Preprint Title: Reactivity of tetrazolo[1,5-a]pyrimidines in click chemistry and hydrogenation

Authors: Elisandra Scapin, Geórgia C. Zimmer, Jean C. B. Vieira, Catarina A. B. Rodrigues, Carlos A. M. Afonso, Nilo Zanatta, Helio G. Bonacorso, Clarissa P. Frizzo and Marcos A. P. Martins

Publication Date: 16 Aug. 2021

Article Type: Full Research Paper

Supporting Information File 1: BJOC Supporting Information.docx; 22.9 MB

ORCID® iDs: Elisandra Scapin - https://orcid.org/0000-0001-7506-308X; Jean C. B. Vieira - https://orcid.org/0000-0002-2209-9297; Nilo Zanatta - https://orcid.org/0000-0002-0959-9043; Marcos A. P. Martins - https://orcid.org/0000-0003-2379-220X
Reactivity of tetrazolo[1,5-a]pyrimidines in click chemistry and hydrogenation

Elisandra Scapin¹,², Geórgia C. Zimmer¹, Jean C. B. Vieira¹, Catarina A. B. Rodrigues³, Carlos A. M. Afonso³, Nilo Zanatta¹, Helio G. Bonacorso¹, Clarissa P. Frizzo¹*, and Marcos A. P. Martins¹*

Address:
¹Núcleo de Química de Heterociclos (NUQUIMHE), Departamento de Química, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil.
²Laboratório de Química, Universidade Federal do Tocantins, 77001-900, Palmas, TO, Brazil.
³Research Institute for Medicines (iMed.ULisboa) Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal.

Email:
Clarissa Piccinin Frizzo - clarissa.frizzo@gmail.com
Marcos Antonio Pinto Martins - marcos.nuquimhe@gmail.com

Abstract

Herein, we explore the synthetic potential of tetrazolo[1,5-a]pyrimidines to obtain new pyrimidine derivatives by click chemistry and hydrogenation. Click chemistry reactions of the trifluoromethyltetrazolo[1,5-a]pyrimidines with terminal acetylenes produced unprecedented trifluoromethylated triazolylpyrimidines in excellent yields (84-98 %) in which one of them was active against all tested microorganisms, presenting moderate MIC values (62.5-15.62 μg/ml). Hydrogenation was carried out using Pd/C-H₂ in MeOH under conventional, photochemical, and pressure (5 bar)
conditions. The hydrogenation was an excellent method to obtain 2-amino-6-aryl-4-trifluoromethyl pyrimidines and/or 2-amino-6-aryl-4-trifluoromethyltetrahydro pyrimidines with a preference for 2-aminopyrimidine formation. The photochemical hydrogenation was the fastest and only pathway to reduce aryl-brominated substrate for the product without dehalogenation. Trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines reacted to 2-amino-6-aryl-4-trifluoromethyl pyrimidine formation in preference to the formation of the corresponding tetrahydropyrimidines. However, the hydrogenation of non-trifluoromethylated tetrazolo[1,5-a]pyrimidines showed a preference for tetrahydropyrimidine formation.

Keywords

Pyrimidines; hydrogenation; click chemistry, trifluoromethyl, photochemical reaction

Introduction

Pyrimidines and their derivatives, such as tetrazolo-[1,5-a]pyrimidines, are extremely important heterocycles and have received special attention from researchers due to their significant biological and pharmaceutical properties [1,2]. Tetrazolopyrimidines were reported for the first time in the 1960s and 1970s, including an azide–tetrazole equilibrium. At the same time, tetrazoles and azides were reported to have different physical and chemical properties [3–11]. Therefore, pharmacokinetics and biological properties may arise from the differences in the chemical structure, and because of this, the azide–tetrazole equilibrium is of great interest from a pharmacological point of view. Recently, we published a study on the synthesis of trifluoromethyl tetrazolo[1,5-a]pyrimidine/2-azidopyrimidines and demonstrated the effects of substituents on regiochemistry and equilibrium [2]. Our findings revealed that when
precursor compounds (α,β-unsaturated ketones) were trifluoromethyl- or trichloromethyl- substituted, tetrazolo[1,5-a]pyrimidines were formed in high regioselectivity. When precursor compounds are substituted with aryl or methyl, it leads to a mixture of compounds, tetrazolo[1,5-a]pyrimidines (R in the 5-position of the ring) and 2-azidopyrimidines (R in the 4-position of the ring), which was attributed to an equilibrium of azide–tetrazole. In that study, we demonstrated that tetrazolo[1,5-a]pyrimidines reacted with terminal alkynes in a 1,3-dipolar cycloaddition catalyzed by copper salts (CuAAC) [12–14], forming 1,2,3-triazole and confirming that an azide intermediate is formed in solution.

Additionally, trifluoromethyl tetrazolo[1,5-a]pyrimidines can set up an important route to synthesize dihydro- or tetrahydropyrimidines through hydrogenation reactions. Dihydro- or tetrahydropyrimidines are crucial for drug discovery due to transitions from aromatic to more flexible and three-dimensional structures. Synthetic pathways for these structures are less common than their aromatic analogs, and because of this, N-heterocycle hydrogenation has been an age-old concern. In fact, tetrazolopyrimidine hydrogenation is particularly rare, and only one example is found in the literature.

Desenko et al. [15] prepared 4,7-dihydotetrazolo[1,5-a]pyrimidines and hydrogenated them using NaBH₄, and the produced tetrahydrotetrazolopyrimidines were obtained in 10-75 % yields and formed as one stereoisomer, although the presence of two chiral centers in the molecules suggests the possible formation of a mixture of diastereoisomers. Reducing aminopyrimidines is more commonly found in the literature, for example, Baskaran et al. [16] reduced 2-aminopyrimidines using triethylsilane (TES) in trifluoroacetic acid (TFA). 2-Aminodihydro pyrimidine formation occurred at lower temperatures, and aminotetra hydropyrimidines were observed
when the reaction was run in refluxing TFA for 24 h. In 2014, Shaw et al. [17] reduced 2-arylamino-pyrimidines using palladium on carbon (Pd/C) in MeOH, forming 2-arylamino-tetrahydro pyrimidines in excellent yields (71-98%). Asymmetric hydrogenation of pyrimidines is an efficient method of synthesizing chiral dihydro- or tetrahydropyrimidines despite the asymmetric hydrogenation of pyrimidines being a novel theme in organic synthesis, and only recently have papers been published on this topic [18–20]. Asymmetric hydrogenation of 2-arylpypyrimidine-4-substitution using [IrCl(cod)]_2−Josiphos−I as a catalytic system and the addition of Yb(OTf)_3 allowed a broad range of pyrimidines to be converted into the corresponding tetrahydropyrimidines with a remarkable improvement in stereoselectivity and high yields (68-99%) [18]. In the sequence, palladium-catalyzed asymmetric hydrogenation of 2-hydroxypyrimidines to corresponding tetrahydropyrimidines was developed with up to 99% of ee. The catalytic system works for mono-, di-, and trisubstituted 2-hydroxypyrimidines [19]. In 2019, Chirik et al. developed a catalyst (rhodium precatalysts) for asymmetric hydrogenation of N-heterocycles, and a diverse array of unsubstituted N-heteroarenes including pyridine, pyrrole, and pyrazine, traditionally challenging substrates for hydrogenation, were successfully hydrogenated using the organometallic precatalysts. The hydrogenation of polyaromatic N-heteroarenes exhibited uncommon chemoselectivity, although only one pyrimidine was reduced in 53% yield [20]. Nevertheless, advances in pyrimidine synthesis have been made, including asymmetric hydrogenation [18–20], and pyrazolopyrimidine hydrogenation still remains challenging. Thermodynamic stability, kinetic inertia of the heteroaromatic ring, highly coordinative nitrogen atoms, and the presence of weak C−H bonds adjacent to the nitrogen atoms, which promote deleterious side reactions, have so far remained unresolved goals [21–23].
The pattern of pyrimidine substitution in relation to hydrogenation reaction is limited to 2-amino-, 2-hydroxy-, and 2-arylpyrimidines, and sparse examples of these substrates with other substituted positions can be found in the literature. Synthesizing 2-amino-trifluoromethylpyrimidines and their respective hydrogenated dihydro- and tetrahydropyrimidines from hydrogenation reactions are practically inexistent in the literature or have very low yields [16]. To the best of our knowledge, hydrogenating trifluoromethyl-substituted pyrazolopyrimidines has yet to be performed.

Considering the recent and growing interest in click chemistry of the versatile 1,2,3-triazoles and the lack of hydrogenation methods for trifluoromethyl-substituted pyrazolopyrimidines, this study aimed to extend knowledge on the reactivity of trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines in the heterocyclic chemistry context. The scope and goals of this paper are given in Scheme 1.

