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

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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-H_2 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-trifluoro methyltetrahydro 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 tethaydropyrimidines. 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

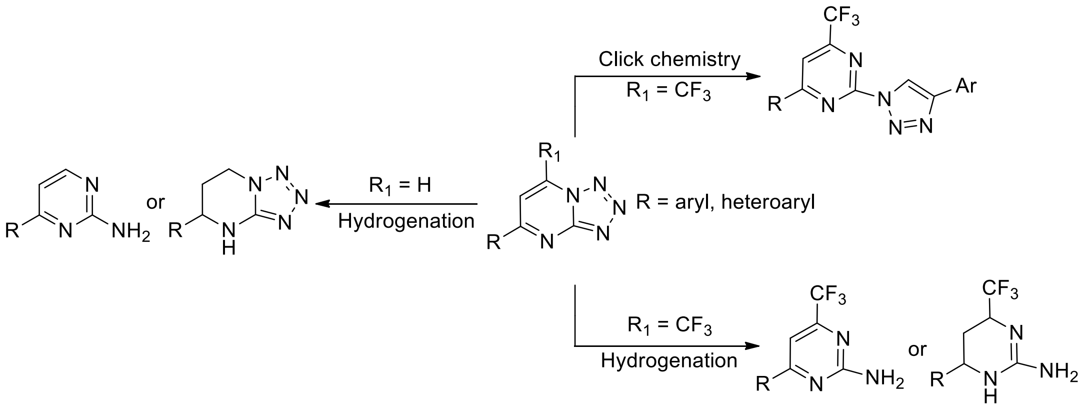
Pyrimidines and their derivatives, such as tetrazolo-[1,5-*a*]pyrimidines, are extremely important heterocycles and have received special attention from researchers due to their significant biological and pharmaceutical properties [1,2]. Tetrazolopyrimidines were reported for the first time in the 1960s 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-dihydrotetrazolo[1,5-*a*]pyrimidines and hydrogenated them using $NaBH_4$, 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-arylaminopyrimidines 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-arylpyrimidine-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

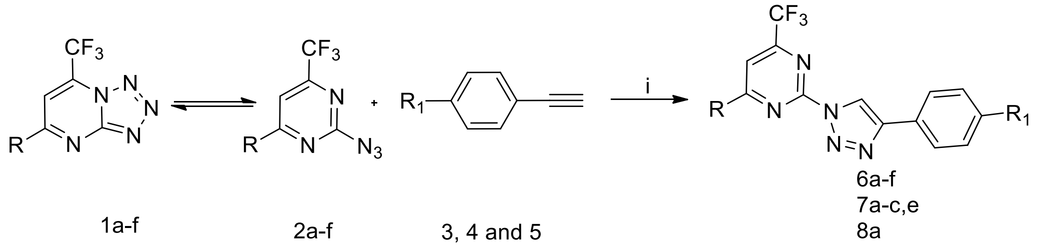
Click reaction

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-1*H*-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-1*H*-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 R^1 groups did not influence the formation of the product, and 1,2,3-triazolylpyrimidine 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 ^{1}H, ^{13}C NMR, and 2D experiments, such as HMQC ^{1}H-^{13}C (heteronuclear multiple-quantum coherence), HMBC ^{1}H-^{13}C (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 δ 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**.



i: $CuSO_4 \cdot 5H_2O$ (10 mol%), sodium ascorbate (20 mol%), $tBuOH/H_2O$ (1:1), 60 °C, 24 h.

Product	R	R^1	Yield (%)^a
6a	Ph	H	91
6b	4-F-C_6H_4	H	90
6c	4-Br-C_6H_4	H	93
6d	4-I-C_6H_4	H	88
6e	4-MeO-C_6H_4	H	93
6f	Tien-2-yl	H	84
7a	Ph	OMe	98
7b	4-F-C_6H_4	OMe	94
7c	4-Br-C_6H_4	OMe	91
7e	4-MeO-C_6H_4	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 δ 163.9-139.7 and 169.6-163.7, respectively. The C5, C6, and CF_3 carbons appeared in the quartet form at δ 113.2-109.9, 158.4-157.7, and 120.8-120.0, respectively, due to the influence of the CF_3 group. The coupling constants of C5, C6, and CF_3 were $^3J_{F\text{-}C}$ = 2.5 Hz, $^2J_{F\text{-}C}$ = 37.0 Hz, and $^1J_{F\text{-}C}$ = 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.

