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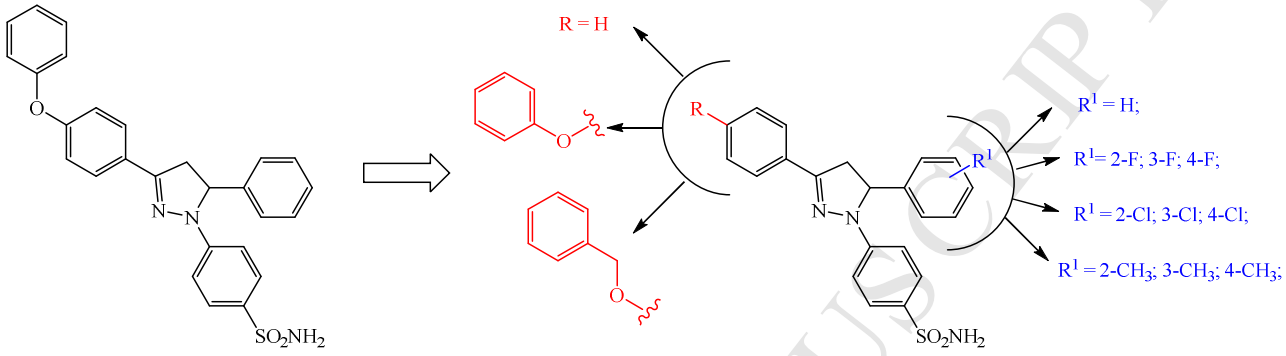

5a

Inhibitors of Yellow Fever Virus replication based on 1,3,5-triphenyl-4,5-dihydropyrazole scaffold: design, synthesis and antiviral evaluation

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ABSTRACT

By the antiviral screening of an in house library of pyrazoline compounds, 4-(3-(4-phenoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**5a**) was identified as a promising hit compound for the development of anti- Yellow Fever Virus (YFV) agents. Structural optimization studies were focused on the development of **5a** analogues which retain the potency as YFV inhibitors and show a reduced cytotoxicity. The synthesized 1-3,5-triphenyl-pyrazolines (**4a-j**, **5a-j**, **6a-j**) were evaluated in cell based assays for cytotoxicity and antiviral activity against representative viruses of two of the three genera of the Flaviviridae family, i.e.: Pestivirus (BVDV) and Flavivirus (YFV). These compounds were also tested against a large panel of different pathogenic RNA and DNA viruses. Most of the new 1-3,5-triphenyl-pyrazolines (**4a-j**, **5a-j**, **6a-j**) exhibited a specific activity against YFV, showing EC_{50} values in the low micromolar range with almost a 10-fold improvement in potency compared to the reference inhibitor 6-azauridine. However, the selectivity indexes of the unsubstituted (**4a-j**) and the phenoxy (**5a-j**) analogues were generally modest due to the pronounced cytotoxicity against BHK-21 cells. Otherwise, the benzyloxy derivatives (**6a-j**) generally coupled high potency and selectivity. On the basis of both anti-YFV activity and selectivity index, pyrazolines **6a** and **6b** were chosen for time of addition experiments. The selected pyrazolines and the reference inhibitor 6-azauridine displayed maximal inhibition when added in the pretreatment or during the infection.

Keywords:
4,5-Dihydropyrazole derivatives;
Antiviral activity;
YFV;
BVDV;
RNA and DNA viruses

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1. Introduction

Viruses belonging to the *Flaviviridae* family are a major cause of human and veterinary infectious diseases of global importance. The family consists of four genera: Flavivirus, Pestivirus, Hepacivirus and Pegivirus. The largest genus Flavivirus comprises more than 50 species of arthropod-borne viruses that can infect mammalians and birds, usually transmitted to vertebrate by ticks or mosquitoes, and causing severe diseases and even mortality. Yellow fever virus (YFV) is the type member of the genus Flavivirus and was the first human pathogenic virus isolated in Ghana in 1927. West-Nile Virus (WNV), Dengue Virus (DENV), Japanese Encephalitis Virus (JEV) and Tick-Borne Encephalitis Virus (TBEV) are other human emerging or reemerging pathogens belonging to Flavivirus genus. The flaviviral infections result in symptoms that range from mild febrile illness to severe hemorrhagic fever or neurological disease. [1]

The Pestivirus genus comprises three important animal pathogens causing significant economic losses to the livestock industry: Bovine Viral Diarrhea Virus (BVDV), Classical Swine Fever Virus (CSFV), and Border Disease Virus (BDV). [2] BVDV, the type member of this genus, can infect cattle, swine and wild ruminant causing clinical manifestations including severe mucosal ulceration of mouth, nose and intestine, in addition to immune system dysfunction with predisposition to secondary infections, abortion and teratogenesis.

The Hepacivirus genus includes the hepatitis C virus (HCV), an important human pathogen targeting the liver. HCV initially causes an acute infection, generally asymptomatic, the 55-85% of infected patients develop chronic hepatitis C infection with high risk of cirrhosis or liver cancer. The WHO estimates that between 130–150 million people worldwide have chronic hepatitis C infection and that about 700,000 people die each year for the consequence of HCV infection. [3]

Human Pegivirus (HPgV), previously reported as GB virus C (GBV-C) or hepatitis G virus (HGV), has been recently classified as a member of the species Pegivirus C in Pegivirus genus. [4] Other members of Pegivirus C and members of Pegivirus A - K can persistently infect a range of mammalian species. However, their infections are not clearly related with the development of any disease, with the exception of Theiler's disease in horses infected with Theiler's disease associated virus, Pegivirus D. [5]

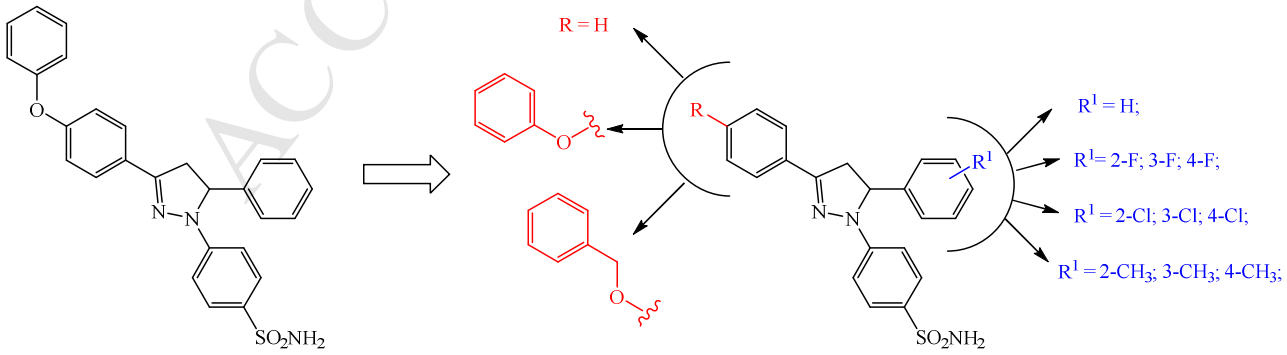
To date, human vaccines are available only for YFV, TBEV and JEV. However, their utilization encounters limitations and difficulties, and many thousands of Flaviviridae infections continue to occur each year in the endemic areas. In particular, YF vaccine is both highly effective and relatively safe, conferring a life-long protection against YF disease. Vaccine associated side effects are usually mild while severe adverse reactions to the liver, kidneys or nervous system are uncommon (less than 1 case per 100,000 people vaccinated). However, YF vaccine is contro-

indicated for immune-suppressed individuals, people with severe allergies to egg proteins, infants under 9 months of age and pregnant women. In 1988, WHO and UNICEF recommended that YF vaccine be involved in routine immunization program in countries at risk in Africa and Americas. As of 2016, YF vaccine had been introduced in routine infant immunization programs in 35 of the 42 countries at risk. In these 42 countries, coverage is estimated at 45%. The goal is to achieve a vaccine coverage > 80% conducting preventive mass vaccination campaigns of the populations at risk. [6, 7]

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. [3] However, to date no specific drug has been approved to combat Flavivirus and Pestivirus infections, and patient care remains symptomatic.

By the antiviral screening of an in house library of pyrazoline compounds, 4-(3-(4-phenoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**5a**) (Figure 1) was identified as a potent inhibitor of YFV replication (EC_{50} = 2.1 μM). However, its selectivity index (SI = 6.2) was modest due to the considerable cytotoxicity (CC_{50} = 13 μM) (data not published).

As a part of our research on Flaviviridae inhibitors [8, 9], in an effort to identify low cytotoxic compounds and to retain or to improve the anti-flavivirus activity of the hit compound, **5a**, the 4-phenoxy substituent on the phenyl ring at the 3 position of pyrazoline moiety was removed or replaced by a bulkier benzyloxy substituent in the same position. Moreover, either electron-donor or withdrawing groups were introduced at the *ortho*, *meta* or *para* position of the 5-phenyl substituent (Figure 1).



