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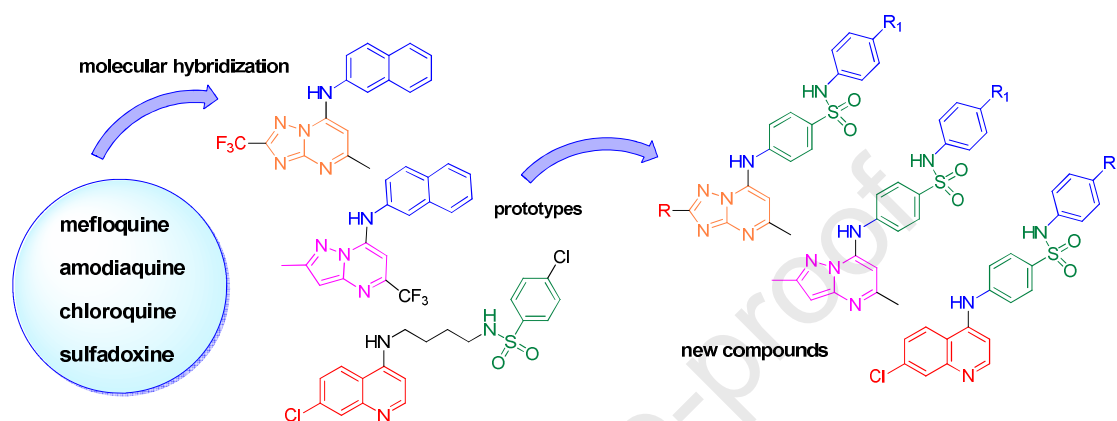
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Flávia F. Silveira^{1,2#}, Juliana O. de Souza^{3#}, Lucas V. B. Hoelz¹, Vinícius R. Campos⁴, Valquíria A. P. Jabor⁵, Anna C. C. Aguiar³, M. Cristina Nonato⁵, Magaly G. Albuquerque², Rafael V. C. Guido^{3*}, Nubia Boechat^{1,2,*} and Luiz C. S. Pinheiro^{1*}

¹ Laboratório de Síntese de Fármacos, Instituto de Tecnologia em Fármacos, Farmanguinhos - FIOCRUZ, Fundação Oswaldo Cruz. Rua Sizenando Nabuco 100, Mangunhos, Rio de Janeiro, RJ, 21041-250, Brazil.

² Programa de Pós-Graduação em Química, PGQu Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

³ Instituto de Física de São Carlos, Universidade de São Paulo, Av. João Dagnone, 1.100, Jd. Santa Angelina São Carlos-SP, Brazil.

⁴ Departamento de Química Orgânica, Programa de Pós-Graduação em Química, Instituto de Química, Universidade Federal Fluminense, Niterói, RJ, Brazil.

⁵ Laboratório de Cristalografia de Proteínas, Departamento de Ciências BioMoleculares, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café s/n Monte Alegre, 14040-903 Ribeirão Preto, SP, Brazil.

#These authors contributed equally to the work

*Correspondence: nubia.boechat@far.fiocruz.br; luiz.pinheiro@far.fiocruz.br; rvcguido@ifsc.usp.br Tel.: + 55-21-3977-2464 (N.B.; L.C.S.P.); +55-16-3373-8673 (R.V.C.G.)

ABSTRACT

In this work, we designed and synthesized 35 new triazolopyrimidine, pyrazolopyrimidine and quinoline derivatives as *P. falciparum* inhibitors (3D7 strain). Thirty compounds exhibited anti-*P. falciparum* activity, with IC₅₀ values ranging from 0.030 to 9.1 μM. The [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives were more potent than the pyrazolo[1,5-*a*]pyrimidine and quinoline analogues. Compounds **20**, **21**, **23** and **24** were the most potent inhibitors, with IC₅₀ values in the range of 0.030 - 0.086 μM and were equipotent to chloroquine. In addition, the compounds were selective, showing no cytotoxic activity against the human hepatoma cell line HepG2. All [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives inhibited *PfDHODH* activity in the low micromolar to low nanomolar range (IC₅₀ values of 0.08 - 1.3 μM) and did not show significant inhibition against the *HsDHODH* homologue (0 - 30% at 50 μM). Molecular docking studies indicated the binding mode of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives to *PfDHODH*, and the highest interaction affinities for the *PfDHODH* enzyme were in agreement with the *in vitro* experimental evaluation. Thus, the most active compounds against *P. falciparum* parasites **20** (R = CF₃, R₁ = F; IC₅₀ = 0.086 μM), **21** (R = CF₃; R₁ = CH₃; IC₅₀ = 0.032 μM), **23**, (R = CF₃, R₁ = CF₃; IC₅₀ = 0.030 μM) and **24** (R = CF₃, 2-naphthyl; IC₅₀ = 0.050 μM) and the most active inhibitor against *PfDHODH* **19** (R = CF₃, R₁ = Cl; IC₅₀ = 0.08 μM - *PfDHODH*) stood out as new lead compounds for antimalarial drug discovery. Their potent *in vitro* activity against *P. falciparum* and the selective inhibition of the *PfDHODH* enzyme strongly suggest that this is the mechanism of action underlying this series of new [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives.

Keywords: triazolopyrimidine, pyrazolopyrimidine, quinoline, malaria, *P. falciparum*, *PfDHODH*

1. Introduction

The World Health Organization (WHO) has reported a reduction of approximately 23 million cases of malaria since 2010, when 251 million cases were reported with 585 000 deaths. However, the number of cases remain alarming. In 2018, there were approximately 405 000 deaths, mainly children under 5 years of age, representing 67% of all malaria deaths in the world and the death of one child every 2 minutes [1]. The development of new and effective antimalarials is considered necessary due to the resistance of the malaria parasite to existing drugs. Thus, the identification of drugs with new mechanisms of action is imperative for malaria chemotherapy [2].

The WHO recommends artemisinin-based combination therapy (ACT) as the gold-standard treatment for this disease. This therapy combines a fast-acting artemisinin derivative with a long-acting antimalarial with a different mode of action. However, the future of this treatment is at risk due to the development of resistance to both drugs, and this has already led to some treatment failures [3,4].

There are still many challenges to overcome, regardless of the advancement in the control and elimination of malaria in recent years. With the emergence of *Plasmodium falciparum* drug resistance and insecticide-resistant mosquitoes in addition to the difficulty of formulating a potent malaria vaccine and the existence of many areas where there are populations that are not appropriately cared for by the health system, it is not difficult to understand why malaria is still considered a global public health emergency [1,5,6]. Moreover, there is a need to develop antimalarials to decrease the high number of deaths caused by severe malaria [5]. Therefore, new strategies to achieve malaria eradication are needed [7,8].

Currently, researchers have increased interest in exploiting dihydroorotate dehydrogenase enzyme (DHODH) inhibition as a strategy to treat malaria [9]. Pyrimidines are important metabolites that are vital for DNA and RNA biosynthesis. The malaria parasite cannot save preformed pyrimidine bases or nucleosides, and pyrimidines must be developed through a *de novo* biosynthetic pathway. This biosynthesis is catalysed by DHODH and shows the importance of DHODH to parasite survival [10]. An example of a *P. falciparum* dihydroorotate dehydrogenase enzyme (*Pf*DHODH) inhibitor is the [1,2,4]-triazolo[1,5-*a*]pyrimidine derivative DSM265, which was discovered by Phillips and co-workers and has advanced to clinical development [11,12].

Molecular hybridization has been used in current medicinal chemistry and is based on hybrid derivatives that combine two or more different pharmacophoric fragments in a single molecule with possible dual or multiple activities [13,14]. Our research group has been synthesizing several hybrids that have shown anti-*P. falciparum* activity [15–17]. One example is a series of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives, which are hybrid derivatives of mefloquine and amodiaquine (Fig. 1). *In vitro* test results against chloroquine-resistant (CQR) *P. falciparum* W2 strains showed that compound 1,

which contains the bulky 2-naphthylamine group has an IC_{50} of 0.023 μM but showed no toxicity to the human hepatoma cell line HepG2 and was 10-fold more potent than chloroquine ($IC_{50} = 0.22 \mu\text{M}$) (**Fig. 1**). Enzymatic assays confirmed that compound **1** is a *Pf*DHODH inhibitor ($IC_{50} = 0.70 \mu\text{M}$) [18]. Another example is the pyrazolo[1,5-*a*]pyrimidine series system synthesized using ring isosteric replacement and molecular hybridization based on compound **1**. These derivatives were active against the *P. falciparum* W2 CQR strain and showed no toxicity to the kidney epithelial cell line BGM. Compound **2** displayed the highest and most selective inhibition of the *Pf*DHODH enzyme with an IC_{50} value of 0.16 μM (**Fig. 1**) [19].

A quinoline-sulfadoxine hybrid series was active *in vitro* against the *P. falciparum* W2 CQR clone and not toxic to mammalian kidney BGM cells. Compound **3** (**Fig. 1**), with an IC_{50} value of 0.09 μM , was more potent than chloroquine ($IC_{50} = 0.22 \mu\text{M}$). An *in vivo* activity test (*P. berghei* model) showed that compound **3** reduced parasitaemia by 49% 5 days postinfection, contributing to the discovery of a new prototype with antimalarial activity [17]. Inspired by the promising results obtained by our group with prototypes **1–3**, the [1,2,4]triazolo[1,5-*a*]pyrimidine scaffold was chosen for the design of new compounds as antimalarial candidates.

In this work, new [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4–24** were designed based on the concepts of molecular hybridization. The study focused on maintaining the pharmacophoric groups: arylamines present in prototype **1** (blue) and the benzenesulfonamide moiety present in prototype **3** (green) (**Fig. 1**). At the 7 position of the [1,2,4]triazolo[1,5-*a*]pyrimidine ring, the 4-amino-*N*-arylbenzenesulfonamide moiety was added in order to insert the benzenesulfonamide moiety between the heterocyclic system and arylamines, continuing the study of the anti-*P. falciparum* activity of this system [18]. In previous works [17,19], we observed that the pyrazolo[1,5-*a*]pyrimidine and quinoline rings play important roles in antiplasmodial activity. The 4-amino-*N*-arylbenzenesulfonamide moiety was also added to this system in compounds **25–31** and **32–38** to investigate their anti-*P. falciparum* activity.

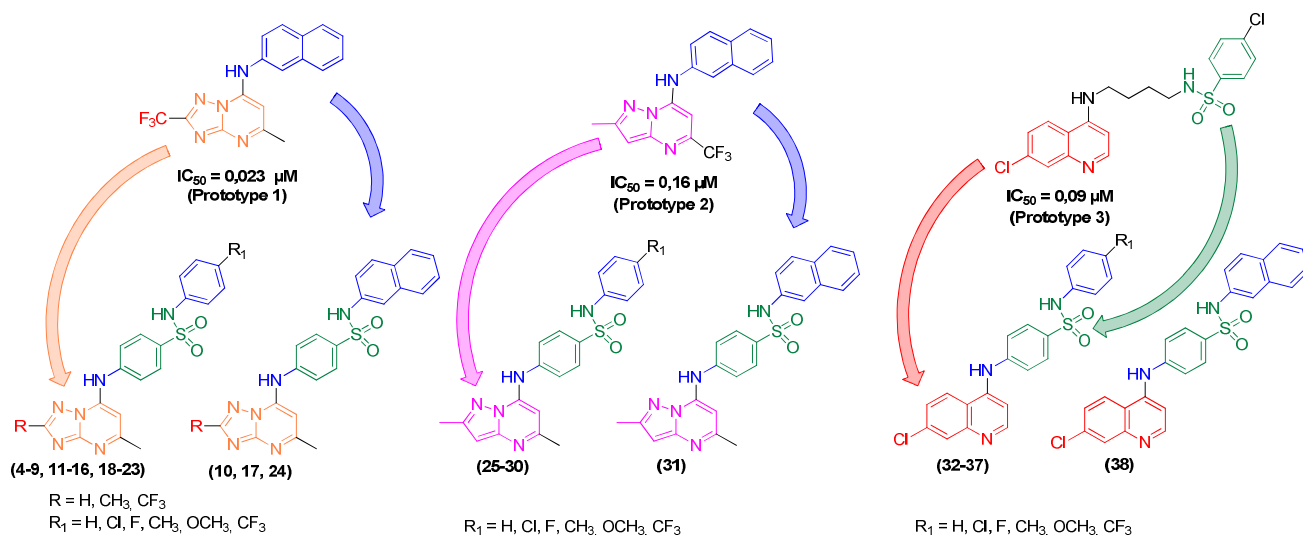


Fig. 1: Rational approach to the design of compounds **4-38**.

2. Results and discussion

2.1. Chemistry

The synthetic route used to prepare the 35 new compounds **4-38** was developed in 3 steps according to **Scheme 1**. In the first step, the condensation reaction of aminoguanidine bicarbonate with the appropriate carboxylic acid in refluxing toluene (110 °C) for 24 h afforded the respective 1*H*-1,2,4-triazoles-5-amines **39a-c** in 70-97% yield [20]. 5-Methyl-1*H*-pyrazol-3-amine (**39d**) was obtained commercially. The reaction of **39a-d** with ethyl acetoacetate in toluene at 110 °C for 20 h in the presence of catalytic *p*-toluenesulfonic acid gave 5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-ones **40a-c** in 71-95% yields and the 2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**40d**) in 98% yield [18]. Compounds **40a-d** were treated with phosphorus oxychloride (POCl₃) at 105 °C for 4 h to produce key intermediaries **41a-d** in 51-97% yield [18,19].

In the second step, the chlorosulfonation reaction of acetanilide was carried out without solvent at 0 °C for 1 h and then heated to 60 °C for another hour to obtain 4-acetamidobenzene-1-sulfonyl chloride (**42**) in 77% yield without purification [21]. The addition-elimination reaction between **42** and the respective aniline using chloroform, triethylamine (TEA) under reflux for 5 h gave protected sulfonamides **43a-g** in 42-87% yield. The hydrolysis reactions of **43a-g** with 6N HCl at 100 °C for 6 h followed by neutralization with 20% NaOH, produced 4-amino-*N*-arylbenzenesulfonamides **44a-g** in 63-89% yield without purification [22].

The third step is a convergent synthesis was a nucleophilic substitution reaction between **44a-g** and the appropriate intermediary **41a-d** was performed in ethanol at 78 °C for 2 h to obtain target compounds **4-31** [18,19]. To obtain quinolinic derivatives **32-38**, the reaction between 4,7-dichloroquinoline (**45**) and the appropriate counterpart **44a-g** was carried out under the same conditions. Purification was performed by recrystallization or thin layer chromatography (TLC). After purification, target compounds **4-38** were obtained in yields of 24 to 98%.

The derivatives **4-38** were isolated as hydrochloride and their structures were confirmed by IR, NMR (¹H, ¹³C and ¹⁹F) and HRMS. The ¹H and ¹³C NMR assignments were confirmed by COSY (*homonuclear CO*rrelation *Sp*ectroscop*Y*), HSQC (*Heteronuclear Single Quantum Coherence – Editing*) and HMBC (*Heteronuclear MultipleBond Coherence*) experiments.

For [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4-10**, compound **5** was used to exemplify the NMR assignments. In the ¹H NMR spectrum, singlets at 8.79 ppm, 6.78 ppm and 2.50 ppm was identified as H2, H6, and methyl hydrogens (CH₃), respectively. The phenyl hydrogens showed signals

at 7.17 - 7.86 ppm. For the hydrogens of the sulfonamide group (SO₂NH) a singlet around 10 ppm was assigned. In the ¹³C NMR spectrum, C2, methyl carbon (CH₃) and C6 were assigned as signals at a signal at 152.4 ppm, 23.5 ppm and 91.7 ppm, respectively.

For derivatives **11-17**, two singlets around 2.43 ppm and 2.48 ppm referring to the hydrogen of the CH₃ group can be observed in the spectrum of ¹H NMR.

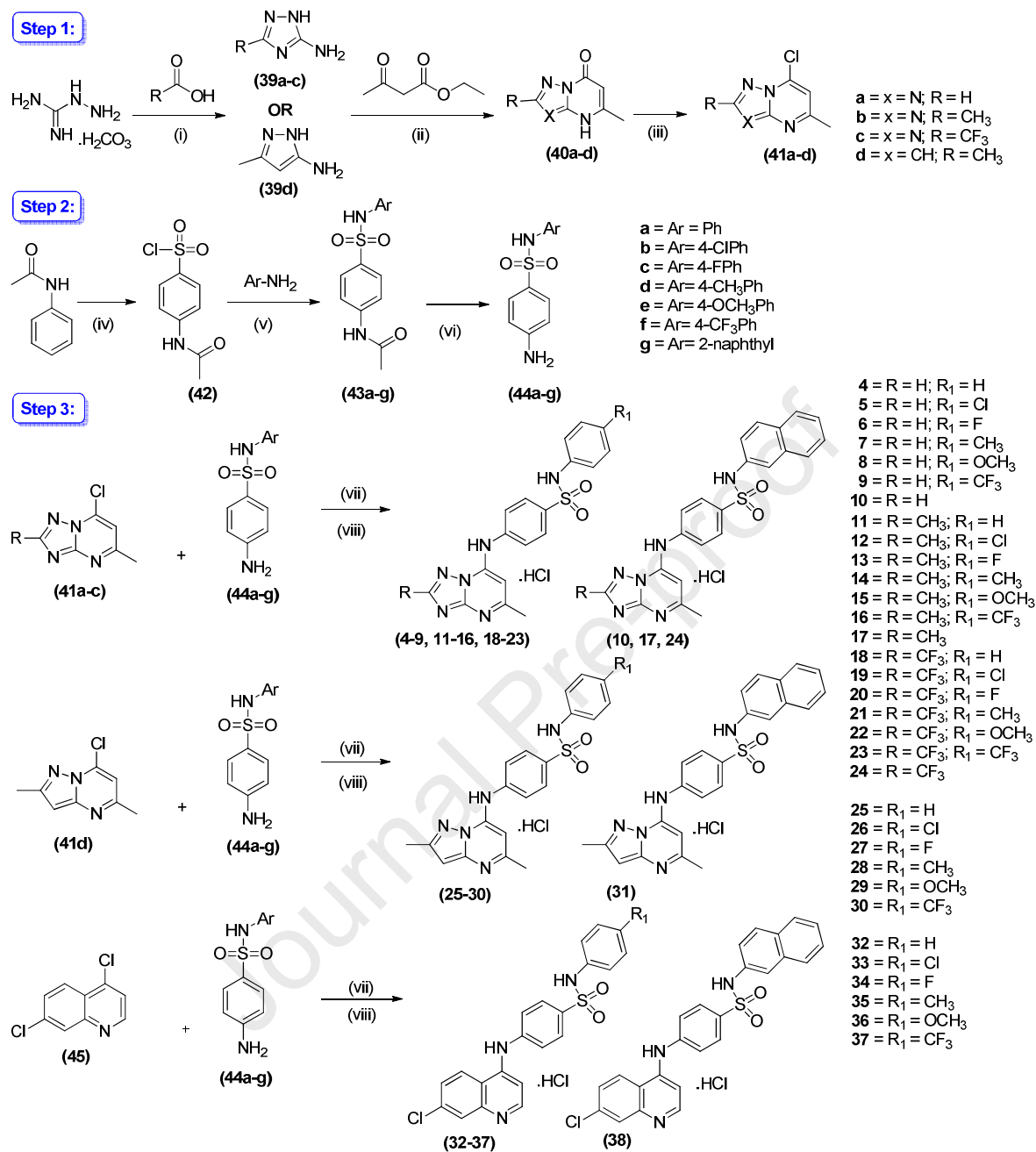
In the ¹³C NMR spectrum of derivatives **18-24**, quartets with $J = 269$ Hz referring to the C-F coupling of the CF₃ group, and a quartet with $J = 38$ Hz by the coupling of the fluorine atoms with C2 were observed. In the ¹⁹F NMR spectrum, the signal can be observed at -64.30 ppm for the CF₃ group.

