 19.24, N 6.74; found C 69.61, H 4.62, Br 19.31, N 6.76. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.14 (s, 1H, pyrazole-H5), 7.76 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 7.6 Hz), 7.64 – 7.58 (m, 4H, H2, H6, H3', H5'), 7.46 (t, 2H, H3, H5, $J_{2\text{-}3}$ = 7.6 Hz), 7.34 – 7.28 (m, 3H, H4, H2'', H6''), 7.15 (d, 2H, H3'', H5'', $J_{2''\text{-}3''}$ = 8.0 Hz), 6.68 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 6.91 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 2.35 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.49, 139.78, 137.48, 134.51, 121.19, 131.74, 130.02, 129.47, 129.44, 129.42, 126.67, 126.11, 124.54, 122.29, 120.41, 119.05, 117.25, 21.24.

5.1.3.2.1.9.4. (*E*)-3-(4-Chlorophenyl)-1-phenyl-4-styryl-1*H*-pyrazole (18d). Yield: 13%, m.p. = 124 – 125 °C from n-Hexane; Anal. Calc. for $C_{23}H_{17}ClN_2$: C 77.41, H 4.80, Cl 9.94, N 7.85; found C 77.12, H 4.81, Cl 9.97, N 7.83. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.16 (s, 1H, pyrazole-H5), 7.77 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 7.8 Hz), 7.69 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.4 Hz), 7.49 – 7.43 (m, 6H, H3, H5, H3', H5', H2'', H6''), 7.36 – 7.29 (m, 3H, H4, H3'', H5''), 7.27 – 7.24 (m, 1H, H4''), 7.04 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 6.95 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ =

16.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.62, 139.78, 137.33, 134.16, 131.69, 129.78, 129.52, 129.41, 128.86, 128.77, 127.58, 126.75, 126.22, 124.66, 120.27, 119.11, 118.31.

5.1.3.2.1.9.5. (*E*)-3-(4-Chlorophenyl)-4-(4-chlorostyryl)-1-phenyl-1*H*-pyrazole (18e). Yield: 23%, m.p. = 169 – 170 °C from n-Hexane; Anal. Calc. for $C_{23}H_{16}Cl_2N_2$: C 70.60, H 4.12, Cl 18.12, N 7.16; found C 70.88, H 4.12, Cl 18.19, N 7.18. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.17 (s, 1H, pyrazole-H5), 7.77 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.0 Hz), 7.67 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.8 Hz), 7.5 – 7.44 (m, 4H, H3, H5, H2", H6"), 7.37 – 7.29(m, 5H, H4, H3', H5', H3", H5"), 7.01 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 6.90 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.66, 139.68, 135.80, 134.21, 133.07, 131.55, 129.74, 129.51, 128.87, 128.85, 127.98, 127.32, 126.81, 124.67, 119.94, 119.08, 118.89.

5.1.3.2.1.9.6. (*E*)-3-(4-Chlorophenyl)-4-(4-methylstyryl)-1-phenyl-1*H*-pyrazole (18f). Yield: 15%, m.p. = 119 – 120 °C from n-Hexane; Anal. Calc. for $C_{24}H_{19}ClN_2$: C 77.72, H 5.16, Cl 9.56, N 7.55; found C 77.50, H 5.18, Cl 9.59, N 7.57. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.15 (s, 1H, pyrazole-H5), 7.77 (d, 2H, H2, H6, $J_{2\text{-}3}$ = 8.8 Hz), 7.69 (d, 2H, H2', H6', $J_{2'\text{-}3'}$ = 8.6 Hz), 7.5. – 7.43 (m, 4H, H3, H5, H2", H6"), 7.35 – 7.29 (m, 3H, H4, H3', H5'), 7.15(d, 2H, H3", H5", $J_{2"\text{-}3"}$ = 7.8 Hz), 6.99 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 6.92 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 2.36 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 150.51, 139.80, 137.48, 135.55, 134.08, 131.74, 129.74, 129.49, 129.44, 129.40, 128.81, 126.67, 126.12, 124.53, 120.43, 119.07, 117.30, 21.24.

5.1.3.2.1.9.7. (*E*)-1-Phenyl-4-styryl-3-(p-tolyl)-1*H*-pyrazole (18g). Yield: 30%, m.p. = 142-144 °C from n-Hexane; Anal. Calc. for $C_{24}H_{20}N_2$: C 85.68, H 5.99, N 8.33; found C 86.01, H 6.01, N 8.30. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.17 (s, 1H, pyrazole-H5), 7.78 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.64 (d, 2H, H2, H6, J_{2-3} = 8.0 Hz), 7.48 – 7.43 (m, 4H, H3, H5, H2", H6"), 7.35 – 7.21 (m, 6H, H4, H3', H5', H4", H2", H6"),7.10 (d, 1H, Hα, $J_{\alpha-\beta}$ = 16.4 Hz), 6.95 (d, 1H, Hβ, $J_{\alpha-\beta}$ = 16.4 Hz), 2.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 151.15, 139.82, 138.00, 137.70, 130.42, 130.04, 129.81, 129.30, 129.26, 128.47, 127.82, 126.85, 126.49, 126.43, 120.08, 118.80, 118.73, 21.35.

5.1.3.2.1.9.8. (*E*)-4-(4-Chlorostyryl)-1-phenyl-3-(p-tolyl)-1*H*-pyrazole (18h). Yield: 33%, m.p. = 99 – 100 °C from n-Hexane; Anal. Calc. for $C_{24}H_{19}ClN_2$: C 77.72, H 5.16, Cl 9.56, N 7.55; found C 77.41, H 5.17, Cl 9.59, N 7.53. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.14 (s, 1H, pyrazole-H5), 7.76 (d, 2H, H2", H6", $J_{2"-3"}$ = 7.8 Hz), 7.61 (d, 2H, H2', H6', $J_{2'-3'}$ = 7.8 Hz), 7.45 (t, 2H, H3, H5 J_{3-4} = 7.7 Hz), 7.34 – 7.27 (m, 7H, H2, H6, H4, H3', H5', H3", H5"), 7.05 (d, 1H, Hα, $J_{\alpha-\beta}$ = 16.4 Hz), 6.87 (d, 1H, Hβ, $J_{\alpha-\beta}$ = 16.4 Hz), 2.41 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 151.22, 139.78, 138.03, 136.65, 132.06, 130.34, 130.04, 129.81, 129.21, 128.49, 128.15, 127.92, 126.90, 126.57, 119.89, 119.73, 118.75, 21.34.

5.1.3.2.1.9.9. (*E*)-4-(4-Methylstyryl)-1-phenyl-3-(p-tolyl)-1*H*-pyrazole (18i). Yield: 40%, m.p. = 140 – 141 °C from n-Hexane; Anal. Calc. for $C_{25}H_{22}N_2$: C 85.68, H 6.33, N 7.99; found C 86.01, H 6.34, N 7.96. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 8.14 (s, 1H, pyrazole-H5), 7.77 (d, 2H, H2, H6, J_{2-3} = 7.6 Hz), 7.64 (d, 2H, H2', H6', $J_{2'-3'}$ = 8.0 Hz), 7.45 (t, 2H, H3, H5 J_{3-4} = 7.6 Hz), 7.33 (d, 2H, H2", H6", $J_{2"-3"}$ = 8.0 Hz), 7.29 – 7.26 (m, 3H, H4, H3', H5'), 7.13 (d, 2H, H3", H5", $J_{2"-3"}$ = 8.0 Hz), 7.00 (d, 1H, Hα, $J_{\alpha-\beta}$ = 16.4 Hz), 6.92 (d, 1H,

Hβ, $J_{\alpha-\beta}$ = 16.4 Hz), 2.41 (s, 3H, CH_3), 2.34 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 151.77, 139.87, 137.90, 137.21, 134.76, 130.26, 129.40, 129.37, 129.29, 128.71, 128.42, 126.41, 126.08, 124.22, 120.29, 119.01, 117.87, 21.33, 21.22.

5.1.3.2.2. Biology

The cytotoxicity and antiviral assays were performed by Prof. Roberta Loddo, Department of Biomedical Sciences, Microbiology and Virology Section, University of Cagliari.

The new compounds were evaluated following the experimental protocol previously described in chapter 5.1.1.3. and 5.1.2.1.

References

1. Sidique S, Shiryaev SA, Ratnikov BI, Herath A, Su Y, Strongin AY, Cosford NDP. Structure–activity relationship and improved hydrolytic stability of pyrazole derivatives that are allosteric inhibitors of West Nile Virus NS2B-NS3 proteinase. *Bioorg. Med. Chem. Lett*. (**2009**) 19, 5773-5777.
2. Fioravanti R, Desideri N, Biava M, Droghini P, Atzori EM, Ibba C, Collu G, Sanna G, Delogu I, Loddo R. *N*-((1,3-Diphenyl-1*H*-pyrazol-4-yl)methyl)anilines: A novel class of anti-RSV agents. *Bioorg. Med. Chem. Lett*. (**2015**) 25, 2401-2404.
3. Vila JM, Pereira T, Ortigueira JM, Lopez-Torres M, Castineiras A, Lata, Ferandez JJ, Fernandez A. *J. Organomet. Chem.* (**1998**) 556, 21-30
4. Lebedev AV, Lebedeva AB, Sheludyakov VD, Kovaleva EA, Ustinova OL, Kozhevnikov IB. *Russ. J. Gen. Chem.* (**2005**) 75, 782-789.

5.1.4. Conclusions and perspectives

The initial hits identified by the antiviral screening of a library of our compounds, have been structurally modified in order to enhance their anti-

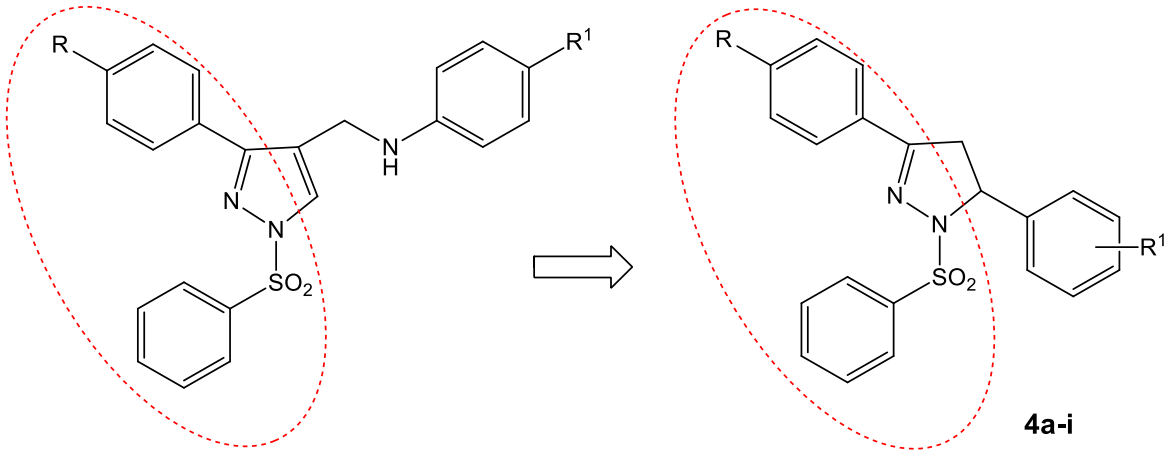
flavivirus or anti-pestivirus potency. Results of the SAR studies on the novel series of 1,3,4-trisubstituted-1*H*-pyrazoles (**11a–p**, **12a-l**, **15a–l** and **18a–i**) designed and synthesized, allowed the selection of N-((3-phenyl-1-(phenylsulfonyl)-1*H*-pyrazol-4-yl)methyl)anilines (**11a–p**) as a new generation of hits for the development of potent and selective inhibitors of YFV replication. Time of addition studies are in progress in order to determine the possible step(s) of YFV replication cycle which are inhibited by compound **11k**, selected for its high potency and low cytotoxicity. The identification of the molecular target for these compounds is necessary to address a further systematic optimization process.

5.2. Pyrazoline-based antiviral agents

5.2.1. Design, synthesis and antiviral evaluation of new 1,3,5-trisubstituted 4,5-dihydropyrazoles

Pursuing our research on anti-*Flaviridae* compounds, we devoted our attention to pyrazoline analogues of previously described pyrazole derivatives. The first series of pyrazolines synthesized and tested (**4a-i**) retained the N-phenylsulfonyl and the 3-phenyl substituent present in previously studied pyrazole analogues, while a phenyl ring was introduced at C5 pyrazoline ring position (Figure 1).

FIGURE 1



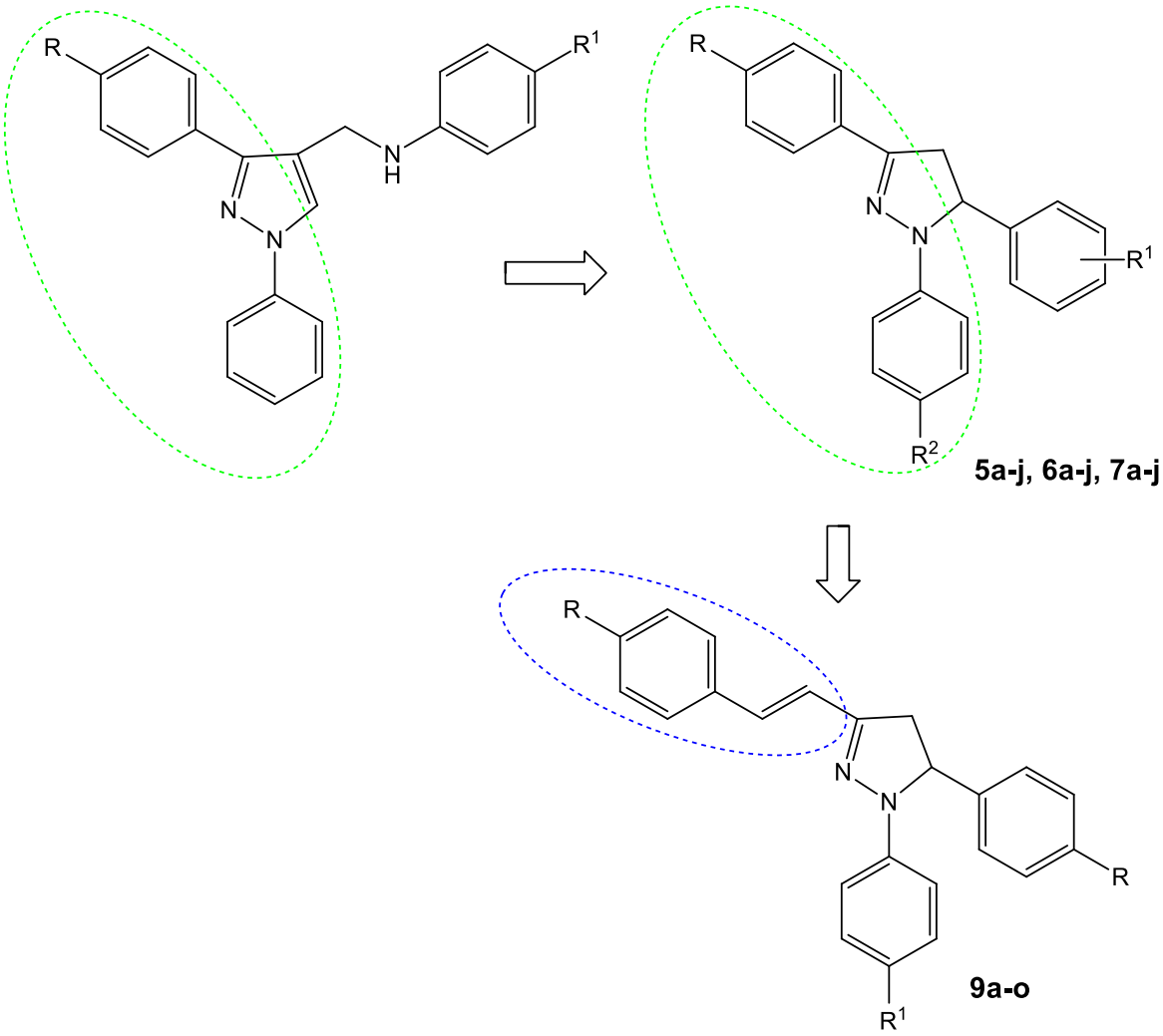
Unfortunately, these pyrazoline derivatives (**4a-i**) were inactive against all the viruses tested.