5a

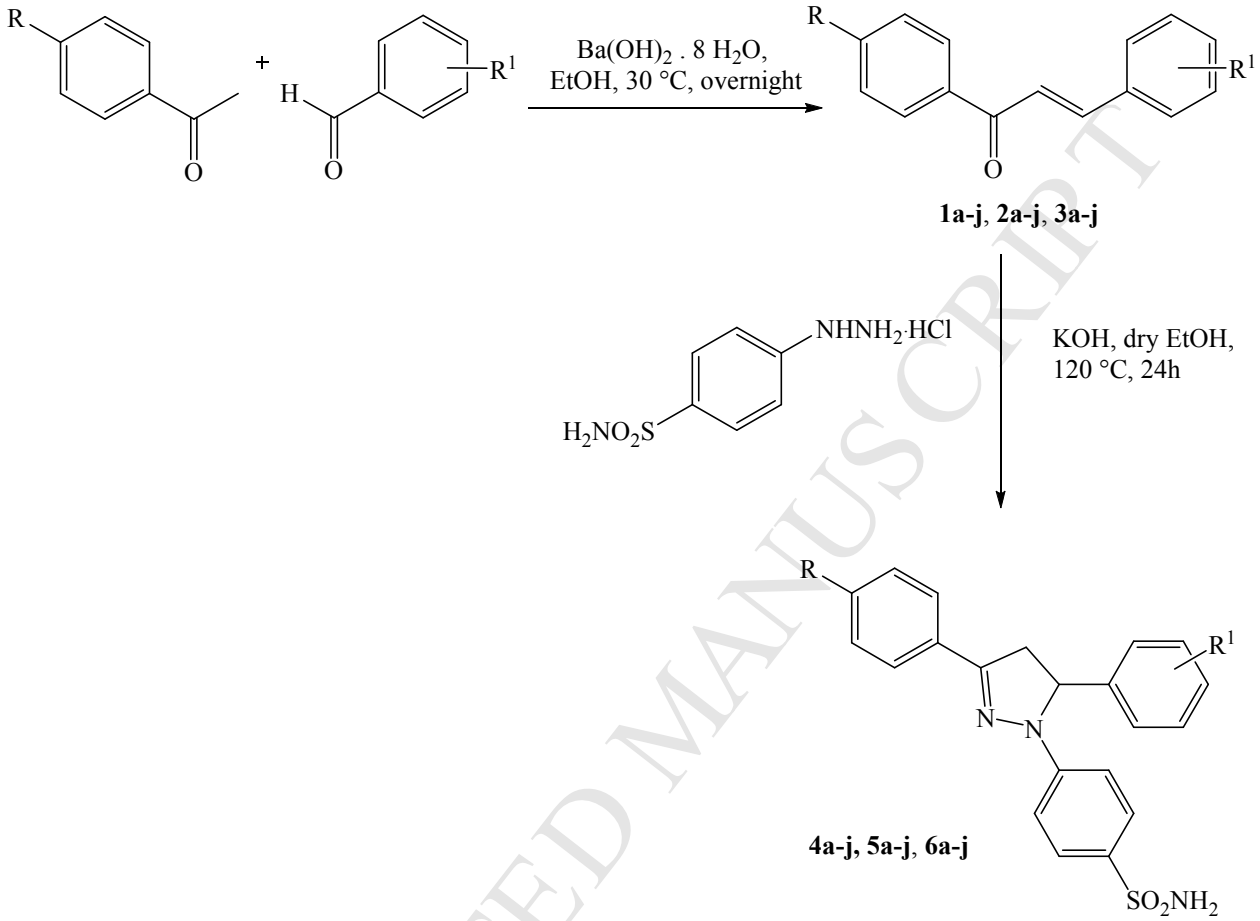
Figure 1. Structure of **5a** based 1,3,5-triphenylpirazolines designed for the development of new inhibitors of YFV replication.

2. Results and discussion

2.1. Chemistry

The 1,3,5-trisubstituted 4,5-dihydropyrazoles (**4a-i, 5a-j, 6a-j**) were synthesized according to Scheme 1. Chalcones **1a-l, 2a-j** and **3a-j** were obtained by Claisen-Schmidt condensation between substituted benzaldehydes and suitable acetophenones in basic alcoholic medium. The subsequent reaction between the appropriate chalcone with 4-hydrazinylbenzenesulfonamide hydrochloride in basic alcoholic medium provided compounds **4a-i**, **5a-j** and **6a-j**. 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 7.2 – 8.2 ppm) and the presence of three double doublets for the two methylene protons in position 4 (~ 4.0 ppm and ~ 3.1 ppm) and for the proton in position 5 (~ 5.6 ppm).

Scheme 1. Synthesis of 4-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamides (**4a-j**, **5a-j**, **6a-j**).



1a, 4a: R = R^1 = H
1b, 4b: R = H, R^1 = 2-F
1c, 4c: R = H, R^1 = 3-F
1d, 4d: R = H, R^1 = 4-F
1e, 4e: R = H, R^1 = 2-Cl
1f, 4f: R = H, R^1 = 3-Cl
1g, 4g: R = H, R^1 = 4-Cl
1h, 4h: R = H, R^1 = 2-CH_3
11, 4i: R = H, R^1 = 3-CH_3
1j, 4j: R = H, R^1 = 4-CH_3

2a, 5a: R = OPh, R^1 = H
2b, 5b: R = OPh, R^1 = 2-F
2c, 5c: R = OPh, R^1 = 3-F
2d, 5d: R = OPh, R^1 = 4-F
2e, 5e: R = OPh, R^1 = 2-Cl
2f, 5f: R = OPh, R^1 = 3-Cl
2g, 5g: R = OPh, R^1 = 4-Cl
2h, 5h: R = OPh, R^1 = 2-CH_3
21, 5i: R = OPh, R^1 = 3-CH_3
2j, 5j: R = OPh, R^1 = 4-CH_3

3a, 6a: R = OBn, R^1 = H
3b, 6b: R = OBn, R^1 = 2-F
3c, 6c: R = OBn, R^1 = 3-F
3d, 6d: R = OBn, R^1 = 4-F
3e, 6e: R = OBn, R^1 = 2-Cl
3f, 6f: R = OBn, R^1 = 3-Cl
3g, 6g: R = OBn, R^1 = 4-Cl
3h, 6h: R = OBn, R^1 = 2-CH_3
31, 6i: R = OBn, R^1 = 3-CH_3
3j, 6j: R = OBn, R^1 = 4-CH_3

2.2. Antiviral tests

Cytotoxicity and antiviral activity of the new compounds (**4a-j, 5a-j** and **6a-j**) and reference inhibitors are reported in Tables 1 and 2.

Each compound was initially evaluated in cell based assays for its cytotoxicity and antiviral activity against Yellow Fever Virus (YFV) and Bovine Viral Diarrhea Virus (BVDV) representative members of the Flavivirus genus and the Pestivirus genus, respectively. The derivatives able to interfere with YFV and/or BVDV replication were also tested against two other viruses belonging to the Flavivirus genus of the *Flaviviridae* family (Dengue Virus, DENV-2 and West-Nile Virus, WNV). 6-Azauridine, ribavirin and 2′-C-methyl-guanosin (NM108) were employed as reference inhibitors of these single-stranded, positive RNA ($ssRNA^+$) viruses.

Moreover, in order to verify the specific inhibition of our pyrazoline derivatives on *Flaviviridae* replication, all the analogues were tested against a larger panel of RNA and DNA viruses. Among $ssRNA^+$ viruses, a retrovirus (Human Immunodeficiency Virus type 1, HIV-1), and two Picornaviruses (Coxsackie Virus type-5, CVB-5, and Poliovirus type-1, Sabin strain, Sb-1) were also included. Among single-stranded, negative RNA ($ssRNA^-$) viruses a *Paramyxoviridae* (Respiratory Syncytial Virus, RSV) and a *Rhabdoviridae* (Vesicular Stomatitis Virus, VSV) were considered. Among double-stranded RNA (dsRNA) viruses, a *Reoviridae* family member (Reo-1) was selected. Finally, two representatives of DNA virus families were considered: Herpes Simplex Virus type-1, HSV-1 (*Herpesviridae*) and Vaccinia Virus, VV (*Poxviridae*). 6-Azauridine, ribavirin, efavirenz (EFV), pleconaril, acyclovir (ACG), and mycophenolic acid (M5255) were used as reference inhibitors.

As far as the antiviral activity reported in Table 1, the majority of the new 1-3,5-triphenyl-pyrazolines (**4a-j, 5a-j, 6a-j**) interfered with YFV replication in the low micromolar concentrations (EC_{50} ranging from 1.8 μM to 2.6 μM) providing almost a 10-fold improvement in potency compared to the reference inhibitor 6-azauridine (EC_{50} = 20.0 μM). However, the unsubstituted (**4a-j**) and the phenoxy (**5a-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 (**6a-j**) showed lower cytotoxicity and higher selectivity indexes, as a consequence. In particular, **6a** (R^1 = H) and the fluoro substituted derivatives **6b** and **6d** coupled high potency and selectivity. In addition, the majority of the benzyloxy derivatives (**6b**, **6c**, **6e-g**, **6i**, **6j**) inhibited also the BVDV replication, generally showing higher activity and selectivity than the reference compound ribavirin (EC_{50} = 20.0 μM, SI = 3).