The data of compound **18** will be used as an example for assignments of the pyrazolo[1,5-*a*]pyrimidine derivatives. In the ¹H NMR spectrum, two singlets can be observed at 2.36 ppm and 2.42 ppm referring to the hydrogen of CH₃ group. For H3 and H6, singlets were assigned at 6.22 ppm and 6.40 ppm, respectively. Hydrogens of phenyl rings are in the range of 7.01-7.78 ppm. The simplets at 10.02 ppm and 10.30 ppm were attributed to the hydrogens linked to the NH and SO₂NH groups. In the ¹³C NMR spectra, the two carbons of CH₃ group presented the signals at 14.1 ppm and 24.4 ppm. The C3 and C6 carbons of the pyrazolo[1,5-*a*]pyrimidine ring showed a signal at 87.6 and 93.7 ppm, respectively.

The data of compound **36** will be used as an example for the assignments of the quinoline derivatives. H2 couple with H3 ($J = 6.2$ Hz) and these hydrogens were assigned as a doublet at 8.61 ppm and 7.09 ppm, respectively. H6 was assigned as a double doublet at 7.79 ppm by coupling with H5 and H8 ($J = 9.1$ Hz and $J = 2.0$ Hz). H5 and H8 were assigned as a doublet at 8.64 ppm and 8.08 ppm, respectively. For hydrogen of NH and the sulfonamide group (SO₂NH), singlets were assigned at 10.52 ppm and 9.97 ppm, respectively. For hydrogens of the OCH₃ group, a singlet was assigned at 3.67 ppm. In the NMR ¹³C spectra, the assignments were as follow: C2 at 146,9ppm, C3 at 102,5 ppm, C5 at 125,4 ppm, C6 at 126,8 ppm and C8 122,5 ppm. It was further confirmed by HSQC. Quaternary carbons were identified with HSQC. The spectra are in the supplementary material.

Based on X-ray crystallographic analysis, the molecular structure of the derivative **17** was confirmed and allowed us to determine that the 4-amino-*N*-arylbenzenesulfonamide moiety was introduced at the 7 position of the [1,2,4]triazolo[1,5-*a*]pyrimidine ring. **Fig. 2** shows the Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagram of this compound, and the crystal data and refinements are provided in **Table S1** (Supplementary Information).

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Reagents and conditions: (i) toluene, 110 °C, 24 h, 70-97%; (ii) toluene, cat. *p*-TsOH, 110 °C, 20 h, 71-98%; (iii) POCl₃, 105 °C, 4 h, 51-97%; (iv) HSO₃Cl, 1) 0 °C, 1 h; 2) 60 °C, 1 h, 77%; (v) CHCl₃, TEA, 61 °C, 5 h, 42-87%; (vi) 1) 6N HCl, 100 °C, 6 h; 2) 20% NaOH, 63-89%; (vii) EtOH, 78 °C, 2 h, 24-98%; (viii) HCl/H₂O 1:1, 15 minutes.

Scheme 1: Synthetic route used to prepare compounds 4-38.

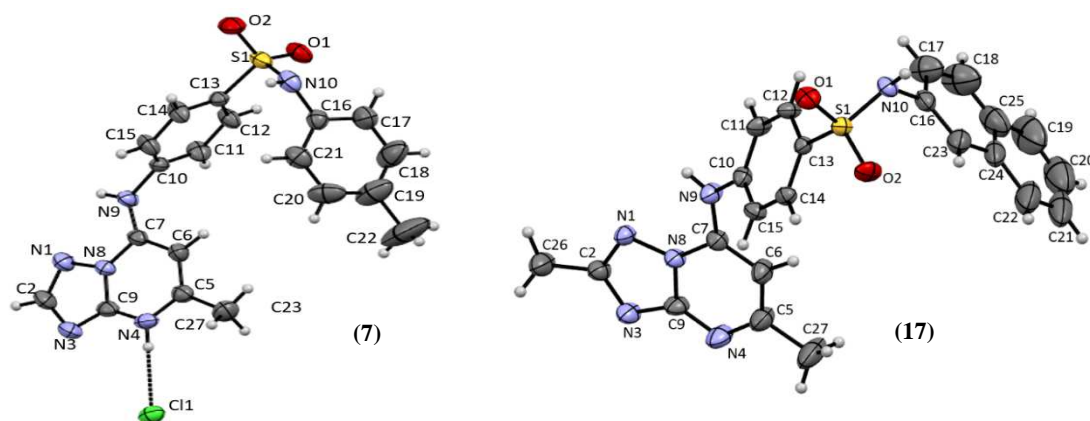


Fig. 2: Asymmetric unit representation of derivatives **7** and **17** (ellipsoids at 50% probability).

In addition, it was possible to identify those molecules that did not need a purification process, such as derivative **7**, which was found in the form of a hydrochloride salt, since the X-ray diffraction study revealed protonation of the nitrogen atom N(4)–H(4)⁺Cl(1) of derivative **7**, as shown Fig. 2. The same did not apply to the purified molecules that have a neutral form, such as derivative **17**.

It was not possible to observe that some compounds were present as hydrochlorides in the analyses previously carried out. Therefore, based on the results of the X-ray analyses, it was decided to standardize all compounds in the form of a hydrochloride. Initially, the molecules were dissolved in a water/methanol (1:1) solution, and the respective pH was measured. The fact that there were molecules in both the hydrochloride and free base forms was confirmed after analysis of the melting points (m.p. values), when it was determined that the molecules had different patterns, some in the range of 174-195 °C and others in the range of 200-288 °C. The molecules with the lower m.p. values (174-195 °C) had a neutral pH, and the molecules with higher m.p. values (200-288 °C) had a more acidic pH.

It was observed that the molecules that did not need to be purified were in the form of a hydrochloride and presented a lower pH range (2.6-5.4), which was the case for derivative **7**. The molecules that were purified, either by recrystallization or by preparative TLC, lost their hydrochloride form and presented a higher pH (6.5-7.8), which was the case for derivative **17**. Thus, molecules with pH values in the range of 6.5-7.8 were treated with a HCl/H₂O (1:1) solution for 15 minutes and then concentrated under vacuum. The respective hydrochlorides were used in biological tests.

2.2. Biological evaluation

New compounds **4-38** were assayed against the *P. falciparum* 3D7 chloroquine-sensitive (CQS) strain and human hepatoma cell line HepG2. Chloroquine and artesunate were used as positive controls for inhibition (**Table 1**).

Comparing the [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4-24**, it can be seen that a substituent at the 2 position of the [1,2,4]triazolo[1,5-*a*]pyrimidine ring influences activity. All compounds containing the 2-trifluoromethyl group **18-24** were more potent than the corresponding unsubstituted **4-10** and methylated **11-17** derivatives. This result reinforced the importance of the trifluoromethyl group at the 2 position for anti-*P. falciparum* activity [18].

Analyses of the importance of the substituents at the 4 position of the 4-amino-*N*-phenylbenzenesulfonamide moiety indicated that they play a significant role in the anti-*P. falciparum* activity. 4-Trifluoromethylated compounds **9** (R = H, R₁ = CF₃; IC₅₀ = 0.15 μM), **16** (R = CH₃, R₁ = CF₃; IC₅₀ = 0.39 μM) and **23** (R = CF₃, R₁ = CF₃; IC₅₀ = 0.03 μM) showed enhanced inhibitory activity (up to 61-fold) compared with unsubstituted analogues **4** (R = H, R₁ = H; IC₅₀ = 9.1 μM), **11** (R = CH₃, R₁ = H; IC₅₀ = 6.9 μM) and **18** (R = CF₃, R₁ = H; IC₅₀ = 1.3 μM), which were the least potent analogues among the triazolopyrimidine derivatives. The 4-trifluoromethylated compounds were also more potent than analogues containing methoxy groups, such as **8** (R = H, R₁ = R = OCH₃; IC₅₀ = 3.5 μM), **15** (R = CH₃, R₁ = OCH₃; IC₅₀ = 4 μM) and **22** (R = CF₃, R₁ = OCH₃; IC₅₀ = 0.8 μM). The greater activity of the trifluoromethylated derivatives was also seen after comparison with other substituents in the series. This leads us to believe that electron withdrawing groups at the 4 position is favourable for inhibitory activity. Another factor to be considered is the greater lipophilicity that these compounds have related to the others, which is another factor attributed to the CF₃ group [23].

Compounds containing a methoxy substituent (R₁ = OCH₃; **8**, **15** and **22**) did not show relevant inhibitory activity when compared to the other compounds in the series; however, introduction of this substituent increased the potency of the compounds 1.6- to 2.6-fold when compared with their unsubstituted analogues. The introduction of a methyl at the 4 position significantly increased the activity of the compounds (up to 40-fold), as seen when comparing the IC₅₀ values of compounds **18** (R = CF₃, R₁ = H; IC₅₀ = 1.3 μM) and **21** (R = CF₃, R₁ = CH₃; IC₅₀ = 0.032 μM). The introduction of a halogen, such as in **19** (R = CF₃, R₁ = Cl; IC₅₀ = 0.4 μM) and **20** (R = CF₃, R₁ = F; IC₅₀ = 0.086 μM), was favourable for inhibitory activity when compared to the unsubstituted analogues. In this sense, fluorinated derivative **20** was 15-fold more potent than **18**.

The bulky *N*-(naphthalen-2-yl)benzenesulfonamide group present in compounds **10** (R = H; IC₅₀ = 0.23 μM), **17** (R = CH₃; IC₅₀ = 0.64 μM) and **24** (R = CF₃; IC₅₀ = 0.05 μM) proved to be very important for anti-*P. falciparum* activity, significantly increasing the activity of these compounds up to 39-fold when compared to compounds **4** (R = H, R₁ = H; IC₅₀ = 9.1 μM), **11** (R = CH₃, R₁ = H; IC₅₀ = 6.9 μM) and **18** (R = CF₃, R₁ = H; IC₅₀ = 1.3 μM). This finding reinforces the importance of the presence of a bulky group with the [1,2,4]triazolo[1,5-*a*]pyrimidine ring for anti-*P. falciparum* activity.

Derivatives containing the pyrazolo[1,5-*a*]pyrimidine ring **25-31** were the least potent inhibitors. Compounds **25** ($R_1 = H$) and **30** ($R_1 = CF_3$), the only active compounds in this series, had IC_{50} values of 2.8 and 8.0 μM , respectively; however, they were less potent than the [1,2,4]triazolo[1,5-*a*]pyrimidine and quinoline analogues. It is interesting to note that among the active compounds, derivative **30** is trifluoromethylated. What is surprising is the activity of **25** and the loss of activity of the other compounds in the series containing compounds **26-29**, including derivative **31** that has the bulky *N*-(naphthalen-2-yl)benzenesulfonamide group. This indicates that the [1,2,4]triazolo[1,5-*a*]pyrimidine ring is important for activity in molecules containing a pattern of bulky substituents. Thus, we can hypothesize that compounds containing a pyrazolo[1,5-*a*]pyrimidine nucleus are not effective with this substitution pattern.

Quinoline derivatives **32-38** showed activity with IC_{50} values ranging from 0.89 to 4.6 μM . Compounds **35** ($R_1 = CH_3$) and **38** (2-naphthyl) were the most potent, with IC_{50} values of 0.97 and 0.89 μM , respectively. Quinoline derivatives **32** ($R_1 = H$; $IC_{50} = 2.0 \mu M$) and **36** ($R_1 = OCH_3$; $IC_{50} = 2.8 \mu M$) were more potent than their respective unsubstituted or 2-methylated [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives; however, all of these derivatives showed decreased inhibitory activity compared to the 2-trifluoromethylated [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **18-24**.

Compound **23** ($IC_{50} = 0.03 \mu M$) was selected as a representative compound from this series for cross-resistance evaluation. In this assay, a panel of drug-resistant strains of *P. falciparum*, including K1 (resistant to chloroquine), DD2 (resistant to chloroquine and mefloquine), IPC4912 (partial resistance to artemisinin) and a laboratory-generated strain resistant to phosphatidylinositol 4-kinases PI4K inhibitors (PI4K^{RES}), was used. The evaluated ratios of IC_{50} values ($IC_{50}^{resistant}/IC_{50}^{3D7}$) were equal to or less than one (Fig. S1 and Table S3, Supplementary Information). These ratios suggest that the mechanism of action of compound **23** is distinct from the standard antimalarials, thereby indicating no cross-resistance against genetically diverse strains of the parasite.

In summary, [1,2,4]triazolo[1,5-*a*]pyrimidine hydrochlorides **4-24** showed *in vitro* inhibitory activity against *P. falciparum* with IC_{50} values ranging from 0.030 to 9.1 μM and were not toxic in the HepG2 cell assay. Compounds **20**, **21**, **23** and **24** were the most potent inhibitors, with IC_{50} values of 0.086, 0.032, 0.03 and 0.05 μM , respectively, being equipotent to chloroquine ($IC_{50} = 0.03 \mu M$). Compound **23** stood out as the most potent and selective in the series with a selectivity index (SI) of 3000 and no signs of cross-resistance in a panel of drug-resistant strains of *P. falciparum*.

The [1,2,4]triazolo[1,5-*a*]pyrimidine scaffold is a privileged scaffold active against DHODH enzymes [10]. Therefore, the *P. falciparum* and human DHODH (*PfDHODH* and *HsDHODH*, respectively) inhibitory activities of compounds **4-31** were assessed (quinoline derivatives **32-38** were not evaluated against *PfDHODH* because it is known that the quinoline moiety is not recognized by this

enzyme). The enzymatic inhibitory activity evaluation was carried out in two stages. The first stage involved the evaluation of each compound's inhibitory activity against both *Hs*DHODH and *Pf*DHODH enzymes at a single concentration (50 μ M), **Table 2S** (Supplementary Information). The compounds that considerably inhibited the enzymatic activity ($\geq 80\%$ inhibition) were evaluated in a second step (**Table 2S**, Supplementary Information). In this stage, IC_{50} values against *Pf*DHODH were determined. Compounds DSM265 [24] and ML390 [25] were used as position controls for *Pf*DHODH and *Hs*DHODH inhibition, respectively. The results of the enzyme inhibition evaluation are shown in **Table 1**.

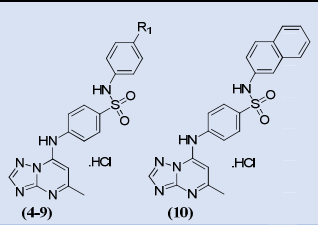
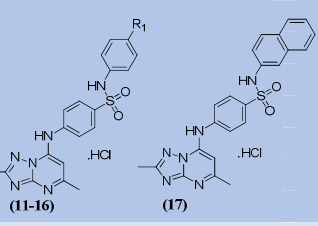
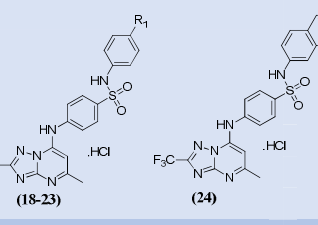
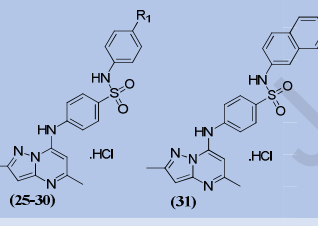
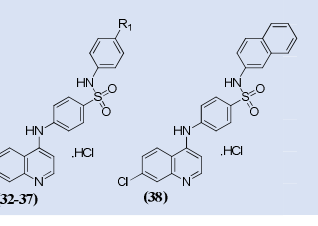
Compound **18** showed *Pf*DHODH inhibition below 80% at 50 μ M; for this reason, this compound was not subjected to IC_{50} evaluation. The IC_{50} values of compounds **5**, **8**, **9**, **11** and **23** were not determined because it was not possible to measure consumption of the substrates at a certain concentration on the IC_{50} curve.

The IC_{50} values of the [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4-24** spanned from 0.08 to 1.3 μ M. On the other hand, pyrazolo[1,5-*a*]pyrimidine derivatives **25-31** did not show significant *Pf*DHODH inhibition (25 to 66% at 50 μ M), which is in line with the *in vitro* inhibitory activity against *P. falciparum*. It is interesting to note that compounds **4-31** showed no significant inhibition against the *Hs*DHODH enzyme (0-30% at 50 μ M), thereby indicating that they are selective inhibitors of the *Pf*DHODH enzyme.

The [1,2,4]triazolo[1,5-*a*]pyrimidines **20** ($R_1 = F$, $IC_{50} = 0.5 \mu$ M) and **21** ($R_1 = CH_3$; $IC_{50} = 0.8 \mu$ M) had very close IC_{50} values to prototype **1** ($IC_{50} = 0.7 \mu$ M) [19], and can be considered equipotent bioactive compounds, whereas **22** ($R_1 = OCH_3$; $IC_{50} = 0.21 \mu$ M), **24** ($R_1 = 2$ -naphthyl; $IC_{50} = 0.22 \mu$ M), and **19** ($R_1 = Cl$; $IC_{50} = 0.08 \mu$ M) were 3.5, 3.5 and 8.75-fold more potent than prototype **1**, respectively.

It is worth mentioning that a reasonable correlation between enzymatic and whole parasite inhibition was verified for the low micromolar and submicromolar inhibitors **4-18**, suggesting that *Pf*DHODH is the main target underlying the inhibitory activity of this series. However, a difference in the IC_{50} values of 5- to 25-fold against *Pf*DHODH and the *P. falciparum* 3D7 strain was observed for the most potent parasite inhibitors (**20**, **21** and **24**, Table 2). These inconsistencies may be due to the lower limit of detection of the enzymatic assay. In the inhibition assay, the *Pf*DHODH enzyme was used at a final concentration of 50 nM. Therefore, the enzyme concentration might have a significant effect on the IC_{50} value assessment of the most potent inhibitors as their IC_{50} values approach the enzyme concentration [26].

Table 1: *In vitro* inhibitory activity against *P. falciparum* parasites (3D7 strain, chloroquine-sensitive), human hepatocarcinoma cells (HepG2), and *Pf*DHODH, and the selectivity index (SI) values for compounds **4-38**. Chloroquine and artesunate were used as positive controls for whole parasite inhibition.