In a second series of compounds, the phenylsulfonyl moiety at the N1 position of the pyrazoline ring was replaced by a phenyl moiety bearing a sulfonamide group at the *para* position (**5a-j**, **6a-j**, **7a-j**). Either electron-donor or withdrawing groups were introduced at the *ortho*, *meta* or *para* position of the 5-phenyl substituent while the phenyl ring at the 3 position

was unsubstituted or bore a bulky substituent such as a phenoxy or a benzyloxy at the *para* position (Figure 2).

Encouraged by the potent anti-YFV activity of several 1-3,5-triphenyl-pyrazolines, we also explored the replacement of the phenyl ring at the 3-position of the pyrazoline ring with a styryl moiety. The sulfonamide group at the *para* position of the N1-phenyl ring was retained (**9k-o**) or replaced with a methyl substituent (**9f-j**). For comparison, the unsubstituted analogues (**9a-e**) were also synthesized. For easy of synthesis, the same substituent was introduced at the *para* position of 3-styryl and 5-phenyl moieties (Figure 2).

FIGURE 2



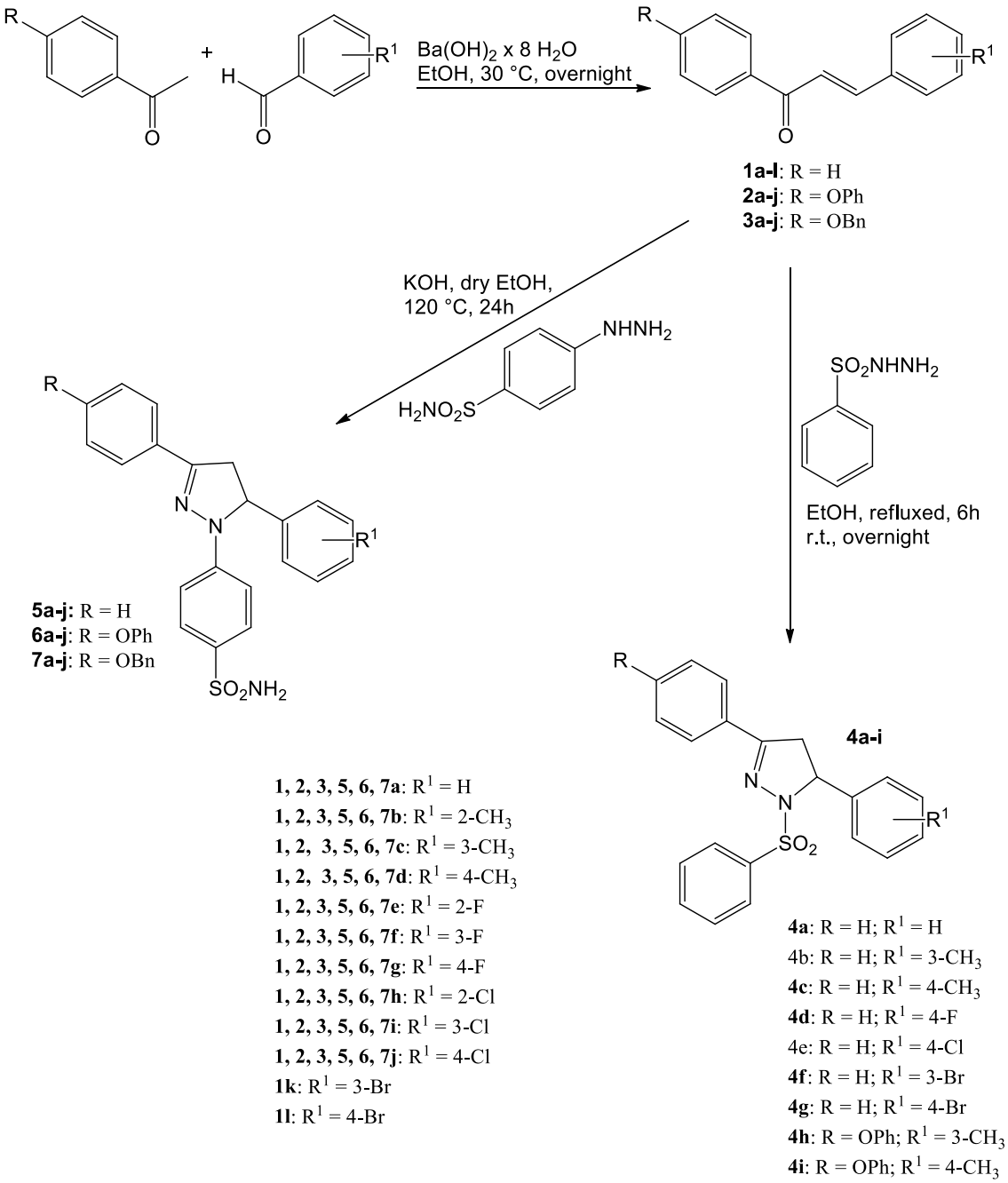
5.2.1.1. Results and discussion

5.2.1.1.1. Chemistry

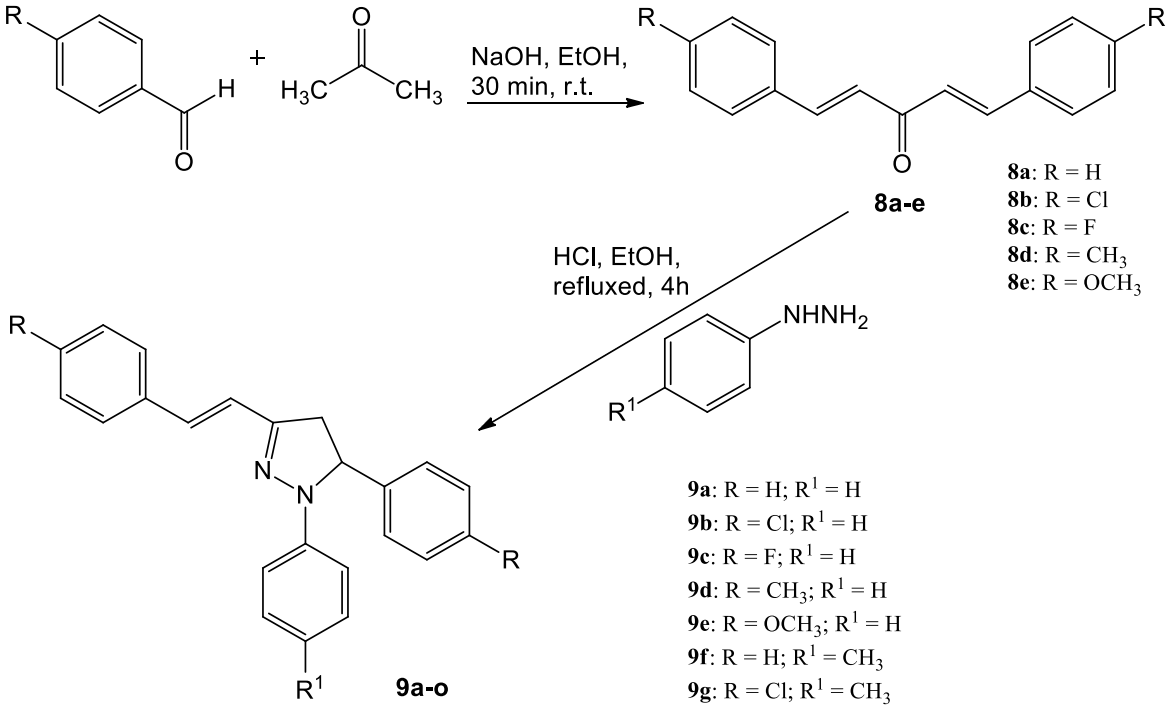
The 1,3,5-trisubstituted 4,5-dihydropyrazoles (**4a-i**, **5a-j**, **6a-j** and **7a-j**) were synthesized according to Scheme 1. Chalcones **1a-l**, **2a-j** and **3a-j** were obtained by Claisen-Schmidt condensation between substituted benzaldehyde and suitable acetophenone in basic alcoholic medium. The subsequent reaction between the appropriate chalcone and commercially available benzenesulfonylhydrazide or synthesized 4-hydrazinylbenzenesulfonamide in ethanol provided compounds **4a-i** and **5a-j**, **6a-j**, **7a-j**, respectively. The reaction proceeds via hydrazone formation and successive cyclization to pyrazolines. The formation of the pyrazoline derivatives was confirmed in ^{1}H NMR spectra by the disappearance of signals for olefinic protons (between 7.20 – 8.20 ppm) and the presence of three double doublets for the two methylene protons in position 4 (~ 4 ppm and ~ 3.9 ppm) and for the proton in position 5 (~ 5 ppm).

As depicted in Scheme 2, (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazoles (**9a-o)** were synthesized in two steps starting from the cross-aldol condensation of the suitable benzaldehyde and acetone (2:1) in basic ethanolic solution. The obtained 1,5-diphenylpenta-1,4-dien-3-ones (**8a-e**) were subsequently refluxed with substituted phenylhydrazine in acidic ethanolic solution in order to obtain the target pyrazolines (**9a-o**).

Scheme 1. Synthesis of 3,5-diphenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazole (**4a-i**) and 4-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**5a-j**, **6a-j**, **7a-j**).



Scheme 2. Synthesis of (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazole (**9a-o**).



5.2.1.1.2. Antiviral tests

Cytotoxicity and antiviral activity of the new compounds (**4a-i**, **5 a-j**, **6 a-j**, **7a-j** and **9a-o**) and reference inhibitors are reported in Tables 1-5. Each compound was evaluated in cell based assays for its cytotoxicity and antiviral activity against the panel of RNA and DNA viruses reported in chapter 5.1.1.2. All the derivatives able to interfere with YFV and/or BVDV replication were tested against two additional significant human pathogens such as DENV-2 and WNV.

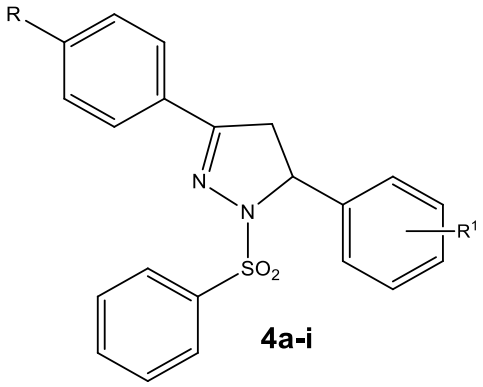
Data listed in Table 1 showed that compounds **4a-i** were devoid of antiviral activity up to the highest concentration tested, corresponding to the CC_{50} for the specific host cells. The only exception was represented by derivatives **4e** (R = H, R' = 4-Cl) that was able to interfere with YFV replication at high concentrations (CC_{50} = 63.5 μM).

As far as the antiviral activity reported in table 2, the majority of the new 1-3,5-triphenyl-pyrazolines (**5a-j**, **6a-j**, **7a-j**) interfered with YFV replication in the low micromolar concentrations (EC_{50}s ranging from 1.8 to 2.2 μM) providing almost a 10-fold improvement in potency compared to the reference inhibitor 6-azauridine (EC_{50} = 20 μM). However, the unsubstituted (**5a-j**) and the phenoxy (**6a-j**) analogues were generally endowed with significant cytotoxicity against BHK-21 cells resulting in compounds with modest selectivity indexes. On the contrary, the benzyloxy derivatives (**7a-j**) showed lower cytotoxicity and higher selectivity indexes, as a consequence. In particular, **7a** (R = H) and the fluoro substituted derivatives **7b** and **7d** coupled high potency and selectivity. In addition, the majority of the benzyloxy derivatives (**7a-j**) inhibited also the BVDV replication, generally showing higher activity and selectivity than the reference compound ribavirin (EC_{50} = 19 μM, SI = 3.2).

All the analogues were inactive against the other two members of the Flavivirus genus (WNV and DENV-2) utilized in the antiviral tests (Table 2).

When tested against HIV-1, Reo-1, Sb-1, VV, HSV-1, and VSV, all the compounds were devoid of antiviral activity up to the highest concentration tested, while five phenoxy derivatives (**6a-e**) and three benzyloxy analogues (**7c**, **7i**, **7j**) inhibited CVB-5 with EC_{50}s ranging from 3.5 μM to 13.0 μM (Table 3).

Table 1. Cytotoxicity and antiviral activity of 3,5-diphenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazoles **4a-i** against ssRNA$^+$ (BVDV, YFV, CVB-5, Sb-1), ssRNA$^-$ (VSV, RSV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

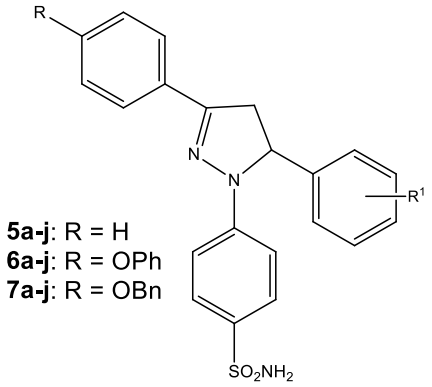


4a-i

Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	BHK-21 [c]CC_{50} (µM)	YFV [d]EC_{50} (µM)	Reo-1 [d]EC_{50} (µM)	Vero76 [c]CC_{50} (µM)	CVB-5	Sb-1	VV	HSV-1	VSV	RSV
									[f]EC_{50} (µM)					
4a	H	H	67	>67	>100	>100	>100	95	>95	>95	>95	>95	>95	>95
4b	H	3-CH_3	44	>44	95	>95	>95	32	>32	>32	>32	>32	>32	>32
4c	H	4-CH_3	>100	>100	>100	>100	>100	90	>90	>90	>90	>90	>90	>90
4d	H	4-F	53	>53	>100	>100	>100	36	>36	>36	>36	>36	>36	>36
4e	H	4-Cl	66	>66	>100	63.5±3.5	>100	70	>70	>70	>70	>70	>70	>70
4f	H	3-Br	>100	>100	>100	>100	>100	80	>80	>80	>80	>80	>80	>80
4g	H	4-Br	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
4h	OPh	3-CH_3	>100	≥100	54	>54	>54	≥100	>100	>100	>100	>100	>100	>100
4i	OPh	4-CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
Ref. compd.														
Ribavirin			>100	16.0±2.0				>100						37.5±2.5
6-Azauridine					>100	41.0±1.5		≥100						1.8±0.25
NM 107					>100		7.5±1.5							
ACG								>100				2.9±0.1		
Pleconaril								70	0.0025 ±0.0005	2.0±0.1				
NM 5255								20			2.0±0.1			

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV and Reo-1 induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1), VSV (Vescicular Stomatitis Virus) and RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

Table 2. Cytotoxicity and antiviral activity of 4-(3,5- diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamides (**5a-j**, **6a-j**, **7a-j**) against ssRNA$^+$ (BVDV, YFV, DENV-2, WNV) viruses.