All the analogues were inactive against the other two members of the Flavivirus genus (WNV and DENV-2) utilized in the antiviral tests (Table 1).

When tested against HIV-1, Reo-1, Sb-1, VV, HSV-1, VSV, and RSV the compounds were generally devoid of antiviral activity up to the highest concentration tested, although five phenoxy derivatives (**5a-e**) and three benzyloxy analogues (**6c**, **6i**, **6j**) inhibited CVB-5 replication with EC_{50}

ranging from 3.5 µM to 13.0 µM (Table 2), while five compounds interfered with HIV-1 replication showing $EC_{50} \geq 10$ µM and modest selectivity (Table 2).

2.3. Time of addition studies

1,3,5-Triphenyl-pyrazolines **6a** and **6b** 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 = 36.4 and 50.0, respectively). Time of addition experiments were performed to determine the possible step(s) in YFV replication cycle that is inhibited by analogues **6a** and **6b**. For this purpose, the test medium containing approximately 10 times higher than the EC_{50} of selected compounds **6a** and **6b** concentration (20 µM) or 4 times higher than the EC_{50} of 6-azauridine concentration (90 µM), used as reference inhibitor, were added for two hours before the infection (pre-treatment), during the two hours of infection of BHK-21 cell cultures with YFV and every two hours post infection (p.i.) from time 0 to 10 hrs p.i (0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10 h). 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). [10]

After each incubation period, the monolayers were washed two times with maintenance medium and incubated with fresh medium until 10 hrs post-infection. Then, after 24–36 hrs post-infection the cytopathogenic effect (CPE) was evaluated and the monolayers were collected, centrifuged and frozen. The viral titre was determined by a plaque reduction assay.

Data represented in Figures 2 and 3 indicate that both pyrazolines **6a** and **6b** retained their inhibitory activity when added in the pretreatment and during the infection, before losing their antiviral efficacy. Indeed, the virus titre decreases when the compound is added to cells during pretreatment or during two hours of infection, while the viral titre is very similar to that of the untreated control when the compound is added in the subsequent phases of the infection. A similar behavior was observed for the reference inhibitor, at higher concentrations. The comparison of the curves obtained for our compounds and for the reference inhibitor could suggest a similar mechanism of action. Like 6-arauridine, both pyrazolines **6a** and **6b** could act by blocking the entry of the virus into the cell or interfere with some mechanism during the early stages of the infection. However, further investigations are necessary for the identification of the anti-YFV target of these pyrazoline derivatives.

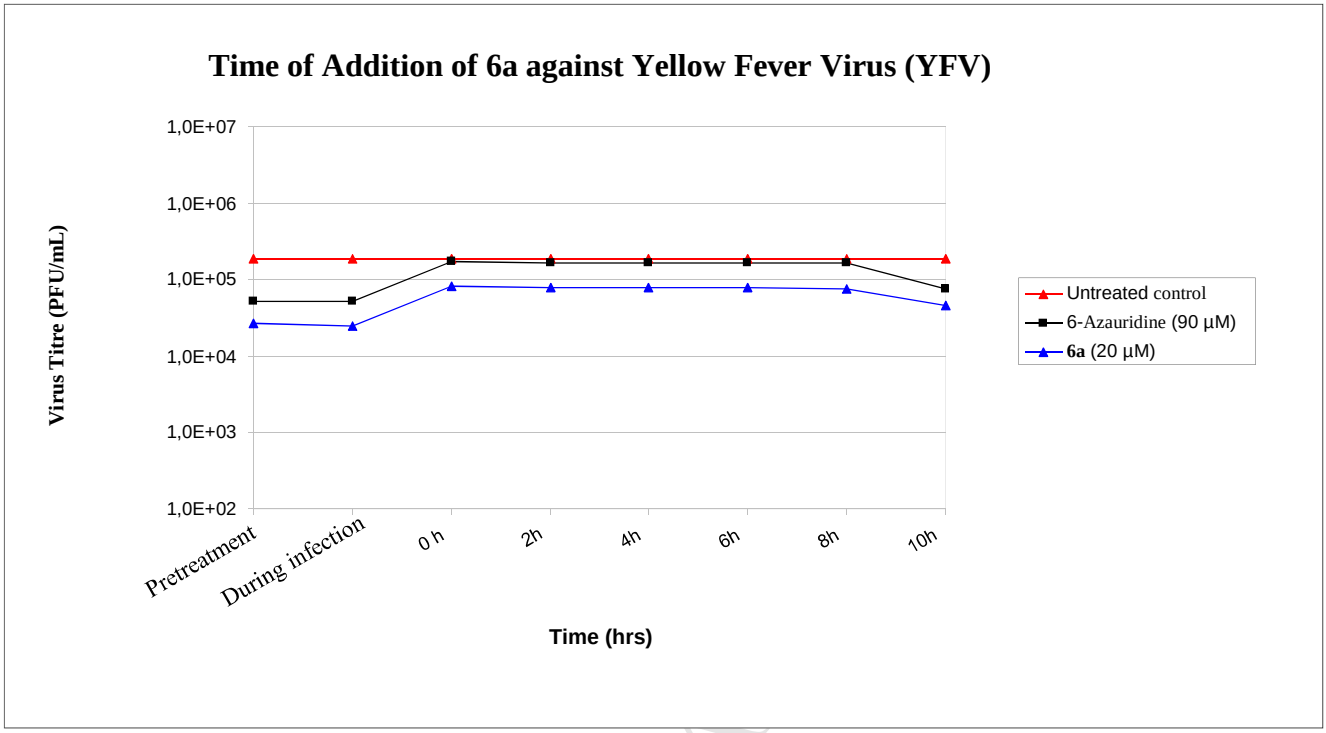


Figure 2. Effect of time of addition on anti-YFV activity of derivative **6a** (blue). For comparison, the same test was performed using the reference compound 6-azauridine (black). In red we observe the untreated control.

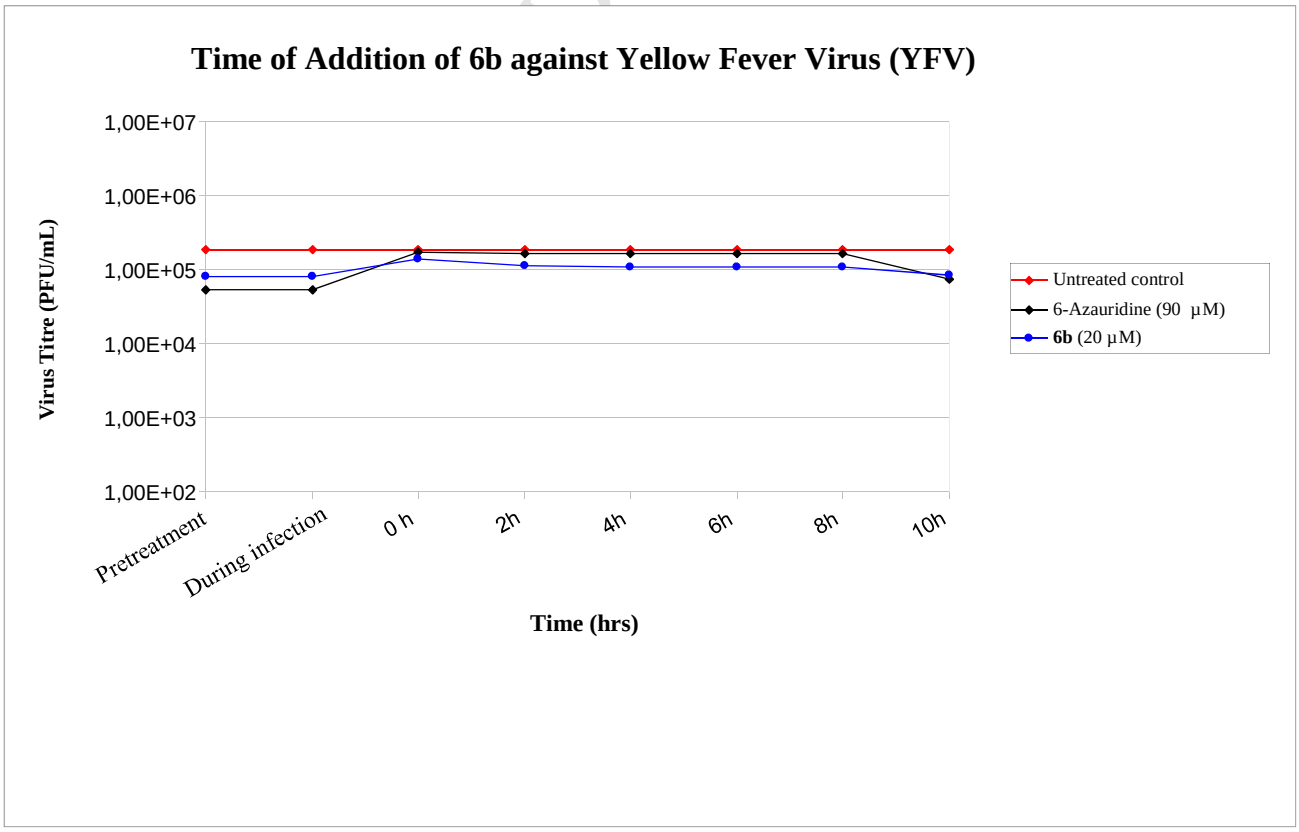


Figure 3. Effect of time of addition on anti-YFV activity of derivative **6b** (blue). For comparison, the same test was performed using the reference compound 6-azauridine (black). In red we observe the untreated control.