**Scheme 1**: Reactivity of tetrazolopyrimidines in 1,3-cycloaddition and hydrogenation reaction.
Results and Discussion

Click reaction

The tetrazolo[1,5-a]pyrimidines (1) were synthesized using the method previously described by our research group [2]. Considering that tetrazolo[1,5-a]pyrimidines, under certain solution conditions, can be detected as an equilibrium of tetrazolo[1,5-a]pyrimidine and 2-azidopyrimidine [24], the click reaction between azides and terminal alkynes catalyzed by CuAAC was explored. From this reaction, 1,2,3-triazolopyrimidines 6a-f, 7a-c,e, and 8a were synthesized. The reaction between 5-aryl-7-trifluoromethyltetrazolo[1,5-a]pyrimidines 1a-f (1 mmol) and the terminal acetylenes 3, 4, and 5 (1 mmol) using sulfate copper pentahydrate (10 mol%) and sodium ascorbate (20 mol%) in tert-butyl alcohol/water (1:1 mL) was carried out at 60 °C for 24 h. The 4-aryl-2-(4-aryl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl)pyrimidines 6a-f, 7a-c,e, and 8a were obtained in excellent yields (84-98 %) (Table 1).

All 4-aryl-2-(4-aryl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl)pyrimidines 6a-f, 7a-c,e, and 8a are unpublished, and the excellent yields proved the efficiency of this method. Our findings showed that the electronic nature of the R and R1 groups did not influence the formation of the product, and 1,2,3-triazolopyrimidine formation confirms that although the azide form detection depends on solution conditions, even when it is not detectable, it is still present, and in the presence of acetylene, the tetrazolopyrimidine is converted into azide by chemical equilibrium displacement, forming the product of click chemistry in high yields.

The synthesis of compounds 6a-f, 7a-c,e, and 8a was confirmed by 1H, 13C NMR, and 2D experiments, such as HMQC 1H-13C (heteronuclear multiple-quantum coherence), HMBC 1H-13C (heteronuclear multiple-bond correlation), and literature
data. The $^1$H NMR spectrum of compounds 6a-f, 7a-c,e, and 8a showed a chemical shift between 8.68–7.85 that correspond to hydrogen H5 of the pyrimidine ring. The chemical shift between $\delta$ 9.75-8.76 (singlet) corresponds to the H5' of the triazole ring.

Table 1: Synthesis of 4-aryl-2-(4-aryl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl) pyrimidines 6a-f, 7a-c,e, and 8a.

| Product | R     | R1 | Yield (%)$^a$ |
|---------|-------|----|---------------|
| 6a      | Ph    | H  | 91            |
| 6b      | 4-F-C₆H₄ | H  | 90            |
| 6c      | 4-Br-C₆H₄ | H  | 93            |
| 6d      | 4-I-C₆H₄ | H  | 88            |
| 6e      | 4-MeO-C₆H₄ | H  | 93            |
| 6f      | Tien-2-yl | H  | 84            |
| 7a      | Ph    | OMe | 98            |
| 7b      | 4-F-C₆H₄ | OMe | 94            |
| 7c      | 4-Br-C₆H₄ | OMe | 91            |
| 7e      | 4-MeO-C₆H₄ | OMe | 86            |
| 8a      | Ph    | CN | 94            |

$^a$ Isolated product.

In the $^{13}$C NMR spectrum of compounds 6a-f, 7a-c,e, and 8a, the signals of the C2 and C4 carbons for the pyrimidine ring were observed at $\delta$ 163.9-139.7 and 169.6-163.7, respectively. The C5, C6, and CF₃ carbons appeared in the quartet form at $\delta$ 113.2-109.9, 158.4-157.7, and 120.8-120.0, respectively, due to the influence of the CF₃ group. The coupling constants of C5, C6, and CF₃ were $^3J_{F-C} = 2.5$ Hz, $^2J_{F-C} = 37.0$ Hz, and $^1J_{F-C} = 275.6$ Hz, respectively. In addition, the signals of the C5' and C4'
carbons in the triazole ring were found at δ 119.1-114.3 and 155.1-148.2, respectively. X-ray diffractometry verified the regiochemistry of these compounds, and the ORTEP® of compound 7b is illustrated in Figure 1.

**Figure 1:** ORTEP® plot of compounds 7b with thermal ellipsoids drawn at 50 % probability level (Z' = 2).

A mechanism for the formation of compounds 6a-f, 7a-c,e, and 8a was proposed based on the most acceptable current mechanism [13,25] (Scheme 2). Initially, the complexation π between Cu(I) and the terminal alkyne results in the formation of copper (II) acetylate. After the formation of copper (II) acetylate, the complexation with the azide generates the azide-acetylene (III) complex. In the azide-acetylide (III) complex, copper makes the terminal azide nitrogen more electrophilic and the β-vinylidene carbon more nucleophilic, causing the formation of the first C-N bond and the consequent formation of copper (IV) metallocycle. This stage is endothermic and defines the regiochemistry of the reaction. In the next step, the ring contraction occurs by associating the non-binding electron pair of N-1 with C-5, providing the copper triazolyl (V-VI). In the last step, protonation of the copper triazolyl intermediate
(V-VI) occurs, leading to the final product VII (4-aryl-2(4-aryl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl)pyrimidine) and regeneration of the copper catalyst.

Scheme 2: Mechanism reaction proposed for formation of the 4-aryl-2-(4-aryl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl)pyrimidines 6a-f, 7a-c,e, and 8a.

Hydrogenation reaction

The hydrogenation of 5-aryl-7-trifluorometltetrazolo[1,5-a]pyrimidines 1a-h to obtain di- or tetrahydropyrimidines was explored using three methods. First, the hydrogenation reaction was carried out using compound 1a and reacting with Pd/C-H₂ in MeOH for 16 h. The 5-phenyl-7-trifluoromethyltetrazolo[1,5-a]pyrimidine 1a was reduced and the 2-amino-4-phenyl-6-trifluoromethylpyrimidine 9a formed in 97%
yield. Subsequently, this method was carried out for the other substituents 1b-h. The reaction time ranged from 16 to 24 h and yields of up to 97 % were obtained. In the case of 1c,g, in addition to the pyrimidine ring reduction, dehalogenation also occurred (loss of chlorine and bromine atoms), and tetrahydropyrimidine 10a was formed (Table 2). This result deviates from Baskaran et al. [16], who reduced 2-aminopyridines to 2-aminodihydropyrimidines using TES in TFA, in which the bromine was retained during hydrogenation. However, this is an expected result considering the hydrogenation reaction was done in the presence of palladium [26–28] and hydride sources such as H₂, which is a well-known condition for aryl halide hydrodehalogenation.

The photochemical hydrogenation of 5-aryl-7-trifluoromethyltetrazolo[1,5-a]pyrimidines 1a-h was performed in EtOH as a solvent, and 2-amino-6-phenyl-4-trifluoromethylpyrimidine 9a was formed in just 3.5 h and in excellent yield (85 %). Having established the conditions, 1b-h was submitted to the same conditions, forming 2-amino-6-aryl-4-trifluoromethylpyrimidines 9b-f,h (Table 2). The products were obtained in moderate (43 %) to high yields (78 %). Nonetheless, the photochemical hydrogenation was the fastest and the yields were the lowest. Interestingly, 6-(4-bromophenyl)-tetrazolo-4-trifluoromethyl-pyrimidines were reduced to the corresponding 2-amino-6-(4-bromophenyl)-4-trifluoromethyl-pyrimidines without dehalogenation. Finally, the reactions using Pd/C, MeOH as a solvent, at room temperature, and in 24 h were performed under high pressure (5 bar) (Table 2). 5-Aryl-7-trifluoromethyltetrazolo[1,5-a]pyrimidines 1a-h were produced in excellent yields (75-95 %). However, the loss of the chlorine and bromine atoms was observed for compounds 1c,g, forming product 10a. Terazolo[1,5-a]pyrimidine 1e led to the formation of 10e.
The $^{13}$C NMR spectrum analysis for compounds 9a-f,h showed that the signals referring to carbons C6 and C2 were observed in $\delta$ 164.3-162.4 and 168.2-163.2, respectively. The CF$_3$ group caused the carbons C5, C4, and CF$_3$ to appear as a quartet at $\delta$ 102.8-100.5 ($^3J = 2.8$ Hz), 157.1-156.2 ($^2J = 35$ Hz), and 121.5-120.7 ($^1J = 275.4$ Hz), respectively. For compounds 10a,e, the $^{13}$C NMR spectra revealed that the signals referring to C5, C6, and C2 were observed in $\delta$ 29.8-29.0, 51.3, and 159.6-155.2, respectively. The C4 and CF$_3$ appeared in $\delta$ 52.7-50.7 ($^2J = 32$ Hz) and 125.9-124.8 ($^1J = 280.3$ Hz), respectively. The regiochemistry of compounds was also verified by X-ray diffraction, as illustrated by the ORTEP® of 9c (Figure 2).

Non-trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines can also exist as an equilibrium with their azide form and were therefore submitted to hydrogenation. Initially, the reaction was carried out using Pd/C-H$_2$ in MeOH for 24 h, and the results showed that when the R was Ph (11a) and 4-F-Ph (11b), 2-amino-4-arylpyrimidines (13a and 13b) were formed in excellent yields (83-86 %) (Table 3). When the R was 4-Cl-Ph (11g) and 4-I-Ph (11d), no product was observed. When R = 4-Br-Ph (11c), the pyrimidine reduction resulted in tetrahydropyrimidine formation (14a) with a loss of bromine, as observed during hydrogenation of 1c.