Compds	R	R[1]	MDBK [a]CC_{50} (µM)	BVDV [b]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	BHK-21 [d]CC_{50} (µM)	YFV [e]EC_{50} (µM)	[c]SI CC_{50}/EC_{50}	DENV-2 [f]EC_{50} (µM)	WNV [g]EC_{50} (µM)
5a	H	H	16	>16	-	15	>10	-	-	-
5b	H	2-F	19	>19	-	21	>22	-	-	-
5c	H	3-F	20	>20	-	15	≥12	-	-	-
5d	H	4-F	18	>18	-	12	2.6±0.4	4.6	>12	>12
5e	H	2-Cl	9	>9	-	13	2.4±0.4	5.4	>13	>13
5f	H	3-Cl	2	>2	-	8	2.5±0.1	3.2	>8	>8
5g	H	4-Cl	11	>11	-	32	>32	-	-	-
5h	H	2-CH_3	18	>18	-	14	2.2±0.2	6.4	>14	>14
5i	H	3-CH_3	23	>23	-	25	>25	-	-	-
5j	H	4-CH_3	32	>32	-	7	2.2±0.2	3.2	>7	>7
6a	OPh	H	5	>5	-	11	2.1±0.1	5.2	>11	>11
6b	OPh	2-F	36	>36	-	32	1.9±0.1	16.8	>32	>32
6c	OPh	3-F	12	>12	-	11	1.8±0.2	6.1	>11	>11
6d	OPh	4-F	6	>6	-	8	1.8±0.2	4.4	>8	>8
6e	OPh	2-Cl	5	>5	-	8	1.8±0.2	4.4	>8	>8
6f	OPh	3-Cl	6	>6	-	9	2.2±0.2	4.1	>9	>9
6g	OPh	4-Cl	19	>19	-	7	1.9±0.1	3.7	>7	>7
6h	OPh	2-CH_3	16	>16	-	7	2.2±0.2	3.2	>7	>7
6i	OPh	3-CH_3	6	>6	-	8	1.8±0.1	4.4	>8	>8
6j	OPh	4-CH_3	21	>21	-	8	1.8±0.1	4.4	>8	>8
7a	OBn	H	72	>72	-	84	2.2±0.2	38.2	>84	>84
7b	OBn	2-F	>100	52.0±10.0	>1.9	92	1.8±0.2	51.1	>92	>92
7c	OBn	3-F	17	10.0±1.0	1.7	9	2.0±0.05	4.5	>9	>9
7d	OBn	4-F	>100	>100	-	69	1.9±0.1	36.3	>69	>69
7e	OBn	2-Cl	55	7.5±3.5	7.3	12	1.9±0.1	6.3	>12	>12
7f	OBn	3-Cl	38	5.5±0.5	6.9	47	2.0±0.05	23.5	>47	>47
7g	OBn	4-Cl	19	4.5±1.5	4.2	16	1.9±0.1	8.4	>16	>16
7h	OBn	2-CH_3	>100	>100	-	20	1.8±0.2	11.1	>20	>20
7i	OBn	3-CH_3	53	12.5±4.5	4.2	8	1.8±0.2	4.4	>8	>8
7j	OBn	4-CH_3	78	2.5±0.5	31.2	32	1.8±0.2	17.8	>32	>32
Ref. Compds.										
Ribavirin			61	19	3.2					
6-Azauridine						>100	20	>5		
NM 108						47	108	0.7		

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC_{50} and EC_{50}. [d]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. . [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method.

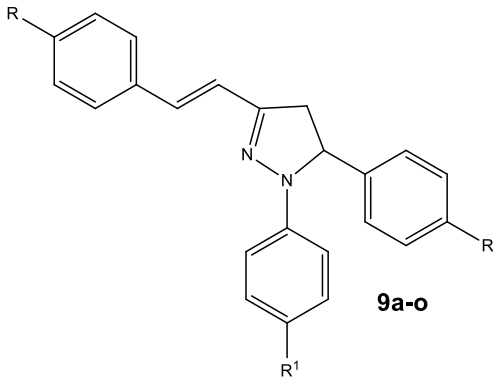
Table 3. Cytotoxicity and antiviral activity of 4-(3,5- diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamides (**5a-j**, **6a-j**, **7a-j**) against ssRNA^{+} (HIV-1, CVB-5, Sb-1), ssRNA^{-} (VSV, RSV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

Compds	R	R[1]	MT-4 [a]CC_{50} (μM)	HIV-1 [b]EC_{50} (μM)	BHK-21 [c]CC_{50} (μM)	Reo-1 [d]EC_{50} (μM)	Vero76 [e]CC_{50} (μM)	CVB-5 [f]EC_{50} (μM)	[g]SI CC_{50}/ EC_{50}	Sb-1	VV	HSV-1	VSV	RSV
										[h]EC_{50} (μM)				
5a	H	H	42	>42	15	>15	22	> 22	-	>22	>22	>22	>22	>22
5b	H	2-F	42	>42	21	>21	28	> 28	-	>28	>28	>28	>28	>28
5c	H	3-F	43	13	15	>15	24	> 24	-	>24	>24	>24	>24	>24
5d	H	4-F	45	>45	12	>12	23	> 23	-	>23	>23	>23	>23	>23
5e	H	2-Cl	44	>44	13	>13	26	> 26	-	>26	>26	>26	>26	>26
5f	H	3-Cl	41	8.5	8	>8	21	> 21	-	>21	>21	>21	>21	>21
5g	H	4-Cl	36	>36	32	>32	21	> 21	-	>21	>21	>21	>21	>21
5h	H	2-CH_3	43	>43	14	>14	24	> 24	-	>24	>24	>24	>24	>24
5i	H	3-CH_3	37	>37	25	>25	25	> 25	-	>25	>25	>25	>25	>25
5j	H	4-CH_3	40	>40	7	>7	20	> 20	-	>20	>20	>20	>20	>20
6a	OPh	H	41	12	11	>11	90/35	3.5±0.5	25.7	>90	>90	>90	>90	>35
6b	OPh	2-F	36	>36	32	>32	97	7.0±1.0	13.8	>97	>97	>97	>97	>97
6c	OPh	3-F	25	>25	11	>11	24	4.2±0.2	5.7	>24	>24	>24	>24	>24
6d	OPh	4-F	33	>33	8	>8	26	6.0±1.0	4.3	>26	>26	>26	>26	>26
6e	OPh	2-Cl	40	>40	8	>8	27	10.5±0.5	2.6	>27	>27	>27	>27	>27
6f	OPh	3-Cl	8	>8	9	>9	88	> 88	-	>88	>88	>88	>88	>88
6g	OPh	4-Cl	9	>9	7	>7	88	> 88	-	>88	>88	>88	>88	>88
6h	OPh	2-CH_3	30	13	7	>7	90/14	> 90	-	>90	>90	>90	>90	>14
6i	OPh	3-CH_3	35	>35	8	>8	81/14	> 81	-	>81	>81	>81	>81	>14
6j	OPh	4-CH_3	7.2	>7.2	8	>8	86/20	> 86	-	>86	>86	>86	>86	>20
7a	OBn	H	>100	>100	84	>84	> 100	> 100	-	>100	>100	>100	>100	>100
7b	OBn	2-F	39	>39	92	>92	> 100	> 100	-	>100	>100	>100	>100	>100
7c	OBn	3-F	65	>65	9	>9	95/25	8.5±2.5	11.2	>95	>95	>95	>95	>25
7d	OBn	4-F	>100	>100	69	>69	90	> 90	-	>90	>90	>90	>90	>90
7e	OBn	2-Cl	26	>26	12	>12	92	> 92	-	>92	>92	>92	>92	>92
7f	OBn	3-Cl	33	13	47	>47	85	> 85	-	>85	>85	>85	>85	>85
7g	OBn	4-Cl	70	>70	16	>16	82	> 82	-	>82	>82	>82	>82	>82
7h	OBn	2-CH_3	≥100	>100	20	>20	100	> 100	-	>100	>100	>100	>100	>100
7i	OBn	3-CH_3	44	>44	8	>8	100	8.0±2.0	12.5	>100	>100	>100	>100	>100
7j	OBn	4-CH_3	35	>35	32	>32	100	13.0±4.5	7.7	>100	>100	>100	>100	>100
Ref. Compds.														
EFV			37	0.002										
Ribavirin							>100							30
6-Azauridine					>100	19	17							2
ACG							>100					1.6		
Pleconaril							80	0.02	4000	2.5				
NM 5255							8				1.3			

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kidney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5) by 50% in VERO76 monolayers. [g]Selectivity index (SI) was the ratio between CC_{50} and EC_{50}. [h]Compound concentration required to reduce the plaque number of Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1), VSV (Vescicular Stomatitis Virus) and RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

Concerning the activity against *Flaviviridae* of pyrazolines **9a-o**, the results reported in Table 4 showed that the replacement of the phenyl ring at the 3-position of the pyrazoline ring with a styryl moiety results in compounds devoid of efficacy or exhibiting a specific activity against YFV. The only exception to this general rule was represented by compound **9o** that in addition to be a potent and selective anti-YFV inhibitor (EC_{50} = 1.05 μM and SI = 17.1) was also able to interfere with the replication of BVDV and DENV-2, although with a lower potency (EC_{50} = 10.0 μM and 6.9 μM, respectively). SAR studies reveal that the sulfonamide group at the *para* position of the N1-phenyl ring (**9k-o**) was necessary for an anti-YFV potency in a low micromolar range (EC_{50}s ranging from 1.05 μM to 2.2 μM). The replacement of the sulfonamide group with a methyl substituent in the same position suppressed the anti-YFV activity (**9f**, **9g**, **9j**) or led to analogues about 10-fold less potent than corresponding benzenesulfonamides (**9h**, **9i**) (EC_{50} = 20.0 μM and 12.0 μM, respectively). Moreover, the lack of substituent at the *para* position completely abolished the anti-YFV activity. It is interesting to note that all the analogues able to interfere with YFV replication, exhibited improved potency and sometimes better selectivity than the reference inhibitor 6-Azauridine (EC_{50} = 41.0 μM and SI = >2.4). Unfortunately, the presence of the sulfonamide group seems to increase the cytotoxicity against all the cell lines utilized. In addition, when tested against viruses representative of the other virus families, the benzenesulfonamides **9k**, **9l** and **9n** affected the HIV-1 replication (Table 5).

Table 4. Cytotoxicity and antiviral activity of (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazoles **9a-o** against ssRNA$^+$ (BVDV, YFV, DENV-2, WNV) viruses.



9a-o

Compds	R	R^1	MDBK [a]CC_{50} (μM)	BVDV [b]EC_{50} (μM)	BHK-21 [c]CC_{50} (μM)	YFV [d]EC_{50} (μM)	[e]SI CC_{50}/EC_{50}	DENV-2 [f]EC_{50} (μM)	WNV [g]EC_{50} (μM)
9a	H	H	>100	>100	>100	>100	-	-	-
9b	Cl	H	>100	>100	>100	>100	-	-	-
9c	F	H	>100	>100	>100	>100	-	-	-
9d	CH_3	H	>100	>100	>100	>100	-	-	-
9e	OCH_3	H	>100	>100	>100	>100	-	-	-
9f	H	CH_3	>100	>100	>100	>100	-	-	-
9g	Cl	CH_3	>100	>100	>100	>100	-	-	-
9h	F	CH_3	>100	>100	>100	20.0±6.0	>5	>100	>100
9i	CH_3	CH_3	>100	>100	>100	12.0±3.5	>8.3	>100	>100
9j	OCH_3	CH_3	>100	>100	>100	>100	-	-	-
9k	H	SO_2NH_2	38	>38	10	2.2±0.65	4.5	>10	>10
9l	Cl	SO_2NH_2	40	>40	8.5	1.1±0.1	7.7	>8.5	>8.5
9m	F	SO_2NH_2	45	>45	7.5	1.75±0.25	4.3	>7.5	>7.5
9n	CH_3	SO_2NH_2	8	>8	8.5	1.05±0.05	8.09	>8.5	>8.5
9o	OCH_3	SO_2NH_2	>100	10.0±1.0	18	1.05±0.05	17.1	6.9±1.0	>18
Ref. Compds.									
Ribavirin			>100	16.0±2.0					
6-Azauridine					>100	41.0±1.5	>2.4		
NM 108					60			1.2	0.65

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [c]Compound concentration required to reduce the viability of mock-infected BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [e]Selectivity index (SI) was the ratio between CC_{50} and EC_{50}. [f]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method

Table 5. Cytotoxicity and antiviral activity of (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazoles **9a-o** against ssRNA$^+$ (HIV-1, CVB-5, Sb-1), ssRNA$^-$ (VSV, RSV), dsRNA (Reo-1) and DNA (VV, HSV-1) viruses.

Compds	R	R[1]	MT-4 [a]CC_{50} (µM)	HIV-1 [b]EC_{50} (µM)	BHK-21 [c]CC_{50} (µM)	Reo-1 [d]EC_{50} (µM)	Vero76 [e]CC_{50} (µM)	CVB-5	Sb-1	VV	HSV-1	VSV	RSV
								[f]EC_{50} (µM)					
9a	H	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9b	Cl	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9c	F	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9d	CH_3	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9e	OCH_3	H	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9f	H	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9g	Cl	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9h	F	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9i	CH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9j	OCH_3	CH_3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
9k	H	SO_2NH_2	35	17	10	>10	65/11	>65	>65	>65	>65	>65	>11
9l	Cl	SO_2NH_2	34	14	8.5	>8	70/12	>60	>70	>70	>70	>70	>12
9m	F	SO_2NH_2	37	>37	7.5	>7	60/6	10.5±0.5	>60	>60	>60	>60	>6
9n	CH_3	SO_2NH_2	42	11	8.5	>8	60/7	>60	>60	>60	>60	>60	>7
9o	OCH_3	SO_2NH_2	14	>14	18	>18	≥100	>100	>100	>100	>100	>100	11.1±1.0
Ref. Compds.													
EFV			39	0.002									
Ribavirin													37.5±2.5
6-Azauridine					>100	9.5±1.5							1.8±0.25
ACG							>100				2.9±0.1		
Pleconaril							70	0.0025 ±0.0005	2.0±0.1				
NM 5255							20			2.0±0.1			

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
[a]Compound concentration required to reduce the viability of mock-infected MT-4 (cd4+ Human T-cells containing an integrated HTL V-1 genome) cells by 50%, as determined by the MTT method. [b]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytioathigenicity, as determined by the MTT method. [c] Compound concentration required to reduce the viability of mock-infected BHK (hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [d]Compound concentration required to achieve 50% protection of BHK cells (kidney fibroblast) from the Reo (Reovirus 1), induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to reduce the viability of mock-infected VERO76 (monkey normal kidney) monolayers by 50%. [f]Compound concentration required to reduce the plaque number of CVB-5 (Coxsackievirus B5), Sb-1 (Poliovirus 1), VV (Vaccina virus), HSV-1 (Herpesvirus 1), VSV (Vescicular Stomatitis Virus) and RSV (Respiratory Syncytial Virus) by 50% in VERO76 monolayers.