3. Conclusion

In conclusion, pursuing our research on antiviral compounds, in this paper we described the design and synthesis of new series of 1,3,5-triphenyl-4,5-dihydropyrazole derivatives that were endowed with a noticeable and generally specific anti-YFV activity. In particular, analogues bearing a bulky 4-benzyloxy substituent on the phenyl ring at the 3 position of pyrazoline moiety, coupled potency in the low micromolar range with low cytotoxicity resulting in selective compounds.

4. Experimental section

4.1. Chemistry

Chemicals were purchased from Sigma-Aldrich or AlfaAesar 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 aluminum oxide fluorescent coated plates (Fluka, DC-Alufolien Kieselgel or aluminum oxide F254).

4.1.1. General procedure for the synthesis of chalcones (1a-j, 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-j**, **2a-j** and **3a-j** are identical to those previously reported. [11-16]

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

A mixture of the appropriate chalcone **1a-j**, **2a-j** and **3a-j** (10 mmol) and 4-hydrazinylbenzenesulfonamide hydrochloride (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.

4.1.2.1. 4-(3,5-Diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4a). Yield: 69%, m.p. = 212 – 214 °C from EtOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.48 - 7.30 (m, 5H), 7.29 - 7.20 (m, 3H), 7.11 – 7.03 (m, 4H), 5.63 (dd, 1H, CH, *J* = 12.0, Hz, *J* = 5.1 Hz), 3.97 (dd, 1H, CH_2, J_{gem} = 17.7 Hz, *J* = 12.2 Hz), 3.16 (dd, 1H, CH_2, J_{gem} = 17.7 Hz, *J* = 5.1 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.2, 146.3, 142.0, 133.3, 132.2, 129.9, 129.6, 129.2, 128.2, 127.6, 126.5, 126.1, 112.5, 62.7, 43.4. MS-ESI: m/z 378 ($M + H^+$).

4.1.2.2. 4-(5-(2-Fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4b). Yield: 27%, m.p. = 260 – 262 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (dd, 2H, *J* = 7.9 Hz, *J* = 1.3 Hz); 7.61 (d, 2H, *J* = 9.0), 7.48 - 7.21 (m, 5H), 7.18 – 6.90- (m, 6H), 5.80 (dd, 1H, *J* = 12.3 Hz, *J* = 5.1 Hz); 4.00 (dd, 1H, J_{gem} = 17.7, *J* = 12.3 Hz); 3.24 (dd, 1H, J_{gem} = 17.7, *J* = 5.1 Hz).^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 160.0 (d, *J* = 244 Hz), 150.5, 146.1, 133.6, 132.0, 130.4 (d, *J* = 8 Hz), 129.9, 129.2, 128.2 (d, *J* = 14 Hz), 128.1, 127.7, 126.6, 125.4 (d, *J* = 4 Hz), 116.6 (d, *J* = 20 Hz), 112.3, 57.4, 42.1. MS-ESI: m/z 396 ($M + H^+$).

4.1.2.3. 4-(5-(3-fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4c). Yield: 20%, m.p. = 158 – 160 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 (d, 2H, *J* = 6.8 Hz); 7.61 (d, 2H, *J* = 8.8 Hz); 7.50-7.34 (m, 5H), 7.14-7.03 (m, 6H), 5.66 (dd, 1H, *J* = 12.0 Hz, *J* = 4.95 Hz), 3.97 (dd, 1H, J_{gem} = 17.7 Hz, *J* = 12.2 Hz), 3.21 (dd, J_{gem} = 17.7 Hz, *J* = 5.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 163.9 (d, *J* = 244 Hz), 150.3, 146.2, 144.9, (d, *J* = 7 Hz), 133.6, 132.0, 131.8 (d, *J* = 8 Hz), 129.9, 129.2, 127.7, 126.6, 122.1, 115.0 (d, *J* = 21 Hz), 113.2 (d, *J* = 22 Hz), 112.5, 62.3, 43.2. MS-ESI: m/z 396 ($M + H^+$).

4.1.2.4. 4-(5-(4-Fluorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4d). Yield: 77%, m.p. = 213 – 218 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 (d, 2H, *J* = 8.4 Hz); 7.59 (d, *J* = 8.9 Hz, 2H), 7.47-7.38 (m, 3H); 7.32-7.26 (m, 2H); 7.16 (t, *J* = 8.9 Hz, 2H); 7.10-7.05 (m, 4H); 5.65 (dd, *J* = 12.0 Hz, 5.07 Hz, 1H), 3.96 (dd, J_{gem} = 17.8 Hz, *J* = 12.1 Hz, 1H); 3.17 (dd, J_{gem} = 17.8, *J* = 5.1 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.9 (d,

J = 242 Hz), 150.1, 146.3, 138.2 (d, *J* = 3 Hz), 133.5, 132.1, 129.9, 129.2, 128.3 (d, *J* = 8 Hz), 127.6, 126.5, 116.4 (d, *J* = 21 Hz), 112.5, 62.1, 43.3. MS-ESI: m/z 396 ($M + H^+$).

4.1.2.5. 4-(5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4e). Yield: 22%, m.p. = 228 – 231 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 2H); 7.62 (d, *J* = 8.9 Hz, 2H); 7.13-7.03 (m, 5H); 7.03-6.97 (m, 6H); 5.91 (dd, *J* = 12.2 Hz, *J* = 5.1 Hz, 1H) 4.16-3.88 (dd, J_{gem} = 17.8 Hz, *J* = 12.2 Hz, 1H); 3.24-2.98 (dd, J_{gem} = 17.8 Hz, *J* = 5.1 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.4, 146.0, 138.3, 133.6, 132.2, 131.9, 131.7, 130.7, 130.04, 129.9, 129.2, 128.9, 128.8, 128.4, 112.2, 60.43, 42.1. MS-ESI: m/z 412 ($M + H^+$).

4.1.2.6. 4-(5-(3-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4f). Yield: 32%, m.p. = 120 – 123 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 (d, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.9 Hz, 2H); 7.48-7.30 (m, 6H); 7.18 (d, *J* = 7.5 Hz, 1H); 7.11-7.05 (m, 4H); 5.66 (dd, *J* = 12.2 Hz, *J* = 5.1 Hz, 1H); 3.96 (dd, J_{gem} = 17.8 Hz, *J* = 12.2 Hz, 1H), 3.20 (dd, J_{gem} = 17.8 Hz, *J* = 5.1 Hz, 1H).^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.3, 146.2, 144.5, 134.1, 133.6, 132.0, 131.6, 130.0, 129.2, 128.2, 127.7, 126.6, 126.1, 124.8, 112.5, 62.2, 43.2. MS-ESI: m/z 412 ($M + H^+$).

4.1.2.7. 4-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4g). Yield: 37%, m.p. = 203 – 205 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 6.7 Hz, 2H); 7.61 (d, *J* = 8.8 Hz, 2H); 7.49-7.39 (m, 5H); 7.28 (d, *J* = 8.4 Hz, 2H); 7.11-7.02 (m, 4H); 5.68 (dd, *J* = 12.0 Hz, *J* = 5.0 Hz, 1H); 3.98 (dd, J_{gem} = 17.7 Hz, *J* = 12.1 Hz, 1H); 3.21 (dd, J_{gem} = 17.7 Hz, *J* = 5.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.2, 146.2, 141.0, 133.7, 132.6, 132.1, 129.9, 129.6, 129.2, 128.2, 127.7, 126.5, 112.5, 62.1, 43.2. MS-ESI: m/z 412 ($M + H^+$).

4.1.2.8. 4-(3-Phenyl-5-(o-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4h). Yield: 23%, m.p. = 204 – 206 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 7.0 Hz, 2H); 7.60 (d, *J* = 8.9 Hz, 2H); 7.49-7.35 (m, 4H); 7.22 (t, *J* = 7.6 Hz, 1H); 7.12-7.01 (m, 6H); 5.58 (dd, *J* = 12.1 Hz, *J* = 5.3 Hz, 1H); 3.97 (dd, J_{gem} = 17.7 Hz, *J* = 12.2 Hz, 1H); 3.17 (dd, J_{gem} = 17.7 Hz, *J* = 5.3 Hz, 1H); 2.26 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.1, 146.4, 142.2,

138.8, 133.5, 132.2, 129.7, 129.5, 129.2, 128.8, 127.6, 126.6, 126.5, 123.2, 112.4, 62.9, 43.5, 21.6. MS-ESI: m/z 392 ($M + H^+$).

4.1.2.9. 4-(3-Phenyl-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4i). Yield: 27%, m.p. = 200 – 203 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.78 (d, *J* = 7.2 Hz, 2H); 7.60 (d, *J* = 8.9 Hz, 2H); 7.49 – 7.25 (m, 6H); 7.15 – 7.00 (m, 5H); 5.59 (dd, *J* = 12.1 Hz, *J* = 5.3 Hz, 1H); 3.95 (dd, J_{gem} = 17.7 Hz, *J* = 12.2 Hz, 1H); 3.20 (dd, J_{gem} = 17.5 Hz, *J* = 5.3 Hz, 1H); 2.24 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.1, 146.4, 140.6, 141.2, 133.5, 132.3, 129.8, 129.7, 129.2, 127.6, 126.5, 125.4, 123.9, 122.8, 112.5, 63.0, 43.4, 21.1. MS-ESI: m/z 392 ($M + H^+$).