Compounds	Substituents R ₁	IC ₅₀ ^{3D7} (μM) <i>P. falciparum</i>	IC ₅₀ ^{HepG2} (μM)	SI*	IC ₅₀ (μM) <i>Pf</i> DHODH		
 (4-9) (10)	4	R ₁ = H	9.1 ± 0.9	365 ± 10	> 40	0.8 ± 0.1	
	5	R ₁ = Cl	3.0 ± 0.5	92 ± 2	>31	>50	
	6	R ₁ = F	2.0 ± 0.4	367 ± 12	>187	0.3 ± 0.1	
	7	R ₁ = CH ₃	0.50 ± 0.05	367 ± 10	>728	0.41 ± 0.04	
	8	R ₁ = OCH ₃	4 ± 1	46 ± 2	>13	>50	
	9	R ₁ = CF ₃	0.15 ± 0.01	>100	>667	>50	
	10	-	0.23 ± 0.01	56 ± 2	243	0.3 ± 0.1	
	 (11-16) (17)	11	R ₁ = H	6.9 ± 0.2	193 ± 9	28	>50
		12	R ₁ = Cl	0.8 ± 0.1	>50	>62.5	0.6 ± 0.1
		13	R ₁ = F	2.7 ± 0.8	150 ± 20	55.6	1.0 ± 0.2
14		R ₁ = CH ₃	0.91 ± 0.09	110 ± 10	121	0.3 ± 0.2	
15		R ₁ = OCH ₃	4 ± 1	170 ± 10	42.5	1.3 ± 0.1	
16		R ₁ = CF ₃	0.39 ± 0.06	125 ± 12	321	0.6 ± 0.3	
17		-	0.64 ± 0.01	56 ± 2	87.4	0.4 ± 0.1	
 (18-23) (24)		18	R ₁ = H	1.3 ± 0.4	92 ± 4	>70	>50
	19	R ₁ = Cl	0.4 ± 0.2	47 ± 2	>123	0.08 ± 0.01	
	20	R ₁ = F	0.086 ± 0.004	101 ± 7	1174	0.5 ± 0.1	
	21	R ₁ = CH ₃	0.032 ± 0.005	54 ± 4	1688	0.8 ± 0.1	
	22	R ₁ = OCH ₃	0.8 ± 0.1	93 ± 6	>114	0.21 ± 0.06	
	23	R ₁ = CF ₃	0.030 ± 0.003	90 ± 10	3000	>50	
	24	-	0.050 ± 0.002	>100	>2000	0.22 ± 0.07	
	 (25-30) (31)	25	R ₁ = H	2.8 ± 0.1	27 ± 4	9.6	>50
26		R ₁ = Cl	>10	ND	ND	>50	
27		R ₁ = F	>10	ND	ND	>50	
28		R ₁ = CH ₃	>10	ND	ND	>50	
29		R ₁ = OCH ₃	>10	ND	ND	>50	
30		R ₁ = CF ₃	8.0 ± 1	42 ± 4	5.25	>50	
31		-	>10	ND	ND	>50	
 (32-37) (38)		32	R = H	2.0 ± 0.2	92 ± 4	>46	-
		33	R = Cl	1.7 ± 0.2	93 ± 5	>56	-
		34	R = F	4.6 ± 0.6	92 ± 2	>21	-
	35	R = CH ₃	0.97 ± 0.08	92 ± 6	>95	-	
	36	R = OCH ₃	2.8 ± 0.4	92 ± 4	>33	-	
	37	R = CF ₃	2.1 ± 0.2	23 ± 1	11	-	
	38	-	0.89 ± 0.06	13 ± 1	15.1	-	
	chloroquine		0.03 ± 0.01	384 ± 62	12,800	-	
artesunate		0.009 ± 0.002	267 ± 21	29,666			

$$*SI = IC_{50}^{HepG2} / IC_{50}^{3D7}$$

2.3. Molecular docking

Molecular docking simulations were performed considering only the triazolo[1,5-*a*]pyrimidine derivatives, which were active against *P. falciparum* and inhibited the *Pf*DHODH enzyme (**Table 1**).

Validation of the docking protocol was carried out using the inhibitor DSM265 complexed to *Pf*DHODH (PDB code: **5BOO**, **Fig. S2A-B**, Supplementary Information) [12]. Thus, redocking of this inhibitor showed a root-mean-square deviation (RMSD) value of 1.16 Å, predicting the co-crystallized binding pose correctly (**Fig. S2A**) with a MolDock score value of -169.45 arbitrary units (a.u.). The inhibitor DSM265 interacts via H-bonds with Arg265 and His185 (H-bond energy = -5.56 a.u.) and presents steric interactions with Arg265, His185 and Leu240 (steric interaction energy = -187.48 a.u.) (**Table S4** and **Fig. S3A**, Supplementary Information).

Consequently, the predicted complexes between the selected triazolo[1,5-*a*]pyrimidine inhibitors and *Pf*DHODH showed their lowest energy poses docked into the same binding pocket as the inhibitor DSM265 (**Fig. S2B**). Hydrogen bonds (H-bonds) and steric interactions were also evaluated (**Table S4** and **Fig. S3A-P**). Among the active compounds, only **19** (**Fig. 3A-C**), the most potent in the enzymatic assay ($IC_{50} = 0.08 \mu\text{M}$), and **21** (**Fig. S4A-D**) (Supplementary Information) presented similar interactions with the enzyme compared to those of DSM265. Thus, here, we described only the interactions concerning these two inhibitors, with the others reported in the supplementary material (**Fig. S3A-P**).

Interestingly, the best superposition of the triazolo[1,5-*a*]pyrimidine and the phenylamine groups occurs between the predicted pose of **19** and the co-crystallized structure of DSM265. Thus, this inhibitor interacts via H-bonds with the same residues as described for the inhibitor DSM265, Arg265 and His185 (H-bond energy = -3.85 a.u.), and shows steric interactions with Arg265, Cys233, His185, Gly192, Leu240, Leu531, Leu197, Met536, and Phe188 (steric interaction energy = -196.20 a.u.) (**Fig. 3B-C**).

Inhibitor **21** also interacts via the same H-bonds as the inhibitor DSM265 (H-bond energy = -3.85 a.u.), and shows steric interactions with Arg265, Cys233, Gly192, His185, Leu197, Leu240, Leu531, Met536, and Phe188 (steric interaction energy = -194.90 a.u.) (**Table S4** and **Fig. S3N**). However, its predicted pose shows superposition only with the triazolo[1,5-*a*]pyrimidine ring when compared to DSM265.

Overall, the molecular docking results suggest that the active triazolo[1,5-*a*]pyrimidine derivatives interact within at same binding site as DSM265. However, among the newly synthesized derivatives, compound **19** presents the most similar binding mode as that described for DSM265, with a higher interaction affinity for the *Pf*DHODH enzyme and a negative interaction energy value (**Table S4**).

Therefore, the molecular docking simulations corroborate the tests carried out on the *Pf*DHODH enzyme since the most active triazolo[1,5-*a*]pyrimidine (**19**), which shows similar interactions and a similar pose to that of DSM265, inhibited *Pf*DHODH activity at low nanomolar concentrations (IC_{50} of 0.08 μM ; **Table 1**).

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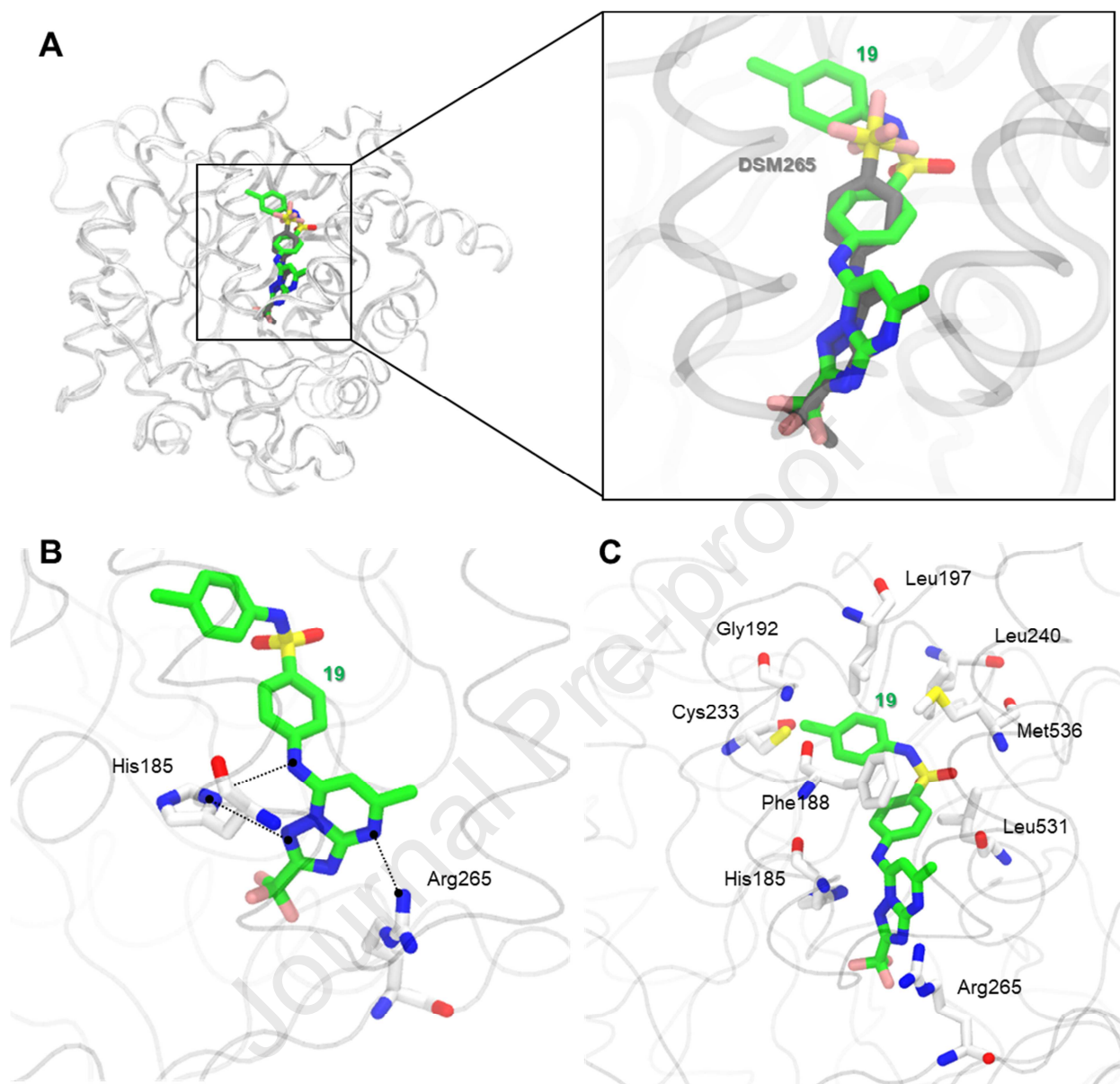


Fig. 3: (A) Representation of the superposition between DSM265 (grey) and **19** (green) complexed to *PfDHODH*. (B) Hydrogen-bonding interactions (B) and steric interactions (C) between **19** and the amino acid residues of *PfDHODH*. The structures are represented as sticks and coloured by atom: nitrogen atoms in blue, sulfur atoms in yellow, fluorine atoms in pink, oxygen atoms in red, and carbon atoms in grey, green or white.

3. Conclusions

Among the 35 synthesized compounds, 30 exhibited *in vitro* inhibitory activity against *P. falciparum* (3D7 strain) with IC_{50} values ranging from 0.030 to 9.1 μ M. Of the three series synthesized, the [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4-24** were the most potent and showed no cytotoxicity. Compounds **20** (R = CF_3 , R_1 = F; IC_{50} = 0.086 μ M), **21** (R = CF_3 , R_1 = CH_3 ; IC_{50} = 0.032 μ M), **23** (R =

CF₃, R₁ = CF₃; IC₅₀ = 0.03 μM), and **24** (R = CF₃, 2-naphthyl; IC₅₀ = 0.05 μM) were equipotent to chloroquine (IC₅₀ = 0.03 μM), and compound **23** stood out as the most active in the series. Compounds with a trifluoromethyl group at the 2 position of the [1,2,4]triazolo[1,5-*a*]pyrimidine ring were the most potent among the four series. This finding is in good agreement with a previous structure-activity relationship study [18].

Compounds **9** (R = H, R₁ = CF₃; IC₅₀ = 0.150 μM), **16** (R = CH₃, R₁ = CF₃; IC₅₀ = 0.39 μM) and **23** (R = CF₃, R₁ = CF₃; IC₅₀ = 0.030 μM) containing a CF₃ group at the 4 position of the 4-amino-*N*-phenylbenzenesulfonamide moiety showed substantially increased activity (up to 61-fold) compared to the unsubstituted analogues. These results indicated that CF₃ groups, with greater lipophilicity at the 2 position of the [1,2,4]triazolo[1,5-*a*]pyrimidine ring and the 4 position of the 4-amino-*N*-phenylbenzenesulfonamide moiety are attractive substituents to increase potency. Compound **20** (R = CF₃, R₁ = F; IC₅₀ = 0.086 μM) and compound **21** (R = CF₃, R₁ = CH₃; IC₅₀ = 0.032 μM) showed an increase in activity by 15- and 40-fold, respectively, when compared with their unsubstituted (R₁ = H) analogues. In addition, the bulky 4-amino-*N*-(naphthalen-2-yl)benzenesulfonamide moiety proved to be important for the inhibitory activity (up to 40-fold more potent when compared to the unsubstituted analogue). Compound **23** (IC₅₀ = 0.03 μM), a representative compound of this series, showed no cross-resistance against genetically diverse strains of the parasite.

The series of pyrazolo[1,5-*a*]pyrimidine derivatives **25-31** were less potent than the [1,2,4]triazolo[1,5-*a*]pyrimidine and quinoline analogues. These results suggest that the pyrazolo[1,5-*a*]pyrimidine ring was not relevant for anti-*P. falciparum* activity among molecules that have this pattern of substituents. A similar trend was observed for quinoline derivatives **32-38**, which showed decreased inhibitory activity related to the [1,2,4]triazolo[1,5-*a*]pyrimidine series. The most potent quinoline derivatives were low micromolar inhibitors (**35**, R₁ = CH₃; IC₅₀ = 0.97 μM and **38**, 2-naphthyl; IC₅₀ = 0.89 μM).

The [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives **4-24** selectively inhibited the *Pf*DHODH enzyme, confirming that this system is active against this target. These derivatives showed potent inhibitory activity of the *Pf*DHODH enzyme with IC₅₀ values in the range of 1.3-0.08 μM. These derivatives did not show significant inhibition against the *Hs*DHODH enzyme (0-30% at 50 μM), thereby indicating that they are selective for the parasite homologue. The potent *in vitro* activity against *P. falciparum* and inhibition of the *Pf*DHODH enzyme strongly suggest that this is the mechanism of action underlying this series of new [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives. Pyrazolo[1,5-*a*]pyrimidines **25-31** did not significantly inhibit the *Pf*DHODH enzyme or the parasite in the *in vitro* tests.

Molecular docking studies suggest that compounds **4-24** interact at the same site as DSM265, a *Pf*DHODH inhibitor, which is in phase II of clinical trials. Among the pyrazolo[1,5-*a*]pyrimidine

inhibitors, compound **19** presented the most similar binding mode when compared to DSM265 but showed a higher interaction energy for the *Pf*DHODH enzyme.

Thus, the most active inhibitor against *Pf*DHODH **19** ($R = CF_3$, $R_1 = Cl$; $IC_{50} = 0.08 \mu M$ - *Pf*DHODH) and the most active compounds against *P. falciparum* parasites **20**, ($R = CF_3$, $R_1 = F$; $IC_{50} = 0.086 \mu M$; **21**, $R = CF_3$, $R_1 = CH_3$; $IC_{50} = 0.032 \mu M$; **23**, $R = CF_3$, $R_1 = CF_3$; $IC_{50} = 0.030 \mu M$; and **24**, $R = CF_3$, 2-naphthyl; $IC_{50} = 0.050 \mu M$) stand out as the new prototypes of the group.

Overall, these results demonstrate the potential of [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives as inhibitors of the *Pf*DHODH enzyme and represent new lead compounds for antimalarial drug discovery.

4. Materials and methods

4.1. Chemistry

All reagents and solvents used were of analytical grade. TLC (thin layer chromatography) was performed using silica gel Merck TLC F-254 PTLC (Preparative TLC) Glass Plates (20×20 cm). The melting points (m.p. values) were determined using a Buchi model B-545 apparatus. Electron-ionization mass spectrometry (EI-MS, scan ES+capillary (3.0 kV)/cone (30 V)/extractor (1 V)/RF lens (1.0 V)/source temperature (150 °C)/desolvation temperature (300 °C)) spectra were recorded using a Micromass/Waters spectrometer (model: ZQ-4000). High-resolution mass spectrometry (HRMS) data were obtained using an LC-MS Bruker Daltonics MicroTOF (time of flight) analyser. Fourier transform infrared (FTIR) absorption spectra were recorded on a Shimadzu mode IR Prestige-21 spectrophotometer using the attenuated total reflection (ATR) technique. 1H , ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were obtained at 400.00, 100.00 and 376.00 MHz, respectively, using a Bruker Avance instrument equipped with a 5 mm probe. Tetramethylsilane was used as the internal standard. The chemical shifts (δ) are reported in ppm, and the coupling constants (J) are reported in Hertz. Analysis by high-performance liquid chromatography (HPLC) was performed on an LC-20AD Shimadzu liquid chromatograph using a Hypersil BDS C18 column (5 μm , 250 × 4.6 mm).

4.2. Synthesis

4.2.1. General procedure for the preparation of 1*H*-1,2,4-triazole-5-amines **39a-c**

A mixture of 6.8 g (0.05 mol) of aminoguanidine bicarbonate and 5 mL (0.066 mol) of the appropriate acid (formic, acetic or trifluoroacetic acid) was kept under magnetic stirring until the release of carbon dioxide was complete. Then, toluene (100 mL) was added, and the reaction mixture was heated to reflux (110 °C) with a Dean-Stark apparatus under stirring for 24 h. A white precipitate formed and was cooled, vacuum filtered and washed with cold toluene [20].

1*H*-1,2,4-triazol-5-amine (**39a**) Yield: 97%. m.p. 152-154 °C. IR (cm⁻¹): 3399-3324; 1590-1533; 1631; 1266. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 5.77 (s; 2H; NH₂); 7.44 (s; 1H; H3); 11.99 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 147.7 (C3); 157.9 (C5). ESI [M + 1]⁺ 85.47.

3-methyl-1*H*-1,2,4-triazol-5-amine (**39b**) Yield: 70%. m.p. 145-146 °C. IR (cm⁻¹): 3419-3189; 1708; 1599-1544; 1255. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.05 (s; 3H; CH₃); 5.49 (s; 2H; NH₂); 11.68 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 13.2 (CH₃); 168.5 (C3); 172.7 (C5). ESI [M+1]⁺ 99.38.

3-(trifluoromethyl)-1*H*-1,2,4-triazol-5-amine (**39c**) Yield: 93%. m.p. 197 °C. IR (cm⁻¹): 3487-3360; 1634; 1580-1490; 1361, 1177, 758. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 6.46 (s; 2H; NH₂); 12.71 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 119.6 (q; *J* = 267.1 Hz; CF₃); 149.9 (q; *J* = 36.8 Hz; C3); 157.8 (C5). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -64.78. ESI [M + 1]⁺ 153.10.

4.2.2. General procedure for the preparation of 5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-7(4*H*)-ones **40a-c** and 2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**40d**)

To 0.01 mol of the appropriate 1*H*-1,2,4-triazole-5-amines **39a-c** or 5-methyl-1*H*-pyrazol-3-amine (**39d**) was added 13 mL of ethyl acetoacetate (0.1 mol), 15 mL of toluene and a catalytic amount of *p*-toluenesulfonic acid. The reaction mixture stirred under reflux (110 °C) for 24 h. The mixture was cooled to room temperature, and the white precipitate formed was vacuum filtered and washed with cold toluene [18,19].