5.2.1.1.3. Time of addition studies

1,3,5-Triphenyl-pyrazolines **7a** and **7b** were selected for time of addition experiments because of their potencies against YFV (EC_{50} = 2.2 µM and 1.8 µM, respectively) and high selectivity indexes (SI = 38.2 and 51.1, respectively). Time of addition experiments were performed to determine the possible step(s) in YFV replication cycle that is inhibited by inhibitors. For

this purpose, the selected compounds, **7a** and **7b**, were added at different time intervals after infection of BHK-21 cell cultures with YFV. A concentration of 20 μM, approximately 10 times the EC_{50} against YFV, was utilized for both derivatives. For comparison, the same test was performed using the reference inhibitor 6-Azauridine, at a concentration of 90 μM. 6-Azauridine is a nucleoside analog inhibitor of the Orotidine 5'-phosphate decarboxylase (OMP-decarboxylase). This enzyme is essential in the biosynthesis of the pyrimidine nucleotides, as it converts the Orotidine monophosphate (OMP) in Uridine monophosphate (UMP) [1]. Data represented in Figures 1-4 indicate that both pyrazolines **7a** and **7b** exhibit maximal inhibition when added in the pretreatment and during infection. A similar behavior was observed for the reference inhibitors, at higher concentrations. The comparison of the curves obtained for our compounds and for the reference inhibitor could suggest a similar mechanism of action, however, further investigations are necessary for the identification of the mechanism of action.

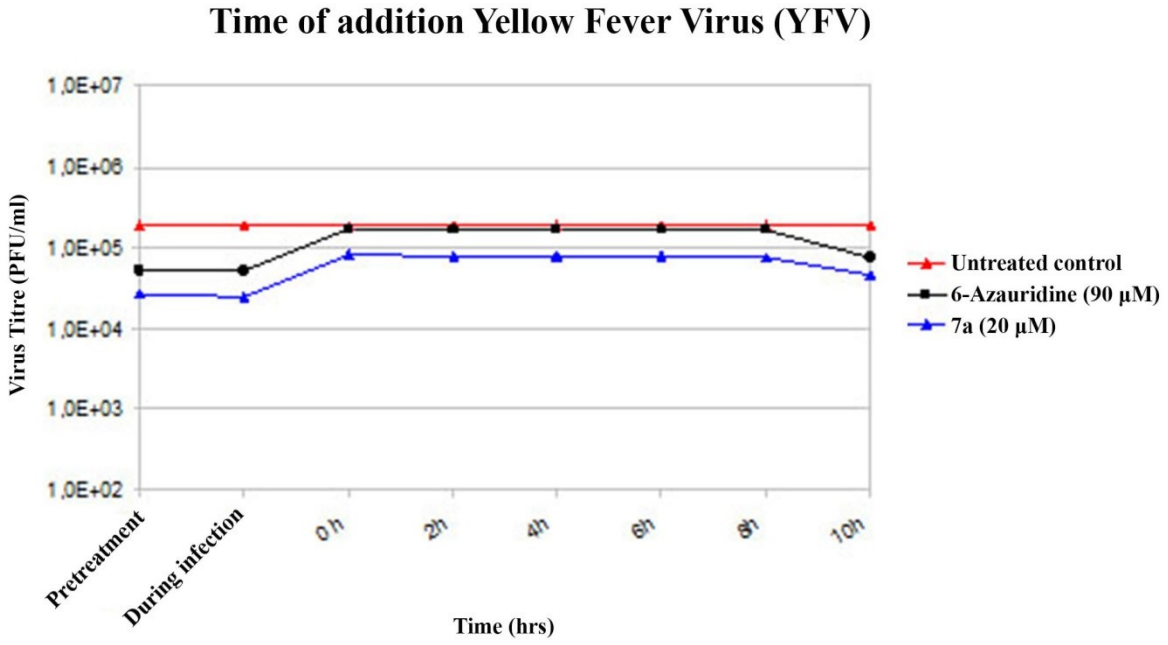


Figure 1. Effect of time of addition on antiviral activity of derivative **7a** (Blue). The same test was performed using the reference compound 6-Azauridine (Black) for comparison. In red we observe the untreated control.

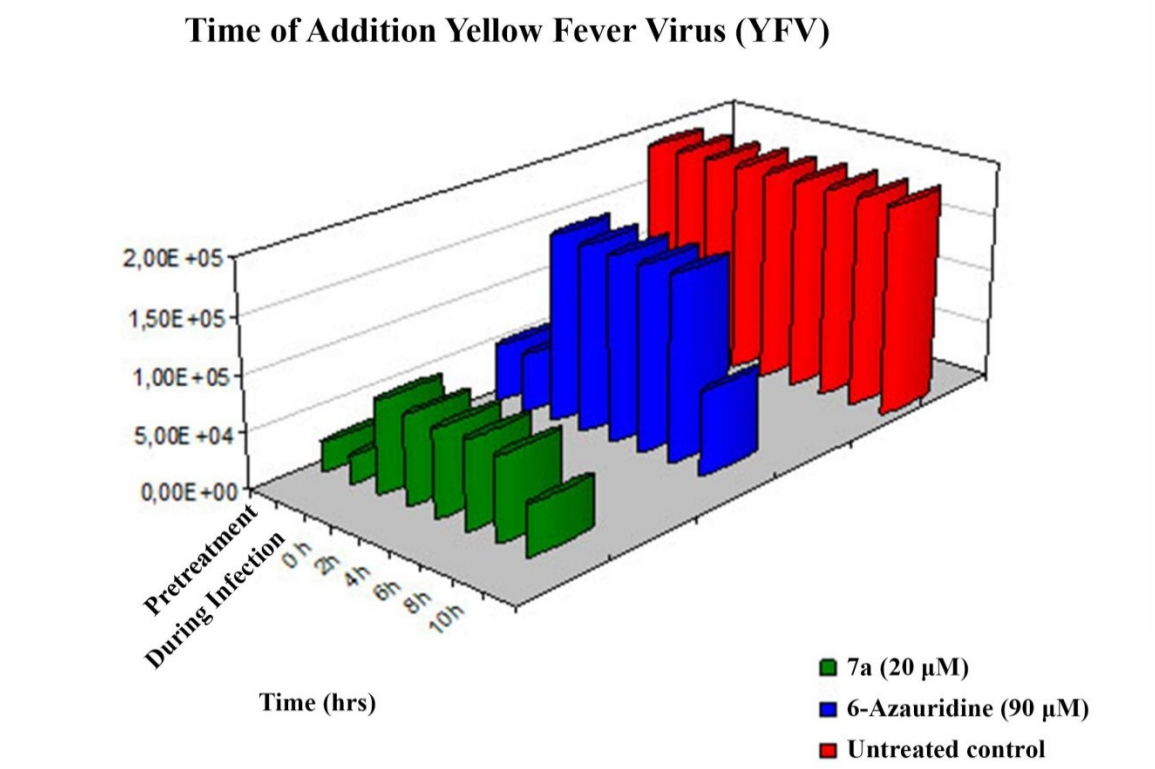


Figure 2. Effect of time of addition on antiviral activity of derivative **7a** (Green). The same test was performed using the reference compound 6-Azauridine (Blue) for comparison. In red we observe the untreated control.

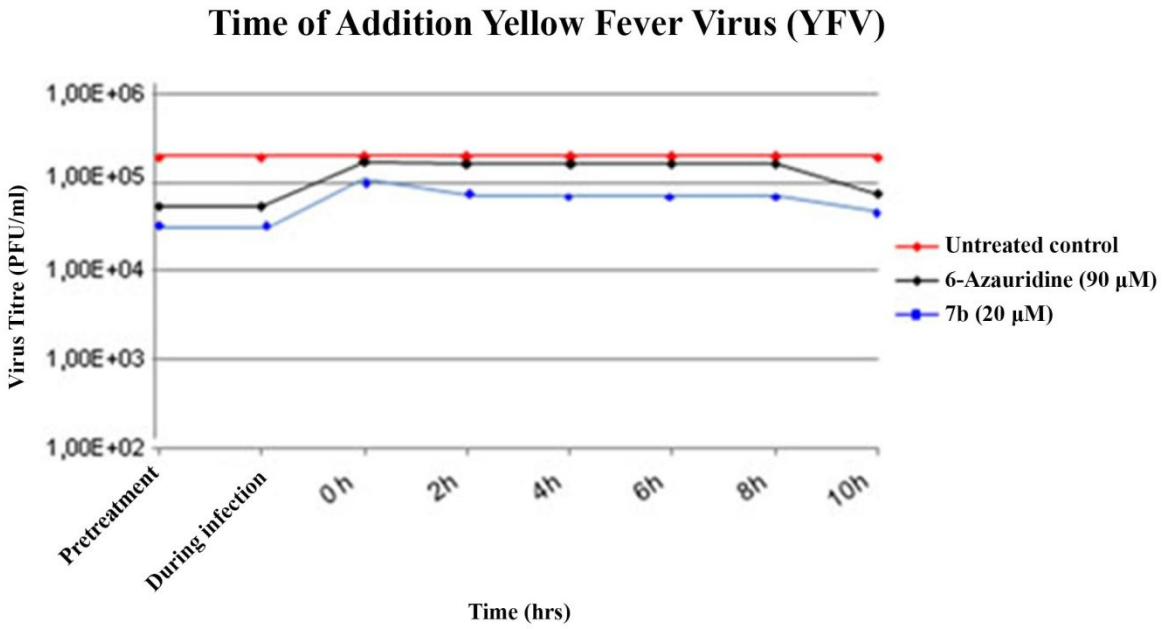


Figure 3. Effect of time of addition on antiviral activity of derivative **7b** (Blue). The same test was performed using the reference compound 6-Azauridine (Black) for comparison. In red we observe the untreated control.

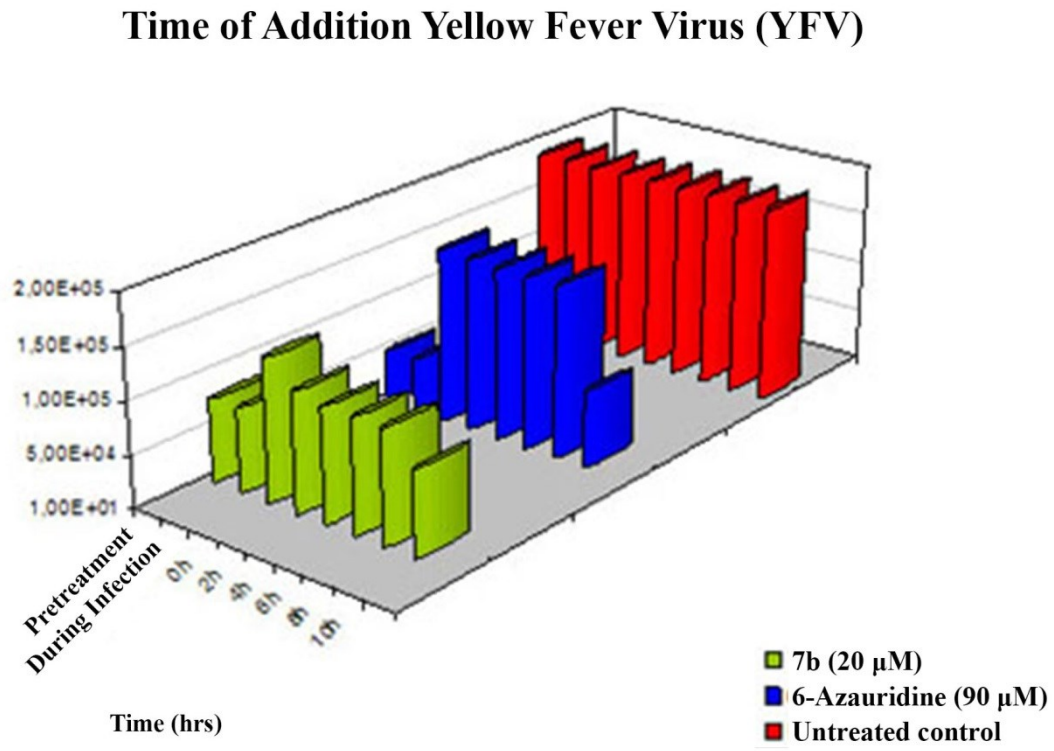


Figure 4. Effect of time of addition on antiviral activity of derivative **7b** (Green). The same test was performed using the reference compound 6-Azauridine (Blue) for comparison. In red we observe the untreated control.

5.2.1.2. Experimental

5.2.1.2.1. Chemistry

Chemicals were purchased from Sigma-Aldrich and used without further purification. 4-Benzyloxyacetophenone and 4-hydrazinylbenzene sulfonamide were synthetized according to the procedures previously reported [2, 3]. Melting points were determined on a Stuar Scientific SMP1 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer in $CDCl_3$ or DMSO-d_6, and chemical shifts were reported in ppm (δ). All compounds were routinely checked by thin-layer chromatography (TLC). TLC was performed on silica gel or aluminium oxide fluorescent coated plates (Fluka, DC-Alufolien Kieselgel or aluminum oxide F254). Compound purity was determined by elemental analysis and was

confirmed to be > 95% for all the tested compounds. Analytical results are within ±0.40% of the theoretical values.

5.2.1.2.1.1. General procedure for the synthesis of chalcones 1a-l, 2a-j and 3a-j.

Barium hydroxide octahydrate (10 mmol) was added to a solution of the suitable acetophenone (10 mmol) and substituted benzaldehyde (12 mmol) in ethanol (150 ml). The reaction mixture was stirred overnight at 30°C; then water was added and the mixture was acidified with 2N HCl, until precipitation was observed. The precipitate was collected by filtration, washed with water and crystallized. Spectroscopic data of chalcones **1a-l**, **2a-j** and **3a-j** are identical to those previously reported [4-10].

5.2.1.2.1.2. General procedure for the synthesis of the 3,5-Diphenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazoles (4a-i).

A solution of the suitable chalcone (3 mmol) and benzenesulfonyl hydrazide (3 mmol) in ethanol (60 ml) was refluxed and stirred for 6h. Subsequently the stirring was carried on at room temperature for 18 hours. Afterward, the mixture was poured into crushed ice and the precipitate was collected by filtration, washed with water and dried. The crude solid was purified by crystallization from suitable solvent (**4b-g**) or by column chromatography on silica gel eluting with a mixture of AcOEt and n-Hexane 1:3 (**4a**, **4h**, **4i**).