4.1.2.10. 4-(3-Phenyl-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (4j). Yield: 27%, m.p. = 219 – 223 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 7.2 Hz, 2H); 7.59 (d, *J* = 8.8 Hz, 2H); 7.48-7.38 (m, 3H); 7.17-7.11 (m, 4H); 7.11-7.01 (m, 4H); 5.60 (dd, *J* = 11.9 Hz, *J* = 4.9 Hz, 1H); 3.95 (dd, J_{gem} = 17.7 Hz, *J* = 12.2 Hz, 1H); 3.16 (dd, J_{gem} = 17.7, *J* = 4.9 Hz, 1H); 2.24 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 150.1, 146.3, 139.1, 137.3, 133.5, 132.3, 130.1, 129.8, 129.2, 127.6, 126.5, 126.2, 112.5, 62.6, 43.4, 21.1. MS-ESI: m/z 392 ($M + H^+$).

4.1.2.11. 4-(3-(4-phenoxyphenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5a). Yield: 36%, m.p. = 175 – 178 °C from EtOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 8.8 Hz, 2H); 7.58 (d, *J* = 8.9 Hz, 2H); 7.45-7.38 (m, 2H); 7.34 (t, *J* = 7.1 Hz, 2H); 7.28 – 7.22 (m, 3H); 7.18 (t, *J* = 7.4 Hz, 1H); 7.08-7.01 (m, 8H); 5.61 (dd, *J* = 12.0 Hz, *J* = 5.1 Hz, 1H); 3.95 (dd, J_{gem} = 17.7 Hz, *J* = 12.0 Hz; 1H); 3.14 (dd, J_{gem} = 17.7 Hz, *J* = 5.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 158.2, 156.5, 149.7, 146.4, 142.0, 133.2, 130.7, 129.6, 128.5, 128.1, 127.6, 127.3, 126.2, 124.5, 119.5, 118.9, 112.4, 62.8, 43.5.. MS-ESI: m/z 470 ($M + H^+$).

4.1.2.12. 4-(5-(2-fluorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5b). Yield: 25%, m.p. = 208 – 212 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.80 (d, *J* = 8.8 Hz, 2H); 7.60 (d, *J* = 9.0 Hz, 2H); 7.45 – 7.39 (m, 2H); 7.37 - 7.30 (m, 1H); 7.29-7.22 (m, 1H); 7.21-7.16 (m, 1H); 7.15-7.10 (m, 2H); 7.09-7.02 (m, 8H); 5.78 (dd, *J* = 12.3 Hz, *J* = 5.1 Hz, 1H), 3.98 (dd, J_{gem} = 17.6 Hz, *J* = 12.2 Hz, 1H), 3.22 (dd, J_{gem} = 17.6 Hz, *J* = 5.0 Hz, 1H,). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 160.0 (d, *J* = 244 Hz), 158.3, 156.4,

150.0, 146.2, 133.4, 130.7, 130.4 (d, *J* = 8 Hz), 128.5, 128.1 (d, *J* = 3 Hz), 128.2 (d, *J* = 20 Hz), 127.7, 127.2, 125.4 (d, *J* = 4 Hz), 124.5, 119.5, 118.9, 116.6 (d, *J* = 21 Hz), 112.2, 57.4, 42.3. MS-ESI: m/z 488 ($M + H^+$).

4.1.2.13. 4-(5-(3-fluorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5c). Yield: 54%, m.p. = 180 – 182 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 8.8 Hz, 2H); 7.60 (d, *J* = 8.9 Hz, 2H); 7.45 - 7.35 (m, 3H); 7.18 (t, *J* = 7.6 Hz, 1H); 7.11-7.02 (m, 11H); 5.65 (dd, *J* = 12.0 Hz, *J* = 4.9 Hz, 1H), 3.95 (dd, J_{gem} = 17.7 Hz, 12.1 Hz, 1H), 3.18 (dd, J_{gem} = 17.7 Hz, *J* = 5.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 162.9 (d, *J* = 244 Hz), 158.3, 156.4, 149.8, 146.3, 144.9 (d, *J* = 6 Hz), 133.4, 131.8 (d, *J* = 8 Hz), 130.7, 128.5, 127.7, 127.2, 124.5, 122.2, 119.5, 118.9, 115.0 (d, *J* = 21 Hz), 113.2 (d, *J* = 22 Hz), 112.4, 62.2, 43.3. MS-ESI: m/z 488 ($M + H^+$).

4.1.2.14. 4-(5-(4-fluorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5d). Yield: 56%, m.p. = 184 – 187 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 8.8, 2H); 7.59 (d, *J* = 9.0, 2H); 7.45 – 7.39 (m, 2H); 7.31 - 7.25 (m, 2H); 7.21 – 7.13 (m, 3H); 7.08-7.02 (m, 8H); 5.63 (dd, *J* = 12.0 Hz, *J* = 4.9 Hz, 1H), 3.94 (dd, J_{gem} = 17.7 Hz, *J* = 12.0 Hz, 1H), 3.15 (dd, J_{gem} = 17.7 Hz, *J* = 5.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.9 (d, *J* = 243 Hz), 158.3, 156.4, 149.7, 146.3, 138.2 (d, *J* = 3 Hz), 133.3, 130.7, 128.5, 128.3 (d, *J* = 8 Hz), 127.6, 127.2, 124.5, 119.5, 118.9, 116.4 (d, *J* = 21 Hz), 112.4, 62.1, 43.5. MS-ESI: m/z 488 ($M + H^+$).

4.1.2.15. 4-(5-(2-chlorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5e). Yield: 27% m.p. = 131 – 135 °C from MeOH. ^{1}H NMR (DMSO-d6, 400 MHz): δ (ppm) 7.79 (d, *J* = 8.7 Hz, 2H); 7.61 (d, *J* = 8.9 Hz, 2H); 7.56 (d, *J* = 7.6 Hz, 1H); 7.42 (t, *J* = 7.9 Hz, 2H); 7.32 (dt, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H); 7.25 (t, *J* = 7.3 Hz, 1H); 7.16 (t, *J* = 7.4 Hz, 1H); 7.11 – 6.93 (m, 9H); 5.80 (dd, *J* = 12.1 Hz, *J* = 5.1 Hz, 1H), 4.04 (dd, J_{gem} = 17.7 Hz, *J* = 12.2 Hz, 1H), 3.13 (dd, J_{gem} = 17.7 Hz, 5.1 Hz, 1H).^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 158.4, 156.4, 150.0, 146.1, 138.3, 133.5, 131.7, 130.7, 130.0, 128.6, 128.4, 127.8, 127.1, 124.5, 119.6, 118.9, 112.1, 60.4, 42.2. MS-ESI: m/z 504 ($M + H^+$).

4.1.2.16. 4-(5-(3-chlorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5f). Yield: 60%, m.p. = 218 – 220 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.79 (d, *J* = 8.7 Hz, 2H); 7.60 (d, *J* = 8.9 Hz, 2H); 7.45 – 7.29 (m, 5H); 7.19 (t, *J* = 7.1 Hz, 2H); 7.10 - 7.04 (m, 8H); 5.65 (dd, *J* = 12.0 Hz, *J* = 4.9 Hz, 1H), 3.95 (dd, J_{gem} = 17.8 Hz, *J* = 12.0 Hz, 1H), 3.19 (dd, J_{gem} = 17.8, *J* = 4.9 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 158.3, 156.4, 149.8, 146.2, 144.5, 134.1, 133.5, 131.6, 130.7, 128.6, 128.2, 127.7, 127.2, 126.1, 124.8, 124.5, 119.5, 118.9, 112.4, 62.1, 43.3. MS-ESI: m/z 504 (M + H^+).

4.1.2.17. 4-(5-(4-chlorophenyl)-3-(4-phenoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5g). Yield: 60%, m.p. = 105 – 107 °C from EtOH. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.60-7.58 (m, 4H); 7.33 - 7.20 (m, 4H); 7.14-7.04 (m, 3H); 7.00-6.90 (m, 6H); 5.24 (dd, *J* = 12.1 Hz, *J* = 5.8 Hz, 1H); 4.28 - 3.90 (bs, 2H); 3.79 (dd, J_{gem} = 17.3 Hz, *J* = 12.2 Hz, 1H); 3.06 (dd, J_{gem} = 17.4 Hz, *J* = 5.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 158.8, 156.4, 149.0, 147.0, 139.7, 133.8, 130.6, 129.9, 129.6, 128.0, 127.8, 127.1, 126.6, 124.0, 119.4, 118.6, 112.5, 62.9, 43.6. MS-ESI: m/z 504 (M + H^+).