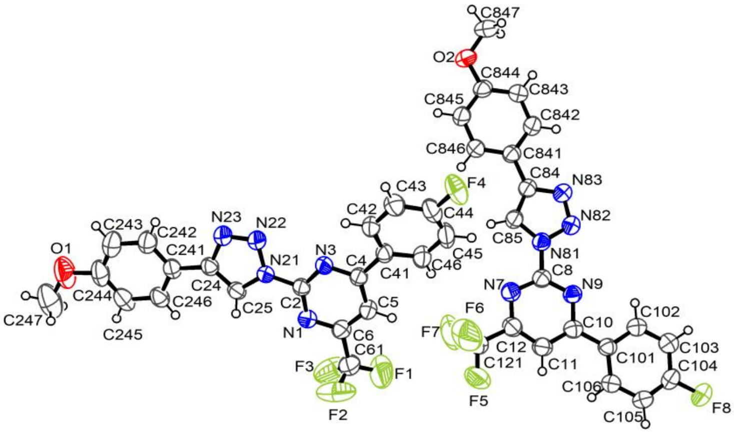
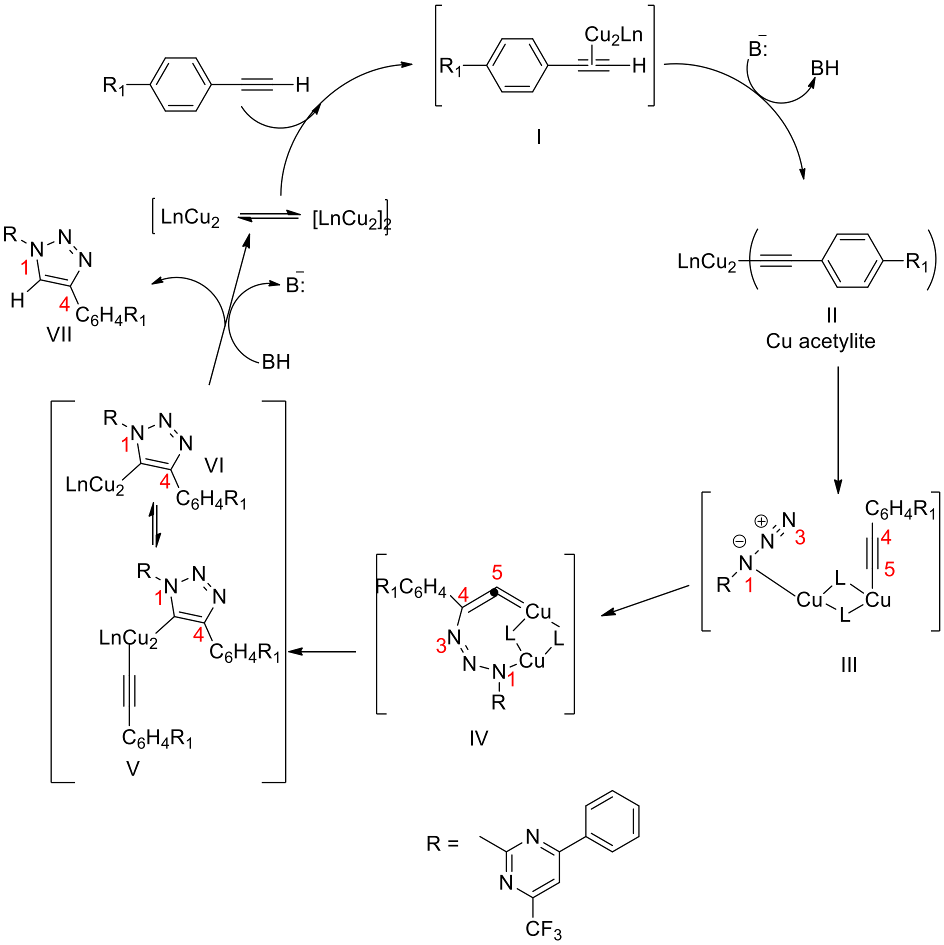


Figure 1: ORTEP® plot of compounds **7b** with thermal ellipsoids drawn at 50 % probability level (Z' = 2).