5.2.1.2.1.2.1. 3,5-Diphenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazole (4a). Yield: 20%, m.p. = 205 – 206 °C (lit. = 205 – 206 °C) [11] from AcOEt/n-Hexane; Anal. Calc. for $C_{21}H_{18}N_2O_2S$: C 69.59, H 5.01, N 7.73, S 8.85; found

C 69.83, H 5.03, N 7.76, S 8.82. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.96 (d, 2H, H2, H6, *J* = 7.6), 7.70 – 7.52 (m, 8H, H3, H4, H5, H2''- H6''), 7.24 – 6.98 (m, 5H, H2'- H6'), 5.08 (dd, 1H, CH, *J* = 10.0 Hz, *J* = 3.2 Hz), 4.04 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 3.2 Hz), 3.91 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 154.7, 144.4, 143.5,136.4, 131.9, 131.0, 130.0, 128.8, 128.5, 128.2, 127.3, 126.9, 126.7, 49.5, 39.0.

5.2.1.2.1.2.2. 3-Phenyl-1-(phenylsulfonyl)-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazole (4b). Yield: 20%, m.p. = 147 – 148 °C from EtOH; Anal. Calc. for $C_{22}H_{20}N_2O_2S$: C 70.19, H 5.35, N 7.44, S 8.52; found C 70.47, H 5.37, N 7.47, S 8.50. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.95 (d, 2H, H2, H6, *J* = 7.6 Hz), 7.59 – 7.55 (m, 4H, H3, H4, H5, H5''), 7.48 – 7.37 (m, 4H, H3', H5', H4'', H6''), 7.10 – 6.96 (m, 4H, H2', H4', H6', H2''), 4.90 (dd, 1H, CH, *J* = 10.0 Hz, *J* = 3.2 Hz), 4.10 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 3.2 Hz), 3.92 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 10.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 151.9, 138.6, 138.1, 137.1, 136.2, 133.6, 132.3, 130.6, 129.6, 129.1, 128.7, 128.7, 128.3, 128.2, 126.8, 66.5, 36.11, 21.2.

5.2.1.2.1.2.3. 3-Phenyl-1-(phenylsulfonyl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (4c). Yield: 18%, m.p. = 160 – 161 °C from EtOH/n-Hexane; Anal. Calc. for $C_{22}H_{20}N_2O_2S$: C 70.19, H 5.35, N 7.44, S 8.52; found C 69.98, H 5.36, N 7.46, S 8.55. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.96 (d, 2H, H3, H6), 7.70 – 7.49 (m, 8H, H3, H4, H5, H2'-H6'), 7.14 (d, 2H, H3', H5'), 7.04 (d, 2H, H2', H6'), 5.03 (dd, 1H, CH, *J* = 10.0 Hz, *J* = 3.2 Hz), 4.01 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 3.2 Hz), 3.87 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 151.7, 144.4, 137.9, 136.8, 135.9, 133.9, 133.6, 129.8, 129.2, 129.0, 128.7, 128.7, 128.1, 65.2, 36.7, 20.6.

5.2.1.2.1.2.4. 5-(4-Fluorophenyl)-3-phenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazole (4d). Yield: 17%, m.p. = 155 – 156 °C from EtOH; Anal. Calc. for $C_{21}H_{17}FN_2O_2S$: C 66.30, H 4.50, F 4.99, N 7.36, S 8.43; found C 66.31, H 4.51, F 4.96, N 7.35, S 8.43. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.93 (d, 2H, H2', H6', J = 8.4 Hz), 7.60 – 7.56 (m, 4H, H3', H5', H2 H6), 7.49 – 7.39 (m, 5H, H4', H3, H4, H5), 7.17 (t, 2H, H2", H6", $J_{2"-3"}$ = 8.4 Hz, $J_{2"-F}$= 6.0 Hz), 6.90 (t, 2H, H3", H5", $J_{2"-3"}$ = $J_{3"-F}$= 8.4 Hz), 4.90 (dd, 1H, CH, J = 10.0 Hz, J = 3.2 Hz), 4.11 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 3.2 Hz), 3.89 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 170.1 (d), 161.0, 145.4, 136.6, 136.0 (d), 135.9, 134.1, 133.7, 132.0 (d), 129.1, 128.7, 128.1, 115.0 (d), 64.6, 36.7.

5.2.1.2.1.2.5. 5-(4-Chlorophenyl)-3-phenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazole (4e). Yield: 20%, m.p. = 157 – 158 °C from EtOH/n-Hexane; Anal. Calc. for $C_{21}H_{17}ClN_2O_2S$: C 63.55, H 4.32, Cl 8.93, N 7.06, S 8.08; found C 63.80, H 4.33, Cl 8.90, N 7.07, S 8.11. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.97 (d, 2H, H2, H6), 7.69 – 7.52 (m, 8H, H3, H4, H5, H2'-H6'), 7.32 (m, 4H, H2", H3", H5", H6"), 5.15 (dd, 1H, CH, J = 10.0 Hz, J = 3.2 Hz), 4.07 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 3.2 Hz), 3.93 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 166.2, 136.6, 135.8, 134.1, 133.6, 133.3, 131.7, 131.5, 129.1, 128.7, 128.1, 124.8, 114.8, 64.7, 36.6.

5.2.1.2.1.2.6. 5-(3-Bromophenyl)-3-phenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazole (4f). Yield: 17%, m.p. = 156 – 157 °C from EtOH/n-Hexane; Anal. Calc. for $C_{21}H_{17}BrN_2O_2S$: C 57.15, H 3.88, Br, 18.10, N 6.35, S 7.27; found C 56.96, H 3.89, Br, 18.04, N 6.36, S 7.29. ^{1}H NMR (DMSO-d_6, 400

MHz): δ (ppm) 7.98 (d, 2H, H2, H6), 7.70 – 7.21 (m, 12H, H3, H4, H5, H2'-H6', H2", H4", H5", H6"), 5.16 (dd, 1H, CH, J = 10.0 Hz, J = 3.2 Hz), 4.11 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 3.2 Hz), 3.93(dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.9, 136.5, 135.8, 135.1, 134.1, 133.6, 132.8, 131.4, 130.1, 129.1, 128.9, 128.8, 128.7, 128.1, 121.2, 64.82, 36.5.

5.2.1.2.1.2.7. 5-(4-Bromophenyl)-3-phenyl-1-(phenylsulfonyl)-4,5-dihydro-1*H*-pyrazole (4g). Yield: 20%, m.p. = 159 – 160 °C from EtOH/n-Hexane; Anal. Calc. for $C_{21}H_{17}BrN_2O_2S$: C 57.15, H 3.88, Br, 18.10, N 6.35, S 7.27; found C 57.35, H 3.90, Br, 18.15, N 6.34, S 7.25. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.96 (d, 2H, H3", H5", J = 7.6 Hz), 7.73 – 7.63 (m, 8H, H2-H6, H4', H2", H6"), 7.45 (d, 2H, H3', H5', J = 8.0 Hz), 7.24 (d, 2H, H2', H6', J = 8.4 Hz), 5.13 (dd, 1H, CH, J = 10.0 Hz, J = 3.2 Hz), 4.06 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 3.2 Hz), 3.91 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 10.0 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 162.9, 137.1, 136.3, 134.6, 134.1, 132.5, 132.4, 131.5, 129.6, 129.2, 128.6, 115.1, 114.9, 65.2, 37.1.

5.2.1.2.1.2.8. 3-(4-Phenoxyphenyl)-1-(phenylsulfonyl)-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazole (4h). Yield: 11%, m.p. = 142 – 143 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{28}H_{24}N_2O_3S$: C 71.77, H 5.16, N 5.98, S 6.84; found C 72.00, H 5.17, N 5.96, S 6.82. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.01 (d, 2H, H2, H6, J = 7.2 Hz), 7.69 (t, 1H, H5", J = 6.8 Hz), 7.64 (d, 2H, H3, H5, J = 7.6 Hz), 7.55 – 7.45 (m, 4H, H3, H5, H3', H5'), 7.26 (t, 1H, H4', J = 7.2 Hz), 7.31 – 7.02 (m, 8H, H2', H6', H2", H4", H6", H2"', H4"', H6"'), 5.0 (dd, 1H, CH, J = 10.0 Hz, J = 3.2 Hz), 4.0 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 3.2 Hz), 3.84 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, J = 10.0 Hz), 2.18 (s, 1H, CH_3). ^{13}C

NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.5, 154.7, 137.1, 136.7, 133.8, 132.1, 130.7, 130.6, 130.5, 130.3, 126.1, 128.9, 128.6, 127.9, 126.9, 124.8, 119.9, 117.0, 65.5, 36.3, 20.7.

5.2.1.2.1.2.9. 3-(4-Phenoxyphenyl)-1-(phenylsulfonyl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (4i). Yield: 12%, m.p. = 164 – 165 °C from AcOEt/n-Hexane; Anal. Calc. for $C_{28}H_{24}N_2O_3S$: C 71.77, H 5.16, N 5.98, S 6.84; found C 71.52, H 5.16, N 6.00, S 6.86. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.97 (d, 2H, H2, H6, *J* = 7.2 Hz), 7.65 – 7.60 (m, 3H, H2', H6', H4'), 7.53 – 7.47 (m, 4H, H3, H5, H3''', H5''') 7.25 – 7.02 (m, 9H, H3', H5', H2'', H3'', H5'', H6'', H2''', H4''', H6'''), 5.0 (dd, 1H, CH, *J* = 10.0 Hz, *J* = 3.2 Hz), 3.98 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 3.2 Hz), 3.81 (dd, 1H, CH_2, J_{gem} = 18.0 Hz, *J* = 10.0 Hz), 2.23 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 166.3, 161.6, 154.7, 150.9, 137.8, 136.8, 133.9, 130.7, 130.3, 129.7, 129.2, 128.9, 128.6, 124.8, 119.9, 117.0, 65.1, 36.3, 20.6.

5.2.1.2.1.3. General procedure for the synthesis of 4-(3,5- diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamides (5a-j, 6a-j, 7a-j).

A solution of the appropriate chalcone **1a-j**, **2a-j** and **3a-j** (10 mmol) and 4-hydrazinylbenzenesulfonamide [3] (20 mmol) in dry ethanol (70 ml) and potassium hydroxide (20 mmol) was refluxed for 24h with stirring. After cooling, the mixture was poured into crushed ice. The precipitate was filtered, washed with water and dried. The crude solid was purified by crystallization from suitable solvent.

5.2.1.2.1.3.1. 4-(3,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzene sulfnamide (5a). Yield: 69%, m.p. = 212 – 214 °C from EtOH; Anal. Calc.

for $C_{21}H_{19}N_3O_2S$: C 66.82, H 5.07, N 11.13, S 8.49; found C 67.03, H 5.09, N 11.09, S 8.52. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.85 – 7.07 (m, 16H, H2-H6, H2', H3', H5', H6', H2"-H6", NH_2), 5.69 (dd, 1H, CH), 4.02(dd, 1H, CH_2), 3.24 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 151.7, 147.0, 143.5, 136.4, 131.0, 130.2, 129.3, 128.8, 128.5, 128.2, 126.9, 126.7, 113.8, 60.4, 40.0.

5.2.1.2.1.3.2. 4-(5-(2-Fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5b). Yield: 27%, m.p. = 260 – 262 °C from MeOH; Anal. Calc. for $C_{21}H_{18}FN_3O_2S$: C 63.78, H 4.59, F 4.80, N 10.63, S 8.11; found C 64.03, H 4.60, F 4.80, N 10.59, S 8.08. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.84 – 7.11 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H3"-H6", NH_2), 5.86 (dd, 1H, CH), 4.06 (dd, 1H, CH_2), 3.35 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.4 (d), 151.7, 146.5, 136.2, 131.01, 130.3, 129.0,128.8, 128.5 (d), 128.3 (d), 128.0, 124.0, 115.3 (d), 113.8, 108.8 (d), 53.6, 40.1.

5.2.1.2.1.3.3. 4-(5-(3-fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5c). Yield: 20%, m.p. = 158 – 160 °C from C_6H_6; Anal. Calc. for $C_{21}H_{18}FN_3O_2S$: C 63.78, H 4.59, F 4.80, N 10.63, S 8.11; found C 64.01, H 4.60, F 4.79, N 10.59, S 8.10. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.85 – 7.09 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H2", H4", H5", H6", NH_2), 5.72 (dd, 1H, CH), 4.02 (dd, 1H, CH_2), 3.27 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 162.7 (d), 151.7, 147.2, 145.1 (d), 136.4, 131.0, 130.5 (d), 130.0, 129.3, 128.8, 128.2, 122.5, 114.2 (d), 113.7 (d), 113.0, 60.4, 40.0.

5.2.1.2.1.3.4. 4-(5-(4-Fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5d). Yield: 77%, m.p. = 213 – 218 °C from MeOH; Anal. Calc. for $C_{21}H_{18}FN_3O_2S$: C 63.78, H 4.59, F 4.80, N 10.63, S 8.11; found C 63.56, H 4.58, F 4.81, N 10.59, S 8.14. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.85 – 7.08 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H2", H3", H5", H6", NH_2), 5.71 (dd, 1H, CH), 4.01 (dd, 1H, CH_2), 3.24 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 160.9 (d), 151.7, 143.7, 139.1 (d), 136.4, 131.0, 129.5, 128.8, 128.5(d), 128.0, 120.8, 116.7, 115.3 (d), 60.8, 40.3.

5.2.1.2.1.3.5. 4-(5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5e). Yield: 22%, m.p. = 228 – 231 °C from MeOH; Anal. Calc. for $C_{21}H_{18}ClN_3O_2S$: C 61.23, H 4.40, Cl 8.61, N 10.20, S 7.78; found C 61.02, H 4.41, Cl 8.59, N 10.17, S 7.81. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.85 – 7.04 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H3"-H6", NH_2), 5.87 (dd, 1H, CH), 4.13(dd, 1H, CH_2), 3.22 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 151.7, 143.8, 142.8, 136.2, 132.2, 131.4, 129.5, 128.8, 128.5, 128.3, 128.1, 127.8, 126.6, 120.8, 116.7, 55.3, 39.5.

5.2.1.2.1.3.6. 4-(5-(3-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5f). Yield: 32%, m.p. = 120 – 123 °C from C_6H_6; Anal. Calc. for $C_{21}H_{18}ClN_3O_2S$: C 61.23, H 4.40, Cl 8.61, N 10.20, S 7.78; found C 61.42, H 4.41, Cl 8.63, N 10.14, S 7.76 ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.80 – 7.11 (m, 13H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H2", H4", H5", H6"), 5.37 (dd, 1H, CH), 4.92 (s, broad, 2H, NH_2), 3.95

(dd, 1H, CH_2), 3.24 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 152.2, 144.9, 143.8, 136.4, 134.1, 131.1, 129.9, 129.5, 128.8, 128.2, 126.8, 126.7, 125.0, 120.9, 116.5, 59.9, 40.0.