4.1.2.18. 4-(3-(4-phenoxyphenyl)-5-(o-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5h). Yield: 28%, m.p. = 200 – 204 °C from EtOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.80 (d, *J* = 8.7 Hz, 2H); 7.60 (d, *J* = 8.8 Hz, 2H); 7.42 (t, *J* = 7.8 Hz, 2H); 7.28 (d, *J* = 7.3 Hz, 1H); 7.21 - 7.13 (m, 2H); 7.11 -7.01 (m, 7H); 6.98 (d, *J* = 8.8 Hz, 2H); 6.84 (d, *J* = 7.4 Hz, 1H); 5.70 (dd, *J* = 12.0 Hz, *J* = 5.1 Hz, 1H); 4.00 (dd, J_{gem} = 17.4 Hz, 12.2 Hz, 1H); 3.06 (dd, J_{gem}= 17.6, *J* = 4.9 Hz, 1H); 2.47 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 158.2, 156.5, 149.8, 146.3, 139.3, 136.2, 135.1, 133.3, 131.6, 130.7, 128.4, 127.9, 127.7, 127.4, 126.8, 124.5, 119.5, 118.9, 112.2, 60.5, 42.2, 19.5. MS-ESI: m/z 484 (M + H^+).

4.1.2.19. 4-(3-(4-phenoxyphenyl)-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5i). Yield: 37%, m.p. = 159 – 163 °C from MeOH. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.66 - 7.57 (m, 4H); 7.32 - 7.25 (m, 2H); 7.14 (t, *J* = 7.7 Hz, 1H); 7.07 (t, *J* = 7.4 Hz, 1H); 7.03 - 6.91 (m, 9H); 5.21 (dd, *J* = 12.2 Hz, *J* = 5.9 Hz, 1H); 4.35 -3.87 (bs, 2H); 3.78 (dd, J_{gem} = 17.3 Hz, *J* = 12.2 Hz, 1H), 3.09 (dd, J_{gem} = 17.4 Hz, *J* = 6.0 Hz, 1H); 2.24 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 158.6, 156.5, 149.0, 147.4, 141.3, 139.3, 130.2, 129.9, 129.3, 128.8, 128.0, 127.7, 126.9, 126.1, 123.9, 122.7, 119.4, 118.6, 112.4, 63.6, 43.8, 21.5. MS-ESI: m/z 484 (M + H^+).

4.1.2.20. 4-(3-(4-phenoxyphenyl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (5j). Yield: 60%, m.p. = 131 – 135 °C from MeOH. ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.70 (d, *J* = 8.9 Hz, 2H); 7.72 (d, *J* = 8.8 Hz, 2H); 7.39 (t, *J* = 7.9 Hz, 2H); 7.21 - 7.14 (m, 5H); 7.10 - 7.01 (m, 6H); 5.34 (dd, *J* = 12.1 Hz, *J* = 5.8 Hz, 1H); 4.94 - 4.50 (bs, 2H); 3.88 (dd, J_{gem} = 17.2 Hz, *J* = 12.2 Hz, 1H), 3.19 (dd, J_{gem} = 17.2 Hz, *J* = 5.8 Hz, 1H,); 2.34 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm) 158.6, 156.5, 149.1, 147.4, 138.2, 137.8, 130.1, 129.9, 128.0, 127.7, 127.4, 126.9, 125.6, 123.9, 119.4, 118.6, 112.4, 63.3, 43.8, 21.1. MS-ESI: m/z 484 ($M + H^+$).

4.1.2.21. 4-(3-(4-(benzyloxy)phenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6a). Yield: 39%, m.p. = 169 – 172 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.71 (d, *J* = 8.7 Hz, 2H); 7.57 (d, *J* = 8.8 Hz, 2H); 7.48 - 7.29 (m, 7H); 7.28 - 7.20 (m, 3H); 7.11 - 7.00 (m, 6H); 5.56 (dd, *J* = 11.9 Hz, *J* = 4.8 Hz, 1H); 5.14 (s, 2H); 3.91 (dd, J_{gem} = 17.6 Hz, *J* = 12.0 Hz, 1H); 3.11 (dd, J_{gem} = 17.6 Hz, *J* = 4.9 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 150.1, 146.5, 142.1, 137.2, 132.9, 129.6, 128.9, 128.4, 128.2, 128.1, 127.6, 126.2, 124.9, 115.6, 112.2, 69.8, 62.6, 43.6. MS-ESI: m/z 484 ($M + H^+$).

4.1.2.22. 4-(3-(4-(benzyloxy)phenyl)-5-(2-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6b). Yield: 25%, m.p. = 188 – 192 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.75 (d, *J* = 8.71 Hz, 2H); 7.60 (d, *J* = 8.79 Hz, 2H); 7.50-7.21 (m, 8H); 7.17-6.93 (m, 7H); 5.61 (dd, *J* = 11.90 Hz, *J* = 4.83 Hz, 1H); 5.15 (s, 2H); 3.89 (dd, J_{gem} = 17.6 Hz, *J* = 11.98 Hz, 1H); 3.12 (dd, J_{gem} = 17.6 Hz, *J* = 4.89 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 159.7 (d, *J* = 245 Hz), 150.2, 146.5, 137.2, 133.1, 130.3 (d, *J* = 10 Hz), 128.9, 128.4, 128.3 (d, *J* = 14 Hz), 128.2, 128.1, 127.9, 127.6, 125.4 (d, *J* = 4 Hz), 124.4, 116.7 (d, *J* = 21 Hz), 115.6, 112.3, 69.8, 61.8, 44.9. MS-ESI: m/z 502 ($M + H^+$).

4.1.2.23. 4-(3-(4-(benzyloxy)phenyl)-5-(3-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6c). Yield: 60%, m.p. = 170 – 173 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.72 (d, *J* = 8.7 Hz, 2H); 7.59 (d, *J* = 8.8 Hz, 2H); 7.45 (d, *J* = 7.1 Hz, 2H); 7.42 - 7.30 (m, 4H); 7.12 - 7.00 (m, 9H); 5.61 (dd, *J* = 11.9 Hz, *J* = 4.9 Hz, 1H); 5.15 (s, 2H); 3.92 (dd, J_{gem} = 17.7 Hz, *J* = 12.1 Hz, 1H); 3.16 (dd, J_{gem} = 17.7 Hz, *J* = 4.8 Hz, 1H).^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 162.8 (d, *J* = 243 Hz), 159.8, 150.2, 146.4, 145.0 (d, *J* = 6 Hz), 137.2, 133.2,

131.8 (d, *J* = 8 Hz), 128.9, 128.4, 128.2, 128.1, 127.7, 124.8, 122.2, 115.6, 114.9 (d, *J* = 20 Hz), 113.2 (d, *J* = 22 Hz), 112.3, 69.8, 62.1, 43.4. MS-ESI: m/z 502 ($M + H^+$).

4.1.2.24. 4-(3-(4-(benzyloxy)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6d). Yield: 45%, m.p. = 136 – 140 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.72 (d, *J* = 8.8 Hz, 2H); 7.58 (d, *J* = 9.0 Hz, 2H); 7.45 (d, *J* = 7.0 Hz, 2H); 7.42 - 7.24 (m, 5 H); 7.15 (t, *J* = 8.8 Hz, 2H); 7.10 - 7.01 (m, 6H); 5.59 (dd, *J* = 12.0 Hz, *J* = 5.0 Hz, 1H); 5.15 (s, 2H); 3.91 (dd, J_{gem} = 17.7 Hz, *J* = 12.0 Hz, 1H); 3.12 (dd, J_{gem} = 17.7 Hz, *J* = 4.9 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 161.9 (d, *J* = 242 Hz), 159.8, 150.1, 146.4, 138.3 (d, *J* = 2 Hz), 137.2, 133.0, 128.9, 128.4, 128.3 (d, *J* = 8 Hz), 128.2, 128.1, 127.6, 124.9, 116.3 (d, *J* = 21 Hz), 115.6, 112.3, 69.8, 61.9, 43.5. MS-ESI: m/z 502 ($M + H^+$).

4.1.2.25. 4-(3-(4-(benzyloxy)phenyl)-5-(2-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6e). Yield: 52%, m.p. = 184 – 188 °C from C_6H_6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm)) 7.71 (d, *J* = 8.8 Hz, 2H); 7.61 (d, *J* = 8.9 Hz, 2H); 7.55 (d, *J* = 7.9 Hz, 1H); 7.44 (d, *J* = 6.8 Hz, 2H); 7.41 - 7.28 (m, 5H); 7.24 (t, *J* = 7.2 Hz, 1H); 7.09 - 7.04 (m, 4 H); 7.01 - 6.92 (m, 2H); 5.75 (dd, *J* = 12.1 Hz, *J* = 5.1 Hz, 1H); 5.14 (s, 2H); 4.00 (dd, J_{gem} = 17.6 Hz, *J* = 12.1 Hz, 1H); 3.10 (dd, J_{gem} = 17.6 Hz, *J* = 5.1 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 150.3, 146.2, 138.4, 137.2, 133.3, 131.7, 130.7, 130.0, 128.9, 128.80, 128.79, 128.4, 128.2, 128.1, 127.8, 124.7, 115.6, 112.0, 69.7, 60.3, 42.3. MS-ESI: m/z 518 ($M + H^+$).