5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**40a**) Yield: 71%. m.p. 278-279 °C. IR (cm⁻¹): 2807; 1697; 1620; 1335. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.32 (s; 3H; CH₃); 5.83 (s; 1H; H6); 8.18 (s; 1H; H2). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 18.6 (CH₃); 98.1 (C6); 150.6 (C5); 151.7 (C3a); 151.8 (C2); 155.8 (C7). ESI [M-1]⁻ 149.17.

2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**40b**) Yield: 95%. m.p. 313-314 °C. IR (cm⁻¹): 2703; 1692; 1641; 1323. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.29 (s; 3H; CH₃); 2.33 (s; 3H; CH₃); 5.76 (d; 1H; *J* = 0.56 Hz; H6); 13.00 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.2 (CH₃); 18.6 (CH₃); 98.1 (C6); 150.8 (C5); 151.0 (C3a); 155.5 (C2); 160.7 (C7). ESI [M-1]⁻ 163.18.

5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**40c**) Yield: 84%. m.p. 262-263 °C. IR (cm⁻¹): 2887; 1694; 1605; 1399; 751. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.35 (d; 3H; *J* = 0.60 Hz; CH₃); 5.99 (d; 1H; *J* = 0.72 Hz; H6); 13.58 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 18.6 (CH₃); 99.1 (C6); 119.1 (q; *J* = 269.0 Hz; CF₃); 151.4 (C5); 151.8 (q; *J* = 38.5 Hz; C2); 152.6 (C3a); 155.1 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -64.96. ESI [M-1]⁻ 217.06.

2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7(4*H*)-one (**40d**) Yield: 98%. m.p. 253-255 °C. IR (cm⁻¹): 1664; 1612; 1330. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.26 (d; 3H; *J* = 0.36 Hz; CH₃); 2.27 (s; 3H; CH₃); 5.50 (d; 1H; *J* = 0.56 Hz; H6); 5.91 (s; 1H; H3); 12.10 (s; 1H; NH). ¹³C NMR (100 MHz,

DMSO-d₆, TMS, δ in ppm): 14.0 (CH₃'); 18.5 (CH₃); 87.9 (C3); 94.8 (C6); 142.0 (C3a); 149.5 (C5); 151.6 (C2); 156.0 (C7). ESI [M+23]⁺ 186.0687.

4.2.3. General procedure for the preparation of 7-chloro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidines **41a-c** and 7-chloro-2,5-dimethylpyrazolo[1,5-a]pyrimidine (**41d**)

A mixture of 0.006 mol of the appropriate carbonylated intermediate **40a-d** and 10 mL of POCl₃ was stirred under reflux (105 °C) for 4 h. The reaction mixture was carefully poured into ice water and made alkaline with 20% NaOH to reach pH 9-10. The mixture was diluted with water (50 mL) and extracted with ethyl acetate (3 × 50 mL). The organic phase was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated under vacuum [18,19].

7-chloro-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine (**41a**) Yield: 51%. m.p. 139-140 °C. IR (cm⁻¹): 1619; 1525; 868. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.63 (s; 3H; CH₃); 7.66 (s; 1H; H6). 8.67 (s; 1H; H2). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 4.4 (CH₃); 111.8 (C6); 138.0 (C5); 155.2 (C3a); 155.5 (C2); 165.4 (C7). ESI [M+1]⁺ 169.10.

7-chloro-2,5-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidine (**41b**) Yield: 67%. m.p. 143-145 °C. IR (cm⁻¹): 3065; 1610; 1519; 862. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.50 (s; 3H; CH₃); 3.60 (s; 3H; CH₃); 7.55 (s; 1H; H6). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.6 (CH₃); 24.3 (CH₃); 110.9 (C6); 137.2 (C5); 155.5 (C3a); 164.7 (C2); 165.0 (C7). ESI [M+1]⁺ 182.97.

7-chloro-5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-a]pyrimidine (**41c**) Yield: 79%. m.p. 107-108 °C. IR (cm⁻¹): 3061; 1613; 1522; 877; 748. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.68 (s; 3H; CH₃); 7.88 (s; 1H; H6). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.6 (CH₃); 113.9 (C6); 119.1 (q; J = 269.0 Hz; CF₃); 138.8 (C5); 154.5 (q; J = 38.6 Hz; C2); 155.3 (C3a); 167.8 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -64.60. HRMS (ESI): [M+1]⁺ 237.0142.

7-chloro-2,5-dimethylpyrazolo[1,5-a]pyrimidine (**41d**) Yield: 97%. m.p. 51-53 °C. IR (cm⁻¹): 1603; 1543; 831. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.43 (s; 3H; CH₃); 2.50 (s; 3H; CH₃); 6.51 (s; 1H; H3); 7.22 (s; 1H; H6). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.1 (CH₃); 23.8 (CH₃); 95.9 (C3); 108.2 (C6); 136.3 (C3a); 149.4 (C5); 154.5 (C2); 158.4 (C7). ESI [M+1]⁺ 182.0451.

4.2.4. General procedure for the preparation of 4-acetamidobenzene-1-sulfonyl chloride (**42**)

Ten grams (0.073 mol) of *N*-phenylacetamide was slowly added to 24,6 mL of chlorosulfonic acid (0.37 mol) with magnetic stirring. During the addition, an ice-water bath was used to keep the temperature at approximately 0 °C; then, the bath was removed, and the temperature was raised to 60 °C. The reaction mixture was stirred and heated for 1 h. The mixture was then slowly poured into ice, and the precipitate formed was vacuum filtered and washed with water. The product was used without purification [21].

4-acetamidobenzene-1-sulfonyl chloride (**42**) Yield: 77%. HRMS (ESI): calc. for C₈H₇ClNO₃S 231.9841; found [M-1]⁻ 231.9861.

4.2.5. General procedure for the preparation of *N*-(4-(*N*-arylsulfamoyl)phenyl)acetamides **43a-g**

A mixture of 2.3 g (0.01 mol) of 4-acetamidobenzenesulfonyl chloride (**42**), 1 equivalent of the respective aniline, 5 mL of TEA (2.5 equivalents), and 25 mL of chloroform was kept with stirring under reflux for 5 h. Then, the solvent was evaporated, and ice water was added to the mixture. The precipitate formed was vacuum filtered, washed with distilled water and recrystallized from ethanol/water (1:1).

N-(4-(*N*-phenylsulfamoyl)phenyl)acetamide (**43a**) Yield: 67%. m.p. 197-199 °C. IR (cm⁻¹): 3445-3249; 1669; 1592; 1314-1150. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.04 (s; 3H; CH₃); 6.93 (t; 1H; *J* = 7.4 Hz; H4'); 7.02 (dd; 2H; *J* = 1.2 Hz; *J* = 7.4 Hz; H2', H6'); 7.16 (t; 2H; *J* = 7.4 Hz; H3', H5'); 7.66 (s; 4H; H2, H3, H5, H6); 10.26 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 23.9 (CH₃); 118.3 (C3, C5); 119.9 (C2', C6'); 122.8 (C4'); 127.6 (C2, C6); 128.8 (C3', C5'); 134.0 (C1); 139.2 (C1'); 142.5 (C4); 168.8 (C=O). HRMS (ESI): [M+23]⁺ 313.0612.

N-(4-(*N*-(4-chlorophenyl)sulfamoyl)phenyl)acetamide (**43b**) Yield: 42%. m.p. 194-195 °C. IR (cm⁻¹): 3358-3192; 1682; 1601; 1326-1150; 845. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.05 (s; 3H; CH₃); 7.07 (d; 2H; *J* = 8.8 Hz; H2', H6'); 7.28 (d; 2H; *J* = 8.8 Hz; H3', H5'); 7.67 (d; 4H; *J* = 9.1 Hz; H2, H6); 7.70 (d; 4H; *J* = 9.1 Hz; H3, H5); 10.30 (s; 1H; NH); 10.31 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in p.p.m): 24.0 (CH₃); 118.4 (C3, C5); 121.4 (C2', C6'); 127.84 (C2, C6); 127.88 (C4'); 128.9 (C3', C5'); 132.4 (C1); 136.7 (C1'); 143.1 (C4); 168.9 (C=O). HRMS (ESI): [M-1]⁻ 323.0143.

N-(4-(*N*-(4-fluorophenyl)sulfamoyl)phenyl)acetamide (**43c**) Yield: 59%. m.p. 177-179 °C. IR (cm⁻¹): 3356-3229; 1688; 1592; 1324-1147; 1091. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.05 (s; 3H; CH₃); 7.07-7.06 (m; 4H; H2', H3', H5', H6'); 7.63 (d; 2H; *J* = 7.0 Hz; H2, H6); 7.68 (d; 2H; *J* = 7.0 Hz; H3, H5); 10.09 (s; 1H; SO₂NH); 10.29 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.1 (CH₃); 115.8 (d; *J* = 22.3 Hz; C3', C5'); 118.4 (C3, C5); 122.7 (d; *J* = 8.1 Hz; C2', C6'); 127.9 (C2, C6); 132.6 (C1); 134.0 (d; *J* = 2.3 Hz; C1'); 143.1 (C4); 158.9 (d; *J* = 239.2 Hz; C4'); 169.0 (C=O). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -118.57. HRMS (ESI): [M+23]⁺ 331.0462.

N-(4-(*N*-(*p*-tolyl)sulfamoyl)phenyl)acetamide (**43d**) Yield: 74%. m.p. 190-192 °C. IR (cm⁻¹): 3352-3199; 1683; 1594; 1397; 1327-1145. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.05 (s; 3H; CH₃); 2.17 (s; 3H; CH₃'); 6.94 (d; 2H; *J* = 8.4 Hz; H2', H6'); 7.01 (d; 2H; *J* = 8.4 Hz; H3', H5'); 7.63 (d; 4H; *J* = 9.0 Hz; H2, H6); 7.67 (d; 4H; *J* = 9.0 Hz; H3, H5); 9.97 (s; 1H; SO₂NH); 10.27 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 20.2 (CH₃'); 24.0 (CH₃); 118.4 (C3, C5); 120.5 (C2', C6'); 127.8 (C2, C6); 129.4 (C3', C5'); 133.0 (C4'); 133.2 (C1); 135.1 (C1'); 142.9 (C4); 168.9 (C=O). HRMS (ESI): [M+23]⁺ 327.0778.

N-(4-(*N*-(4-methoxyphenyl)sulfamoyl)phenyl)acetamide (**43e**) Yield: 87%. m.p. 195-197 °C. IR (cm⁻¹): 3302-3242; 1668; 1586; 1315-1156; 1032. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.05 (s; 3H; CH₃); 3.66 (s; 3H; OCH₃); 6.78 (d; 2H; *J* = 9.0 Hz; H2', H6'); 6.95 (d; 2H; *J* = 9.0 Hz; H3', H5'); 7.59 (d; 2H; *J* = 7.0 Hz; H2, H6); 7.67 (d; 2H; *J* = 7.0 Hz; H3, H5); 9.76 (s; 1H; SO₂NH); 10.27 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.1 (CH₃); 55.1 (OCH₃); 114.2 (C3', C5');

118.4 (C3, C5); 123.4 (C2', C6'); 127.9 (C2, C6); 130.2 (C1); 133.0 (C1'); 142.9 (C4); 156.4 (C4'); 168.9 (C=O). HRMS (ESI): $[M+23]^+$ 343.0702.

N-(4-(*N*-(4-(trifluoromethyl)phenyl)sulfamoyl)phenyl)acetamide (**43f**) Yield: 44%. m.p. 185-188 °C. IR (cm⁻¹): 1673; 1591; 1320-1152. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.05 (s; 3H; CH₃); 7.27 (d; 2H; *J* = 8.4 Hz; H2', H6'); 7.59 (d; 2H; *J* = 8.6 Hz; H3', H5'); 7.72 (d; 4H; *J* = 9.2 Hz; H2, H6); 7.76 (d; 4H; *J* = 9.2 Hz; H3, H5); 10.33 (s; 1H; SO₂NH); 10.77 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.0 (CH₃); 118.52 (C3, C5); 118.58 (C2', C6'); 123.4 (q; *J* = 31.8 Hz; C4'); 124.1 (q; *J* = 269.7 Hz; CF₃); 126.4 (q; *J* = 3.7 Hz; C3', C5'); 127.9 (C2, C6) 132.4 (C1); 141.5 (C1'); 143.3 (C4); 168.9 (C=O). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -60.42. HRMS (ESI): $[M-1]^-$ 357.0459.

N-(4-(*N*-(naphthalen-2-yl)sulfamoyl)phenyl)acetamide (**43g**) Gross Yield: 61%. HRMS (ESI): calc. for $[M-1]^-$ C₁₈H₁₅N₂O₃S 339,0803; found $[M-1]^-$ 339,0812 (used without purification).

4.2.6. General procedure for the preparation of 4-amino-*N*-arylsulfonamides (**44a-g**)

A mixture of 0.006 mol of the respective *N*-(4-(*N*-arylsulfamoyl)phenyl)acetamide **43a-g** in 30 mL of 6N HCl was kept with stirring under reflux (100 °C) for 6 h. Then, the solution was cooled and neutralized with 20% NaOH. The precipitate formed was vacuum filtered, washed with water and recrystallized from ethanol/water (1:1).

4-amino-*N*-phenylbenzenesulfonamide (**44a**) Yield: 81%. m.p. 189 °C. IR (cm⁻¹): 3250-3219; 1593; 1300-1147. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 5.95 (s; 2H; NH₂); 6.51 (d; 2H; *J* = 8.7 Hz; H3, H5); 6.96 (t; 1H; *J* = 7.5 Hz; H4'); 7.05 (dd; 2H; *J* = 0.9 Hz; *J* = 7.5 Hz; H2', H6') 7.19 (t; 2H; *J* = 7.5 Hz; H3', H5'); 7.37 (d; 2H; *J* = 8.7 Hz; H2, H6); 9.83 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 112.5 (C3, C5); 119.3 (C2', C6'); 123.2 (C4'); 124.3 (C1); 128.6 (C3', C5'); 128.9 (C2, C6); 138.4 (C1'); 152.7 (C4). HRMS (ESI): $[M+23]^+$ 271.0531.

4-amino-*N*-(4-chlorophenyl)benzenesulfonamide (**44b**) Yield: 75%. m.p. 191-193 °C. IR (cm⁻¹): 3413-3341; 1594; 1312-1147; 817. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 5.99 (s; 2H; NH₂); 6.53 (d; 2H; *J* = 8.1 Hz; H3, H5); 7.06 (d; 2H; *J* = 8.2 Hz; H2', H6'); 7.24 (d; 2H; *J* = 8.2 Hz; H3', H5'); 7.37 (d; 2H; *J* = 8.1 Hz; H2, H6); 10.00 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 112.4 (C3, C5); 120.8 (C2', C6'); 123.8 (C1); 127.1 (C4'); 128.6 (C2, C6); 128.8 (C3', C5'); 137.3 (C1'); 152.8 (C4). HRMS (ESI): $[M+23]^+$ 305.0214.

4-amino-*N*-(4-fluorophenyl)benzenesulfonamide (**44c**) Yield: 84%. m.p. 163-164 °C. IR (cm⁻¹): 3391-3345; 1593; 1308-1146; 1088. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 5.96 (s; 2H; NH₂); 6.51 (d; 2H; *J* = 8.7 Hz; H3, H5); 7.05 (d; 4H; *J* = 6.8 Hz; H2', H3', H5', H6'); 7.33 (d; 2H; *J* = 8.7 Hz; H2, H6); 9.77 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 112.4 (C3, C5); 115.5 (d; *J* = 22.4 Hz; C3', C5'); 121.9 (d; *J* = 8.1 Hz; C2', C6'); 123.9 (C1); 128.5 (C2, C6); 134.6 (d; *J* = 2.2 Hz; C1'); 152.7 (C4); 158.5 (d; *J* = 238.5 Hz; C4'). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -119.43. HRMS (ESI): $[M+23]^+$ 289.0442.

4-amino-*N*-(*p*-tolyl)benzenesulfonamide (**44d**) Yield: 89%. m.p. 187-188 °C. IR (cm⁻¹): 3411-3343; 1594; 1397; 1317-1147. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.17 (s; 3H; CH₃); 5.92 (s; 2H; NH₂); 6.50 (d; 2H; *J* = 8.7 Hz; H3, H5); 6.93 (d; 2H; *J* = 8.4 Hz; H2', H6'); 6.99 (d; 2H; *J* = 8.4 Hz;

H3', H5'); 7.34 (d; 2H; $J = 8.7$ Hz; H2, H6); 9.65 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 20.2 (CH₃); 112.4 (C3, C5); 119.9 (C2', C6'); 124.4 (C1); 128.6 (C3', C5'); 129.3 (C2, C6); 132.4 (C4'); 135.8 (C1'); 152.6 (C4). HRMS (ESI): [M+23]⁺ 285.0681.

4-amino-*N*-(4-methoxyphenyl)benzenesulfonamide (**44e**) Yield: 66%. m.p. 194-195 °C. IR (cm⁻¹): 3401-3258; 1589; 1317-1149; 1031. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 3.65 (s; 3H; OCH₃); 5.91 (s; 2H; NH₂); 6.50 (d; 2H; $J = 8.7$ Hz; H3, H5); 6.76 (d; 2H; $J = 8.9$ Hz; H2', H6'); 6.94 (d; 2H; $J = 8.9$ Hz; H3', H5'); 7.2 (d; 2H; $J = 8.7$ Hz; H2, H6); 9.44 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 55.0 (OCH₃); 112.4 (C3, C5); 114.0 (C3', C5'); 122.8 (C2', C6'); 124.4 (C1); 128.6 (C2, C6); 131.0 (C1'); 152.6 (C4); 156.0 (C4'). HRMS (ESI): [M+23]⁺ 301.0631.

4-amino-*N*-(4-(trifluoromethyl)phenyl)benzenesulfonamide (**44f**) Yield: 63%. m.p. 165-167 °C. IR (cm⁻¹): 3348; 1592; 1316-1144; 731. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 6.04 (s; 2H; NH₂); 6.54 (d; 2H; $J = 8.7$ Hz; H3, H5); 7.23 (d; 2H; $J = 8.5$ Hz; H2', H6'); 7.44 (d; 2H; $J = 8.7$ Hz; H2, H6); 7.57 (d; 2H; $J = 8.6$ Hz; H3', H5'); 10.45 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 112.5 (C3, C5); 117.9 (C2', C6'); 122.7 (q; $J = 31.8$ Hz; C4'); 123.5 (C1); 124.2 (q; $J = 269.6$ Hz; CF₃); 126.2 (q; $J = 3.8$ Hz; C3', C5'); 128.6 (C2, C6); 142.1 (C1'); 153.0 (C4). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -60.29. HRMS (ESI): [M-1]⁻ 315.0314.