5.2.1.2.1.3.7. 4-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5g). Yield: 37%, m.p. = 203 – 205 °C from C_6H_6; Anal. Calc. for $C_{21}H_{18}ClN_3O_2S$: C 61.23, H 4.40, Cl 8.61, N 10.20, S 7.78; found C 61.45, H 4.39, Cl 8.64, N 10.16, S 7.80. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.84 – 7.08 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H2", H3", H5", H6", NH_2), 5.72 (dd, 1H, CH), 4.02 (dd, 1H, CH_2), 3.24 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 151.7, 143.8, 141.6, 136.4, 132.3, 131.0, 129.5, 128.8, 128.6, 128.2, 127.2, 120.8, 116.7, 60.4, 40.2.

5.2.1.2.1.3.8. 4-(3-Phenyl-5-(o-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzene sulfonamide (5h). Yield: 23%, m.p. = 204 – 206 °C from C_6H_6; Anal. Calc. for $C_{22}H_{21}N_3O_2S$: C 67.50, H 5.41, N 10.73, S 8.19; found C 67.25, H 5.39, N 10.69, S 8.21. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 – 7.02 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H3"-H6", NH_2), 5.56 – 5.52 (dd, 1H, CH), 3.98 – 3.90 (dd, 1H, CH_2), 3.17 – 3.11 (dd, 1H, CH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 152.1, 143.6, 141.8, 136.4, 134.7, 131.0, 130.2, 129.5, 128.8, 128.2, 126.6, 125.5, 123.1, 120.8, 116.8, 57.9, 40.3, 19.6.

5.2.1.2.1.3.9. 4-(3-Phenyl-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzene sulfonamide (5i). Yield: 27%, m.p. = 200 – 203 °C from C_6H_6; Anal. Calc. for $C_{22}H_{21}N_3O_2S$: C 67.50, H 5.41, N 10.73, S 8.19; found C 67.73, H 5.40, N

10.68, S 8.16. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 8.24 – 6.89 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H2'', H4'', H5'', H6'', NH_2), 5.75 (dd, 1H, CH); 4.07(dd, 1H, CH_2), 3.12 (dd, 1H, CH_2), 2.49 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 151.7, 143.8, 141.8, 136.4, 134.7, 131.0, 130.2, 129.5, 128.8, 128.2, 126.6, 125.5, 123.1, 120.8, 116.7, 57.7, 40.4, 21.6.

5.2.1.2.1.3.10. 4-(3-Phenyl-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzene sulfonamide (5j). Yield: 27%, m.p. = 219 – 223 °C from C_6H_6; Anal. Calc. for $C_{22}H_{21}N_3O_2S$: C 67.50, H 5.41, N 10.73, S 8.19; found C 67.26, H 5.40, N 10.76, S 8.22. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.84 – 7.07 (m, 15H, H2-H6, H2', H3', H5', H6', H2', H3', H5', H6', H2'', H3'', H5'', H6'', NH_2), 5.63 (dd, 1H, CH), 3.99 (dd, 1H, CH_2), 3.2 (dd, 1H, CH_2), 2.29 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 151.7, 143.4, 140.5, 136.4, 136.2, 131.1, 129.5, 128.8, 128.6, 128.2, 125.3, 120.7, 116.4, 60.4, 40.1, 22.2.

5.2.1.2.1.3.11. 4-(3-(4-phenoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6a). Yield: 36%, m.p. = 175 – 178 °C from EtOH; Anal. Calc. for $C_{27}H_{23}N_3O_3S$: C 69.06, H 4.94, N 8.95, S 6.83; found C 68.80, H 4.96, N 8.92, S 6.78. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.86 – 7.07 (m, 20H, H2, H3, H5, H6, H2', H3', H5', H6', H2''-H6'', H2'''-H6''', NH_2), 5.67 (dd, 1H, CH), 4.0 (dd, 1H, CH_2), 3.22 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.3, 157.0, 151.7, 147.0, 143.8, 143.5, 130.0, 129.8, 129.5, 128.5, 128.2, 126.9, 126.6, 121.8, 118.9, 117.6, 113.8, 60.4, 40.1.

5.2.1.2.1.3.12. 4-(5-(2-fluorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6b). Yield: 25%, m.p. = 208 – 212 °C from C_6H_6; Anal. Calc. for $C_{27}H_{22}FN_3O_3S$: C 66.51, H 4.55, F 3.90, N 8.62, S 6.58; found C 66.75, H 4.56, F 3.90, N 8.59, S 6.60. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.87 – 7.11 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H3"-6", H2"'-H6"', NH_2), 5.85 (dd, 1H, CH), 4.05 (dd, 1H, CH_2), 2.50 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.5(d), 159.3, 157.1, 151.6, 143.8, 129.8, 129.5, 128.5 (d), 128.3 (d), 127.4, 124.1, 121.8, 120.8, 118.9, 117.6, 116.7, 115.3 (d), 113.6, 108.8 (d), 53.6. 40.0.

5.2.1.2.1.3.13. 4-(5-(3-fluorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6c). Yield: 54%, m.p. = 180 – 182 °C from EtOH; Anal. Calc. for $C_{27}H_{22}FN_3O_3S$: C 66.51, H 4.55, F 3.90, N 8.62, S 6.58; found C 66.76, H 4.55, F 3.89, N 8.60, S 6.59. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.86 – 7.12 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H4", H5", H6", H2"'-H6"', NH_2), 5.71(dd, 1H, CH), 4.01(dd, 1H, CH_2), 3.26 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 162.7 (d), 159.3, 157.0, 151.7, 147.0, 151.7, 145.0 (d), 143.8, 130.4, 130.2 (d), 128.4, 127.4, 122.5, 121.8, 118.9, 117.6, 113.9 (d), 113.7, 113.5 (d), 60.4, 40.2.

5.2.1.2.1.3.14. 4-(5-(4-fluorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6d). Yield: 56%, m.p. = 184 – 187 °C from MeOH; Anal. Calc. for $C_{27}H_{22}FN_3O_3S$: C 66.51, H 4.55, F 3.90, N 8.62, S 6.58; found C 66.26, H 4.56, F 3.89, N 8.65, S 6.57. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.86 – 7.08 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", H2"'-H6"', NH_2), 5.70 (dd, 1H, CH), 3.99 (dd, 1H, CH_2), 3.21(dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm)

160.9 (d), 159.3, 157.2, 151.7, 147.0, 143.8, 139.2 (d), 130.1, 129.4, 128.5 (d), 128.3, 127.4, 121.8, 120.8, 117.7, 115.3 (d), 113.8, 60.4, 39.9.

5.2.1.2.1.3.15. 4-(5-(2-chlorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6e). Yield: 27% m.p. = 131 – 135 °C from MeOH; Anal. Calc. for $C_{27}H_{22}ClN_3O_3S$: C 64.34, H 4.40, Cl 7.03, N 8.34, S 6.36; found C 64.12, H 4.41, Cl 7.06, N 8.37, S 6.38. ^{1}H NMR (DMSO-d6, 400 MHz): δ (ppm) 7.86 – 7.02 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H3"-6", H2'"-H6'", NH_2), 5.86 (dd, 1H, CH), 4.11 (dd, 1H, CH_2), 3.21(dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.3, 157.2, 151.6, 143.9, 143.5, 132.2, 130.5, 129.5, 128.8, 128.6, 128.4, 128.1, 127.4, 126.6, 121.8, 120.7, 118.9, 117.6, 113.2, 55.3, 39.5.

5.2.1.2.1.3.16. 4-(5-(3-chlorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6f). Yield: 60%, m.p. = 218 – 220 °C from MeOH; Anal. Calc. for $C_{27}H_{22}ClN_3O_3S$: C 64.34, H 4.40, Cl 7.03, N 8.34, S 6.36; found C 64.58, H 4.41, Cl 7.01, N 8.36, S 6.37. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.66 – 7.10 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H4", H5", H6", H2'"-H6'", NH_2), 5.70 (dd, 1H, CH), 3.99 (dd, 1H, CH_2); 3.27 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.3, 157.4, 152.0, 144.9, 143.9, 134.1, 130.0, 129.7, 129.4, 128.4, 127.4, 126.7, 126.6, 125.0, 121.8, 120.8, 118.9, 117.6, 116.7, 59.9, 40.3.

5.2.1.2.1.3.17. 4-(5-(4-chlorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6g). Yield: 60%, m.p. = 105 – 107 °C from EtOH; Anal. Calc. for $C_{27}H_{22}ClN_3O_3S$: C 64.34, H 4.40, Cl 7.03, N

8.34, S 6.36; found C 64.53, H 4.40, Cl 7.05, N 8.36, S 6.37. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.76 – 7.07 (m, 17H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", H2"'-H6"'), 5.37 (dd, 1H, CH), 4.96 (s, 2H, NH_2), 3.92 (dd, 1H, CH_2), 3.19 (s, 1H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 159.3, 157.0, 151.7, 143.7, 141.6, 132.3, 130.0, 129.4, 128.6, 128.3, 127.4, 127.0, 121.8, 120.8, 118.9, 117.4, 114.1, 60.7, 40.1.

5.2.1.2.1.3.18. 4-(3-(4-phenoxyphenyl)-5-(o-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6h). Yield: 28%, m.p. = 200 – 204 °C from EtOH; Anal. Calc. for $C_{28}H_{25}N_3O_3S$: C 69.54, H 5.21, N 8.69, S 6.63; found C 69.80, H 5.19, N 8.66, S 6.61. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.85 – 6.90 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H3"-6", H2"'-H6"', NH_2), 5.74(dd, 1H, CH), 4.05 (dd, 1H, CH_2), 3.10 (dd, 1H, CH_2), 2.51(s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.3, 157.0, 151.7, 143.5, 141.8, 134.7, 130.2, 129.5, 129.3, 128.4, 127.4, 126.6, 125.5, 123.1, 121.8, 120.8, 118.9, 117.6, 113.8, 57.9, 40.3, 19.1.

5.2.1.2.1.3.19. 4-(3-(4-phenoxyphenyl)-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6i). Yield: 37%, m.p. = 159 – 163 °C from MeOH; Anal. Calc. for $C_{28}H_{25}N_3O_3S$: C 69.54, H 5.21, N 8.69, S 6.63; found C 69.78, H 5.20, N 8.72, S 6.65. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.78 – 7.07 (m, 17H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H4", H5", H6", H2"'-H6"'), 5.36 (dd, 1H, CH), 4.74 (s, 2H, NH_2), 3.93 (dd, 1H, CH_2), 3.24 (dd, 1H, CH_2), 2.23 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 159.3, 156.8, 151.7, 143.8, 143.4, 138.2, 129.8, 129.5, 128.6, 128.4, 127.4, 126.7, 123.8, 121.8, 120.8, 118.9, 117.6, 113.8, 60.7, 40.0, 21.6.

5.2.1.2.1.3.20. 4-(3-(4-phenoxyphenyl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6j). Yield: 60%, m.p. = 131 – 135 °C from MeOH; Anal. Calc. for $C_{28}H_{25}N_3O_3S$: C 69.54, H 5.21, N 8.69, S 6.63; found C 69.80, H 5.23, N 8.67, S 6.64. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.94 – 7.07 (m, 17H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", H2"'-H6"'), 5.37 (dd, 1H, CH), 4.80 (s, 2H, NH_2), 3.86 (dd, 1H, CH_2), 3.22(dd, 1H, CH_2), 2.38 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 158.8, 156.8, 152.0, 143.8, 140.5, 136.4, 130.0, 129.5, 129.3, 128.8, 128.4, 127.4, 125.5, 121.8, 118.9, 117.6, 113.8, 60.1, 40.2, 21.3.

5.2.1.2.1.3.21. 4-(3-(4-(benzyloxy)phenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7a). Yield: 39%, m.p. = 169 – 172 °C from C_6H_6; Anal. Calc. for $C_{28}H_{25}N_3O_3S$: C 69.54, H 5.21, N 8.69, S 6.63; found C 69.29, H 5.19, N 8.72, S 6.65. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.74 – 7.01 (m, 20H, H2, H3, H5, H6, H2', H3', H5', H6', H2"-H6", H2"'-H6"', NH_2), 5.59 (dd, 1H, CH), 5.57 (s, 2H, CH_2), 3.93 (dd, 1H, CH_2), 3.15 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 151.7, 143.8, 143.5, 136.7, 129.5, 128.9, 128.7, 128.5, 128.4, 127.6, 127.1, 126.8, 126.4, 120.8, 114.4, 113.7, 70.8, 60.2, 40.1.

5.2.1.2.1.3.22. 4-(3-(4-(benzyloxy)phenyl)-5-(2-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7b). Yield: 25%, m.p. = 188 – 192 °C from MeOH; Anal. Calc. for $C_{28}H_{24}FN_3O_3S$: C 67.05, H 4.82, F 3.79, N 8.38, S 6.39; found C 66.81, H 4.83, F 3.80, N 8.40, S 6.41. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 – 7.08 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H3"-6", H2"'-H6"', NH_2), 5.79 (dd, 1H, CH), 5.20 (s, 2H, CH_2),

4.01 (dd, 1H, CH_2), 3.26 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 159.4 (d), 151.7, 143.8, 136.5, 129.5, 128.3, 128.7, 128.6, 128.5(d), 128.2 (d), 127.6, 127.1, 124.1, 120.8, 115.3 (d), 114.4, 113.8, 108.8 (d), 70.5, 53.6, 40.0.

5.2.1.2.1.3.23. 4-(3-(4-(benzyloxy)phenyl)-5-(3-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7c). Yield: 60%, m.p. = 170 – 173 °C from C_6H_6; Anal. Calc. for $C_{28}H_{24}FN_3O_3S$: C 67.05, H 4.82, F 3.79, N 8.38, S 6.39; found C 66.84, H 4.1, F 3.80, N 8.35, S 6.37. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 – 7.07 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H4", H5", H6", H2'"-H6'", NH_2), 5.66 (dd, 1H, CH), 5.21 (s, 2H, CH_2), 3.96 (dd, 1H, CH_2), 3.23 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 162.7 (d), 161.3, 151.5, 145.1 (d), 143.8, 136.7, 130.1 (d), 129.5, 128.9, 128.7, 128.6, 127.6, 127.2, 122.5, 120.8, 116.7, 114.4, 113.9 (d), 113.5 (d), 70.1, 60.1, 40.3.

5.2.1.2.1.3.24. 4-(3-(4-(benzyloxy)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7d). Yield: 45%, m.p. = 136 – 140 °C from MeOH; Anal. Calc. for $C_{28}H_{24}FN_3O_3S$: C 67.05, H 4.82, F 3.79, N 8.38, S 6.39; found C 67.27, H 4.83, F 3.79, N 8.41, S 6.41. ^1H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 – 7.07 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", H2'"-H6'", NH_2), 5.65 (dd, 1H, CH), 5.21 (s, 2H, CH_2), 3.94 (dd, 1H, CH_2), 3.54 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 160.8 (d), 151.7, 143.1, 139.1 (d), 136.7, 129.5, 129.8, 128.7, 128.6, 128.4 (d), 127.6, 127.1, 120.8, 115.3 (d), 114.3, 113.7, 70.4, 60.1, 39.8.