4.1.2.26. 4-(3-(4-(benzyloxy)phenyl)-5-(3-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6f). Yield: 63%, m.p. = 134 – 136 °C from C6H6. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.71 (d, *J* = 8.8 Hz, 2H); 7.59 (d, *J* = 8.9 Hz, 2H); 7.45 (d, *J* = 7.1 Hz, 2H); 7.41 - 7.28 (m, 6 H); 7.17 (d, *J* = 7.5 Hz, 1H) 7.10 - 7.01 (m, 6H); 5.60 (dd, *J* = 11.9 Hz, *J* = 4.9 Hz, 1H); 5.15 (s, 2H); 3.91 (dd, J_{gem} = 17.7 Hz, *J* = 12.0 Hz, 1H); 3.16 (dd, J_{gem} = 17.7 Hz, *J* = 4.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 150.2, 146.3, 144.6, 137.2, 134.0, 133.2, 131.6, 129.0, 128.4, 128.3, 128.2, 127.7, 126.1, 124.8, 124.7, 115.6, 112.3, 69.7, 62.0, 43.4. MS-ESI: m/z 518 ($M + H^+$).

4.1.2.27. 4-(3-(4-(benzyloxy)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6g). Yield: 40%, m.p. = 98 – 102 °C from C_6H_6. ^{1}H NMR (DMSO-d_6): δ (ppm) 7.71 (d, *J* = 8.8 Hz, 2H); 7.57 (d, *J* = 9.0 Hz, 2H); 7.45 (d, *J* = 7.0 Hz, 2H); 7.42 - 7.30 (m,

5H); 7.25 (d, *J* = 8.6 Hz, 2H); 7.10 - 7.00 (m, 6 H); 5.60 (dd, *J* = 12.0 Hz, *J* = 5.0 Hz, 1H); 5.15 (s, 2H); 3.91 (dd, J_{gem} = 17.6 Hz, *J* = 12.0 Hz, 1H); 3.13 (dd, J_{gem} = 17.6 Hz, *J* = 5.0 Hz, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 150.2, 146.3, 141.1, 137.2, 133.1, 132.6, 129.5, 128.9, 128.4, 128.21, 128.17, 127.6, 124.8, 115.6, 112.3, 69.8, 62.0, 43.4. MS-ESI: m/z 518 (M + H^+).

4.1.2.28. 4-(3-(4-(benzyloxy)phenyl)-5-(o-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6h). Yield: 33%, m.p. = 230 – 234 °C from CH_3CN. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.71 (d, *J* = 8.8 Hz, 2H); 7.58 (d, *J* = 9.0 Hz, 2H); 7.44 (d, *J* = 8.4 Hz, 2H); 7.41 - 7.30 (m, 3H); 7.26 (d, *J* = 7.5 Hz, 1H); 7.15 (t, *J* = 7.5 Hz, 1H); 7.09 - 7.02 (m, 5H); 6.95 (d, *J* = 8.8 Hz, 2H); 6.82 (d, *J* = 6.4 Hz, 1H); 5.63 (dd, *J* = 12.1 Hz, *J* = 5.3 Hz, 1H) 5.13 (s, 2H); 3.96 (dd, J_{gem} = 17.5 Hz, *J* = 12.1 Hz, 1H); 3.00 (dd, J_{gem} = 17.5 Hz, *J* = 5.1 Hz, 1H); 2.44 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 150.0, 146.5, 139.2, 137.3, 133.1, 131.3, 130.1, 130.0, 129.0, 128.9, 128.2, 127.6, 126.2, 125.1, 115.6, 112.3, 69.8, 62.5, 43.6, 21.1. MS-ESI: m/z 498 (M + H^+).

4.1.2.29. 4-(3-(4-(benzyloxy)phenyl)-5-(m-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6i). Yield: 29%, m.p. = 131 – 134 °C from EtOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.71 (d, *J* = 8.7 Hz, 2H); 7.57 (d, *J* = 9.0 Hz, 2H); 7.47 - 7.29 (m 5H); 7.21 (t, *J* = 7.7 Hz, 1H); 7.10 - 6.98 (m, 9H); 5.49 (dd, *J* = 12.0 Hz, *J* = 5.3 Hz, 1H); 5.14 (s, 2H); 3.90 (dd, J_{gem} = 17.7 Hz, *J* = 12.0 Hz, 1H); 3.09 (dd, J_{gem} = 17.7 Hz, *J* = 5.2 Hz, 1H); 2.23 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.7, 150.0, 146.6, 142.3, 138.8, 137.2, 132.9, 129.5, 128.9, 128.8, 128.4, 128.3, 128.2, 127.6, 126.6, 124.9, 123.2, 115.6, 112.2, 69.8, 62.7, 43.6, 21.5. MS-ESI: m/z 498 (M + H^+).

4.1.2.30. 4-(3-(4-(benzyloxy)phenyl)-5-(p-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (6j). Yield: 39%, m.p. = 135 – 137 °C from MeOH. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 7.73 (d, *J* = 8.7 Hz, 2H); 7.58 (d, *J* = 8.8 Hz, 2H); 7.49 - 7.37 (m, 5H); 7.15 - 7.00 (m, 10H); 5.54 (dd, *J* = 11.9 Hz, *J* = 5.0 Hz, 1H); 5.17 (s, 2H); 3.91 (dd, J_{gem} = 17.5 Hz, *J* = 12.0 Hz, 1H); 3.12 (dd, J_{gem} = 17.5 Hz, *J* = 5.0 Hz, 1H); 2.25 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 159.8, 150.0, 146.5, 139.2, 137.3, 133.1, 131.3, 130.1, 130.0, 129.0, 128.9, 128.2, 127.6, 126.2, 125.1, 115.6, 112.3, 69.8, 62.5, 43.6, 21.1. MS-ESI: m/z 498 (M + H^+).

4.2. Biology

4.2.1. Test compounds

Compounds were solubilized in DMSO at a concentration of 100 mM, and subsequently subjected to a serial dilutions in the culture medium. The final concentrations of test compound, diluted with ratio 1: 5, are: 100, 20, 4 and 0.8 μM. The first dilution, with ratio 1:50, diluted the compounds from 100 mM to a concentration of 2 mM, reducing the percentage of DMSO from 100% to 2%. The second dilution, with ratio 1:20, leads the compounds from 2 mM to a concentration of 100 μM and reduces the concentration of DMSO from 2% to 0.1%, a non-toxic concentration for cells. Next dilutions, performed with ratio 1: 5, result in a further decrease in the percentage of DMSO in contact with the cells.

6-Azauridine, 2′-C-methyl-guanosin (NM108), ribavirin, efavirenz (EFV), acyclovir (ACG), pleconaril and mycophenolic acid (M5255) were employed as reference inhibitors and were solubilized in DMSO at a concentration of 100 mM, and subsequently subjected to serial dilutions in the culture medium. The final concentrations of 6-azauridine, ribavirin and ACG are: 100, 20, 4 and 0.8 μM. The final concentrations of NM108, EFV, pleconaril and M5255 diluted are: 100, 20, 4, 0.8, 0.16, 0.032, 0.0064 and 0.00128 μM.

4.2.2. 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), Dengue virus type 2 (DENV-2), West Nile virus (WNV) 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)], Dengue virus type 2 (DENV-2) [clinical isolate], West Nile virus (WNV) [clinical isolate] 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)].

4.2.3. 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. [17] 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 MTT method. [17]

4.2.4. 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. [15] Antiviral activity against YFV, DENV-2, WNV 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. [17]

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.

4.2.5. 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 **6a** and **6b** concentration or 4 × EC_{50} 6-azauridine concentration was added before the infection (pre-treatment), during the two hours of infection of BHK-21 cell cultures with YFV and every two hours post infection (p.i.) from time 0 to 10 hrs p.i (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 10 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.

4.2.6. 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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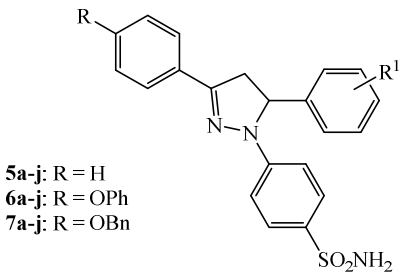
Table 1. Cytotoxicity and antiviral activity of 4-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl) benzenesulfonamides (**4a-j, 5a-j, 6a-j**) against ssRNA$^+$ (YFV, DENV-2, WNV, BVDV) viruses.