**Table 2:** Synthesis of 2-amino-6-aryl-4-trifluoromethylpyrimidines 9a-f,h and 2-amino-6-aryl-4-(trifluoromethyl)-tetrahydropyrimidines 10a,e.

| Comp. | R          | Pd/C-H$_2$, MeOH, r.t., 16-24 h | EtOH, hv, 3.5-5 h | Pd/C-H$_2$, MeOH, r.t., 5 bar, 24 h |
|-------|------------|-------------------------------|-------------------|-----------------------------------|
| 1a-h  |            |                               |                   |                                   |
| 2a-h  |            |                               |                   |                                   |
| 9a-f,h|            |                               |                   |                                   |
| 10a,e |            |                               |                   |                                   |

i: Pd/C-H$_2$, MeOH, r.t., 16-24 h. ii: EtOH, hv, 3.5-5 h. iii: Pd/C-H$_2$, MeOH, r.t., 5 bar, 24 h.
Table 3: Synthesis of 2-amino-4-arylpyrimidines \(13a-b\) and 5-aryl-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidines \(14a-b,e\).

| Comp. | R                  | Prod. | Yield\(^a\) (%) | Prod. | Yield\(^a\) (%) | Prod. | Yield\(^a\) (%) |
|-------|--------------------|-------|------------------|-------|------------------|-------|------------------|
| 11a   | Ph                 | 13a   | 86               | 13a   | 45               | 14a   | 88               |
| 11b   | 4-F-C\(_6\)H\(_4\) | 13b   | 83               | -     | -                | 14b   | 81               |
| 11c   | 4-Br-C\(_6\)H\(_4\) | 13a   | 47               | -     | -                | 14a   | 58               |
| 11d   | 4-I-C\(_6\)H\(_4\) | \(\text{b}\) | -               | \(\text{b}\) | -                | \(\text{b}\) | -                |
| 11e   | 4-OCH\(_3\) - C\(_6\)H\(_4\) | 14e   | 85               | -     | -                | 14e   | 79               |
| 11g   | 4-Cl-C\(_6\)H\(_4\) | \(\text{b}\) | -               | \(\text{b}\) | -                | 14a   | 40               |

\(^a\)Yield of isolated product. \(^b\)No product was observed.
**Figure 2:** ORTEP® plot of compounds 9c and 14a with thermal ellipsoids drawn at the 50 % probability level.

All compounds were characterized by $^1$H and $^{13}$C NMR. Compounds 9a-f,h showed the signal of the NH$_2$ group in the $^1$H NMR spectrum at 5.71 ppm. The H5 of the pyrimidine ring can be seen as a singlet at 7.35 ppm (Figure 3). For compounds 10a,e, the $^1$H NMR spectrum showed a signal at 7.49 ppm corresponding to NH and two singlets at 8.94 and 9.18 ppm corresponding to NH$_2$. The signals between 1.88-1.77 and 2.39-2.43 ppm refer to the tetrahydropyrimidine diasterotopic hydrogens H5 and H5'. The hydrogen H6 appears as a multiplet between 4.63-4.56 ppm, and the H4 appears as a double doublet at 4.72 ppm. The $^1$H NMR spectrum of compounds 9a and 10a is shown in Figure 3. As we can see, evidence for an additional doubling of signals was absent in the $^1$H NMR spectra, confirming that, despite the presence of the two stereogenic centers in 10a,e, only one diastereomer was formed. The values for the spin-spin coupling H4 and H6 (11 and 3 Hz and 12 and 6 Hz) are typical for a $^3$JA-A type constant and indicate a diequatorial orientation for the CF$_3$ substituents and aryl in the predominant conformers, which by similarity with data reported previously for triazolotetrahydropyrimidines [15], were assigned to the cis-isomer 4S6R/4R6S (Figure 3c).
When R = 4-OMe-Ph (11e), tetrahydropyrimidine (14e) was formed in excellent yield (85 %) (Table 3). The photochemical condition also was tested. After 24 h, a reduction of 11a led to the formation of 2-aminopyrimidine 13a in low yield (45 %). No product was obtained during attempts of photochemical reduction of 11b-e,g (Table 3). The third condition tested was Pd/C-H₂ under high pressure (5 bar), in MeOH, at room temperature, and for 24 h. Reduction of compounds 11a-e,g resulted in the formation of 5-aryl-tetrahydrotetrazolo[1,5-a]pyrimidines 14a-b,e with moderate to excellent yields (40-88 %). Similar to previous results under the other two tested conditions, 11c,g reduction led to the 2-amino-4-phenylpyrimidines 14a due to the loss of the halogen group, and no product was obtained when 11d was submitted to hydrogenation conditions. Compounds 13a-b and 14a-b,e were characterized by ¹H and ¹³C NMR. The NH₂ group for compounds 13a-b appeared in the ¹H NMR spectrum at 6.70-6.65 ppm as a singlet. The chemical shifts at 7.11 and 8.31 ppm referred to hydrogens H5 and H6, respectively, and appeared as two doublets. Hydrogens H5 and H6 have a coupling constant of 5.2 Hz. In the ¹H NMR spectrum of compounds 14a-b,e, we observed a singlet referring to the NH of the tetrahydrotetrazolo[1,5-a]pyrimidine ring at 8.68-6.89 ppm.
Figure 3: (a) $^1$H NMR of compound 9a in CDCl$_3$, (b) $^1$H NMR of compound 10a in DMSO-d$_6$, and (c) stereochemistry of tetrahydropyrimidines 10a,e.

The signals between 2.86-2.02 and 2.96-2.20 ppm correspond to the diasterotopic hydrogens H6 and H6’ of the tetrahydrotetrazolo[1,5-a]pyrimidine. The signals between 5.07-4.14 ppm refer to the diasterotopic hydrogens H7 and H7’ of the tetrahydrotetrazolo[1,5-a]pyrimidine. The H5 appears as a doublet of doublets at 5.35-4.69 ppm. In the $^{13}$C NMR spectrum of compounds 13a-b, the signals referring to carbons C6, C5, C4, and C2 were observed at 159.5, 106.3, 164.4-162.9, and 164.3 ppm, respectively. For compounds 14a-b,e, the carbons C6, C7, C5, and C3 were observed at 29.7, 41.7, 54.3-53.0, and 159.2-154.1 ppm, respectively. The regiochemistry for compounds 14 was verified by X-ray diffractometry. The ORTEP® of compound 14a is shown in Figure 2. Although the photochemical method was efficient in hydrogenating the trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines, it did not reduce the non-trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines. Considering that the method was the same, a reasonable explanation for this is low
compound solubility in EtOH. Additionally, the reduction of 5-arylterazolopyrimidine with R= 4-I-Ph failed in all methods, and our findings revealed that trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines undergo hydrogenation much more easily. This fact corroborates Baskaran et al. [16], who reduced 4-aryl-2-aminopyrimidine using TES and TFA and observed that 4-aryl-2-aminopyrimidines were readily converted into 4-aryl-2-aminotetrahydropyrimidines. Nevertheless, the reduction of 4,6-disubstituted-2-aminopyrimidines was much slower, and only ~50 % conversions to the corresponding tetrahydropyrimidines were achieved after reflux in TFA for 24 h.

**Antimicrobial activity**

Considering the biological and pharmacological potential of heterocycles reported herein, some of them (6a-f, 7a-c,e, 8a, 9e, 13a, and 14a-b,e) were screened against Gram-positive and Gram-negative bacteria and yeast using the well diffusion test (Table 4). The antimicrobial screening results involved measuring the average diameter of the inhibition zones (in mm), and we found that only compound 6c had growth inhibition compared to the respective positive control. The remaining compounds exhibited inhibition zones of 5 mm as the negative control (DMSO) and no growth inhibition for each microorganism tested.

Additionally, compound 6c was submitted to the microdilution method against the Gram-positive bacterial strains, and the MICs were evaluated (Table 5). The panel of bacteria was increased, and the Gram-positive *Mycobacterium smegmatis* and *Bacillus subtilis* and Gram-negative *Klebsiella pneumoniae* were included in the microorganisms tested by the microdilution method (Table 5). Compound 6c was active in all tested microorganisms and presented moderate MIC values (MIC 62.5-
15.62 \(\mu g/ml\), being highly active against \textit{Staphylococcus aureus} and \textit{Enterococcus faecalis} (7.81 \(\mu g/ml\)).

\textbf{Table 4:} Antimicrobial activity of compound 6c expressed as inhibition zone and using the well diffusion test (inhibition zone/mm).

| Microorg.\(^a\) | Compound | 6c | Positive control\(^b\) | Negative control (DMSO) |
|-----------------|----------|----|------------------------|-------------------------|
| S.a.            | 14       | 18 | 5                      |
| MRSA            | 13       | 17 | 5                      |
| \textit{E.f.}   | 11       | 15 | 5                      |
| VRE             | 9        | 19 | 5                      |
| \textit{P.a.}   | 5        | 35 | 5                      |
| \textit{E.c.}   | 5        | 35 | 5                      |
| \textit{C.a.}   | 5        | 17 | 5                      |

\(^a\)\textit{S.a.} = \textit{Staphylococcus aureus}, \textit{MRSA} = \text{methicillin-resistant S. aureus}, \textit{E.f.} = \textit{Enterococcus faecalis}, \textit{VRE} = \text{vancomycin-resistant Enterococci}, \textit{P.a.} = \text{Psedomonas auruginosa}, \textit{E.c.} = \textit{Escherichia coli}, and \textit{C.a.} = \textit{Candida albicans}. \(^b\)Positive controls were described in the Experimental Section.