4-amino-*N*-(naphthalen-2-yl)benzenesulfonamide (**44g**) Yield: 67%. m.p. 207-208 °C. IR (cm⁻¹): 3412-3341; 1594; 1307-1142. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 5.93 (s; 2H; NH₂); 6.49 (d; 2H; $J = 8.7$ Hz; H3, H5); 7.27 (dd; 1H; $J = 2.1$ Hz; $J = 8.8$ Hz; H4'); 7.38-7.34 (m; 1H; H5'); 7.43 (d; 3H; $J = 8.7$ Hz; H2, H6); 7.45-7.40 (m; 3H; H8'); 7.51 (d; 1H; $J = 1.76$ Hz; H1'); 7.78-7.72 (m; 3H; H3', H6', H7'); 10.10 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 112.5 (C3, C5); 114.9 (C1'); 120.0 (C4'); 124.2 (C1); 124.6 (C5'); 126.5 (C8'); 127.0 (C6' or C7'); 127.4 (C6' or C7'); 128.7 (C2, C6, C3'); 129.6 (C4a'); 133.2 (C8a'); 136.1 (C2'); 152.8 (C4). EM (ESI): [M-1]⁻ 296.97.

4.2.7. General procedure for the preparation of 4-((5-methyl-[1,2,4]triazolo[1,5-a]pyrimidin-7-yl)amino)-N-arylbenzenesulfonamide hydrochlorides (4-24), 4-((2,5-dimethylpyrazolo[1,5-a]pyrimidin-7-yl)amino)-N-arylbenzenesulfonamide hydrochlorides (25-31) and 4-((7-chloroquinolin-4-yl)amino)-N-arylbenzenesulfonamide hydrochlorides (32-38)

A mixture of 0.001 mol of compound (**41a-d** or **45**), 1 equivalent of the respective 4-amino-*N*-arylbenzenesulfonamides (**44a-g**) and 3 mL of ethanol was stirred and refluxed (78 °C) for 2 h. The reaction mixture was poured into ice water, and the precipitate formed was vacuum filtered and washed with water. The precipitate was purified by recrystallization from methanol/water (2:1) or chromatography TLC Glass Plates (Merck TLC Silica gel 60 RP-18 F₂₅₄S) in chloroform/methanol (9.5:0.5). After purification, the respective hydrochloride was prepared, and the molecules were solubilized and stirred for 15 minutes in a 1:1 solution of HCl/H₂O and concentrated under vacuum [17–19].

4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-phenylbenzenesulfonamide hydrochloride (**4**) Yield: 48%. m.p. 250-252 °C. IR (cm⁻¹): 3036; 1655; 1608; 1332-1153. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.49 (s; 3H; CH₃); 6.75 (s; 1H; H₆); 7.04 (t; 1H; *J* = 7.3 Hz; H₄''); 7.15 (d; 2H; *J* = 7.5 Hz; H₂'', H₆''); 7.25 (t; 2H; *J* = 7.4 Hz; H₃'', H₅''); 7.63 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.86 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 8.76 (s; 1H; H₂) 10.45 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 23.5 (CH₃); 91.6 (C₆); 119.8 (C₂'', C₆''); 123.6 (C₂', C₆'); 123.9 (C₄''); 128.2 (C₃', C₅'); 129.1 (C₃'', C₅''); 136.3 (C₁'); 137.5 (C₁''); 140.5 (C₄'); 145.5 (C₅); 152.5 (C_{3a}); 152.6 (C₂); 164.1 (C₇). HRMS (ESI): calc. for C₁₈H₁₇N₆O₂S 381.1128; found [M+1]⁺ 381.1115. HPLC: 98%.

N-(4-chlorophenyl)-4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**5**) Yield: 98%. m.p. 286-288 °C. IR (cm⁻¹): 3100-3034; 1655; 1605; 1327-1156; 826. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.50 (s; 3H; CH₃); 6.78 (s; 1H; H₆); 7.17 (d; 2H; *J* = 8.9 Hz; H₂'', H₆''); 7.32 (d; 2H; *J* = 8.9 Hz; H₃'', H₅''); 7.64 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.86 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 8.79 (s; 1H; H₂) 10.65 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 23.5 (CH₃); 91.7 (C₆); 121.4 (C₂'', C₆''); 123.6 (C₂', C₆'); 128.0 (C₄''); 128.2 (C₃', C₅'); 129.0 (C₃'', C₅''); 135.9 (C₁'); 136.5 (C₁''); 140.6 (C₄'); 145.5 (C₅); 152.41 (C_{3a}); 152.48 (C₂); 164.1 (C₇). HRMS (ESI): calc. for C₁₈H₁₆ClN₆O₂S 415.0738; found [M+1]⁺ 415.0729. HPLC: 98.5%.

N-(4-fluorophenyl)-4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**6**) Yield: 79%. m.p. 274-276 °C. IR (cm⁻¹): 3293; 1624; 1587; 1333-1154; 1090. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.46 (s; 3H; CH₃); 6.68 (s; 1H; H₆); 7.14-7.08 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.63 (d; 2H; *J* = 8.5 Hz; H₂', H₆'); 7.77 (d; 2H; *J* = 8.5 Hz; H₃', H₅'); 8.53 (s; 1H; H₂) 10.29 (s; 1H; SO₂NH); 10.47 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 24.6 (CH₃); 90.6 (C₆); 115.8 (d; *J* = 22.4 Hz; C₃'', C₅'') 122.5 (d; *J* = 8.2 Hz; C₂'', C₆''); 122.7 (C₂', C₆'); 128.1 (C₃', C₅'); 133.7 (d; *J* = 2.5 Hz; C₁''); 134.9 (C₁'); 141.3 (C₄'); 144.3 (C₅); 154.3 (C₂); 155.3 (C_{3a}); 158.9 (d; *J* = 239.5 Hz; C₄''); 164.6 (C₇). ¹⁹F NMR (376 MHz, DMSO-*d*₆, TMS, δ in ppm): -118.41. HRMS (ESI): calc. for C₁₈H₁₆FN₆O₂S 399.1033; found [M+1]⁺ 399.1035. HPLC: 99.7%.

4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(*p*-tolyl)benzenesulfonamide hydrochloride (**7**) Yield: 83%. m.p. 266-267 °C. IR (cm⁻¹): 3103; 1659; 1614; 1385; 1339-1190. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.19 (s; 3H; CH₃'); 2.50 (s; 3H; CH₃); 6.76 (s; 1H; H₆); 7.04 (d; 4H; *J* = 9.0 Hz; H₂'', H₃'', H₅'', H₆''); 7.62 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.83 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 8.79 (s; 1H; H₂) 10.28 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 20.3 (CH₃') 23.5 (CH₃); 91.8 (C₆); 120.5 (C₂'', C₆''); 123.7 (C₂', C₆'); 128.3 (C₃', C₅'); 129.6 (C₃'', C₅'') 133.4 (C₄''); 134.9 (C₁'); 136.5 (C₁''); 140.4 (C₄'); 145.7 (C₅); 152.4 (C_{3a}); 152.5 (C₂); 164.2 (C₇). HRMS (ESI): calc. for C₁₉H₁₉N₆O₂S 395.1284; found [M+1]⁺ 395.1283. HPLC: 98.5%.

N-(4-methoxyphenyl)-4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**8**) Yield: 53%. m.p. 261-262 °C. IR (cm⁻¹): 3100-3034; 1655; 1605; 1327-1156; 826. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.50 (s; 3H; CH₃); 3.67 (s; 3H; OCH₃); 6.75 (s; 1H; H₆); 6.82 (d; 2H; *J* = 9.0 Hz; H₂'', H₆''); 7.03 (d; 2H; *J* = 9.0 Hz; H₃'', H₅''); 7.62 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.79 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 8.78 (s; 1H; H₂) 10.07 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 23.6 (CH₃); 55.1 (OCH₃); 91.7 (C₆); 114.3 (C₃'', C₅''); 123.4 (C₂', C₆'); 123.6 (C₂'', C₆''); 128.3 (C₃', C₅'); 130.0 (C₁'); 136.4 (C₁''); 140.3 (C₄'); 145.7 (C₅); 152.5 (C_{3a});

152.6 (C2); 156.5 (C4''); 164.2 (C7). HRMS (ESI): calc. for C₁₉H₁₉N₆O₃S 411.1233; found [M+1]⁺ 411.1236. HPLC: 99.2%.

4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-(trifluoromethyl)phenyl)benzenesulfonamide hydrochloride (**9**) Yield: 25%. m.p. 265-268 °C. IR (cm⁻¹): 3266; 1621; 1589; 1325-1150; 773. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.45 (s; 3H; CH₃); 6.68 (s; 1H; H6); 7.27 (d; 2H; *J* = 8.5 Hz; H2'', H6''); 7.58 (d; 2H; *J* = 8.6 Hz; H3'', H5''); 7.63 (d; 2H; *J* = 8.7 Hz; H2', H6'); 7.87 (d; 2H; *J* = 8.7 Hz; H3', H5'); 8.52 (s; 1H; H2) 10.45 (s; 1H; NH); 10.60 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.6 (CH₃); 90.5 (C6); 118.6 (C2'', C6''); 122.4 (q; *J* = 32.8 Hz; C4''); 122.8 (C2', C6'); 124.2 (q; *J* = 269.5; CF₃); 126.3 (q; *J* = 3.7 Hz; C3'', C5''); 128.0 (C3', C5'); 135.9 (C1'); 141.4 (C4'); 143.1 (C1''); 144.4 (C5); 154.3 (C2); 155.3 (C3a); 164.5 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -60.21. HRMS (ESI): calc. C₁₉H₁₆F₃N₆O₂S 449.1002; found [M+1]⁺ 449.1010. HPLC: 99.3%.

4-((5-methyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(naphthalen-2-yl)benzenesulfonamide hydrochloride (**10**) Yield: 32%. m.p. 264-265 °C. IR (cm⁻¹): 3287; 1621; 1569; 1320-1153. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.43 (s; 3H; CH₃); 6.65 (s; 1H; H6); 7.34 (dd; 1H; *J* = 2.16 Hz; *J* = 8.8 Hz; H4''); 7.41-7.37 (m; 1H; H5''); 7.47-7.43 (m; 1H; H8''); 7.62-7.61 (m; 3H; H2', H6', H1''); 7.80-7.78 (m; 3H; H3'', H6'', H7''); 7.87 (d; 2H; *J* = 8.7 Hz; H3', H5'); 8.50 (s; 1H; H2); 10.43 (s; 1H; NH); 10.58 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.6 (CH₃); 90.5 (C6); 115.7 (C1''); 120.1 (C4''); 122.7 (C2', C6'); 124.9 (C5''); 126.5 (C8''); 127.0 (C6'' ou C7''); 127.4 (C6'' ou C7''); 128.1 (C3', C5'); 129.0 (C3''); 129.8 (C4a''); 133.1 (C8a''); 135.20 (C1'); 135.25 (C2''); 141.3 (C4'); 144.2 (C5); 154.3 (C2); 155.3 (C3a); 164.6 (C7). HRMS (ESI): calc. for C₂₂H₁₈N₆O₂S 431.1285; found [M+1]⁺ 431.1286. HPLC: 98.3%

4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-phenylbenzenesulfonamide hydrochloride (**11**) Yield: 27%. m.p. 256-258 °C. IR (cm⁻¹): 3380; 1626; 1586; 1339-1162. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.42 (s; 3H; CH₃') 2.47 (s; 3H; CH₃); 6.62 (s; 1H; H6); 7.03 (t; 1H; *J* = 7.4 Hz; H4''); 7.12 (d; 2H; *J* = 7.6 Hz; H2'', H6''); 7.24 (t; 2H; *J* = 7.5 Hz; H3'', H5''); 7.60 (d; 2H; *J* = 8.7 Hz; H2', H6'); 7.80 (d; 2H; *J* = 8.7 Hz; H3', H5'); 10.33 (s; 1H; SO₂NH); 10.40 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.7 (CH₃') 24.7 (CH₃); 90.3 (C6); 119.8 (C2'', C6''); 122.6 (C2', C6'); 124.0 (C4''); 128.2 (C3', C5'); 129.2 (C3'', C5''); 135.2 (C1'); 137.7 (C1''); 141.5 (C4'); 143.8 (C5); 155.8 (C3a); 163.6 (C2); 164.1 (C7). HRMS (ESI): calc. for C₁₉H₁₉N₆O₂S 395.1284; found [M+1]⁺ 395.1269. HPLC: 99.9%

N-(4-chlorophenyl)-4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**12**) Yield: 44%. m.p. 270-271 °C. IR (cm⁻¹): 3371-3099; 1620; 1586; 1338-1160; 833. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.43 (s; 3H; CH₃') 2.48 (s; 3H; CH₃); 6.64 (s; 1H; H6); 7.14 (d; 2H; *J* = 8.8 Hz; H2'', H6''); 7.32 (d; 2H; *J* = 8.8 Hz; H3'', H5''); 7.62 (d; 2H; *J* = 8.6 Hz; H2', H6'); 7.80 (d; 2H; *J* = 8.7 Hz; H3', H5'); 10.43 (s; 1H; NH); 10.50 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.5 (CH₃') 24.6 (CH₃); 90.3 (C6); 121.2 (C2'', C6''); 122.5 (C2', C6'); 127.9 (C4''); 128.1 (C3', C5'); 129.1 (C3'', C5''); 134.7 (C1'); 136.6 (C1''); 141.6 (C4'); 143.7 (C5); 155.7 (C3a); 163.5 (C2); 164.0 (C7). HRMS (ESI): calc. for C₁₉H₁₈ClN₆O₂S 429.0894; found [M+1]⁺ 429.0901. HPLC: 100%.

4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-fluorophenyl)benzenesulfonamide hydrochloride (**13**) Yield: 25%. m.p. 252-254 °C. IR (cm⁻¹): 1625; 1559; 1328-1157; 1095. ¹H NMR

(400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.43 (s; 3H; CH₃'); 2.48 (s; 3H; CH₃); 6.62 (s; 1H; H₆); 7.15-7.08 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.60 (d; 2H; $J = 8.7$ Hz; H₂', H₆'); 7.76 (d; 2H; $J = 8.7$ Hz; H₃', H₅'); 10.29 (s; 1H; SO₂NH); 10.43 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 14.5 (CH₃'); 24.5 (CH₃); 90.2 (C₆); 115.8 (d; $J = 22.5$ Hz; C₃'', C₅'') 122.4 (d; $J = 7.1$ Hz; C₂'', C₆''); 122.5 (C₂', C₆'); 128.1 (C₃', C₅'); 133.7 (d; $J = 2.5$ Hz; C₁''); 134.8 (C₁'); 141.4 (C₄'); 143.7 (C₅); 155.6 (C_{3a}); 158.9 (d; $J = 239.5$ Hz; C₄''); 163.4 (C₂); 164.0 (C₇). ¹⁹F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -118.45. HRMS (ESI): calc. for C₁₉H₁₈FN₆O₂S 413.1190; found [M+1]⁺ 413.1184. HPLC: 100%.

4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(*p*-tolyl)benzenesulfonamide hydrochloride (**14**) Yield: 29%. m.p. 257-260 °C. IR (cm⁻¹): 3372-3128; 1621; 1562; 1387; 1337-1158. ¹H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.18 (s; 3H; CH₃'); 2.42 (s; 3H; CH₃); 2.47 (s; 3H; CH₃); 6.61 (s; 1H; H₆); 7.05-6.99 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.59 (d; 2H; $J = 8.6$ Hz; H₂', H₆'); 7.76 (d; 2H; $J = 8.6$ Hz; H₃', H₅'); 10.16 (s; 1H; SO₂NH); 10.40 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 14.6 (CH₃'); 20.2 (CH₃'') 24.7 (CH₃); 90.2 (C₆); 120.3 (C₂'', C₆''); 122.6 (C₂', C₆'); 128.1 (C₃', C₅'); 129.6 (C₃'', C₅'') 133.3 (C₄''); 135.0 (C₁'); 135.2 (C₁''); 141.4 (C₄'); 143.8 (C₅); 155.9 (C_{3a}); 163.6 (C₂); 164.1 (C₇). HRMS (ESI): calc. for C₂₀H₂₁N₆O₂S 409.1441; found [M+1]⁺ 409.1441. HPLC: 98%.

4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-methoxyphenyl)benzenesulfonamide hydrochloride (**15**) Yield: 82%. m.p. 281-284 °C. IR (cm⁻¹): 1656; 1578; 1332-1159; 1025. ¹H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.49 (s; 3H; CH₃'); 2.56 (s; 3H; CH₃); 3.67 (s; 3H; OCH₃); 6.76 (s; 1H; H₆); 6.82 (d; 2H; $J = 9.0$ Hz; H₂'', H₆''); 7.03 (d; 2H; $J = 9.0$ Hz; H₃'', H₅''); 7.59 (d; 2H; $J = 8.7$ Hz; H₂', H₆'); 7.79 (d; 2H; $J = 8.7$ Hz; H₃', H₅'); 10.09 (s; 1H; SO₂NH); 11.09 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 13.4 (CH₃'); 23.5 (CH₃); 55.0 (OCH₃); 92.2 (C₆); 114.2 (C₃'', C₅''); 123.2 (C₂', C₆'); 123.5 (C₂'', C₆''); 128.2 (C₃', C₅'); 129.9 (C₁'); 136.4 (C₁''); 140.0 (C₄'); 145.2 (C₅); 151.5 (C₄''); 156.4 (C_{3a}); 160.3 (C₂); 164.4 (C₇). HRMS (ESI): calc. for C₂₀H₂₁N₆O₃S 425.1390; found [M+1]⁺ 425.1387. HPLC: 99.2%.

4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-(trifluoromethyl)phenyl)benzenesulfonamide hydrochloride (**16**) Yield: 30%. m.p. 250-252 °C. IR (cm⁻¹): 3369; 1621; 1562; 1321-1157; 846. ¹H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.42 (s; 3H; CH₃'); 2.47 (s; 3H; CH₃); 6.63 (s; 1H; H₆); 7.30 (d; 2H; $J = 8.4$ Hz; H₂'', H₆''); 7.61 (d; 2H; $J = 8.4$ Hz; H₃'', H₅''); 7.62 (d; 2H; $J = 8.6$ Hz; H₂', H₆'); 7.87 (d; 2H; $J = 8.8$ Hz; H₃', H₅'); 10.43 (s; 1H; SO₂NH); 10.91 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 14.7 (CH₃'); 24.6 (CH₃); 90.4 (C₆); 118.6 (C₂'', C₆''); 122.7 (C₂', C₆'); 123.2 (q; $J = 32.0$ Hz; C₄''); 124.2 (q; $J = 269.6$; CF₃); 126.5 (q; $J = 3.7$ Hz; C₃'', C₅''); 128.2 (C₃', C₅'); 135.1 (C₁'); 141.8 (C₄'); 142.2 (C₁''); 143.8 (C₅); 155.8 (C_{3a}); 163.6 (C₂); 164.1 (C₇). ¹⁹F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -60.28. HRMS (ESI): calc. for C₂₀H₁₈F₃N₆O₂S 463.1158; found [M+1]⁺ 463.1147. HPLC: 98%.