5a-j: R = H
6a-j: R = OPh
7a-j: R = OBn

Compds	R	R[1]	BHK-21 [a]CC_{50} (µM)	YFV [b]EC_{50} (µM)	[c]SI CC_{50} BHK-21/ EC_{50} YFV	DENV-2 [d]EC_{50} (µM)	WNV [e]EC_{50} (µM)	MDBK [f]CC_{50} (µM)	BVDV [g]EC_{50} (µM)	[h]SI CC_{50} MDBK/ EC_{50} BVDV
4a	H	H	15	>15	-	-	-	16	>16	-
4b	H	2-F	21	>21	-	-	-	19	>19	-
4c	H	3-F	15	≥15	-	-	-	20	>20	-
4d	H	4-F	13±2	2.6±0.4	5.0	>13	>13	18	>18	-
4e	H	2-Cl	14±2	2.4±0.4	5.8	>14	>14	9	>9	-
4f	H	3-Cl	9±1	2.5±0.1	3.6	>9	>9	2	>2	-
4g	H	4-Cl	32	>32	-	-	-	11	>11	-
4h	H	2-CH_3	14±0.001	2.2±0.2	6.4	>14	>14	18	>18	-
4i	H	3-CH_3	25	>25	-	-	-	23	>23	-
4j	H	4-CH_3	8±1	2.2±0.2	3.6	>8	>8	32	>32	-
5a	OPh	H	13±1	2.1±0.1	6.2	>13	>13	5	>5	-
5b	OPh	2-F	34±3	1.9±0.1	17.9	>34	>34	36	>36	-
5c	OPh	3-F	10±2	1.8±0.2	5.6	>10	>10	12	>12	-
5d	OPh	4-F	9±0.5	1.8±0.2	5.0	>9	>9	6	>6	-
5e	OPh	2-Cl	9±1	1.8±0.2	5.0	>9	>9	5	>5	-
5f	OPh	3-Cl	10±0.3	2.2±0.2	4.5	>10	>10	6	>6	-
5g	OPh	4-Cl	8±0.8	1.9±0.1	4.2	>8	>8	19	>19	-
5h	OPh	2-CH_3	8±1	2.2±0.2	3.6	>8	>8	16	>16	-
5i	OPh	3-CH_3	9±1	1.8±0.1	5.0	>9	>9	6	>6	-
5j	OPh	4-CH_3	9±0.8	1.8±0.1	5.0	>9	>9	21	>21	-
6a	OBn	H	80±4	2.2±0.2	36.4	>80	>80	72	>72	-
6b	OBn	2-F	90±5	1.8±0.2	50.0	>90	>90	>100	52.0±8	>1.9
6c	OBn	3-F	11±1	2.0±0.05	5.5	>11	>11	15±2	10.0±1	1.5
6d	OBn	4-F	60±8	1.9±0.1	31.6	>60	>60	>100	>100	-
6e	OBn	2-Cl	12±0.5	1.9±0.1	6.3	>12	>12	52±5	7.5±2	6.9
6f	OBn	3-Cl	45±3	2.0±0.05	22.5	>45	>45	40±5	5.5±0.5	7.3
6g	OBn	4-Cl	15±1	1.9±0.1	7.9	>15	>15	16±1	4.5±1.5	3.6
6h	OBn	2-CH_3	22±3	1.8±0.2	12.2	>22	>22	>100	>100	-
6i	OBn	3-CH_3	9±0.3	1.8±0.2	5.0	>9	>9	56±5	12.5±4	4.5
6j	OBn	4-CH_3	36±4	1.8±0.2	20.0	>36	>36	80±7	2.5±0.5	32
Ref. Compds.										
6-Azauridine			>100	20.0±5	>5					
NM 108			48±6			1.7±0.2	0.8±0.1			
Ribavirin								62.0±1	20.0±2	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 BHK (Hamster normal kidney fibroblast) monolayers by 50%, as determined by the MTT method. [b]Compound concentration required to reduce the viability of mock-infected BHK cells from the YFV-induced cytopathogenicity, as determined by the MTT method. [c]Selectivity index (SI) was the ratio between CC_{50} against BHK-21 and EC_{50} against YFV. [d]Compound concentration required to achieve 50% protection of BHK cells from DENV-2 induced cytopathogenicity, as determined by the MTT method. [e]Compound concentration required to achieve 50% protection of BHK cells from WNV induced cytopathogenicity, as determined by the MTT method. [f]Compound concentration required to reduce the viability of mock-infected MDBK (Bovine normal kidney) cells by 50%, as determined by the MTT method. [g]Compound concentration required to achieve 50% protection of MDBK cells from the BVDV-induced cytopathogenicity, as determined by the MTT method. [h]Selectivity index (SI) was the ratio between CC_{50} against MDBK and EC_{50} against BVDV.
The sign '>' indicates that the EC_{50} is higher than the CC_{50} for the corresponding host cell line or beyond the highest tested concentration.

Table 2. Cytotoxicity and antiviral activity of 4-(3,5-diphenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamides (**4a-j, 5a-j, 6a-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} Vero76/ EC_{50} CVB-5	Sb-1	VV	HSV-1	VSV	RSV
										[h]EC_{50} (µM)				
4a	H	H	42	>42	15	>15	22	> 22	-	>22	>22	>22	>22	>22
4b	H	2-F	42	>42	21	>21	28	> 28	-	>28	>28	>28	>28	>28
4c	H	3-F	44±1	13.0±0.5	15	>15	24	> 24	-	>24	>24	>24	>24	>24
4d	H	4-F	45	>45	12	>12	23	> 23	-	>23	>23	>23	>23	>23
4e	H	2-Cl	44	>44	13	>13	26	> 26	-	>26	>26	>26	>26	>26
4f	H	3-Cl	41±0.1	10.0±0.8	8	>8	21	> 21	-	>21	>21	>21	>21	>21
4g	H	4-Cl	36	>36	32	>32	21	> 21	-	>21	>21	>21	>21	>21
4h	H	2-CH_3	43	>43	14	>14	24	> 24	-	>24	>24	>24	>24	>24
4i	H	3-CH_3	37	>37	25	>25	25	> 25	-	>25	>25	>25	>25	>25
4j	H	4-CH_3	40	>40	7	>7	20	> 20	-	>20	>20	>20	>20	>20
5a	OPh	H	42±2	13.0±1	11	>11	85±5/35	3.5±0.5	24.3	>85	>85	>85	>85	>35
5b	OPh	2-F	36	>36	32	>32	96±1	7.0±1	13.7	>96	>96	>96	>96	>96
5c	OPh	3-F	25	>25	11	>11	28±4	4.2±0.2	6.7	>28	>28	>28	>28	>28
5d	OPh	4-F	33	>33	8	>8	25±2	6.0±1	4.2	>25	>25	>25	>25	>25
5e	OPh	2-Cl	40	>40	8	>8	29±3	10.5±0.5	2.8	>29	>29	>29	>29	>29
5f	OPh	3-Cl	8	>8	9	>9	88	> 88	-	>88	>88	>88	>88	>88
5g	OPh	4-Cl	9	>9	7	>7	88	> 88	-	>88	>88	>88	>88	>88
5h	OPh	2-CH_3	36±4	13.0±0.6	7	>7	90/14	> 90	-	>90	>90	>90	>90	>14
5i	OPh	3-CH_3	35	>35	8	>8	81/14	> 81	-	>81	>81	>81	>81	>14
5j	OPh	4-CH_3	7.2	>7.2	8	>8	86/20	> 86	-	>86	>86	>86	>86	>20
6a	OBn	H	>100	>100	84	>84	> 100	> 100	-	>100	>100	>100	>100	>100
6b	OBn	2-F	39	>39	92	>92	> 100	> 100	-	>100	>100	>100	>100	>100
6c	OBn	3-F	65	>65	9	>9	90±5/25	8.5±2.5	10.6	>90	>90	>90	>90	>25
6d	OBn	4-F	>100	>100	69	>69	90	> 90	-	>90	>90	>90	>90	>90
6e	OBn	2-Cl	26	>26	12	>12	92	> 92	-	>92	>92	>92	>92	>92
6f	OBn	3-Cl	36±4	11.0±1	47	>47	85	> 85	-	>85	>85	>85	>85	>85
6g	OBn	4-Cl	70	>70	16	>16	82	> 82	-	>82	>82	>82	>82	>82
6h	OBn	2-CH_3	≥100	>100	20	>20	100	> 100	-	>100	>100	>100	>100	>100
6i	OBn	3-CH_3	44	>44	8	>8	100±0.1	8.0±2	12.5	>100	>100	>100	>100	>100
6j	OBn	4-CH_3	35	>35	32	>32	100±0.2	13±4.5	7.7	>100	>100	>100	>100	>100
Ref. Compds.														
EFV			40±2	0.002±0.001										
Ribavirin							>100							40.0±5
6-Azauridine					>100	18.0±2	16±2							1.2±0.2
ACG							>100					1.8±0.5		
Pleconaril							83±3	0.005±0.002	16000	2.7±0.6				
M5255							9±1				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 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%. The two values of cytotoxicity reported in some cases for the cytotoxicity of Vero 76 cell lines are due to the fact that for the RSV the compound remains in contact with the cells for 5 days (data reported in red) instead of 2 days for Sb-1 and VSV and 3 days for CVB-5, VV and HSV-1 (data reported in black), therefore the cytotoxicity is higher. [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} against Vero 76 cells (data reported in black) and EC_{50} against CVB-5. [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.
The sign '>' indicates that the EC_{50} is higher than the CC_{50} for the corresponding host cell line or beyond the highest tested concentration.

- A series of 1,3,5-triphenyl-4,5-dihydropyrazole derivatives were designed and synthesized as YFV inhibitors
- All compounds were tested against a large panel of RNA and DNA viruses
- Most of the analogues exhibited a potent and specific activity against YFV
- The benzyloxy derivatives (**6a-j**) generally coupled potency in the low micromolar range and high selectivity.