\textbf{Table 5:} Minimal inhibitory concentrations (\(\mu g/mL\)) of compound 6c using the microdilution assay.

| Microorg.\(^a\) | Compound | 6c | Positive control\(^b\) | Negative control (DMSO) |
|-----------------|----------|----|------------------------|-------------------------|
| S.a.            | 7.81     | 7.81 | 250                    |
| MRSA            | 15.62   | >250 (Met)\(^c\) | 250                    |
|                 |         | 3.91 (Vanc)\(^c\) |
| \textit{E.f.}   | 7.81    | 1.95 | 125                    |
| VRE             | 15.62   | 3.91 | 125                    |
| \textit{M.s.}   | 15.62   | < 0.48 | 250             |
| \textit{B.s.}   | 15.62   | < 0.48 | 125             |
| \textit{K.p.}   | 31.25   | 15.62 | 250                    |
| \textit{C.a.}   | 62.5    | < 0.48 | 125             |

\(^a\)\textit{S.a.} = \textit{Staphylococcus aureus}, \textit{MRSA} = \text{methicillin-resistant S. aureus}, \textit{E.f.} = \textit{Enterococcus faecalis}, \textit{VRE} = \text{vancomycin-resistant enterococci}, \textit{M.s.} = \textit{Mycobacterium smegmatis}, \textit{B.s.} = \textit{Bacillus subtilis}, \textit{K.p.} = \text{Klebsiella pneumoniae}, and \textit{C.a.} = \textit{Candida albicans}. \(^b\)Positive controls were described in the Experimental Section. \(^c\) Met = Meticillina and Vanc. = vancomicina
Conclusion

In summary, the results of click chemistry reaction (1,3-dipolar cycloaddition) showed that although the azide form detection depends on the solution condition, it is present even when it is not detectable, and in the presence of acetylene, the tetrazolopyrimidines are converted into their azide form by displacement of chemical equilibrium. Additionally, this reaction resulted in a series of unprecedented trifluoromethylated triazolylpyrimidines, in which one of them was active against all tested microorganisms and presented moderate MIC values (62.5-15.62 µg/ml).

The hydrogenation of trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines using Pd/C-H₂ and MeOH was an excellent method to obtain 2-amino-6-aryl-4-trifluoromethylpyrimidines or 2-amino-6-aryl-4-trifluoromethyltetrahydropyrimidines with a preference for 2-aminopyrimidine formation. The photochemical hydrogenation was the fastest and only pathway to reduce aryl-brominated substrate for the product without dehalogenation. The presence of the strong electron-withdrawing trifluoromethyl group affected hydrogenation chemoselectivity. Trifluoromethyl-substituted tetrazolo[1,5-a]pyrimidines reacted to form 2-amino-6-aryl-4-trifluoromethylpyrimidines in preference to the formation of the corresponding tetrahydropyrimidines. Nonetheless, the hydrogenation of non-trifluoromethylated tetrazolo[1,5-a]pyrimidines showed a preference for tetrahydropyrimidine formation.

Experimental

The reagents and solvents used were obtained from commercial suppliers without further purification. ¹H and ¹³C NMR spectra were recorded on Bruker DPX 400 (¹H at 400.13 MHz and ¹³C at 100.62 MHz) and Bruker DPX-200 (¹H at 200.13 MHz and
\(^{13}\text{C}\) at 50.32 MHz) spectrometers in CDCl\(_3\)/TMS solutions at 298 K and in DMSO-
\(d_6\)/TMS solutions at 298 K. All spectra were acquired in a 5 mm tube at natural
abundance. The chemical shifts (\(\delta\)) are reported in ppm and \(J\) values are given in Hz.
The melting points were measured using a Microquímica MQAPF 301 apparatus or
differential scanning calorimetry (DSC). The DSC experiments were performed using
a MDSC Q2000 (TA Instruments, US), with a heating rate of 5 °C/min under a \(N_2\) flux
of 50 mL/min. Elemental analyses were performed using a Perkin Elmer 2400
apparatus. X-ray diffraction measurements were performed using graphite
monochromatized Mo Ka radiation with \(k = 0.71073\) Å on a Bruker SMART CCD
diffractometer. The structures were solved with direct methods using the SHELXS
software and refined on F2 by full-matrix least-squares with the SHELXL package
[29]. Molecular graphs were prepared using ORTEP for Windows [30]. Data
collection and structure refinement for the structures of \(7b, 9c\), and \(14a\) are given in
Table S1 in the Supporting Information File 1. High-resolution mass spectrometry
(HRMS) was performed using HPLC/MICROTOF ESI-MS equipment. Additional
information regarding the experimental data for the synthesized compounds is
presented in the Supporting Information File 1.

**General procedure to synthesize tetrazolo[1,5-\(a\)]pyrimidines 1a–h**

The tetrazolo[1,5-\(a\)]pyrimidines 1a–h were synthesized from the reaction of 5-
aminotetrazole with 1,1,1-trifluor-4-metoxi-4-aryl-3-alquen-2-onas according to the
method developed in our laboratory [31].

**General procedure to synthesize 4-aryl-2-(4-aryl-1\(H\)-1,2,3-triazol-1-yl)-6-
(trifluoromethyl)pyrimidines 6a-f, 7a-c,e, and 8a**
A mixture of 5-aryl-7-trifluoromethyltetrazolo[1,5-a]pyrimidine 1a-h (1.0 mmol), acetylenes 3, 4, and 5 (1 mmol), copper sulfate pentahydrate (10 mol%), and sodium ascorbate (20 mol%) in tert-butyl alcohol/water (1:1 mL) were placed in a round-bottomed flask and magnetically stirred at 60 °C for 24 h. After the reaction time was reached, chloroform (30 mL) was added and the resulting mixture was washed with distilled water (3 × 10 mL), dried over sodium sulfate (Na$_2$SO$_4$), and the solvent was then removed under reduced pressure. Compounds were obtained in pure form.

**General procedure to synthesize tetrazolo[1,5-a]pyrimidines 11a–e,g**

A mixture of the 5-aminotetrazoles and precursor β-enaminones in [HMIM][TsO] (1.0 mmol) and HCl was performed according to the method developed in our laboratory [2].

**General procedure to synthesize 2-amino-6-aryl-4-trifluoromethylpyrimidines 9a-b,d-f,h, 2-amino-6-aryl-4-(trifluoromethyl)-1,4,5,6-tetrahydropyrimidines 10a, 2-amino-4-arylpyrimidines 13a-b, and 5-aryl-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidines 14a,e using Pd/C-H$_2$**

The mixture of compounds 11a-e,g (1 mmol) or 1a-h (1 mmol), by Pd/C (1.4 mmol) in MeOH, was initially deoxygenated for 1 h using nitrogen gas (or argon). Then, H$_2$ was added and the mixture was stirred at room temperature for 16-24 h. After the reaction time, the source of H$_2$ gas was removed and the resulting mixture was deoxygenated again using nitrogen gas (or argon). The resulting mixture was then filtered under reduced pressure using celite, and the solvent was removed under reduced pressure. The products were obtained in pure form.
General procedure to synthesize 2-amino-6-aryl-4-trifluormethylpyrimidines 9a-f,h, 2-amino-6-aryl-4-(trifluoromethyl)-1,4,5,6-tetrahydropyrimidines 10a, and 2-amino-4-arylpyrimidines 13a using photochemical reactor

The mixture of compounds 1a-h (1 mmol) or 11a-e,g (1 mmol) in EtOH was initially deoxygenated for 1 h using nitrogen gas (or argon). Then, the mixture was subjected to photochemical irradiation using a photochemical reactor equipped with 16 lamps (254 nm) for 3.5-5 h. After the reaction time, the resulting mixture was deoxygenated again using nitrogen gas (or argon). The solvent was removed using reduced pressure. All compounds synthesized using this method needed to be purified using a hexane eluent:ethyl acetate (8:2) preparative plate.

General procedure to synthesize 2-amino-6-aryl-4-trifluormethylpyrimidines 9b,d,f,h, 2-amino-6-aryl-4-(trifluoromethyl)-1,4,5,6-tetrahydropyrimidines 10a,e, and 5-aryl-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidines 14a-b,e using Pd/C-H₂ under 5 bar

The mixture of compounds 11a-e,g (1 mmol) or 1a-h (1 mmol), by Pd/C (1.4 mmol) in MeOH, was initially deoxygenated for 1 h using nitrogen gas (or argon). Then, in a closed system, H₂ was added up to 5 bar of pressure and the mixture was stirred at room temperature for 16-24 h. After the reaction time, the source of H₂ gas was removed, and the resulting mixture was deoxygenated again using nitrogen gas (or argon). The resulting mixture was then filtered under reduced pressure using celite, and the solvent was removed under reduced pressure. The products were obtained in pure form.

General procedure to obtain single crystals
The single crystals were obtained by slow evaporation of the solvents at 25 °C. Suitable monocrystals for compounds 6d,h, 7c, 9d, and 14a were obtained from solvent mixtures of ethyl acetate and EtOH (3:2).