5.2.1.2.1.3.25. 4-(3-(4-(benzyloxy)phenyl)-5-(2-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7e). Yield: 52%, m.p. = 184 – 188 °C from C_6H_6; Anal. Calc. for $C_{28}H_{24}ClN_3O_3S$: C 64.92, H 4.67, Cl 6.84, N 8.11, S 6.19; found C 65.15, H 4.68, Cl 6.82, N 8.08, S 6.21. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 – 7.00 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H3"-6", H2"'-H6"', NH_2), 5.81(dd, 1H, CH), 5.20 (s, 2H, CH_2), 4.04 (dd, 1H, CH_2), 3.17 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 151.6, 143.8, 143.5, 136.7, 132.2, 129.6, 128.9, 128.7, 128.6, 128.4, 128.3, 128.0, 127.6, 127.0, 126.6, 120.8, 116.7, 114.2, 70.8, 55.3, 39.4.

5.2.1.2.1.3.26. 4-(3-(4-(benzyloxy)phenyl)-5-(3-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7f). Yield: 63%, m.p. = 134 – 136 °C from C6H6; Anal. Calc. for $C_{28}H_{24}ClN_3O_3S$: C 64.92, H 4.67, Cl 6.84, N 8.11, S 6.19; found C 65.13, H 4.66, Cl 6.86, N 8.14, S 6.17. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.77 – 7.06 (m, 17H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H4", H5", H6", H2"'-H6"'), 5.34 (dd, 1H, CH), 5.17 (s, 2H, CH_2), 4.76 (s, 2H, NH_2), 3.91(dd, 1H, CH_2), 3.21(dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 151.7, 144.9, 143.8, 136.6, 134.1, 129.9, 129.5, 128.9, 128.7, 128.6, 127.6, 127.1, 126.8, 126.7, 125.1, 120.8, 116.7, 114.4, 70.2, 59.9, 40.1.

5.2.1.2.1.3.27. 4-(3-(4-(benzyloxy)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7g). Yield: 40%, m.p. = 98 – 102 °C from C_6H_6; Anal. Calc. for $C_{28}H_{24}ClN_3O_3S$: C 64.92, H 4.67, Cl 6.84, N 8.11, S 6.19; found C 64.69, H 4.66, Cl 6.82, N 8.13, S 6.17. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.55 – 7.05 (m, 17H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", H2"'-H6"'), 5.34 (dd, 1H, CH), 5.16 (s, 2H, CH_2), 4.83 (s, 2H, NH_2), 3.90 (dd, 1H, CH_2), 3.18 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100

MHz): δ (ppm) 161.3, 151.7, 143.8, 141.6, 136.7, 132.3, 129.5, 128.9, 128.7, 128.5, 128.2, 127.6, 127.1, 127.0, 120.8, 116.7, 114.3, 70.8, 60.4, 40.1.

5.2.1.2.1.3.28. 4-(3-(4-(benzyloxy)phenyl)-5-(o-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7h). Yield: 33%, m.p. = 230 – 234 °C from CH_3CN; Anal. Calc. for $C_{29}H_{27}N_3O_3S$: C 70.00, H 5.47, N 8.44, S 6.44; found C 69.76, H 5.48, N 8.41, S 6.46. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.77 – 6.88 (m, 19H, Ar, NH_2), 5.69 (dd, 1H, CH), 5.19 (s, 2H, CH_2), 4.02 (dd, 1H, CH_2), 3.07 (dd, 1H, CH_2), 2.50 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.4, 151.7, 143.8, 141.8, 136.7, 134.7, 130.1, 129.5, 128.9, 128.7, 128.6, 127.6, 127.1, 126.6, 125.5, 123.1, 120.8, 116.7, 114.4, 70.7, 60.1, 40.5, 19.0.

5.2.1.2.1.3.29. 4-(3-(4-(benzyloxy)phenyl)-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7i). Yield: 29%, m.p. = 131 – 134 °C from EtOH; Anal. Calc. for $C_{29}H_{27}N_3O_3S$: C 70.00, H 5.47, N 8.44, S 6.44; found C 69.75, H 5.48, N 8.42, S 6.43. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 – 7.05 (m, 19H, H2, H3, H5, H6, H2', H3', H5', H6', H3"-6", H2"'-H6"', NH_2), 5.56 (dd, 1H, CH), 5.21 (s, 2H, CH_2), 3.26 (dd, 1H, CH_2), 2.55 (s, 3H, CH_3), 2.52 (dd, 1H, CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 151.7, 143.8, 143.4, 138.2, 129.5, 128.9, 128.7, 128.6, 128.6, 128.4, 127.6, 127.1, 126.9, 126.7, 123.9, 120.8, 116.7, 114.4, 70.8, 57.9, 40.3, 21.6.

5.2.1.2.1.3.30. 4-(3-(4-(benzyloxy)phenyl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (7j). Yield: 39%, m.p. = 135 – 137 °C from MeOH; Anal. Calc. for $C_{29}H_{27}N_3O_3S$: C 70.00, H 5.47, N 8.44, S 6.44; found C 70.27, H 5.47, N 8.47, S 6.46. ^{1}H NMR (DMSO-d_6, 400 MHz): δ

(ppm) 8.11 – 7.05 (m, 17H, H2, H3, H5, H6, H2', H3', H5', H6', H2'', H3'', H5'', H6'', H2'''-H6'''), 5.19 (dd, 1H, CH), 5.16 (s, 2H, CH_2), 4.77 (s, 2H, NH_2), 3.88 (dd, 1H, CH_2), 3.21(dd, 1H, CH_2), 2.46 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.3, 151.7, 143.8, 140.5, 136.7, 136.4, 129.5, 128.9, 128.8, 128.7, 128.6, 127.6, 127.1, 125.3, 120.8, 116.7, 114.4, 70.9, 60.4, 40.0, 21.4.

5.2.1.2.1.4. General procedure for the synthesis of the (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-ones (8a-c).

The appropriate benzaldehyde (18 mmol) and acetone (9 mmol) were added to a solution of potassium hydroxide (45 mml) in water (20 ml) and ethanol (15 ml). The mixture was stirred for 30 min at room temperature. After this period, the precipitate was filtered, washed with water, dried and crystallized from suitable solvent.

5.2.1.2.1.4.1. (1*E*,4*E*)-1,5-diphenylpenta-1,4-dien-3-one (8a). Yield 90%, m.p. 105 – 107 °C from EtOH. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.79 (d, 2H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.0 Hz), 7.65 – 7.64 (m, 4H, H2, H6, H2', H6'), 7.45 – 7.43 (m, 6H, H3, H4, H5, H3', H4', H5'), 7.13 (d, 2H, Hα, $J_{\alpha\text{-}\beta}$ = 16.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 188.6, 142.2, 135.2, 128.6, 128.5, 127.9, 123.3.

5.2.1.2.1.4.2. (1*E*,4*E*)-1,5-bis(4-chlorophenyl)penta-1,4-dien-3-one (8b). Yield 85%, m.p. 121 – 125 °C from EtOH. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.72 (d, 2H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.0 Hz), 7.56 – 7.48(m, 4H, H2, H6, H2', H6'), 7.41 – 7.39 (m, 4H, 4H, H3, H5, H3', H5'), 7.07 (d, 2H, Hα, $J_{\alpha\text{-}\beta}$ = 16.0 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 189.1, 142.8, 133.5, 133.3, 129.0, 128.7, 122.9.

5.2.1.2.1.4.3. (1*E*,4*E*)-1,5-bis(4-fluorophenyl)penta-1,4-dien-3-one (8c). Yield 95%, m.p. 120 – 123 °C from EtOH. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.71 (d, 2H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.0 Hz), 7.71 – 7.61 (m, 4H, H2, H6, H2', H6'), 7.15 – 7.10 (m, 4H, H3, H5, H3', H5'), 7.05 (d, 2H, Hα, $J_{\alpha\text{-}\beta}$ = 15.6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 188.8, 162.1, 142.2, 130.8, 130.4, 123.3, 115.4.

5.2.1.2.1.4.4. (1*E*,4*E*)-1,5-di-p-tolylpenta-1,4-dien-3-one (8d). Yield 95%, m.p. 112 – 115 °C from C_6H_6. ^{1}H-NMR ($CDCl_3$, 400 MHz, 400 MHz): δ (ppm) 7.76 (d, 2H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.0 Hz), 7.54 – 7.33 (m, 4H, H2, H6, H2', H6'), 7.29 – 7.04 (m, 4H, H3, H5, H3', H5'), 6.73 (d, 2H, Hα, $J_{\alpha\text{-}\beta}$ = 15.6 Hz), 2.41 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 188.8, 142.4, 137.6, 132.5, 129.9, 128.5, 123.3, 21.4.

5.2.1.2.1.4.5. (1*E*,4*E*)-1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one (8e). Yield 88%, m.p. 127 – 130 ° C from EtOH. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.74 (d, 2H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.0 Hz), 7.60 – 7.58 (m, 4H, H2, H6, H2', H6'), 6.99 – 6.94 (m, 6H, H3, H5, H3', H5', Hα), 3.87 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 188.6, 159.8, 142.4, 130.2, 127.5, 123.3, 114.2, 55.8.

5.2.1.2.1.5. General procedure for the synthesis of the (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazoles (9a-o).

A solution of the suitable chalcone (**8a-e**) (18 mmol) and the appropriate phenylhydrazine (27 mmol) in ethanol (70 ml) and HCl 2N (0.2 ml) was refluxed for 10h with stirring. After cooling, the solid was filtered, washed with water, dried and crystallized from suitable solvent.

5.2.1.2.1.5.1. (*E*)-1,5-diphenyl-3-styryl-4,5-dihydro-1*H*-pyrazole (9a). Yield 29%, m.p. 170 – 173 °C from EtOH; Anal. Calc. for $C_{23}H_{20}N_2$: C 85.15, H 6.21, N 8.63; found C 85.46, H 6.23, N 8.60. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.57 – 7.20 (m, 13H, H2- H6, H3', H5', H2"- H6", Hβ), 7.11 (d, 2H, H2', H6', J = 7.6 Hz), 6.86 (t, 1H, H4', J = 6.8 Hz), 6.55 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.28 (dd, 1H, CH, J = 12.0 Hz, J = 6.8 Hz), 3.74 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.0 Hz), 3.05 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.9, 143.8, 143.5, 135.9, 135.2, 129.5, 128.6, 128.5, 128.1, 127.9, 126.9, 126.7, 116.7, 116.3, 120.8, 60.9, 40.9.

5.2.1.2.1.5.2. (*E*)-5-(4-chlorophenyl)-3-(4-chlorostyryl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (9b). Yield 30%, m.p. 221 – 223 °C from EtOH; Anal. Calc. for $C_{23}H_{18}Cl_2N_2$: C 74.24, H 4.61, Cl 18.03, N 7.12; found C 74.00, H 4.61, Cl 18.08, N 7.10. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.37 – 7.23 (m, 11H, H2, H3, H5, H6, H3', H5', H2", H3", H5", H6", Hβ), 6.99 (d, 2H, H2', H6', J = 7.6 Hz), 6.83 (t, 1H, H4', J = 6.8 Hz), 6.52 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.26 (dd, 1H, CH, J = 12.0 Hz, J = 6.8 Hz), 3.71 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.0 Hz), 2.98 (d, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.9, 143.8, 141.6, 135.9, 133.5, 133.3, 132.3, 129.5, 129.0, 128.7, 128.6, 127.2, 120.8, 120.5, 116.7, 60.9, 40.9.

5.2.1.2.1.5.3. (*E*)-5-(4-fluorophenyl)-3-(4-fluorostyryl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (9c). Yield 75%, m.p. 195 – 199 °C from EtOH; Anal. Calc. for $C_{23}H_{18}F_2N_2$: C 76.85, H 5.03, F 10.54, N 7.77; found C 76.63, H 5.05, F 10.52, N 7.77. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.43 – 7.02 (m, 13H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", Hβ), 6.85 (t, 1H, H4', J = 6.8 Hz), 6.54 (d,1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.26 (dd, 1H, CH, J =

12.0 Hz, J = 6.8 Hz), 3.73(dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.0 Hz), 3.0 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 162.1 (d), 160.9 (d), 154.9, 143.8, 139.1, 135.7, 130.8, 130.4 (d), 129.5, 128.5 (d), 120.9, 120.7, 116.7, 115.4 (d), 115.1 (d), 60.8, 40.5.

5.2.1.2.1.5.4. (*E*)-3-(4-methylstyryl)-1-phenyl-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (9d). Yield 76%, m.p. 184 – 187 °C from EtOH; Anal. Calc.$C_{25}H_{24}N_2$: C 85.19, H 6.86, N 7.95; found C 84.88, H 6.87, N 7.93. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7,36 (d, 2H, H2", H6", J = 7.2 Hz), 7.28 – 7.18 (m, 9H, H2, H3, H5, H6, H3', H5', H3", H5", Hβ), 7.03 (d, 2H, H2', H6', J = 7.6 Hz), 6.79 (t, 1H, H4', J = 6.8 Hz) 6.55 (d,1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.24 (dd, 1H, CH, J = 12.0 Hz, J = 6.8 Hz), 3.71 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.0 Hz), 3.01 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.8 Hz), 2.38 (s, 3H, CH_3), 2.35 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 155.1, 144.0, 140.5, 139.2, 137.5, 136.4, 135.9, 129.5, 128.9, 128.9, 125.3, 120.8, 120.2, 116.7, 115.1, 60.9, 40.8, 21.3.

5.2.1.2.1.5.5. (*E*)-5-(4-methoxyphenyl)-3-(4-methoxystyryl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (9e). Yield 60%, m.p. 150 – 152 °C from EtOH; Anal. Calc. for $C_{25}H_{24}N_2O_2$: C 78.10, H 6.29, N 7.29; found C 78.38, H 6.30, N 7.27. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.43 – 6.79 (m, 14H, H2, H3, H5, H6, H2', H3', H4', H5', H6', H2", H3", H5", H6", Hβ), 6.54 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4), 5.21(dd, 1H, CH, J = 12.0 Hz, J = 6.8 Hz), 3.75 (m, 7H, OCH_3, OCH_3, CH2), 2.64 (d, 1H, CH, J_{gem} = 16.8 Hz, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 159.8, 158.6, 154.9, 143.8, 135.9, 135.8, 130.2, 129.5, 127.5, 126.6, 119.9, 120.8, 116.7, 114.2, 114.1, 59.9, 55.8, 40.9,

5.2.1.2.1.5.6. (*E*)-3-(4-methylstyryl)-1-phenyl-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (9f). Yield 77%, m.p. 170 – 174 °C from EtOH; Anal. Calc. for $C_{24}H_{22}N_2$: C 85.17, H 6.55, N 8.28; found C 84.89, H 6.56, N 8.31. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.46 (d, 2H, H2", H6", J = 7.6 Hz), 7.38 – 7.23 (m, 9H, H2-, H6, H3", H4", H5", Hβ), 7.0 (d, 2H, H2', H6', J = 8.0 Hz) 6.94 (d, 2H, H3', H5', J = 8.4 Hz), 6.55 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.25 (dd, 1H, CH, J = 12.0 Hz, J = 6.8 Hz), 3.73 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.0 Hz), 3.03 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.8 Hz), 2.25 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.9, 143.5, 140.8, 135.9, 135.2, 129.8, 129.6, 128.6, 128.5, 127.9, 126.9, 126.7, 120.8, 120.1, 113.4, 60.7, 40.1, 21.3.