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-trifluorometiltetrazolo[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-H_2 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 %

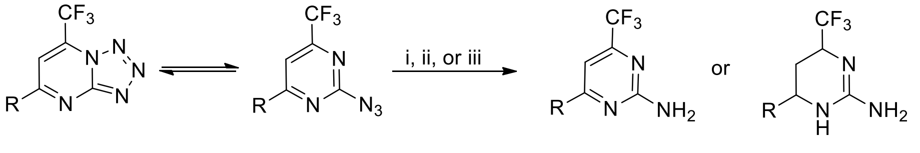
yield. Subsequently, this method was carried out for the other substituents **1b-h**. The reaction time ranged from 16 to 24 h and yields of up to 97 % were obtained. In the case of **1c,g**, in addition to the pyrimidine ring reduction, dehalogenation also occurred (loss of chlorine and bromine atoms), 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 H_2, 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-trifluormethylpyrimidine **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 δ 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 δ 102.8-100.5 (^{3}J = 2.8 Hz), 157.1-156.2 (^{2}J = 35 Hz), and 121.5-120.7 (^{1}J = 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 δ 29.8-29.0, 51.3, and 159.6-155.2, respectively. The C4 and CF_3 appeared in δ 52.7-50.7 (^{2}J = 32 Hz) and 125.9-124.8 (^{1}J = 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**.



1a-h 2a-h 9a-f,h 10a,e

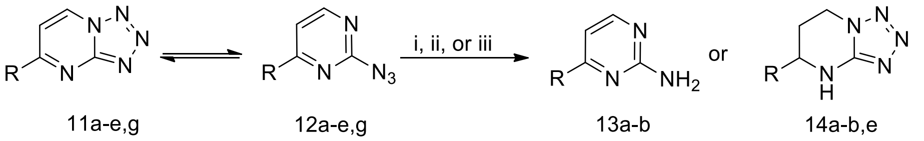
i: Pd/C-H_2, MeOH, r. t., 16-24 h. ii: EtOH, hν, 3.5-5 h. iii: Pd/C-H_2, MeOH, r. t., 5 bar, 24 h.

Comp.	R	Pd/C-H_2, MeOH, r.t., 16-24 h	EtOH, hν, 3.5-5 h	Pd/C-H_2, MeOH, r.t., 5 bar, 24 h

		Prod.	Yield[a] (%)	Prod.	Yield[a] (%)	Prod.	Yield[a](%)
1a	Ph	**9a**	97	**9a**	85	**10a**	87
1b	4-F-C_6H_4	**9b**	97	**9b**	55	**9b**	95
1c	4-Br-C_6H_4	**10a**	40	**9c**	78	**10a**	79
1d	4-I-C_6H_4	**9d**	78	**9d**	43	**9d**	75
1e	4-OCH_3-C_6H_4	**9e**	80	**9e**	56	**10e**	80
1f	Tien-2-yl	**9f**	94	**9f**	73	**9f**	91
1g	4-Cl-C_6H_4	**9a**	80	**10a**	85	**10a**	92
1h	4-CH_3-C_6H_4	**9h**	93	**9h**	76	**9h**	85

[a]Yield of isolated product

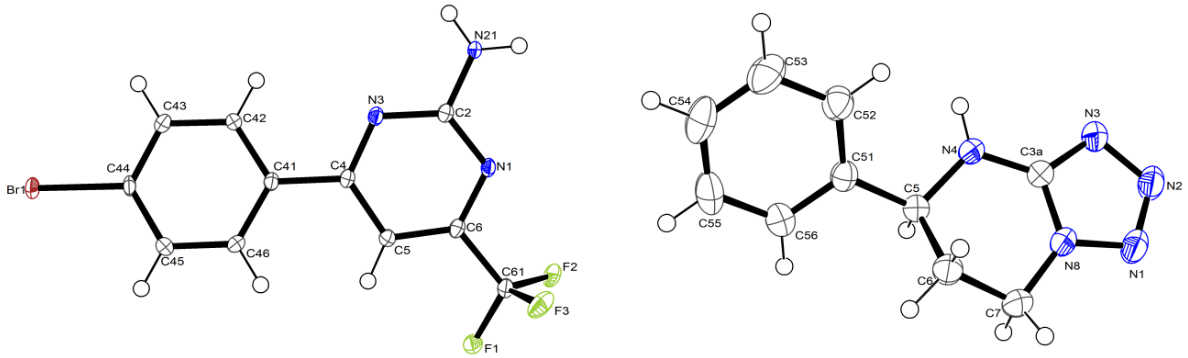
Table 3: Synthesis of 2-amino-4-arylpyrimidines **13a-b** and 5-aryl-4,5,6,7-tetrahydrotetrazolo[1,5-a]pyrimidines **14a-b,e**.