**Spectral data**

4-Phenyl-2-(4-phenyl-1H-1,2,3-triazol-1-yl)-6 (trifluoromethyl)pyrimidine 6a:

C_{19}H_{12}F_{3}N_{5} (367.33). Yield: 91%; m.p. 179-181 °C. ¹H NMR (200 MHz, CDCl₃): δ = 8.88 (s, 1H, H-triazol), 8.30 (d, 2H, H-Ar), 8.07 (s, 1H, H5), 8.00 (d, 2H, ³J 7, H-Ar), 7.59-7.67 (m, 3H, H-Ar), 7.49 (t, 2H, H-Ar), 7.40 (t, 1H, H-Ar). ¹³C NMR (400 MHz, CDCl₃): δ = 169.5 (C₂), 158.1 (q, ¹J 36, C₆), 154.9 (C₄), 148.4 (C₄ - triazol), 134.2, 133.1, 129.6, 129.4, 128.9, 128.8, 127.9 (C-Ar), 120.1 (q, ²J 275, CF₃), 118.6 (C₅ - triazol), 111.2 (q, ³J 2, C₅). Anal. Calcd.: C 62.13; H 3.29; N 19.07. Found: C 61.76; H 3.42; N 18.63.

4-(4-Fluorophenyl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl)pyrimidine 6b:

C_{19}H_{11}F_{4}N_{5} (385.32). Yield: 90%, m.p. 157-159 °C. ¹H NMR (200 MHz, CDCl₃): δ = 7.29 (dd, 2H, ³J 8, ³J 9, H-Ar), 7.40 (t, 1H, H-Ar), 7.48 (t, 2H, H-Ar), 7.99 (dd, 2H, ³J 1, ³J 8, H-Ar), 8.03 (s, 1H, H5), 8.35 (dd, 2H, ³J 5, ³J 9, H-Ar), 8.86 (s, 1H, H5'). ¹³C NMR (400 MHz, CDCl₃): δ = 111.0 (q, ³J 2.5, C₅), 118.5 (C₅'), 120.1 (q, ¹J 275.6, CF₃), 126.1, 128.8, 128.9, 129.6, 130.3 (d, ²J 9), 130.4 (d, ³J 3), 165.9 (d, ¹J 255) (C-Ar), 148.3 (C₄'), 154.9 (C₂), 158.3 (q, ²J 37, C₆), 168.4 (C₄). Anal. Calcd.: C 59.22; H 2.88; N 18.18. Found: C 58.93; H 2.86; N 18.31.

4-(4-Bromophenyl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)-6-(trifluoromethyl)pyrimidine 6c:

C_{19}H_{11}BrF_{3}N_{5} (446.22). Yield: 93%, m.p. 177-179 °C. ¹H NMR (200 MHz, CDCl₃): δ = 7.28 (d, ³J 4, 1H, H-Ar), 7.40 (t, ³J 7, 1H, H-Ar), 7.49 (t, ³J 7, 2H, H-Ar), 7.74 (d, ³J 4,
1H, H-Ar), 7.85 (s, 1H, H5), 7.99 (d, 3J 7, 2H, H-Ar), 8.06 (d, 3J 3, 1H, H-Ar), 8.84 (s, 1H, H5'). 13C NMR (400 MHz, CDCl3): δ = 111.2 (q, 3J 2.5, C5), 118.6 (C5'), 120.1 (q, 1J 276, CF3), 126.1, 128.3, 128.8, 128.9, 129.3, 129.5, 132.7, 133.0, (C-Ar), 148.4 (C4'), 154.9 (C2), 158.4 (q, 2J 37, C6), 168.5 (C4). HRMS (ESI-TOF): requires 446.0228; found 446.0231.

4-(4-Iodophenyl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine 6d:
C19H11IF3N5 (493.22). Yield: 88%, m.p. 172-175 °C. 1H NMR (200 MHz, CDCl3): δ = 7.39 (t, 3J 7, 1H, H-Ar), 7.47 (t, 3J 7, 2H, H-Ar), 7.98-7.93 (m, 4H, H-Ar), 8.00 (s, 1H, H5), 8.02 (d, 2H, H-Ar), 8.84 (s, 1H, H5'). 13C NMR (400 MHz, CDCl3): δ = 111.1 (d, 3J 2, C5), 118.5 (C5'), 120.0 (q, 1J 276, CF3), 126.1, 128.3, 128.8, 128.9, 129.1, 129.5, 133.6, 138.7, (C-Ar), 148.2 (C4'), 154.8 (C2), 158.4 (q, 2J 37, C6), 168.6 (C4). Anal. Calcd.: C 46.27; H 2.25; N 14.20. Found: C 45.67; H 2.31; N 13.66. HRMS (ESI-TOF): requires 494.0089; found 494.0071.

4-(4-Metoxiphenyl)-2-(4-phenyl-1H-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine 6e:
C20H14F3N5O (397.35). Yield: 93%, m.p. 132-134 C. 1H NMR (200 MHz, CDCl3): δ = 3.87 (s, 3H, OCH3), 7.01 (d, 2H, 3J 9, H-Ar), 7.48-7.35 (m, 3H, H-Ar), 7.91 (s, 1H, H5), 7.95 (d, 2H, 3J 7, H-Ar), 8.21 (d, 2H, 3J 9, H-Ar), 8.81 (s, 1H, H5'). 13C NMR (400 MHz, CDCl3): δ = 55.5 (OCH3), 110.3 (q, 3J 2, C5), 114.8 (C5'), 120.3 (q, 1J 275, CF3), 126.1, 126.6, 128.6, 128.8, 129.7, 129.8 (C-Ar), 154.8 (C4'), 157.7 (q, 2J 36, C6), 163.9 (C2), 168.8 (C4). HRMS (ESI-TOF): requires 398.1229; found 398.1233.

2-(4-Phenyl-1H-1,2,3-triazol-1-yl)-6-(trifluormethyl)-4-(tiofen-2-yl)pyrimidine 6f:
C17H10F3N5S (373.06). Yield: 84%, m.p. 169-170 °C. 1H NMR (200 MHz, CDCl3): δ = 7.41 (t, 1H, H-Ar), 7.49 (t, 2H, H-Ar), 7.75 (d, 2H, 3J 8, H-Ar), 7.91 (s, 1H, H5), 8.00
(d, 2H, $^3J$ 7, H-Ar), 8.19 (d, 2H, $^3J$ 8, H-Ar), 8.89 (s, 1H, H5'). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ = 110.3 (q, $^3J$ 3, C5), 118.6 (C5'), 120.1 (q, $^1J$ 275, CF$_3$), 126.1, 128.7, 128.9, 129.2, 129.7, 130.9 (C-Ar), 139.7 (C2), 154.7 (C4'), 157.8 (q, $^2J$ 37, C6), 163.7 (C4). HRMS (ESI-TOF): requires 374.3629; found 374.3679.

2-(4-(4-Metoxiphenyl)-1H-1,2,3-triazol-1-yl)-4-phenyl-6-(trifluormethyl)pyrimidine 7a: C$_{20}$H$_{14}$F$_3$N$_5$O (397.12). Yield: 98%, m.p. 189-190 °C. $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ = 3.85 (s, 3H, OCH$_3$), 6.99 (d, 2H, $^3J$ 9, H-Ar), 7.66-7.58 (m, 3H, H-Ar), 7.90 (d, $^3J$ 9, 2H, H-Ar), 8.05 (d, 1H, H5), 8.29 (d, $^3J$ 7, H-Ar), 8.76 (s, 1H, H5'). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ = 55.3 (OCH$_3$), 111.2 (q, $^3J$ 2.5, C5), 114.4 (C5'), 120.2 (q, $^1J$ 275.5, CF$_3$), 127.5, 127.9, 129.3, 132.9, 134.4, 148.1 (C-Ar), 155.0 (C4'), 158.2 (q, $^2J$ 37, C6), 160.2 (C2), 169.6 (C4). HRMS (ESI-TOF): requires 398.1229; found 398.1230.

4-(4-Fluorophenyl)-2-(4-(4-metoxiphenyl)-1H-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine 7b: C$_{20}$H$_{13}$F$_4$N$_5$O (415.11). Yield: 94%, m.p. 168-169 °C. $^1$H NMR (200 MHz, CDCl$_3$): $\delta$ = 3.84 (s, 3H, OCH$_3$), 6.97 (d, 2H, $^3J$ 9, H-Ar), 7.27 (t, 2H, H-Ar), 7.88 (d, 2H, $^3J$ 8, H-Ar), 7.99 (s, 1H, H5), 8.32 (dd, 2H, $^3J$ 5, $^3J$ 8, H-Ar), 8.76 (s, 1H, H5'). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ = 55.3 (OCH$_3$), 110.9 (q, $^3J$ 2.5, C5), 114.4 (C5'), 120.1 (q, $^1J$ 276, CF$_3$), 116.5 (d, $^2J$ 22), 127.4, 130.3 (d, $^3J$ 9), 130.4 (d, $^4J$ 3), 165.9 (d, $^1J$ 255) (C-Ar), 154.8 (C4'), 158.1 (q, $^2J$ 37, C6), 160.1 (C2), 168.3 (C4).