4-((2,5-dimethyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(naphthalen-2-yl)benzenesulfonamide hydrochloride (**17**) Yield: 27%. m.p. 246-248 °C. IR (cm⁻¹): 3335; 1620; 1563; 1308-1151. ¹H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.40 (s; 3H; CH₃'); 2.45 (s; 3H; CH₃); 6.58 (s; 1H; H₆); 7.33 (dd; 1H; $J = 2.16$ Hz; $J = 8.8$ Hz; H₄''); 7.41-7.37 (m; 1H; H₅''); 7.47-7.43 (m; 1H; H₈''); 7.61-7.58 (m; 3H; H₂', H₆', H₁''); 7.83-7.78 (m; 3H; H₃'', H₆'', H₇''); 7.86 (d; 2H; $J = 8.7$ Hz; H₃', H₅'); 10.38 (s; 1H; NH); 10.57 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 14.6 (CH₃); 24.7

(CH₃); 90.3 (C6); 115.7 (C1''); 120.2 (C4''); 122.6 (C2', C6'); 125.0 (C5''); 126.6 (C8''); 127.2 (C6'' or C7''); 127.5 (C6'' or C7''); 128.2 (C3', C5'); 129.1 (C3''); 129.9 (C4a''); 133.2 (C8a''); 135.1 (C1'); 135.3 (C2''); 141.5 (C4'); 143.7 (C5); 155.8 (C3a); 163.6 (C2); 164.1 (C7). HRMS (ESI): calc. for C₂₃H₂₀N₆O₂S 445.1437; found [M+1]⁺ 445.1438. HPLC: 99.9%.

4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-phenylbenzenesulfonamide hydrochloride (**18**) Yield: 27%. m.p. 201-203 °C. IR (cm⁻¹): 3350; 1629; 1562; 1296-1163. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.49 (s; 3H; CH₃); 6.81 (s; 1H; H₆); 7.06-7.02 (m; 1H; H₄''); 7.14-7.12 (m; 2H; H₂'', H₆''); 7.27-7.23 (m; 2H; H₃'', H₅''); 7.63 (d; 2H; *J* = 8.6 Hz; H₂', H₆'); 7.83 (d; 2H; *J* = 8.6 Hz; H₃', H₅'); 10.35 (s; 1H; SO₂NH); 10.72 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.7 (CH₃); 92.5 (C6); 119.4 (q; *J* = 269.1; CF₃); 119.8 (C2'', C6''); 123.2 (C2', C6'); 123.9 (C4''); 128.1 (C3', C5'); 129.1 (C3'', C5''); 135.8 (C1'); 137.5 (C1''); 140.8 (C4'); 145.2 (C5); 154.1 (q; *J* = 38.3; C2); 155.6 (C3a); 166.7 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -64.30. HRMS (ESI): calc. for C₁₉H₁₅F₃N₆NaO₂S 471.0821; found [M+23]⁺ 471.0811. HPLC: 97.1%.

N-(4-chlorophenyl)-4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**19**) Yield: 31%. m.p. 216-218 °C. IR (cm⁻¹): 1628; 1566; 1290-1155; 828. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.49 (s; 3H; CH₃); 6.82 (s; 1H; H₆); 7.13 (d; 2H; *J* = 8.8 Hz; H₂'', H₆''); 7.32 (d; 2H; *J* = 8.8 Hz; H₃'', H₅''); 7.64 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.82 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 10.51 (s; 1H; SO₂NH); 10.72 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.5 (CH₃); 24.9 (CH₃); 92.7 (C6); 119.5 (q; *J* = 269.1; CF₃); 121.4 (C2'', C6''); 123.3 (C2', C6'); 128.1 (C4''); 128.2 (C3', C5'); 129.2 (C3'', C5''); 135.5 (C1'); 136.6 (C1''); 141.1 (C4'); 145.3 (C5); 154.2 (q; *J* = 38.1; C2); 155.7 (C3a); 166.8 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -64.31. HRMS (ESI): calc. for C₁₉H₁₄ClF₃N₆NaO₂S 505.0431; found [M+23]⁺ 505.0409. HPLC: 95.4%.

N-(4-fluorophenyl)-4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**20**) Yield: 36%. m.p. 206-209 °C. IR (cm⁻¹): 3356; 1629; 1562; 1297-1151; 1132. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.49 (s; 3H; CH₃); 6.80 (s; 1H; H₆); 7.12-7.10 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.62 (d; 2H; *J* = 8.6 Hz; H₂', H₆'); 7.78 (d; 2H; *J* = 8.6 Hz; H₃', H₅'); 10.30 (s; 1H; SO₂NH); 10.72 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 24.9 (CH₃); 92.6 (C6); 115.9 (d; *J* = 22.5 Hz; C3'', C5'') 119.5 (q; *J* = 269.0; CF₃); 122.7 (d; *J* = 8.1 Hz; C2'', C6''); 123.3 (C2', C6'); 128.2 (C3', C5'); 133.8 (d; *J* = 2.4 Hz; C1''); 135.6 (C1'); 141.0 (C4'); 145.3 (C5); 154.2 (q; *J* = 38.3; C2); 155.7 (C3a); 159.0 (d; *J* = 239.6 Hz; C4''); 166.8 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -64.31 (CF₃); -118.37 (F). HRMS (ESI): calc. for C₁₉H₁₄F₄N₆NaO₂S 489.0727; found [M+23]⁺ 489.0706. HPLC: 97.1%.

4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(*p*-tolyl)benzenesulfonamide hydrochloride (**21**) Yield: 25%. m.p. 210-212 °C. IR (cm⁻¹): 1627; 1565; 1328; 1288-1150. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.19 (s; 3H; CH₃); 2.38 (s; 3H; CH₃); 6.47 (s; 1H; H₆); 7.05-6.99 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.42 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.72 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 10.10 (s; 1H; SO₂NH); 10.70 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 20.1 (CH₃); 24.5 (CH₃); 91.5 (C6); 119.6 (q; *J* = 268.9; CF₃); 120.3 (C2'', C6''); 122.8 (C2', C6'); 128.1 (C3', C5'); 129.4 (C3'', C5'') 133.1 (C4''); 134.1 (C1'); 135.0 (C1''); 145.0 (C4'); 146.2 (C5); 156.1 (C3a); 153.5 (q; *J* = 37.9; C2); 164.9 (C7). ¹⁹F NMR (376 MHz, DMSO-

d_6 , TMS, δ in ppm): δ -64.35. HRMS (ESI): calc. for $C_{20}H_{17}F_3N_6NaO_2S$ 485.0970; found $[M+23]^+$ 485.0970. HPLC: 98.5%.

N-(4-methoxyphenyl)-4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**22**) Yield: 67%. m.p. 201-202 °C. IR (cm^{-1}): 3322-3263; 1628; 1569; 1312-1144; 1092. 1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.49 (s; 3H; CH_3); 3.67 (s; 3H; OCH_3); 6.79 (s; 1H; H6); 6.82 (d; 2H; $J = 9.0$ Hz; H2'', H6''); 7.02 (d; 2H; $J = 9.0$ Hz; H3'', H5''); 7.61 (d; 2H; $J = 8.7$ Hz; H2', H6'); 7.75 (d; 2H; $J = 8.7$ Hz; H3', H5'); 9.97 (s; 1H; SO_2NH); 10.71 (s; 1H; NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 24.8 (CH_3); 55.0 (OCH_3); 92.4 (C6); 114.2 (C3'', C5''); 119.4 (q; $J = 269.0$; CF_3); 123.2 (C2', C6'); 123.4 (C2'', C6''); 128.1 (C3', C5'); 129.9 (C1'); 135.8 (C1''); 140.6 (C4'); 145.2 (C5); 154.1 (q; $J = 38.2$; C2); 155.6 (C3a); 156.4 (C4''); 166.7 (C7). ^{19}F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -64.31. HRMS (ESI): calc. for $C_{20}H_{17}F_3N_6NaO_3S$ 501.0927; found $[M+23]^+$ 501.0932. HPLC: 99.1%.

4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-(trifluoromethyl)phenyl)benzenesulfonamide hydrochloride (**23**) Yield: 28%. m.p. 230-231 °C. IR (cm^{-1}): 1628; 1566; 1323-1150; 749. 1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.41 (s; 3H; CH_3); 6.60 (s; 1H; H6); 7.30 (d; 2H; $J = 8.5$ Hz; H2'', H6''); 7.52 (d; 2H; $J = 8.5$ Hz; H3'', H5''); 7.59 (d; 2H; $J = 8.6$ Hz; H2', H6'); 7.85 (d; 2H; $J = 8.6$ Hz; H3', H5'); 10.83 (s; 1H; NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 24.6 (CH_3); 91.9 (C6); 118.6 (C2'', C6''); 119.5 (q; $J = 268.9$; CF_3); 123.1 (C2', C6'); 122.7 (q; $J = 32.3$ Hz; C4''); 124.2 (q; $J = 269.4$; CF_3); 126.3 (q; $J = 3.6$ Hz; C3'', C5''); 128.1 (C3', C5'); 135.0 (C1'); 142.6 (C4'); 143.8 (C1''); 145.8 (C5); 155.9 (C3a); 153.7 (q; $J = 37.9$ Hz; C2); 165.5 (C7). ^{19}F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -60.28 (CF_3'); -64.34 (CF_3); HRMS (ESI): calc. for $C_{20}H_{14}F_6N_6O_2S$ 516.0803; found $[M+23]^+$ 539.0686. HPLC: 98.8%.

4-((5-methyl-2-(trifluoromethyl)-[1,2,4]triazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(naphthalen-2-yl)benzenesulfonamide hydrochloride (**24**) Yield: 26%. m.p. 225-226 °C. IR (cm^{-1}): 3272; 1629; 1560; 1354-1151. 1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.19 (s; 3H; CH_3); 6.02 (s; 1H; H6); 7.17 (d; 2H; $J = 8.1$ Hz; H2', H6'); 7.32 (dd; 1H; $J = 2.1$ Hz; $J = 8.8$ Hz; H4''); 7.40-7.36 (m; 1H; H5''); 7.46-7.42 (m; 1H; H8''); 7.56 (d; 1H; $J = 1.8$ Hz; H1''); 7.81-7.76 (m; 3H; H3'', H6'', H7''); 7.72 (d; 2H; $J = 8.6$ Hz; H3', H5'); 10.45 (s; 1H; NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 24.3 (CH_3); 90.3 (C6); 115.6 (C1''); 119.9 (q; $J = 268.5$ Hz; CF_3); 120.3 (C4''); 122.3 (C2', C6'); 125.7 (C5''); 126.4 (C8''); 126.9 (C6'' or C7''); 127.3 (C6'' or C7''); 128.1 (C3', C5'); 128.8 (C3''); 129.7 (C4a''); 131.8 (C8a''); 133.1 (C1'); 135.7 (C2''); 147.4 (C4'); 152.8 (q; $J = 37.4$ Hz; C2); 156.9 (C5); 162.7 (C3a); 171.9 (C7). ^{19}F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -64.42 (CF_3). HRMS (ESI): calc. for $C_{23}H_{17}F_3N_6NaO_2S$ 521.0966; found $[M+23]^+$ 521.0967. HPLC: 100%.

4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-phenylbenzenesulfonamide hydrochloride (**25**) Yield: 27%. m.p. 255-257 °C. IR (cm^{-1}): 1617; 1551; 1340-1153. 1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.36 (s; 3H; CH_3'); 2.42 (s; 3H; CH_3); 6.22 (s; 1H; H3); 6.40 (s; 1H; H6); 7.05-7.01 (m; 1H; H4''); 7.14-7.11 (m; 2H; H2'', H6''); 7.27-7.22 (m; 2H; H3'', H5''); 7.61 (d; 2H; $J = 8.8$ Hz; H2', H6'); 7.78 (d; 2H; $J = 8.8$ Hz; H3', H5'); 10.02 (s; 1H; NH); 10.30 (s; 1H; SO_2NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 14.1 (CH_3'); 24.4 (CH_3); 87.6 (C3); 93.7 (C6); 119.6 (C2'', C6''); 122.0 (C2', C6'); 123.8 (C4''); 128.0 (C3', C5'); 129.1 (C3'', C5''); 134.3 (C1'); 137.6 (C1''); 142.1 (C4'); 142.5 (C3a); 149.4 (C5); 152.9 (C2); 158.7 (C7). HRMS (ESI): calc. for $C_{20}H_{19}N_5O_2S$ 394.1330; found $[M+1]^+$ 394.1331. HPLC: 96.4%.

N-(4-chlorophenyl)-4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)benzenesulfonamide hydrochloride (**26**) Yield: 25%. m.p. 218-220 °C. IR (cm⁻¹): 1612; 1551; 1328-1154; 827. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.37 (s; 3H; CH₃') 2.42 (s; 3H; CH₃); 6.22 (s; 1H; H₃); 6.42 (s; 1H; H₆); 7.14 (d; 2H; *J* = 8.9 Hz; H₂'', H₆''); 7.32 (d; 2H; *J* = 8.9 Hz; H₃'', H₅''); 7.62 (d; 2H; *J* = 8.6 Hz; H₂', H₆'); 7.78 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 10.04 (s; 1H; NH); 10.46 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 14.1 (CH₃') ; 24.4 (CH₃); 87.7 (C₃); 93.8 (C₆); 121.2 (C₂'', C₆''); 122.0 (C₂', C₆'); 127.9 (C₄''); 128.0 (C₃', C₅'); 129.0 (C₃'', C₅''); 133.9 (C₁'); 136.6 (C₁''); 142.3 (C₄'); 142.4 (C_{3a}); 149.4 (C₅); 152.9 (C₂); 158.7 (C₇). HRMS (ESI): calc. for C₂₀H₁₈ClN₅O₂S 428.0924; found [M+1]⁺ 428.0925. HPLC: 99.2%.

4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-fluorophenyl)benzenesulfonamide hydrochloride (**27**) Yield: 33%. m.p. 225-227 °C. IR (cm⁻¹): 3073; 1622; 1552; 1326-1152; 1091. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.36 (s; 3H; CH₃') ; 2.42 (s; 3H; CH₃); 6.21 (s; 1H; H₃); 6.36 (s; 1H; H₆); 7.07-7.01 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.57 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.73 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 9.98 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-*d*₆): 14.1 (CH₃') ; 24.3 (CH₃); 87.4 (C₃); 93.7 (C₆); 115.5 (d; *J* = 22.1 Hz; C₃'', C₅'') 122.0 (C₂', C₆'); 122.1 (d; *J* = 8.0 Hz; C₂'', C₆''); 127.9 (C₃', C₅'); 135.8 (C₁'); 136.1 (C₁''); 141.5 (C₄'); 142.7 (C_{3a}); 149.4 (C₅); 152.8 (C₂); 158.1 (d; *J* = 237.7 Hz; C₄''); 158.6 (C₇). ¹⁹F NMR (376 MHz, DMSO-*d*₆): -120.38. HRMS (ESI): calc. for C₂₀H₁₉FN₅O₂S 412.1247; found [M+1]⁺ 412.1248. HPLC: 99%.

4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(*p*-tolyl)benzenesulfonamide hydrochloride (**28**) Yield: 31%. m.p. 253-255 °C. IR (cm⁻¹): 3265; 1622; 1551; 1382; 1330-1153. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.18 (s; 3H; CH₃''); 2.36 (s; 3H; CH₃') ; 2.42 (s; 3H; CH₃); 6.21 (s; 1H; H₃); 6.37 (s; 1H; H₆); 7.04-6.98 (m; 4H; H₂'', H₃'', H₅'', H₆''); 7.58 (d; 2H; *J* = 8.7 Hz; H₂', H₆'); 7.74 (d; 2H; *J* = 8.7 Hz; H₃', H₅'); 10.02 (s; 2H; SO₂NH; NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 14.1 (CH₃') ; 20.1 (CH₃''); 24.3 (CH₃); 87.4 (C₃); 93.7 (C₆); 120.2 (C₂'', C₆''); 122.0 (C₂', C₆'); 127.9 (C₃', C₅'); 129.4 (C₃'', C₅'') 132.6 (C₄''); 134.9 (C₁'); 135.7 (C₁''); 141.9 (C₄'); 142.6 (C_{3a}); 149.4 (C₅); 152.8 (C₂); 158.6 (C₇). HRMS (ESI): calc. for C₂₁H₂₂N₅O₂S 408.1476; found [M+1]⁺ 408.1476 . HPLC: 100%

4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-methoxyphenyl)benzenesulfonamide hydrochloride (**29**) Yield: 79%. m.p. 261-262 °C. IR (cm⁻¹): 1655; 1606; 1335-1160; 1025. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.49 (s; 3H; CH₃') ; 2.51 (s; 3H; CH₃); 3.67 (s; 3H; OCH₃); 6.42 (s; 1H; H₃); 6.48 (s; 1H; H₆); 6.83 (d; 2H; *J* = 8.9 Hz; H₂'', H₆''); 7.05 (d; 2H; *J* = 8.9 Hz; H₃'', H₅''); 7.63 (d; 2H; *J* = 8.6 Hz; H₂', H₆'); 7.82 (d; 2H; *J* = 8.6 Hz; H₃', H₅'); 10.15 (s; 1H; SO₂NH); 11.62 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 14.0 (CH₃') ; 19.7 (CH₃); 55.0 (OCH₃); 91.3 (C₆); 114.2 (C₃'', C₅''); 123.2 (C₂', C₆'); 125.1 (C₂'', C₆''); 128.1 (C₃', C₅'); 129.8 (C₁'); 137.6 (C₁''); 139.2 (C₄'); 141.0 (C_{3a}); 146.8 (C₄''); 155.3 (C₅); 155.8 (C₂); 156.4 (C₇). HRMS (ESI): calc. for C₂₁H₂₁N₅O₃S 424.1432; found [M+1]⁺ 424.1433. HPLC: 96.4%.

4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(4-(trifluoromethyl)phenyl)benzenesulfonamide hydrochloride (**30**) Yield: 29%. m.p. 230-232 °C. IR (cm⁻¹): 1589; 1556; 1324-1105; 838. ¹H NMR (400 MHz, DMSO-*d*₆, TMS, δ in ppm): 2.34 (s; 3H; CH₃') ; 2.42 (s; 3H; CH₃); 6.19 (s; 1H; H₃); 6.30 (s; 1H; H₆); 7.05 (d; 2H; *J* = 8.5 Hz; H₂'', H₆''); 7.35 (d; 2H; *J* = 8.5 Hz; H₃'', H₅''); 7.50 (d; 2H; *J* = 8.6 Hz; H₂', H₆'); 7.78 (d; 2H; *J* = 8.6 Hz; H₃', H₅'); 9.87 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-*d*₆, TMS, δ in ppm): 14.2 (CH₃') ; 24.3 (CH₃); 86.9 (C₃); 93.5

(C6); 118.1 (q; $J = 31.8$ Hz; C4''); 119.1 (C2'', C6''); 122.3 (C2', C6'); 125.0 (q; $J = 268.7$; CF₃); 125.5 (q; $J = 3.6$ Hz; C3'', C5''); 127.5 (C3', C5'); 139.8 (C1'); 139.9 (C4'); 143.1 (C3a); 149.4 (C1''); 149.8 (C5); 152.8 (C2); 158.6 (C7). ¹⁹F NMR (376 MHz, DMSO-d₆, TMS, δ in ppm): -59.32. HRMS (ESI): calc. for C₂₁H₁₉F₃N₅O₂S 462.1194; found [M+1]⁺ 462.1195. HPLC: 98%.