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.

Comp.	R	Pd/C-H_2, MeOH, r.t. 16-24 h		EtOH, $h\nu$, 3.5-5 h		Pd/C-H_2, MeOH, r.t., 5 bar, 24 h	
		Prod.	Yield[a] (%)	Prod.	Yield[a] (%)	Prod.	Yield[a](%)
11a	Ph	**13a**	86	**13a**	45	**14a**	88
11b	4-F-C_6H_4	**14b**	83	**-**[b]	-	**14b**	81
11c	4-Br-C_6H_4	**13a**	47	**-**[b]	-	**14a**	58
11d	4-I-C_6H_4	**-**[b]	-	**-**[b]	-	**-**[b]	-
11e	4-OCH_3-C_6H_4	**14e**	85	**-**[b]	-	**14e**	79
11g	4-Cl-C_6H_4	**-**[b]	-	**-**[b]	-	**14a**	40

[a]Yield of isolated product. [b]No product was observed.



9c

14a

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-aminipyrimidine **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-H_2 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 ^{1}H and ^{13}C NMR. The NH_2 group for compounds **13a-b** appeared in the ^{1}H 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 ^{1}H 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.

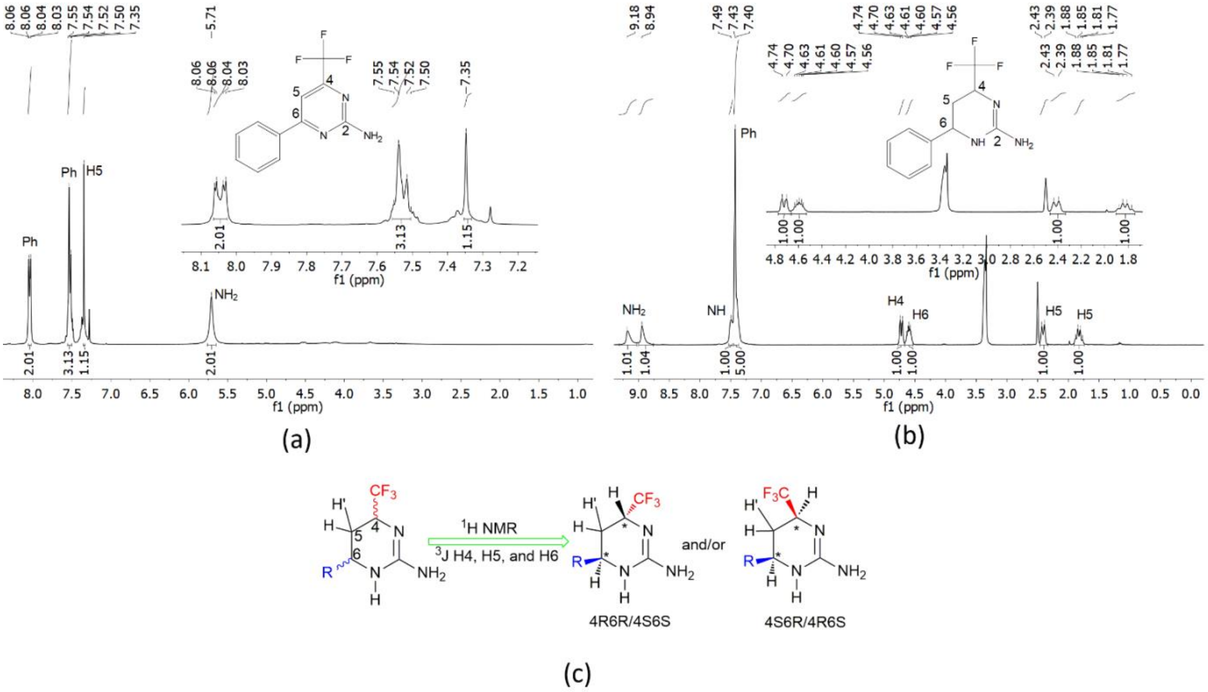


Figure 3: (a) ^{1}H NMR of compound **9a** in CDCl$_3$, (b) ^{1}H NMR of compound **10a** in DMSO-d$_6$, and (c) stereochemistry of tetrahydropyrimidines **10a,e**.