Anal. Calcd.: C 57.83; H 3.15; N 16.86. Found: C 57.87; H 3.37; N 16.60.

4-(4-Bromophenyl)-2-(4-(4-metoxiphenyl)-1H-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine 7c: C$_{20}$H$_{13}$BrF$_3$N$_5$O (476.25). Yield: 91%, m.p. 165-167°C.

$^1$H NMR (200 MHz, CDCl$_3$): $\delta$ = 3.86 (s, 3H, OCH$_3$), 6.99 (d, 2H, $^3J$ 6, H-Ar), 7.73 (d, 2H, $^3J$ 6, H-Ar), 7.90 (d, 2H, $^3J$ 7, H-Ar), 8.04 (s, 1H, H5), 8.19 (d, 2H, $^3J$ 7, H-Ar), 8.48 (d, 2H, $^3J$ 8, H-Ar), 8.89 (s, 1H, H5). $^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ = 55.3 (OCH$_3$), 110.9 (q, $^3J$ 2.5, C5), 114.4 (C5'), 120.1 (q, $^1J$ 276, CF$_3$), 116.5 (d, $^2J$ 22), 127.4, 130.3 (d, $^3J$ 9), 130.4 (d, $^4J$ 3), 165.9 (d, $^1J$ 255) (C-Ar), 154.8 (C4'), 158.1 (q, $^2J$ 37, C6), 160.1 (C2), 168.3 (C4). HRMS (ESI-TOF): requires 477.1278; found 477.1279.
8.93 (s, 1H, H5'). \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \(\delta = 55.3 \text{ (OCH}_3\text{)}, 111.1 \text{ (q, } ^{3}J 2, \text{ C5), 114.4 (C5')}\), 120.1 (q, \(^{1}J 276, \text{ CF}_3\)), 122.4, 127.5, 128.3, 129.3, 132.7, 133.1 (C-Ar), 155.1 (C4'), 158.4 (q, \(^{2}J 37, \text{ C6}\)), 160.1 (C2), 168.4 (C4). Anal. Calcd.: C 50.44; H 2.75; N 14.71. Found: C 50.12; H 2.83; N 14.39.

4-(4-Metoxiphenyl)-2-(4-(4-metoxiphenyl)-1H-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine 7e: C\(_{21}\)H\(_{16}\)F\(_3\)N\(_5\)O\(_2\) (427.13). Yield: 86%, m.p. 169-171 °C. \(^{1}\)H NMR (200 MHz, CDCl\(_3\)): \(\delta = 3.87 \text{ (s, 3H, OCH}_3\text{)}, 3.92 \text{ (s, 3H, OCH}_3\text{)}, 7.00 \text{ (d, 2H, } ^{3}J 8.0, \text{ H-Ar)}, 7.07 \text{ (d, 2H, } ^{3}J 8, \text{ H-Ar)}, 7.91 \text{ (d, 2H, } ^{3}J 8, \text{ H-Ar)}, 7.95 \text{ (s, 1H, H5)}, 8.27 \text{ (d, 2H, } ^{3}J 8, \text{ H-Ar)}, 8.78 \text{ (s, 1H, H5')}\). \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \(\delta = 55.3 \text{ (OCH}_3\text{)}, 55.5 \text{ (OCH}_3\text{)}, 110.2 \text{ (q, } ^{3}J 2, \text{ C5)}, 114.4 \text{ (C5')}\), 120.4 (q, \(^{1}J 275, \text{ CF}_3\)), 114.8, 121.6, 126.7, 127.5, 129.8, 160.1 (C-Ar), 154.9 (C4'), 157.7 (q, \(^{2}J 37, \text{ C6}\)), 163.9 (C2), 168.8 (C4). Anal. Calcd.: C 59.02; H 3.77; N 16.39. Found: C 59.06; H 3.98; N 16.09.

4-(1-(4-Phenyl-6-(trifluormethyl)pyrimidine-2-yl)-1H-1,2,3-triazol-4-yl)benzonitrile 8a: C\(_{20}\)H\(_{11}\)F\(_3\)N\(_6\) . (392.10) Yield: 94%, m.p. 218-220 °C. \(^{1}\)H NMR (200 MHz, CDCl\(_3\)): \(\delta = 7.62-7.68 \text{ (m, 3H, H-Ar)}, 7.91 \text{ (d, 2H, } ^{3}J 8, \text{ H-Ar)}, 8.25 \text{ (d, 2H, } ^{3}J 8, \text{ H-Ar)}, 8.54 \text{ (d, 2H, } ^{3}J 7, \text{ H-Ar)}, 8.68 \text{ (s, 1H, H5)}, 9.75 \text{ (s, 1H, H5')}\). \(^{13}\)C NMR (400 MHz, CDCl\(_3\)): \(\delta = 111.2 \text{ (CN)}, 113.2 \text{ (q, } ^{3}J 2, \text{ C5)}, 119.1 \text{ (C5')}\), 120.8 (q, \(^{1}J 277, \text{ CF}_3\)), 122.5, 126.7, 128.7, 129.6, 133.4, 133.7, 134.2, 134.5, 145.9(C-Ar), 154.4 (C4'), 157.7 (q, \(^{2}J 36, \text{ C6}\)), 162.6 (C2), 169.1 (C4). HRMS (ESI-TOF): requires 393.1087; found 393.1087.

2-Amine-6-phenyl-4-trifluormethylpyrimidine 9a: C\(_{11}\)H\(_{8}\)F\(_3\)N\(_3\) (239.07). m.p. 129.8 °C and m.p. 130-132 °C (lit.\(^{32}\)). \(^{1}\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 5.71 \text{ (s, 2H, NH}_2\text{)}, 7.35 \text{ (s, 1H, H5)}, 7.50-7.55 \text{ (m, 3H, H-Ar)}, 8.05 \text{ (dd, 2H, H-Ar)}, \). \(^{13}\)C NMR (300 MHz, CDCl\(_3\)): \(\delta = 102.8 \text{ (q, } ^{3}J 3; \text{ C5)}, 120.8 \text{ (q, } ^{1}J 275, \text{ CF}_3\) 127.3, 128.9, 131.5, 136.2 (C-
2-Amine-6-(4-fluorophenyl)-4-trifluormethylpyrimidine 9b: C₁₁H₇F₄N₃ (257.06). m.p. 170-172 °C, m.p. 158 °C (lit.³³). ¹H NMR (300 MHz, DMSO-δ₆): δ = 7.32 (s, 2H, NH₂), 7.35 - 7.37 (m, 2H, H-Ar), 7.51 (s, 1H, H₅), 8.24 (dd, 2H, H-Ar). ¹³C NMR (300 MHz, DMSO-δ₆): δ = 101.1 (q, ³J 3; C₅), 121.3 (q, ¹J 275; CF₃), 116.2 (d, ²J 22), 130.2 (d, ³J 9), 132.8 (d, ⁴J 3), 163 (C-Ar), 156.6 (q, ²J 34; C₄), 164.3 (C₆), 166.2 (C₂). Anal. Calcd.: C 51.37; H 2.74; N 16.34. Found: C 51.02; H 3.84; N 15.22.

2-Amine-6-(4-bromophenyl)-4-trifluormethylpyrimidine 9c: C₁₁H₇BrF₃N₃ (316.98). m.p. 189-190 °C. ¹H NMR (300 MHz, DMSO-δ₆): δ = 7.46 (s, 2H, NH₂), 7.60 (s, 1H, H₅), 7.79 (d, 2H, H-Ar), 8.18 (d, 2H, H-Ar). ¹³C NMR (300 MHz, DMSO-δ₆): δ = 101.2 (q, ³J 7; C₅), 121.3 (q, ¹J 275; CF₃), 125.7; 129.7; 132.3; 135.5 (C-Ar), 156.7 (q, ²J 34; C₄), 164.3 (C₆), 166.3 (C₂). Anal. Calcd.: C 41.53; H 2.22; N 13.21. Found: C 42.48; H 2.87; N 12.26.

2-Amine-6-(4-iodophenyl)-4-trifluormethylpyrimidine 9d: C₁₁H₇F₃IN₃ (364.96). m.p. 133-135 °C. ¹H NMR (300 MHz, CDCl₃): δ = 5.80 (s, 2H, NH₂), 7.32 (s, 1H, H₅), 7.47-7.54 (m, 2H, H-Ar), 8.02 (dd, 2H, H-Ar). ¹³C NMR (300 MHz, CDCl₃): δ = 102.2 (q, ³J 3, C₅), 120.7 (q, ¹J 275, CF₃) 127.7, 128.9, 131.5, 136.2 (C-Ar), 157.1 (q, ²J 35, C₄), 163.5 (C₆), 168.2 (C₂). Anal. Calcd.: C 36.19; H 1.93; N 11.51. Found: C 54.98; H 4.19; N 16.95.