4-((2,5-dimethylpyrazolo[1,5-*a*]pyrimidin-7-yl)amino)-*N*-(naphthalen-2-yl)benzenesulfonamide hydrochloride (**31**) Yield: 80%. m.p. 194-196 °C. IR (cm⁻¹): 3055; 2651; 1652; 1589; 1352-1153. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 2.46 (s; 6H; CH₃'); 2.46 (s; 6H; CH₃); 6.39 (s; 1H; H6); 6.44 (s; 1H; H3); 7.36 (dd; 1H; $J = 2.1$ Hz; $J = 8.8$ Hz; H4''); 7.41-7.39 (m; 1H; H5''); 7.47-7.43 (m; 1H; H8''); 7.63-7.61 (m; 3H; H2', H6', H1''); 7.83-7.78 (m; 3H; H3'', H6'', H7''); 7.94 (d; 2H; $J = 8.7$ Hz; H3', H5'); 10.74 (s; 1H; NH); 11.46 (s; 1H; SO₂NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 14.0 (CH₃'); 20.2 (CH₃); 87.7 (C3); 91.5 (C6); 115.7 (C1''); 120.1 (C4''); 124.93 (C5''); 124.99 (C2', C6'); 126.6 (C8''); 127.0 (C6'' or C7''); 127.4 (C6'' or C7''); 128.2 (C3', C5'); 129.0 (C3''); 129.8 (C4a''); 133.1 (C8a''); 135.1 (C1'); 137.2 (C2''); 139.7 (C4'); 141.8 (C3a); 146.3 (C5); 155.4 (C2); 155.7 (C7). HRMS (ESI): calc. for C₂₄H₂₂N₅O₂S 444.1481; found [M+1]⁺ 444.1481. HPLC: 95.3%.

4-((7-chloroquinolin-4-yl)amino)-*N*-phenylbenzenesulfonamide hydrochloride (**32**) Yield: 77%. m.p. 291-293 °C. IR (cm⁻¹): 3088; 1610; 1580; 1333-1148. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 7.01 (d; 1H; $J = 6.9$ Hz; H3); 7.05 (t; 1H; $J = 7.3$ Hz; H4''); 7.14 (d; 2H; $J = 7.5$ Hz; H2'', H6''); 7.26 (t; 2H; $J = 7.5$ Hz; H3'', H5''); 7.68 (d; 2H; $J = 8.6$ Hz; H2', H6'); 7.89 (dd; 1H; $J = 2.1$ Hz; $J = 9.1$ Hz; H6); 7.91 (d; 2H; $J = 8.7$ Hz; H3', H5'); 8.21 (d; 1H; $J = 2.0$ Hz; H8); 8.60 (d; 1H; $J = 6.9$ Hz; H2); 8.88 (d; 1H; $J = 9.1$ Hz; H5); 10.44 (s; 1H; SO₂NH); 11.30 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 101.2 (C3); 116.5 (C4a); 119.3 (C8); 120.2 (C2'', C6''); 124.2 (C4''); 124.8 (C2', C6'); 126.2 (C5); 127.6 (C6); 128.5 (C3', C5'); 129.2 (C3'', C5''); 137.3 (C1''); 137.5 (C1'); 138.5 (C7); 139.2 (C8a); 141.2 (C4'); 143.9 (C2); 154.0 (C4). HRMS (ESI): calc. for C₂₁H₁₇ClN₃O₂S 410.0724; found [M+1]⁺ 410.0705. HPLC: 97.8%.

N-(4-chlorophenyl)-4-((7-chloroquinolin-4-yl)amino)benzenesulfonamide hydrochloride (**33**) Yield: 33%. m.p. 254-256 °C. IR (cm⁻¹): 3409; 1613; 1580; 1335-1158; 811. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 7.05 (d; 1H; $J = 6.8$ Hz; H3); 7.16 (d; 2H; $J = 8.8$ Hz; H2'', H6''); 7.33 (d; 2H; $J = 8.8$ Hz; H3'', H5''); 7.69 (d; 2H; $J = 8.7$ Hz; H2', H6'); 7.92-7.89 (m; 3H; H6, H3', H5'); 8.18 (d; 1H; $J = 2.0$ Hz; H8); 8.62 (d; 1H; $J = 6.8$ Hz; H2); 8.83 (d; 1H; $J = 9.1$ Hz; H5); 10.61 (s; 1H; SO₂NH); 11.20 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 101.3 (C3); 116.4 (C4a); 119.5 (C8); 121.6 (C2'', C6''); 124.6 (C2', C6'); 126.0 (C5); 127.5 (C6); 128.2 (C4''); 128.4 (C3', C5'); 129.1 (C3'', C5''); 136.4 (C1''); 136.7 (C1'); 138.3 (C7); 139.3 (C8a); 141.4 (C4'); 144.1 (C2); 153.7 (C4). HRMS (ESI): calc. for C₂₁H₁₆Cl₂N₃O₂S 444.0334; found [M+1]⁺ 444.0325. HPLC: 98.5%.

4-((7-chloroquinolin-4-yl)amino)-*N*-(4-fluorophenyl)benzenesulfonamide hydrochloride (**34**) Yield: 24%. m.p. 264-267 °C. IR (cm⁻¹): 3021; 1612; 1581; 1326-1152; 1092. ¹H NMR (400 MHz, DMSO-d₆, TMS, δ in ppm): 7.16-7.08 (m; 5H; H3, H2'', H3'', H5'', H6''); 7.60 (d; 2H; $J = 8.7$ Hz; H2', H6'); 7.83-7.80 (m; 3H; H6, H3', H5'); 8.10 (d; 1H; $J = 2.1$ Hz; H8); 8.61 (d; 1H; $J = 6.4$ Hz; H2); 8.68 (d; 1H; $J = 9.1$ Hz; H5); 10.33 (s; 1H; SO₂NH); 10.68 (s; 1H; NH). ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ in ppm): 102.3 (C3); 115.8 (d; $J = 22.5$ Hz; C3'', C5''); 117.2 (C4a); 121.8 (C8); 122.8 (d; $J = 8.2$ Hz; C2'', C6''); 123.0 (C2', C6'); 125.6 (C5); 126.9 (C6); 128.4 (C3', C5'); 133.7 (d; $J = 2.6$ Hz; C1''); 135.3 (C1'); 137.1 (C7); 142.2 (C8a); 142.4 (C4'); 146.3 (C2); 151.4 (C4); 159.0 (q; $J = 239.6$ Hz;

C4''). ^{19}F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -118.2314. HRMS (ESI): calc. for $\text{C}_{21}\text{H}_{16}\text{ClFN}_3\text{O}_2\text{S}$ 428.0630; found $[\text{M}+1]^+$ 428.0652. HPLC: 95.8%.

4-((7-chloroquinolin-4-yl)amino)-*N*-(*p*-tolyl)benzenesulfonamide hydrochloride (**35**) Yield: 84%. m.p. 278-280 °C. IR (cm^{-1}): 3166; 1614; 1581; 1329-1153. ^1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 2.19 (s; 3H; CH_3); 7.07-7.00 (m; 5H; H3, H2'', H3'', H5'', H6''); 7.65 (d; 2H; $J = 8.7$ Hz; H2', H6'); 7.88-7.84 (m; 3H; H6, H3', H5'); 8.17 (d; 1H; $J = 2.0$ Hz; H8); 8.60 (d; 1H; $J = 6.7$ Hz; H2); 8.81 (d; 1H; $J = 9.1$ Hz; H5); 10.23 (s; 1H; SO_2NH); 11.08 (s; 1H; NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 20.2 (CH_3); 101.5 (C3); 116.6 (C4a); 120.1 (C8); 120.6 (C2'', C6''); 124.1 (C2', C6'); 125.9 (C5); 127.3 (C6); 128.4 (C3', C5'); 129.5 (C3'', C5''); 133.4 (C4''); 134.7 (C1''); 136.7 (C1'); 138.0 (C7); 140.1 (C8a); 141.4 (C4'); 144.7 (C2); 153.0 (C4). HRMS (ESI): calc. for $\text{C}_{22}\text{H}_{19}\text{ClN}_3\text{O}_2\text{S}$ 424.0881; found $[\text{M}+1]^+$ 424.0865. HPLC: 96.9%.

4-((7-chloroquinolin-4-yl)amino)-*N*-(4-methoxyphenyl)benzenesulfonamide hydrochloride (**36**) Yield: 47%. m.p. 271-272 °C. IR (cm^{-1}): 3244; 1612; 1567; 1320-1154; 1092. ^1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 3.67 (s; 3H; OCH_3); 6.82 (d; 2H; $J = 9.0$ Hz; H2'', H6''); 7.02 (d; 2H; $J = 9.0$ Hz; H3'', H5''); 7.09 (d; 1H; $J = 6.2$ Hz; H3); 7.57 (d; 2H; $J = 8.7$ Hz; H2', H6'); 7.76 (d; 2H; $J = 8.7$ Hz; H3', H5'); 7.79 (dd; 1H; $J = 2.1$ Hz; $J = 9.1$ Hz; H6); 8.08 (d; 1H; $J = 2.1$ Hz; H8); 8.61 (d; 1H; $J = 6.2$ Hz; H2); 8.64 (d; 1H; $J = 9.1$ Hz; H5); 9.97 (s; 1H; SO_2NH); 10.52 (s; 1H; NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 55.0 (OCH_3); 102.5 (C3); 114.2 (C3'', C5''); 117.4 (C4a); 122.5 (C8); 122.6 (C2'', C6''); 123.4 (C2', C6'); 125.4 (C5); 126.8 (C6); 128.4 (C3', C5'); 129.9 (C1''); 135.3 (C1'); 136.8 (C7); 142.4 (C4'); 143.0 (C8a); 146.9 (C2); 150.8 (C4); 156.4 (C4''). HRMS (ESI): calc. for $\text{C}_{22}\text{H}_{19}\text{ClN}_3\text{O}_3\text{S}$ 440.0830; found $[\text{M}+1]^+$ 440.0813. HPLC: 98.9%.

4-((7-chloroquinolin-4-yl)amino)-*N*-(4-(trifluoromethyl)phenyl)benzenesulfonamide hydrochloride (**37**) Yield: 29%. m.p. >300 °C. IR (cm^{-1}): 1614; 1582; 1322-1154; 815. ^1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 7.14 (d; 1H; $J = 6.3$ Hz; H3); 7.65-7.62 (m; 4H; H2', H6', H3'', H5''); 7.34 (d; 2H; $J = 8.4$ Hz; H2'', H6''); 7.79 (dd; 1H; $J = 2.1$ Hz; $J = 9.0$ Hz; H6); 7.92 (d; 2H; $J = 8.7$ Hz; H3', H5'); 8.09 (d; 1H; $J = 2.1$ Hz; H8); 8.61 (d; 1H; $J = 6.3$ Hz; H2); 8.65 (d; 1H; $J = 9.0$ Hz; H5); 10.99 (s; 1H; SO_2NH); 10.60 (s; 1H; NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 102.8 (C3); 117.5 (C4a); 118.6 (C2'', C6''); 122.4 (C5); 122.6 (C2', C6'); 123.5 (q; $J = 31.9$ Hz; C4''); 124.1 (q; $J = 269.7$ Hz; CF_3); 125.5 (C8); 126.4 (q; $J = 3.6$ Hz; C3'', C5''); 126.8 (C6); 128.4 (C3', C5'); 134.7 (C1'); 136.8 (C7); 141.5 (C1''); 143.0 (C8a); 143.1 (C4'); 146.9 (C2); 150.7 (C4). ^{19}F NMR (376 MHz, DMSO- d_6 , TMS, δ in ppm): -60.4125. HRMS (ESI): calc. for $\text{C}_{22}\text{H}_{16}\text{ClF}_3\text{N}_3\text{O}_2\text{S}$ 478.0598; found $[\text{M}+1]^+$ 478.0600. HPLC: 98.6%.

4-((7-chloroquinolin-4-yl)amino)-*N*-(naphthalen-2-yl)benzenesulfonamide hydrochloride (**38**) Yield: 28%. m.p. 265-268 °C. IR (cm^{-1}): 3232; 1582; 1354-1153. ^1H NMR (400 MHz, DMSO- d_6 , TMS, δ in ppm): 7.09 (d; 1H; $J = 6.0$ Hz; H3); 7.33 (dd; 1H; $J = 2.16$ Hz; $J = 8.8$ Hz; H4''); 7.41-7.37 (m; 1H; H5'') 7.47-7.43 (m; 1H; H8''); 7.53 (d; 2H; $J = 8.7$ Hz; H2', H6'); 7.61 (d; 1H; $J = 2.0$ Hz; H1''); 7.73 (dd; 1H; $J = 2.0$ Hz; $J = 9.0$ Hz; H6); 7.83-7.78 (m; 3H; H3'', H6'', H7''); 7.88 (d; 2H; $J = 8.7$ Hz; H3', H5'); 8.04 (d; 1H; $J = 2.0$ Hz; H8); 8.53 (d; 1H; $J = 9.1$ Hz; H5); 8.57 (d; 1H; $J = 6.0$ Hz; H2); 10.27 (s; 1H; NH); 10.57 (s; 1H; SO_2NH). ^{13}C NMR (100 MHz, DMSO- d_6 , TMS, δ in ppm): 103.1 (C3); 115.8 (C1''); 117.7 (C4a); 120.2 (C4''); 122.0 (C2', C6'); 123.4 (C8); 124.9 (C5''); 125.2 (C5); 126.5 (C6, C8''); 127.0 (C6'' or C7''); 127.4 (C6'' or C7''); 128.4 (C3', C5'); 128.9 (C3''); 129.8 (C4a''); 133.1

(C8a''); 134.6 (C1'); 135.3 (C2''); 136.3 (C7); 143.2 (C4'); 144.1 (C8a); 147.8 (C2); 149.8 (C4).HRMS (ESI): calc. for $C_{25}H_{19}N_3O_2S$ 460.0880; found $[M+1]^+$ 460.0881. HPLC: 97.3%.

4.3. X-ray data collection and structure refinement

Single-crystal X-ray data for **7** and **17** were collected on a Bruker D8 Venture diffractometer using graphite-monochromated MoK α radiation ($\lambda = 0.71073 \text{ \AA}$) at 298 K. Data collection, cell refinement and data reduction were performed with Bruker Instrument Service v4.2.2, APEX2 [27] and SAINT [28], respectively. The absorption correction using equivalent reflections was performed with the SADABS program [29]. The structure solutions and full-matrix least-squares refinements based on F^2 were performed with the SHELX package [30,31]. All H atoms were refined with fixed individual displacement parameters [$U_{iso}(H) = 1.2 U_{eq}(C_{sp^2})$ and C_{ar}] or $1.5 U_{eq}(C_{sp^3})$ using a riding model. All non-hydrogen atoms were refined anisotropically. Structure illustrations were generated using ORTEP-3 for Mercury [32], and crystallographic tables were constructed using Olex2 [33].

X-ray crystallographic data in cif format are available at the CCDC 2023181 and 2023182 and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif

4.4. Biological evaluation

4.4.1. Antiplasmodial in vitro assays against *P. falciparum* blood parasites

P. falciparum blood parasites were cultured as previously described [34]. Parasite strains cultured for this study included 3D7 (chloroquine-sensitive strain), K1 (chloroquine-resistant), DD2 (chloroquine- and mefloquine-resistant), IPC4912 (partial resistance to artemisinin) and a laboratory-generated strain resistant to PI4K inhibitors (PI4K^{RES}). Fresh sorbitol synchronized ring stages [35] were incubated with the test samples at various concentrations previously dissolved in 0.05% DMSO (v/v). Each assay was performed in triplicate. The results were compared with control cultures in complete medium with no sample. Chloroquine was used in each experiment as an antimalarial control. The activity of the test samples was measured using the SYBR green assay [36]. Briefly, the plates were centrifuged at 700 g for 5 minutes at room temperature to remove the medium, washed with $1 \times$ PBS, and incubated for 30 minutes with lysis buffer solution [2.4228 g of TRIS, ultrapure (for a 20 mM solution), pH 7.5; 1.8612 g of EDTA, (for a 5 mM solution); 80 μ g of saponin (0.008% w/v); 800 μ L of Triton X-100 (0.08% v/v); H₂O Type I] and SYBR green I DNA stain (1:20 000). The fluorescence of uninfected erythrocytes was considered as the background. Fluorescence was measured on a SpectraMax340PC384 fluorimeter at 485/535 nm. The half-maximal compound inhibitory concentration (IC₅₀) was estimated by curve fitting using software from the OriginLab Corporation and compared to

the parasite growth in test sample-free medium. Compounds with IC_{50} values below 10 μ M were considered active against *P. falciparum*.

4.4.2. Cytotoxicity tests using immortalized cells

The cytotoxicity of the tested compounds was evaluated in a human hepatoma cell line (HepG2) using cells cultured in 75 cm^2 sterile flasks containing RPMI-1640 medium (supplemented with 10% heat-inactivated foetal bovine serum and 40 mg/L gentamicin) under a 5% CO_2 atmosphere at 37 °C. When confluent, the cell monolayer was washed with culture medium, trypsinized, distributed in a flat-bottomed 96-well plate (5×10^3 cells/well), and incubated for 18 h at 37 °C for cell adherence [37]. The compounds (in 20 μ L of solution) at various concentrations (1000–1 μ g/mL) were placed in 96-well plates and incubated with the cultured cells for 72 h under a 5% CO_2 atmosphere at 37 °C. Then, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL; 20 μ L/well for 3 h) was used to evaluate mitochondrial viability. The supernatants were carefully removed, and 100 μ L of DMSO was added to each well followed by mixing to solubilize the formazan crystals. The optical density was determined at 570 nm. Cell viability was expressed as a percentage of the control absorbance in the untreated cells after subtracting the appropriate background. The half-maximal compound inhibitory concentration (IC_{50}^{HepG2}) was estimated by curve fitting using software from the OriginLab Corporation. The ratio between the IC_{50}^{3D7} and IC_{50}^{HepG2} values was used to determine the selectivity index (SI) of the compounds. Compounds with values above 10 were considered non-toxic.

4.4.3. Recombinant production of human and *Plasmodium falciparum* DHODH and DHODH activity assays were performed as previously described [19,38]

4.4.4. Molecular docking

The molecular structures of compounds **4-24** were built using Spartan'14 software (Wavefunction, Inc., Irvine, CA). Docking of the inhibitors into *Pf*DHODH was performed using the Molegro Virtual Docker 6.0 (MVD) program (CLC Bio, Aarhus, Denmark) [39], which uses a heuristic search algorithm that combines differential evolution with a cavity prediction algorithm. The MolDock scoring function used is based on a modified piecewise linear potential (PLP) with new hydrogen bonding and electrostatic terms included. The full description of the algorithm and its reliability compared to other common docking algorithms have been described [39]. The search algorithm MolDock optimizer was used with a minimum of 100 runs, and the parameter settings were as follows: population size=500; maximum iteration=2000; scaling factor=0.50; offspring scheme, Scheme 1; termination scheme, variance-based; crossover rate=0.90. Due to the stochastic nature of the algorithm search, three

independent simulations per ligand were performed to predict the binding mode. Consequently, the complexes with the lowest interaction energy were evaluated. The interactions between *Pf*DHODH and each inhibitor were analysed using the ligand map algorithm, a standard algorithm in the MVD program [39]. The usual threshold values for H-bonds and steric interactions were used.

All figures for *Pf*DHODH modelling and molecular docking results were edited using the Visual Molecular Dynamics 1.9.3 (VMD) program (<http://www.ks.uiuc.edu/Research/vmd/vmd-1.9.3/>) [40].