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 μg/ml), being highly active against *Staphylococcus aureus* and *Enterococcus faecalis* (7.81 μg/ml).

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
E.f.	11	15	5
VRE	9	19	5
P.a.	5	35	5
E.c.	5	35	5
C.a.	5	17	5

[a]***S.a.*** *= Staphylococcus aureus*, **MRSA** = methicillin-resistant *S. aureus*, ***E.f.*** *= Enterococcus faecalis*, **VRE** = vancomycin-resistant *Enterococci*, ***P.a.*** *= Psedomonas auruginosa*, ***E.c.*** *= Escherichia coli*, and ***C.a.*** *= Candida albicans*. [b]Positive controls were described in the Experimental Section.

Table 5: Minimal inhibitory concentrations (μg/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] 3.91 (Vanc)[c]	250
E.f.	7.81	1.95	125
VRE	15.62	3.91	125
M.s.	15.62	< 0.48	250
B.s.	15.62	< 0.48	125
K.p.	31.25	15.62	250
C.a.	62.5	< 0.48	125

[a]***S.a.*** = *Staphylococcus aureus*, **MRSA** = methicillin-resistant *S. aureus*, ***E.f.*** = *Enterococcus faecalis*, **VRE** = vancomycin-resistant *enterococci*, ***M.s.*** = *Mycobacterium smegmatis*, ***B.s.*** = *Bacillus subtilis*, ***K.p.*** = Klebsiella *pneumoniae*, and **C.a.** = *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-H_2 and MeOH was an excellent method to obtain 2-amino-6-aryl-4-trifluoromethylpyrimidines or 2-amino-6-aryl-4-trifluoromethyltetrahydpyrimidines 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 tethaydropyrimidines. 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. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX 400 (^{1}H at 400.13 MHz and ^{13}C at 100.62 MHz) and Bruker DPX-200 (^{1}H at 200.13 MHz and