2-Amine-6-(4-metoxiphenyl)-4-trifluormethylpyrimidine 9e: C₁₂H₁₀F₃N₃O (269.08). m.p. 190-192 °C and m.p. 201-203 °C (lit.³⁴). ¹H NMR (300 MHz, DMSO-δ₆): δ = 3.83
(s, 3H, OCH3), 7.06 (d, 2H, 3J 9, H-Ar), 7.28 (s, 2H, NH2), 7.46 (s, 1H, H5), 8.17 (d, 2H, 3J 9, H-Ar). 13C NMR (300 MHz, DMSO-d6): δ = 55.8 (s, OCH3), 100.5 (q, 3J 3; C5), 121.5 (q, 1J 275, CF3), 114.6, 128.6, 129.4, 162.4 (C-Ar), 156.2 (q, 2J 34, C4), 164.3 (C6), 166.8 (C2).

2-Amine-6-(4-tiofen-2-yl)-4-trifluormethylpyrimidine 9f: C9H6F3N3S (245.02). m.p. 154 °C, m.p. 141-143 °C (lit.35). 1H NMR (300 MHz, DMSO-d6): δ = 5.58 (s, 2H, NH2), 7.18 (dd, 1H, 3J 4; 3J 5, H-Ar), 7.22 (s, 1H, H5), 7.57 (dd, 1H, 3J 1, 3J 5, H-Ar), 7.80 (dd, 1H, 3J 1, 3J 4; H-Ar). 13C NMR (300 MHz, DMSO-d6): δ = 101.4 (q, 3J 3, C5), 120.63 (q, 1J 275, CF3), 128.5, 128.5, 130.9, 141.5 (C-Ar), 156.9 (q, 2J 31, C4), 162.4 (C6), 163.2 (C2). Anal. Calcd.: C 44.08; H 2.47; N 17.14; S 13.08. Found: C 43.22; H 2.73; N 16.34; S 14.26.

2-Amine-6-(4-methylphenyl)-4-trifluormethylpyrimidine 9h: C12H10F3N3 (253.08). m.p. 177-178 °C. 1H NMR (300 MHz, DMSO-d6): δ = 1.52 (s, 3H, CH3), 6.47 (d, 2H, 3J 8; H-Ar), 6.48 (s, 2H, NH2), 6.62 (s, 1H, H5), 7.22 (d, 2H, 3J 8, H-Ar). 13C NMR (300 MHz, DMSO-d6): δ = 21.4 (CH3), 100.9 (q, 3J 3; C5), 121.4 (q, 1J 275, CF3), 127.6, 129.9, 133.5, 142.0 (C-Ar), 156.4 (q, 2J 34, C4), 164.3 (C6), 167.2 (C2). Anal. Calcd.: C 56.92; H 3.98; N 16.59. Found: C 57.04; H 4.58; N 16.40.

2-Amine-6-(phenyl)-4-trifluormethyl-1,4,5,6-tetrahydropyrimidine 10a: C11H12F3N3 (243.10). m.p. 192-195 °C. 1H NMR (300 MHz, DMSO-d6): δ = 1.77-1.88 (m, 1H, H5), 2.41 (d, 1H, H5’), 4.54–4.63 (m, 1H, H6), 4.72 (dd, 1H, H4), 7.40-7.42 (m, 5H, H-Ar), 7.49 (s, 1H, NH), 8.94 (s, 1H, NH2), 9.18 (s, 1H, NH2). 13C NMR (300 MHz, DMSO-d6): δ = 29.0 (C5), 50.7 (q, 2J 32, C4), 51.3 (C6), 124.8 (q, 1J 280, CF3), 127.0, 128.9, 129.3, 139.5 (C-Ar), 155.8 (C2).
2-Amine-6-(4-metoxiphenyl)-4-trifluormethyl-1,4,5,6-tetrahydropyrimidine \textbf{10e}: C_{12}H_{14}F_{3}N_{3}O (273.11). m.p. 185-187 °C. \textit{^1}H NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 1.47-1.58\) (m, 1H, H5), 2.09 (d, 1H, H5'), 4.22 (m, 1H, H6), 4.42 (dd, 1H, H4), 6.83 (d, 2H, H-Ar), 7.20 (d, 2H, H-Ar). \textit{^{13}C} NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 29.8\) (C5), 51.4 (C6), 55.6 (OCH\textsubscript{3}), 52.7 (q, \(^2J = 32\), C4), 125.9 (q, \(^1J = 272\), CF\textsubscript{3}), 114.5, 127.7, 129.4, 131.4, 159.5 (C-Ar), 159.6 (C2).

2-Amine-4-phenylpyrimidine \textbf{13a}: C\textsubscript{10}H\textsubscript{9}N\textsubscript{3} (171.08). m.p. 162-164 °C, m.p. 162-164 °C (lit\textsuperscript{36}). \textit{^1}H NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 8.31\) (d, 1H, H6), 7.11 (d, 1H, H5), 8.08-8.05, 7.50-7.48 (m, 5H, H-Ar), 6.65 (s, 2H, NH\textsubscript{2}). \textit{^{13}C} NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 164.3\) (C2), 164.1 (C4), 159.5 (C6), 137.5, 130.9, 129.1, 127.1 (C-Ar), 106.3 (C5).

2-Amine-4-(4-fluorphenyl)-pyrimidine \textbf{13b}: C\textsubscript{10}H\textsubscript{8}FN\textsubscript{3} (189.07). m.p. 160-162 °C, m.p. 160-162 °C (lit\textsuperscript{36}). \textit{^1}H NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 8.30\) (d, 1H, H6), 7.11 (d, 1H, H5), 8.15-8.10, 7.34-7.28 (m, 5H, H-Ar), 6.70 (s, 2H, NH\textsubscript{2}). \textit{^{13}C} NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 164.2\) (C2), 162.9 (C4), 159.7 (C6), 133.9, 129.5, 129.4, 116.2, 115.9 (C-Ar), 106.3 (C5).

5-Phenyl-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidine \textbf{14a}: C\textsubscript{10}H\textsubscript{11}N\textsubscript{5} (201.10). m.p. 213-215 °C. \textit{^1}H NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 2.15-2.27\) (m, 1H, H6), 2.31-2.40 (m, 1H, H6'), 4.17-4.35 (m, 2H, H7 e H7'), 4.75-4.78 (m, 1H, H5), 6.89 (s, 1H, NH), 7.29-7.41 (m, 5H, H-Ar). \textit{^{13}C} NMR (300 MHz, DMSO-\textit{d}_6): \(\delta = 29.7\) (C6), 41.7 (C7), 54.3 (C5), 126.0, 128.4, 129.1, 140.2 (C-Ar), 154.1 (C3a).
5-(4-Fluorphenyl)-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidine 14b: C$_{10}$H$_{10}$FN$_{5}$ (219.09). m.p. 167-169 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 2.02–2.15 (m, 1H, H6), 2.20–2.27 (m, 1H, H6'), 4.14–4.21 (m, 1H, H7), 4.26–4.35 (m, 1H, H7'), 4.69 (dd, 1H, $^3$J 2.9; $^3$J 8.6; H5), 7.21 (t, 2H, H-Ar), 7.37-7.42 (m, 2H, Ar), 8.02 (s, 1H, NH). $^{13}$C NMR (300 MHz, DMSO-$d_6$): $\delta$ = 29.8 (C6), 41.8 (C7), 53.0 (C5), 115.8 (d, $^2$J 21.4; C-Ar), 128.8 (d, $^3$J 8.2; C-Ar), 138.2 (d, $^3$J 2.8; C-Ar), 154.7 (C3a), 162.0 (d, $^1$J 243.3; C-Ar).

5-(4-Metoxiphenyl)-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidine 14e: C$_{11}$H$_{13}$N$_{5}$O (231.11). m.p. 152-155 °C. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ = 2.74–2.86 (m, 1H, H6), 2.90–2.96 (m, 1H, H6'), 3.75 (s, 3H, OCH$_3$), 4.85–4.92 (m, 1H, H7), 4.98–5.07 (m, 1H, H7'), 5.35 (dd, 1H, H5), 7.67 (d, 2H, $^3$J 8.5; H-Ar), 7.99 (d, 2H, $^3$J 8.5; H-Ar), 8.68 (s, NH). $^{13}$C NMR (300 MHz, DMSO-$d_6$): $\delta$ = 29.8 (C6), 41.8 (C7), 53.1 (C5), 55.6 (OCH$_3$), 114.4, 127.9, 133.9, 154.8 (C-Ar), 159.2 (C3a).

**Antimicrobial activity** [37,38]

**Microbial strains**

The *in vitro* antimicrobial study was done using Gram-positive bacteria (*S. aureus* ATCC 25923, *S. aureus* CIP106760, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *E. faecalis* ATCC 51299, and *Mycobacterium smegmatis* ATCC 607), Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumonia* ATCC 9997), and a yeast (*Candida albicans* ATCC 10231).

**Well diffusion assay**
The well diffusion assay was used to determine the antimicrobial activity of the compounds. Petri dishes containing 20 ml of Mueller-Hinton culture medium were inoculated with 0.1 ml of a bacterial cell suspension matching a 0.5 McFarland standard solution. The suspension was uniformly spread over the surface of the medium using a sterile swab. Wells (~5 mm in diameter) were made in agar plates using a sterile glass Pasteur pipette and 50 µL of each compound (1 mg/ml), which were previously reconstituted by dissolving DMSO, were placed in the wells. The DMSO was used as a negative control, while vancomycin (1 mg/ml), norfloxacin (1 mg/ml), and amphotericin B were positive controls for Gram-positive bacteria, Gram-negative bacteria, and yeast, respectively. The plates were then incubated at 37 °C for 24 h. The antimicrobial activity was assayed by measuring the diameter of the inhibition zone formed around each well. Each assay was performed in triplicate.