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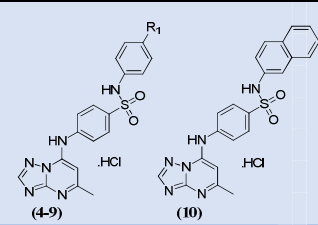
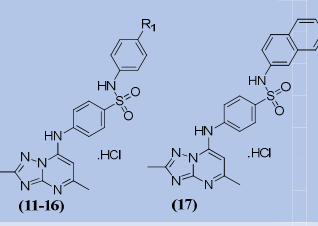
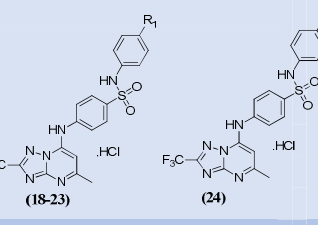
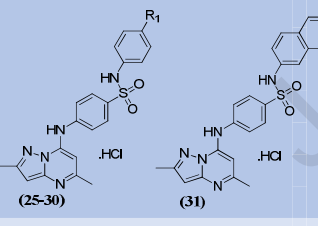
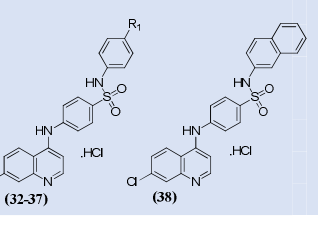
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Table 1: *In vitro* inhibitory activity against *P. falciparum* parasites (3D7 strain, chloroquine-sensitive), human hepatocarcinoma cells (HepG2), and *PfDHODH*, and the selectivity index (SI) values for compounds **4-38**. Chloroquine and artesunate were used as positive controls for whole parasite inhibition.

Compounds	Substituents R ₁	IC ₅₀ ^{3D7} (μM) <i>P. falciparum</i>	IC ₅₀ ^{HepG2} (μM)	SI*	IC ₅₀ (μM) <i>PfDHODH</i>		
 <p>(4-9) (10)</p>	4	R ₁ = H	9.1 ± 0.9	365 ± 10	> 40	0.8 ± 0.1	
	5	R ₁ = Cl	3.0 ± 0.5	92 ± 2	>31	>50	
	6	R ₁ = F	2.0 ± 0.4	367 ± 12	>187	0.3 ± 0.1	
	7	R ₁ = CH ₃	0.50 ± 0.05	367 ± 10	>728	0.41 ± 0.04	
	8	R ₁ = OCH ₃	4 ± 1	46 ± 2	>13	>50	
	9	R ₁ = CF ₃	0.15 ± 0.01	>100	>667	>50	
	10	-	0.23 ± 0.01	56 ± 2	243	0.3 ± 0.1	
	 <p>(11-16) (17)</p>	11	R ₁ = H	6.9 ± 0.2	193 ± 9	28	>50
		12	R ₁ = Cl	0.8 ± 0.1	>50	>62.5	0.6 ± 0.1
		13	R ₁ = F	2.7 ± 0.8	150 ± 20	55.6	1.0 ± 0.2
14		R ₁ = CH ₃	0.91 ± 0.09	110 ± 10	121	0.3 ± 0.2	
15		R ₁ = OCH ₃	4 ± 1	170 ± 10	42.5	1.3 ± 0.1	
16		R ₁ = CF ₃	0.39 ± 0.06	125 ± 12	321	0.6 ± 0.3	
17		-	0.64 ± 0.01	56 ± 2	87.4	0.4 ± 0.1	
 <p>(18-23) (24)</p>		18	R ₁ = H	1.3 ± 0.4	92 ± 4	>70	>50
	19	R ₁ = Cl	0.4 ± 0.2	47 ± 2	>123	0.08 ± 0.01	
	20	R ₁ = F	0.086 ± 0.004	101 ± 7	1174	0.5 ± 0.1	
	21	R ₁ = CH ₃	0.032 ± 0.005	54 ± 4	1688	0.8 ± 0.1	
	22	R ₁ = OCH ₃	0.8 ± 0.1	93 ± 6	>114	0.21 ± 0.06	
	23	R ₁ = CF ₃	0.030 ± 0.003	90 ± 10	3000	>50	
	24	-	0.050 ± 0.002	>100	>2000	0.22 ± 0.07	
	 <p>(25-30) (31)</p>	25	R ₁ = H	2.8 ± 0.1	27 ± 4	9.6	>50
26		R ₁ = Cl	>10	ND	ND	>50	
27		R ₁ = F	>10	ND	ND	>50	
28		R ₁ = CH ₃	>10	ND	ND	>50	
29		R ₁ = OCH ₃	>10	ND	ND	>50	
30		R ₁ = CF ₃	8.0 ± 1	42 ± 4	5.25	>50	
31		-	>10	ND	ND	>50	
 <p>(32-37) (38)</p>		32	R = H	2.0 ± 0.2	92 ± 4	>46	-
		33	R = Cl	1.7 ± 0.2	93 ± 5	>56	-
		34	R = F	4.6 ± 0.6	92 ± 2	>21	-
	35	R = CH ₃	0.97 ± 0.08	92 ± 6	>95	-	
	36	R = OCH ₃	2.8 ± 0.4	92 ± 4	>33	-	
	37	R = CF ₃	2.1 ± 0.2	23 ± 1	11	-	
	38	-	0.89 ± 0.06	13 ± 1	15.1	-	
	chloroquine		0.03 ± 0.01	384 ± 62	12,800	-	
artesunate		0.009 ± 0.002	267 ± 21	29,666	-		

$$*SI = IC_{50}^{HepG2} / IC_{50}^{3D7}$$

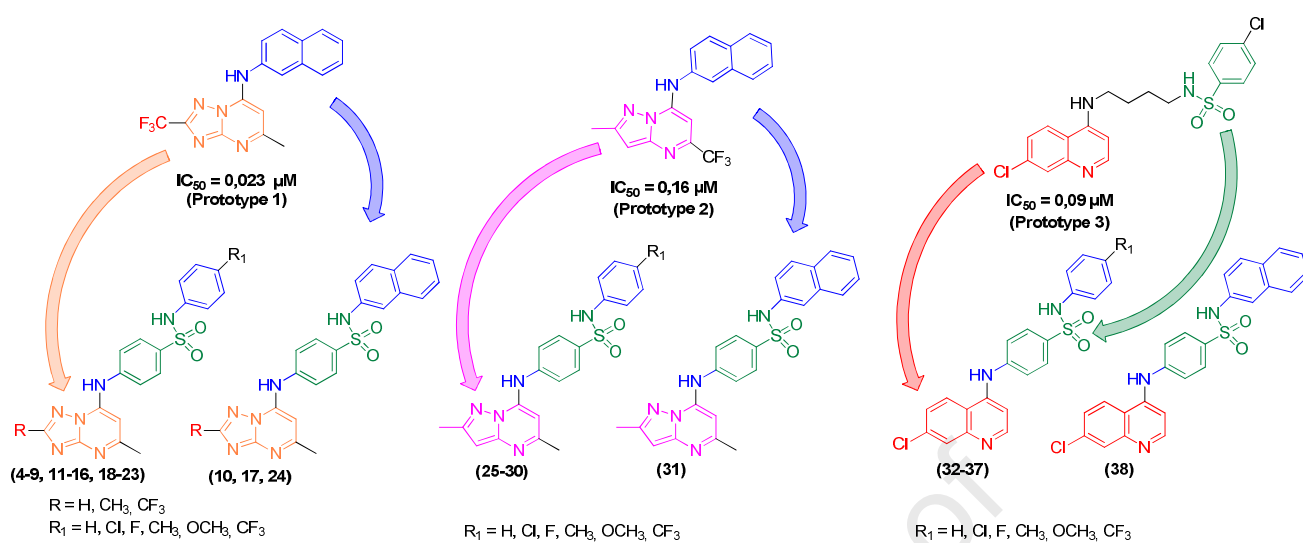


Fig. 1: Rational approach to the design of compounds 4-38.

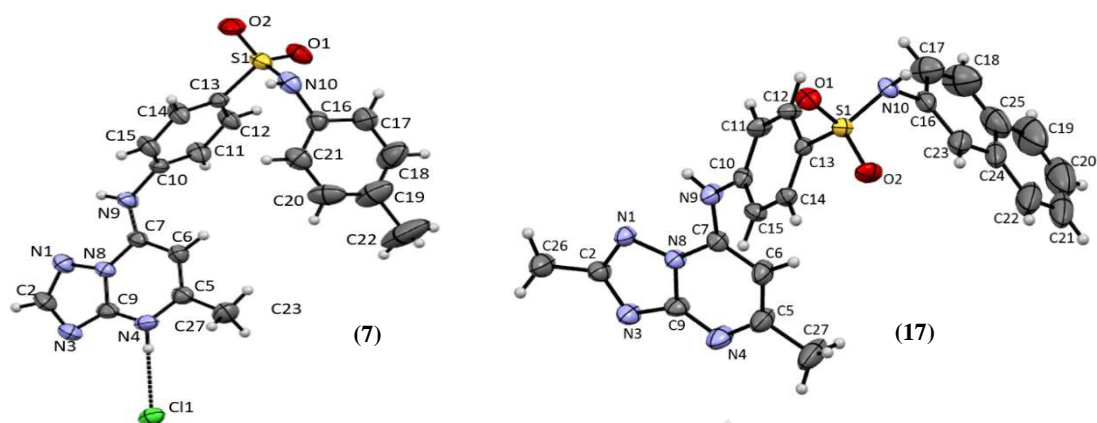


Fig. 2: Asymmetric unit representation of derivatives **7** and **17** (ellipsoids at 50% probability).

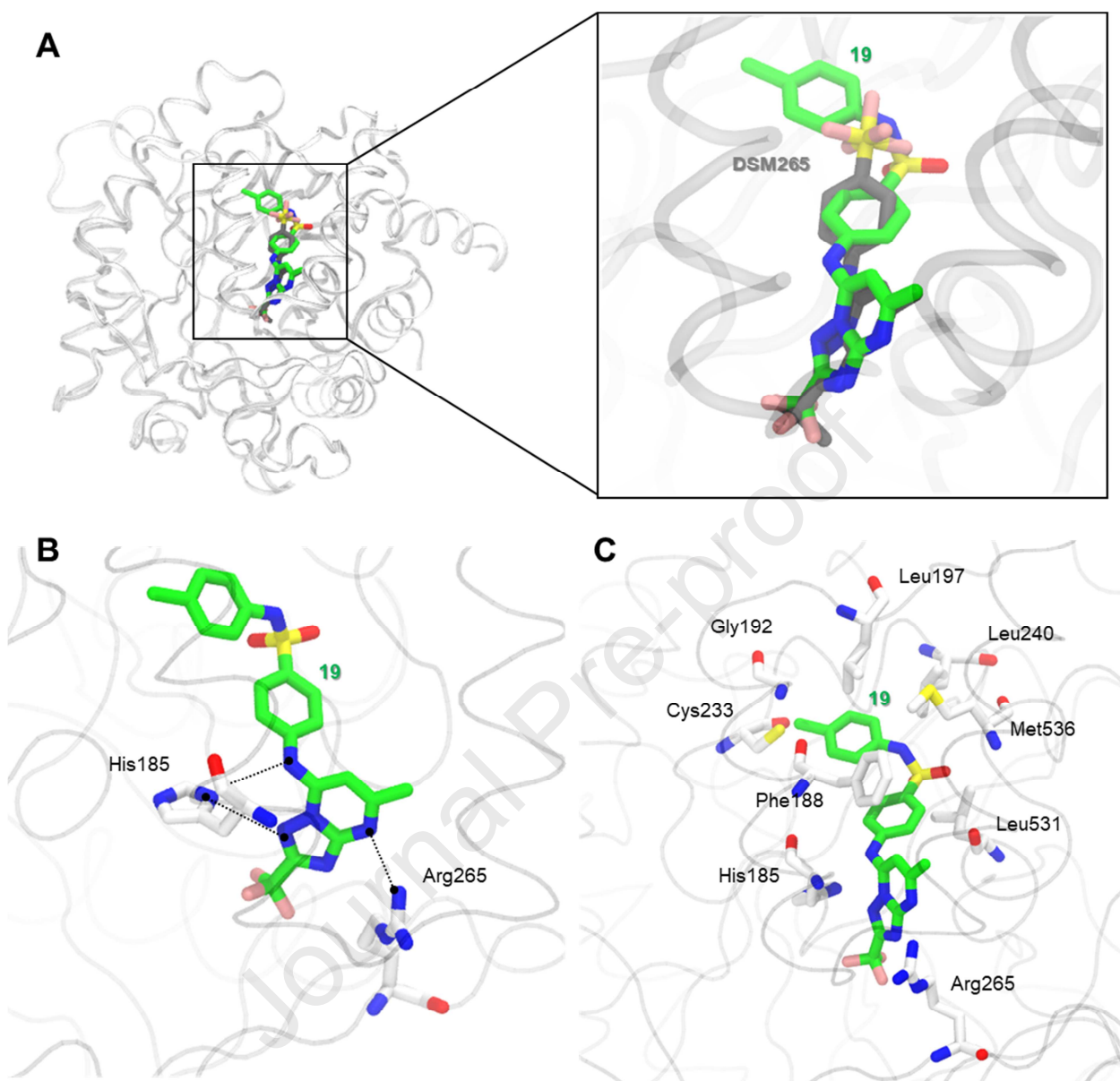
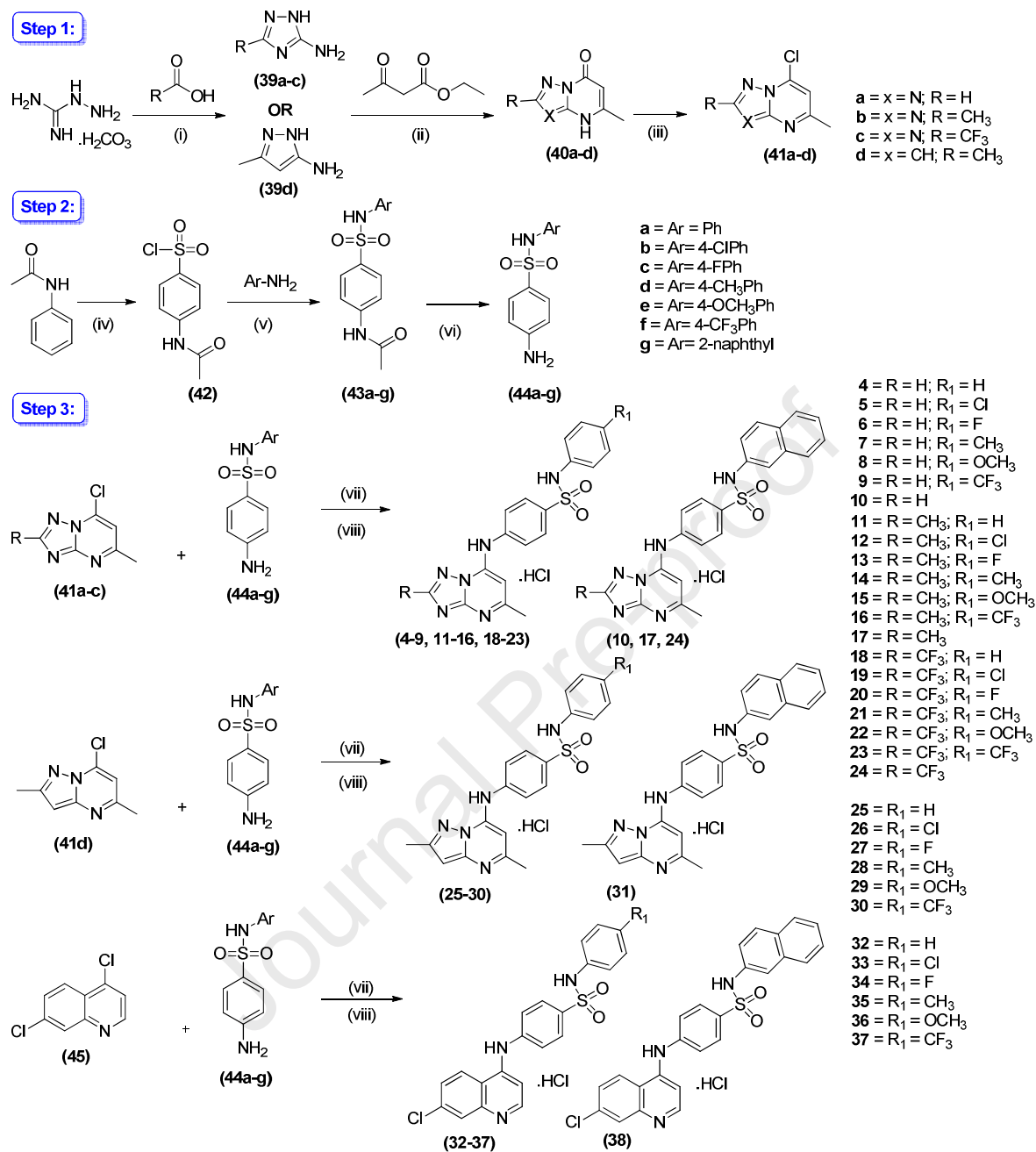


Fig. 3: (A) Representation of the superposition between DSM265 (grey) and 19 (green) complexed to *PfdHODH*. (B) Hydrogen-bonding interactions (B) and steric interactions (C) between 19 and the amino acid residues of *PfdHODH*. The structures are represented as sticks and coloured by atom: nitrogen atoms in blue, sulfur atoms in yellow, fluorine atoms in pink, oxygen atoms in red, and carbon atoms in grey, green or white.



Reagents and conditions: (i) toluene, 110 °C, 24 h, 70-97%; (ii) toluene, cat. *p*-TsOH, 110 °C, 20 h, 71-98%; (iii) POCl₃, 105 °C, 4 h, 51-97%; (iv) HSO₃Cl, 0 °C, 1 h, 60 °C, 1 h, 77%; (v) CHCl₃, TEA, 61 °C, 5 h, 42-87%; (vi) 1) 6N HCl, 100 °C, 6 h; 2) 20% NaOH, 63-89%; (vii) EtOH, 78 °C, 2 h, 24-98%; (viii) HCl/H₂O 1:1, 15 minutes.

Scheme 1: Synthetic route used to prepare compounds **4-38**.

New triazolopyrimidine, pyrazolopyrimidine and quinoline derivatives as *P. falciparum* inhibitors, showing no cytotoxic activity against the human hepatoma cell line HepG2.

The [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives were more potent with IC₅₀ equipotent to chloroquine.

All [1,2,4]triazolo[1,5-*a*]pyrimidine derivatives inhibited *Pf*DHODH activity in the low micromolar and did not show significant inhibition against the *Hs*DHODH homologue.

Molecular docking studies indicated the binding mode of compounds to *Pf*DHODH, and the highest interaction affinities for the *Pf*DHODH enzyme were in agreement with the *in vitro* experimental evaluation.

Their potent *in vitro* activity against *P. falciparum* and the selective inhibition of the *Pf*DHODH enzyme strongly suggest that this is the mechanism of action underlying this series of new compounds.

Comparative study between the anti-*P. falciparum* activity of triazolopyrimidine, pyrazolopyrimidine and quinoline derivatives and the identification of new *Pf*DHODH inhibitors

Flávia F. Silveira, Juliana O. de Souza, Lucas V. B. Hoelz, Vinícius R. Campos, Valquíria A. P. Jabor, Anna C. C. Aguiar, M. Cristina Nonato, Magaly G. Albuquerque, Rafael V. C. Guido, Nubia Boechat and Luiz C. S. Pinheiro

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