^{13}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 (δ) 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-aril-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_2SO_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-H_2 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, H_2 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 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 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**: $C_{19}H_{12}F_3N_5$ (367.33). Yield: 91%; m.p. 179-181 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 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). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 169.5 (C2), 158.1 (q, ^1J36, C6), 154.9 (C4), 148.4 (C4 - triazol), 134.2, 133.1, 129.6, 129.4, 128.9, 128.8, 128.7, 127.9 (C-Ar), 120.1 (q, ^2J275, CF_3), 118.6 (C5 - triazol), 111.2 (q, ^3J2, 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-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **6b**: $C_{19}H_{11}F_4N_5$ (385.32). Yield: 90%, m.p. 157-159 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.29 (dd, 2H, ^{3}J 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'). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 111.0 (q, ^3J 2.5, C5), 118.5 (C5'), 120.1 (q, ^1J 275.6, CF_3), 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-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **6c**: $C_{19}H_{11}BrF_3N_5$ (446.22). Yield: 93%, m.p. 177-179 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 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'). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 111.2 (q, ^3J 2.5, C5), 118.6 (C5'), 120.1 (q, ^1J 276, CF_3), 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-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **6d**: $C_{19}H_{11}IF_3N_5$ (493.22). Yield: 88%, m.p. 172-175 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 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'). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 111.1 (d, ^3J 2, C5), 118.5 (C5'), 120.0 (q, ^1J 276, CF_3), 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-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **6e**: $C_{20}H_{14}F_3N_5O$ (397.35). Yield: 93%, m.p. 132-134 C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.87 (s, 3H, OCH_3), 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'). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 55.5 (OCH_3), 110.3 (q, ^3J 2, C5), 114.8 (C5'), 120.3 (q, ^1J 275, CF_3), 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-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)-4-(tiofen-2-yl)pyrimidine **6f**: $C_{17}H_{10}F_3N_5S$ (373.06). Yield: 84%, m.p. 169-170 °C. ^{1}H NMR (200 MHz, $CDCl_3$): 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, $CDCl_3$): δ = 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)-1*H*-1,2,3-triazol-1-yl)-4-phenyl-6-(trifluormethyl)pyrimidine **7a**: $C_{20}H_{14}F_3N_5O$ (397.12). Yield: 98%, m.p. 189-190 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 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 (s, 1H, H5), 8.29 (d, 2H, ^3J 7, H-Ar), 8.76 (s, 1H, H5'). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 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)-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **7b**: $C_{20}H_{13}F_4N_5O$ (415.11). Yield: 94%, m.p. 168-169 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.84 (s, 3H, OCH_3), 6.97 (d, 2H, ^3J 7, 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, $CDCl_3$): δ = 55.3 (OCH_3), 110.9 (q, ^3J 2, C5), 114.3 (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)-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **7c**: $C_{20}H_{13}BrF_3N_5O$ (476.25). Yield: 91%, m.p. 165-167°C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 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), 8.93 (s, 1H, H5’). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 55.3 (OCH_3), 111.1 (q, ^3J 2, C5), 114.4 (C5’), 120.1 (q, ^1J 276, CF_3), 122.4, 127.5, 128.3, 129.3, 132.7, 133.1 (C-Ar), 155.1 (C4’), 158.4 (q, ^2J 37, 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)-1*H*-1,2,3-triazol-1-yl)-6-(trifluormethyl)pyrimidine **7e**: $C_{21}H_{16}F_3N_5O_2$ (427.13). Yield: 86%, m.p. 169-171 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.87 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 7.00 (d, 2H, ^3J 8.0, H-Ar), 7.07 (d, 2H, ^3J 8, H-Ar), 7.91 (d, 2H, ^3J 8, H-Ar), 7.95 (s, 1H, H5), 8.27 (d, 2H, ^3J 8, H-Ar), 8.78 (s, 1H, H5’). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 55.3 (OCH_3), 55.5 (OCH_3), 110.2 (q, ^3J 2, C5), 114.4 (C5’), 120.4 (q, ^1J 275, CF_3), 114.8, 121.6, 126.7, 127.5, 129.8, 160.1 (C-Ar), 154.9 (C4’), 157.7 (q, ^2J 37, 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)-1*H*-1,2,3-triazol-4-yl)benzonitrile **8a**: $C_{20}H_{11}F_3N_6$. (392.10) Yield: 94%, m.p. 218-220 °C.^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.62-7.68 (m, 3H, H-Ar), 7.91 (d, 2H, ^3J 8, H-Ar), 8.25 (d, 2H, ^3J 8, H-Ar), 8.54 (d, 2H, ^3J 7, H-Ar), 8.68 (s, 1H, H5), 9.75 (s, 1H, H5’). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 111.2 (CN), 113.2 (q, ^3J 2, C5), 119.1 (C5’), 120.8 (q, ^1J 277, 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, ^2J 36, C6), 162.6 (C2), 169.1 (C4). HRMS (ESI-TOF): requires 393.1087; found 393.1087.

2-Amine-6-phenyl-4-trifluormethylpyrimidine **9a**: $C_{11}H_8F_3N_3$ (239.07). m.p. 129.8 °C and m.p. 130-132 °C (lit.[32]). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.71 (s, 2H, NH_2), 7.35 (s, 1H, H5), 7.50-7.55 (m, 3H, H-Ar), 8.05 (dd, 2H, H-Ar). ^{13}C NMR (300 MHz, $CDCl_3$): δ = 102.8 (q, ^3J 3; C5), 120.8 (q, ^1J 275, CF_3) 127.3, 128.9, 131.5, 136.2 (C-

Ar), 157.1 (q, ^2J 35, C6), 163.5 (C6), 168.2 (C2). Anal. Calcd.: C 55.23; H 3.37; N 17.57. Found: C 55.56; H 4.10; N 17.12.