**Microdilution method**

The MIC of the compounds was determined using the two-fold serial broth microdilution assay. The compounds were dissolved in DMSO and diluted with Mueller-Hinton broth medium at concentrations ranging from 500 to 0.488 µg/mL. The antimicrobial activity of the solvent was evaluated. Vancomycin, norfloxacin, and amphotericin B were used as controls. The MIC values, which were taken as the lowest concentration of the compound that inhibited the growth of the microorganisms after 24 h of incubation at 37 °C, are presented in μg/mL. The bacterial growth was measured with an absorbance microplate reader set to 620 nm (Thermo Scientific Multiskan FC). Assays were done in triplicate for each microorganism tested.
Supporting Information

Supporting Information File 1

NMR spectra of the compounds and crystallographic data of new structures reported.

Acknowledgements

The authors acknowledge the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS; Proc. No. 19/2551-0002273-5, 17/2551-0000981-9), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Proc. No. 432201/2018-1, 405071/2016-7), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the financial support. The CNPq (G.C.Z, N.Z., H.G.B., CPF, and M.A.P.M.) and CAPES (E.S., and J.C.B.V.) fellowships are also acknowledged.

References

(1) Raju, C.; Madhaiyan, K.; Uma, R.; Sridhar, R.; Ramakrishna, S. RSC Adv. 2012, 2 (31), 11657. doi:10.1039/c2ra21330c

(2) Scapin, E.; Salbego, P. R. S.; Bender, C. R.; Meyer, A. R.; Pagliari, A. B.; Orlando, T.; Zimmer, G. C.; Frizzo, C. P.; Bonacorso, H. G.; Zanatta, N.; Martins, M. A. P. Beilstein J. Org. Chem. 2017, 13, 2396–2407. doi:10.3762/bjoc.13.237

(3) Kanyalkar, M.; Coutinho, E. C. Tetrahedron 2000, 56 (44), 8775–8777. doi:10.1016/S0040-4020(00)00819-X

(4) Krivopalov, V. P.; Mamatyuk, V. I.; Nikolaenko, E. B. Russ. Chem. Bull. 1995, 44 (8), 1435–1443. doi:10.1007/BF00714425
(5) Bajwa, J. S.; Sykes, P. J. *J. Chem. Soc. Perkin Trans. 1* **1979**, 3085. doi:10.1039/p19790003085

(6) Cubero, E.; Orozco, M.; Luque, F. J. *J. Am. Chem. Soc.* **1998**, **120** (19), 4723–4731. doi:10.1021/ja9726724

(7) Temple, C.; McKee, R. L.; Montgomery, J. A. *J. Org. Chem.* **1962**, **27** (5), 1671–1673. doi:10.1021/jo01052a045

(8) Boyer, J.; Hyde, H. *J. Org. Chem.* **1960**, **25** (3), 458–459. doi:10.1021/jo01073a608

(9) Temple, C.; Thorpe, M. C.; Coburn, W. C.; Montgomery, J. A. *J. Org. Chem.* **1966**, **31** (3), 935–938. doi:10.1021/jo01341a071

(10) Temple, C.; Montgomery, J. A. *J. Org. Chem.* **1965**, **30** (3), 826–829. doi:10.1021/jo01014a041

(11) Pochinok, V. Y.; Avramenko, L. F.; Grigorenko, P. S.; Skopenko, V. N. *Russ. Chem. Rev.* **1975**, **44**(6), 481–492. doi:10.1070/RC1975v044n06ABEH002355

(12) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chemie Int. Ed.* **2002**, **41** (14), 2596–2599. doi:10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4

(13) Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, **39** (4), 1302. doi:10.1039/b904091a

(14) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, **67** (9), 3057–3064. doi:10.1021/jo011148j

(15) Desenko, S. M.; Gladkov, E. S.; Komykhov, S. A.; Shishkln, O. V.; Orlov, V. D. *Khimiya Geterotsiklicheskikh Soedin.* **2001**, **37** (6), 811–818

(16) Baskaran, S.; Hanan, E.; Byun, D.; Shen, W. *Tetrahedron Lett.* **2004**, **45** (10), 2107–2111. doi:10.1016/j.tetlet.2004.01.056

(17) Shaw, J. W.; Grayson, D. H.; Rozas, I. *Arkivoc* **2013**, **2014** (2), 161–174.
doi:10.3998/ark.5550190.p008.222

(18) Kuwano, R.; Hashiguchi, Y.; Ikeda, R.; Ishizuka, K. *Angew. Chemie Int. Ed.* **2015**, *54* (8), 2393–2396. doi:10.1002/anie.201410607

(19) Feng, G.-S.; Chen, M.-W.; Shi, L.; Zhou, Y.-G. *Angew. Chemie Int. Ed.* **2018**, *57* (20), 5853–5857. doi:10.1002/anie.201801485

(20) Kim, S.; Loose, F.; Bezdek, M. J.; Wang, X.; Chirik, P. J. *J. Am. Chem. Soc.* **2019**, *141* (44), 17900–17908. doi:10.1021/jacs.9b09540

(21) Glorius, F. *Org. Biomol. Chem.* **2005**, *3* (23), 4171. doi:10.1039/b512139f

(22) Perrin, L.; Werkema, E. L.; Eisenstein, O.; Andersen, R. A. *Inorg. Chem.* **2014**, *53* (13), 6361–6373. doi:10.1021/ic500133y

(23) Crabtree, R. H. *Chem. Rev.* **2015**, *115* (1), 127–150. doi:10.1021/cr5004375

(24) Cornec, A.-S.; Baudequin, C.; Fiol-Petit, C.; Plé, N.; Dupas, G.; Ramondenc, Y. *European J. Org. Chem.* **2013**, *2013* (10), 1908–1915. doi:10.1002/ejoc.201201033

(25) Freitas, L. B. O.; Ruela, F. A.; Pereira, G. R.; Alves, R. B.; Freitas, R. P. *Quim. Nova* **2011**, *34* (10), 1791–1804

(26) Abazari, R.; Heshmatpour, F.; Balalaie, S. *ACS Catal.* **2013**, *3* (2), 139–149. doi:10.1021/cs300507a

(27) Huang, Y.; Liu, S.; Lin, Z.; Li, W.; Li, X.; Cao, R. *J. Catal.* **2012**, *292*, 111–117. doi:10.1016/j.jcat.2012.05.003

(28) Pyo, A.; Kim, S.; Kumar, M. R.; Byeun, A.; Eom, M. S.; Han, M. S.; Lee, S. *Tetrahedron Lett.* **2013**, *54* (38), 5207–5210. doi:10.1016/j.tetlet.2013.07.071

(29) Sheldrick, G. M. *Acta Crystallogr. Sect. A Found. Crystallogr.* **2008**, *64* (1), 112–122. doi:10.1107/S0108767307043930

(30) Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30* (5), 565–565. doi:10.1107/S0021889897003117
(31) Scapin, E.; Frizzo, C. P.; Rodrigues, L. V.; Zimmer, G. C.; Vaucher, R. A.; Sagrillo, M. R.; Giongo, J. L.; Afonso, C. A. M.; Rijo, P.; Zanatta, N.; Bonacorso, H. G.; Martins, M. A. P. Med. Chem. Res. 2017, 26 (3), 640–649. doi:10.1007/s00044-017-1783-3

(32) Bonacorso, H. G.; Ferla, A.; Cechinel, C. A.; Zanatta, N.; Martins, M. A. P. J. Heterocycl. Chem. 2008, 45 (2), 483–487. doi:10.1002/jhet.5570450228

(33) Sareen, V.; Jain, S. J. Fluor. Chem. 1993, 63 (3), 265–269. doi:10.1016/S0022-1139(00)80570-9

(34) B-Rao, C.; Kulkarni-Almeida, A.; Katkar, K. V.; Khanna, S.; Ghosh, U.; Keche, A.; Shah, P.; Srivastava, A.; Korde, V.; Nemmani, K. V. S.; Deshmukh, N. J.; Dixit, A.; Brahma, M. K.; Bahirat, U.; Doshi, L.; Sharma, R.; Sivaramakrishnan, H. Bioorganic Med. Chem. 2012, 20 (9), 2930–2939. doi:10.1016/j.bmc.2012.03.019

(35) Flores, A. F. C.; Pizzuti, L.; Brondani, S.; Rossato, M.; Zanatta, N.; Martins, M. A. P. J. Braz. Chem. Soc. 2007, 18 (7), 1316–1321. doi:10.1590/S0103-50532007000700004

(36) Kobarfard, F.; Mohebbi, S.; Shirazi, F. H.; Sharifnoa, S. H. Int. J. Drug Discov. 2011, 3 (2), 78–87. doi:10.9735/0975-4423.3.2.78-87

(37) CLSI. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement. Clinical and Laboratory Standards Institute: Wayne, PA 2011, p 172

(38) Balouiri, M.; Sadiki, M.; Ibnsouda, S. K. J. Pharm. Anal. 2016, 6 (2), 71–79. doi:10.1016/j.jpha.2015.11.005