5.2.1.2.1.5.7. (*E*)-5-(4-chlorophenyl)-3-(4-chlorostyryl)-1-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (9g). Yield 82%, m.p. 171 – 172 °C from EtOH; Anal. Calc. for $C_{24}H_{20}Cl_2N_2$: C 70.77, H 4.95, Cl 17.41, N 6.88; found C 70.99, H 4.94, Cl 17.36, N 6.86. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.38 – 7.17 (m, 9H, 9H, H2- H6, H3", H4", H5", Hβ), 7.01 (d, 2H, H2', H6', J = 8.0 Hz), 6.90 (d, 2H, H3', H5', J = 8.4 Hz), 6.49 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.23(dd, 1H, CH, J = 12.0 Hz, J = 6.8 Hz), 3.70 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.0 Hz), 2.97 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.8 Hz), 2.26 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 155.0, 141.6, 140.2, 134.9, 133.5, 133.3, 132.3, 129.8, 129.6, 129.0, 128.7, 128.6, 127.2, 120.6, 113.4, 60.9, 40.9, 22.2.

5.2.1.2.1.5.8. (*E*)-5-(4-fluorophenyl)-3-(4-fluorostyryl)-1-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (9h). Yield 72%, m.p. 155 – 160 °C from EtOH; Anal. Calc. for $C_{24}H_{20}F_2N_2$: C 76.99, H 5.38, F 10.15, N 7.48; found C 77.20, H 5.39, F 10.14, N 7.49. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.44 - 7.25 (m, 4H, H2", H3", H5", H6"), 7.15 (d, 1H, Hβ, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 7.08 – 7.0 (m, 6H, H2, H3, H5, H6, H2', H6'), 6.91 (d, 2H, H3', H5', J = 7.2 Hz) 6.52 (d, 1H, Hα,

$J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.23 (dd, 1H, CH, *J* = 12.4 Hz, *J* = 6.4 Hz), 3.69 (d, 1H, CH_2, J_{gem} = 16.8 Hz, *J* = 12.4 Hz), 2.98 (d, 1H, CH_2, J_{gem} = 16.8 Hz, *J* = 6.4 Hz), 2.25 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 162.1 (d), 160.9 (d), 154.9, 140.8, 139.1, 135.9, 130.8, 130.4 (d), 129.8, 129.6, 128.5 (d), 120.8, 115.4 (d), 115.3 (d), 113.4, 60.9, 40.9, 21.5.

5.2.1.2.1.5.9. (*E*)-3-(4-methylstyryl)-1,5-di-p-tolyl-4,5-dihydro-1*H*-pyrazole (9i). Yield 73%, m.p. 150 – 155 °C from EtOH; Anal. Calc. for $C_{26}H_{26}N_2$: C 85.21, H 7.15, N 7.64; found C 85.00, H 7.17, N 7.61. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.34 (d, 2H, H2", H6", *J* = 7.6 Hz), 7.28 – 7.16 (m, 7H, H2, H3, H5, H6, H3", H5", Hβ), 7.00 (d, 2H, H3', H5', *J* = 8.4 Hz), 6.94 (d, 2H, H2', H6', *J* = 8.4Hz), 6.54, (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.20 (dd, 1H, CH, *J* = 12.4 Hz, *J* = 6.4 Hz), 3.69 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, *J* = 12.4 Hz), 2.99 (dd,1H, CH_2, J_{gem} = 16.8 Hz, *J* = 6.4 Hz), 2.37 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.24 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 154.9, 140.8, 140.5, 139.2, 137.6, 136.4, 135.0, 129.9, 129.4, 128.8, 128.3, 125.3, 119.9, 114.1, 59.8, 39.8, 22.0, 21.9, 21.2.

5.2.1.2.1.5.10. (*E*)-5-(4-methoxyphenyl)-3-(4-methoxystyryl)-1-(p-tolyl)-4,5-dihydro-1*H*-pyrazole (9j). Yield 83%, m.p. 171 – 175 °C from EtOH; Anal. Calc. for $C_{26}H_{26}N_2O_2$: C 78.36, H 6.58, N 7.03; found C 78.64, H 6.58, N 7.01. ^{1}H-NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.39 – 6.89 (m, 13H, H2, H3, H5, H6, H2', H3', H5', H6', H2", H3", H5", H6", Hβ), 6.53 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.15 (dd, 1H, CH, *J* = 12.4 Hz, *J* = 6.4 Hz), 3.76 (m, 7H, OCH_3, OCH_3, CH_2), 2.97 (dd,1H, CH_2, J_{gem} = 16.8 Hz, *J* = 6.4 Hz), 2.31 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ (ppm): 159.8, 158.6, 154.9, 140.8, 135.9, 135.8, 130.2, 129.8, 129.6, 127.5, 126.6, 120.7, 114.2, 114.1, 113.4, 60.9, 55.8, 41.0, 22.2.

5.2.1.2.1.5.11. (*E*)-4-(5-phenyl-3-styryl-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamide (9k). Yield 80%, m.p. 272 – 275 °C from EtOH; Anal. Calc. for $C_{23}H_{21}N_3O_2S$: C 68.46, H 5.25, N 10.41, S 7.95; found C 68.70, H 5.26, N 10.39, S 7.93. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.6 – 7.03 (m,17H, H2- H6, H2', H3', H5', H6', H2''- H6'', Hβ, NH_2), 6.89 (d, 1H, Hα, $J_{\alpha-\beta}$ = 16.4 Hz), 5.62 (dd, 1H, CH, J = 12.4 Hz, J = 6.4 Hz), 3.82 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.4 Hz), 3.06 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 156.2, 147.0, 143.5, 135.9, 135.2, 130.3, 129.3, 128.6, 128.5, 128.2, 127.9, 126.9, 126.7, 120.2, 113.8, 60.7, 41.0.

5.2.1.2.1.5.12. (*E*)-4-(5-(4-chlorophenyl)-3-(4-chlorostyryl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (9l). Yield 50%, m.p. 155 – 157 °C from EtOH; Anal. Calc. for $C_{23}H_{19}Cl_2N_3O_2S$: C 58.48, H 4.05, Cl 15.01, N 8.90, S 6.79; found C 58.65, H 4.06, Cl 14.97, N 8.88, S 6.80. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.74 – 7.59 (m, 4H, H2, H6, H3', H5'),7.44 – 7.41(m, 4H, H3, H5, H2'', H6''), 7.32 – 7.19 (m, 3H, H3'', H5'', Hβ), 7.03 – 7.01 (m, 4H, H2', H6', NH_2), 6.89 (d, 1H, Hα, $J_{\alpha-\beta}$ = 16.4 Hz), 5.67 (dd, 1H, CH, J = 12.4 Hz, J = 6.4 Hz), 3.84 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 12.4 Hz), 3.05 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 154.9, 147.0, 141.6, 135.9, 133.5, 133.3, 132.3, 130.0, 129.3, 129.0, 128.7, 128.6, 127.2, 121.2, 114.0, 408, 61.2.

5.2.1.2.1.5.13. (*E*)-4-(5-(4-fluorophenyl)-3-(4-fluorostyryl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (9m). Yield 46%, m.p. 209 – 211 °C from EtOH; Anal. Calc. for $C_{23}H_{19}F_2N_3O_2S$: C 62.86, H 4.36, F 8.65, N 9.56, S 7.30; found C 63.03, H 4.37, F 8.62, N 9.59, S 7.29. ^{1}H-NMR (DMSO-d_6, 400

MHz): δ (ppm) 7.67 – 7.58 (m, 4H, H3', H5', H2", H6"), 7.26 – 7.16 (m, 7H, H2, H3, H5, H6, H5", H6", Hβ), 7.04 – 7.01 (m, 4H, H2', H6', NH_2), 6.90 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.65 (dd, 1H, CH, *J* = 12.4 Hz, *J* = 6.4 Hz), 3,7(dd, 1H, CH_2, J_{gem} = 16.8 Hz, *J* = 12.4 Hz), 3,0 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, *J* = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 162.11 (d), 160.92 (d), 155.28, 147.87, 139.13, 136.24, 130.89, 130.46 (d), 130.11, 129.37, 128.50 (d) 121.58, 116.19, 115.55 (d), 114.33 (d), 61.6, 41.5.

5.2.1.2.1.5.14. (*E*)-4-(3-(4-methylstyryl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (9n). Yield 77%, m.p. 215 – 218 °C from EtOH; Anal. Calc. for $C_{25}H_{25}N_3O_2S$: C 69.58, H 5.84, N 9.74, S 7.43; found C 69.79, H 5.82, N 9.72, S 7.44. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.58 – 7.48 (m, 4H, H3', H5', H2", H6",), 7.23 – 6.99 (m, 11H, H2, H3, H5, H6, H2', H6', H3", H5", Hβ, NH_2), 6.84 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.55 (dd, 1H, CH, *J* = 12.4 Hz, *J* = 6.4 Hz), 3.77(dd, 1H, CH_2, J_{gem} = 16.8 Hz, *J* = 12.4 Hz), 3.01 (dd,1H, CH_2, J_{gem} = 16.8 Hz, *J* = 6.4 Hz), 2.31 (s, 3H, CH_3), 2.25 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 155.02, 146.91, 140.55, 139.23, 137.68, 136.46, 135.94, 130.07, 129.35, 128.93, 128.81, 128.55, 125.34, 120.81, 115.11, 62.27, 42.12, 24.31.

5.2.1.2.1.5.15. (*E*)-4-(5-(4-methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (9o). Yield 88%, m.p. 200 – 203 °C from EtOH; Anal. Calc. for $C_{25}H_{25}N_3O_4S$: C 64.78, H 5.44, N 9.06, S 6.92; found C 64.99, H 5.44, N 9.03, S 6.93. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.57 – 7.53 (m, 4H, H3', H5', H2", H6"), 7.14 – 6.88 (m, 11H, H2, H3, H5, H6, H2', H6', H3", H5", Hβ, NH_2), 6.83 (d, 1H, Hα, $J_{\alpha\text{-}\beta}$ = 16.4 Hz), 5.54 (dd, 1H, CH, *J* = 12.4 Hz, *J* = 6.4 Hz), 3.73 (m,7H, CH_2, OCH_3,

OCH_3), 3.01 (dd, 1H, CH_2, J_{gem} = 16.8 Hz, J = 6.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.82, 158.65, 154.91, 147.02, 135.97, 135.85, 130.21, 130.03, 129.32, 127.51, 126.64, 120.80, 114.12, 113.87, 60.91, 55.89, 41.14.

5.2.1.2.2. Biology

The cytotoxicity and antiviral assays were performed by Prof. Roberta Loddo, Department of Biomedical Sciences, Microbiology and Virology Section, University of Cagliari.

The new compounds were evaluated following the experimental protocol described in chapter 5.1.1.3. and 5.1.2.1.

5.2.1.2.3. Time of addition assay

A time-of-addition experiment was carried out with BHK-21 cells. The confluent monolayers of BHK-21 cells, seed in 96-well tissue culture plates were inoculated at room temperature with 50000 PFU of YFV, corresponding to a multiplicity of infection of 1 PFU/cell. After adsorption for 60 min, the monolayers were washed two times with maintenance medium in the presence of FBS inactivated and incubated with the same medium at 5% CO_2 and 37 °C. The test medium containing 10 × EC_{50} compound **7a** and **7b** concentration or 4 × EC_{50} 6-Azauridine concentration was added at –1 to 0 (adsorption), 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10. After each incubation period, the monolayers were washed two times with maintenance medium and incubated with fresh medium until 20 hrs post-infection. Then, after 24–36 hrs post-infection the CPE was evaluated and the monolayers were collected, centrifuged and frozen at -80 °C. The viral titre was determined by a plaque reduction assay.

References

1. Neyts J, Meerbach A, McKenna P, De Clercq E. Use of the yellow fever virus vaccine strain 17D for the study of strategies for the treatment of yellow fever virus infections. *Antiviral Res*. (**1996**) 30, 125-32.
2. Lin CF, Yang JS, Chang CY, Kuo SC, Lee MR, Huang LJ. *Bioorgan. Med. Chem.* (**2005**) 13, 1537-1544.
3. Blankenbuehler M, Parkin S. *Heterocycles*. (**2002**) 57, 2383-2391
4. Jiang Q, Jia J, Xu B, Zhao A, Guo C. *J. Org. Chem*. (**2015**) 80, 3586-3596.
5. Jiang Q, Jia J, Xu B, Zhao A, Guo CC. *J. Org. Chem*. (**2015**) Vol. 80, pp. 3586-3596.
6. Larionov VA, Markelova EP, Smol'yakov AF, Savel'yeva TF, Maleev VI, Belokon YN. *RSC Advances*. (**2015**) 5, 72764-72771.
7. Robinson SJ, Petzer JP, Terre'Blanche G, Petzer A, Van Der Walt MM, Bergh JJ, Lourens ACU. *Eur. J. Med. Chem*. (**2015**) 104, 177-188.
8. Huang D, Wang JX, Hu Y, Zhang Y, Tang J. *Synthetic Commun*. (**2002**) 32, 971-979.
9. Wilhelm A, Lopez-Garcia LA, Busschots K, Froehner W, Maurer F, Boettcher S, Zhang H, Schulze JO, Biondi RM, Engel M. *J. Med. Chem*. (**2012**) 55, 9817-9830.
10. Zhu Ji, Srikanth Natarajan, Ng Siu-Choon, Kon Oi-Lian, Sim Keng-Yoow. *J. Chem. Res. Miniprint*. (**1994**) 3, 672-682.
11. Sasaki Tadashi, Eguchi Shoji, Tanaka Yumo. *Tetrahedron*. (**1980**) 36, 1565-1569.

5.2.2. Conclusions and perspectives

The antiviral screening of the novel series of 1,3,5-trisubstituted pyrazolines (**4a-i**, **5a-j**, **6a-j**, **7a-j**, **9a-o**) has led to identification of new hit compounds with promising activity against YFV and BVDV. SAR studies showed that the presence of a sulfonamide group at the *para* position of the N1-phenyl ring is a requirement for anti-YFV potency in a low micromolar range. Several benzenesulfonamides exhibited up to 40-fold higher potency

against YFV than the reference inhibitor 6-Azauridine. However, while compounds **5a-j**, **6a-j** and **9a-o** suffered from significant cytotoxicity, the benzyloxy derivatives **7a-j** presented a better selectivity due to the low cytotoxicity. For the high potency against YFV coupled with low cytotoxicity, compounds **7a** and **7b** were selected for time of addition experiments. These studies showed a similar behavior of **7a**, **7b** and 6-Azauridine, however, further investigations are necessary for the elucidation of the mechanism of action. The new hit compounds will be submitted to a further systematic optimization process addressed not only to the improvement of potency but also to the decrease of cytotoxicity.