2-Amine-6-(4-fluorphenyl)-4-trifluormethylpyrimidine **9b**: $C_{11}H_7F_4N_3$ (257.06). m.p. 170-172 °C, m.p. 158 °C (lit.[33]). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.32 (s, 2H, NH_2), 7.35 -7.37 (m, 2H, H-Ar), 7.51 (s, 1H, H5), 8.24 (dd, 2H, H-Ar). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 101.1 (q, ^3J 3; C5), 121.3 (q, ^1J 275; CF_3), 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**: $C_{11}H_7BrF_3N_3$ (316.98). m.p. 189-190 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.46 (s, 2H, NH_2), 7.60 (s, 1H, H5), 7.79 (d, 2H, ^3J 8, H-Ar), 8.18 (d, 2H, ^3J 8, H-Ar). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 101.2 (q, ^3J 7; C5), 121.3 (q, ^1J 275; CF_3), 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**: $C_{11}H_7F_3IN_3$ (364.96). m.p. 133-135 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.80 (s, 2H, NH_2), 7.32 (s, 1H, H5), 7.47-7.54 (m, 2H, H-Ar), 8.02 (dd, 2H, H-Ar). ^{13}C NMR (300 MHz, $CDCl_3$): δ = 102.2 (q, ^3J 3, C5), 120.7 (q, ^1J 275, CF_3) 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**: $C_{12}H_{10}F_3N_3O$ (269.08). m.p. 190-192 °C and m.p. 201-203 °C (lit.[34]). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.83

(s, 3H, OCH_3), 7.06 (d, 2H, ^3J 9, H-Ar), 7.28 (s, 2H, NH_2), 7.46 (s, 1H, H5), 8.17 (d, 2H, ^3J 9,H-Ar). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 55.8 (s, OCH_3), 100.5 (q, ^3J 3; C5), 121.5 (q, ^1J 275, CF_3), 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**: $C_9H_6F_3N_3S$ (245.02). m.p. 154 °C, m.p. 141-143 °C (lit.[35]). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.58 (s, 2H, NH_2), 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). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 101.4 (q, ^3J 3, C5), 120.63 (q, ^1J 275, CF_3), 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**: $C_{12}H_{10}F_3N_3$ (253.08). m.p. 177-178 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.52 (s, 3H, CH_3), 6.47 (d, 2H, ^3J 8; H-Ar), 6.48 (s, 2H, NH_2), 6.62 (s, 1H, H5), 7.22 (d, 2H, ^3J 8, H-Ar). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 21.4 (CH_3), 100.9 (q, ^3J 3; C5), 121.4 (q, ^1J 275, CF_3), 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**: $C_{11}H_{12}F_3N_3$ (243.10). m.p. 192-195 °C. ^{1}H NMR (300 MHz, DMSO-d_6): 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, NH_2), 9.18 (s, 1H, NH_2). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 29.0 (C5), 50.7 (q, ^2J 32, C4), 51.3 (C6), 124.8 (q, ^1J 280, CF_3), 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 **10e**: $C_{12}H_{14}F_3N_3O$ (273.11). m.p. 185-187 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): *δ* = 29.8 (C5), 51.4 (C6), 55.6 (OCH_3), 52.7 (q, ^2J 32, C4), 125.9 (q, ^1J 272, CF_3,), 114.5, 127.7, 129.4, 131.4, 159.5 (C-Ar), 159.6 (C2).

2-Amine-4-phenylpyrimidine **13a**: $C_{10}H_9N_3$ (171.08). m.p. 162-164 °C, m.p. 162-164 °C (lit.[36]). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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_2). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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 **13b**: $C_{10}H_8FN_3$ (189.07). m.p. 160-162 °C, m.p. 160-162 °C (lit[36]). ^{1}H NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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_2). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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 **14a**: $C_{10}H_{11}N_5$ (201.10). m.p. 213-215 °C. ^{1}H NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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). ^{13}C NMR (300 MHz, DMSO-*d*$_6$): *δ* = 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): δ = 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, ^3J 2.9; ^3J 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): δ = 29.8 (C6), 41.8 (C7), 53.0 (C5), 115.8 (d, ^2J 21.4; C-Ar), 128.8 (d, ^3J 8.2; C-Ar), 138.2 (d, ^3J 2.8; C-Ar), 154.7 (C3a), 162.0 (d, ^1J 243.3; C-Ar).

5-(4-Metoxiphenyl)-4,5,6,7-tetrahydrotetrazolo[1,5-*a*]pyrimidine **14e**: $C_{11}H_{13}N_5O$ (231.11). m.p. 152-155 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 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, ^3J 8.5; H-Ar), 7.99 (d, 2H, ^3J 8.5; H-Ar), 8.68 (s, NH). ^{13}C NMR (300 MHz, DMSO-d_6): δ = 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 ^{o}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 ^{o}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.

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