https://dx.doi.org/10.21577/0103-5053.20200241

J. Braz. Chem. Soc., Vol. 32, No. 4, 900-911, 2021
Printed in Brazil - ©2021 Sociedade Brasileira de Química

Article

Efficient Synthesis and Antimicrobial Activities of Long Alkyl Chain Trifluoromethyl-1*H*-pyrazol-1-(thio)carboxamides and Trifluoromethyl-1*H*-pyrazol-1-yl-thiazoles

Debora L. de Mello,[a] Juliana L. Malavolta,[a] Roberto C. V. Santos,[b] Leonardo Q. S. Lopes,[b] Sidnei Moura,[c] Darlene C. Flores[d] and Alex F. C. Flores*[,d]

[a]*Departamento de Química, Universidade Federal de Santa Maria, 97105-900 Santa Maria-RS, Brazil*

[b]*Centro Universitário Franciscano, 97010-032 Santa Maria-RS, Brazil*

[c]*Instituto de Biotecnologia, Universidade de Caxias do Sul, 95070-560 Caxias do Sul-RS, Brazil*

[d]*Escola de Química e Alimentos, Universidade Federal do Rio Grande, 96203-900 Rio Grande-RS, Brazil*

The synthesis of 3-alkyl-5-trifluoromethyl-1*H*-pyrazole-1-carboxamides, 3-alkyl-5-trifluoromethyl-1*H*-pyrazole-1-thiocarboxamides, and 2-(3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)-thiazoles derivatives are reported. [3 + 2] cyclocondensations for a series of long alkyl chain 1,1,1-trifluoro-4-methoxyalk-3-en-2-ones and semicarbazide or thiosemicarbazide were carried out in ethanol, an eco-friendly medium. The series of trifluoromethyl-1*H*-pyrazol-1-yl-thiocarboxamide following a [3 + 2] cyclocondensation with 2-bromoacetophenone were converted into two series of 2-(3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)-thiazoles. Good yields (69-96%) of the isolated products were obtained. The structures of the new long alkyl chain 1*H*-pyrazoles and 2-(1*H*-pyrazol-1-yl)thiazoles were characterized using ^{1}H, ^{13}C, and ^{19}F nuclear magnetic resonance (NMR) spectroscopy and electrospray ionization tandem mass spectrometric (ESI MS/MS) data. Moreover, some of the products were evaluated for their antimicrobial activity against Gram-negative *Escherichia coli* American Type Culture Collection (ATCC) 35218, *Salmonella enteritidis* ATCC 13076, and *Pseudomonas aeruginosa* ATCC 15692; and Gram-positive *Staphylococcus aureus* ATCC 6538, methicillin resistant *Staphylococcus aureus* (MRSA clinical isolate), *Streptococcus* sp. (clinical isolate), and *Candida albicans* ATCC 14053 and *Candida krusei* ATCC 6258 fungi. All the tested 1*H*-pyrazoles exhibited antibacterial and antifungal activities at the tested concentrations. The compounds from series **4** were found to be powerful against MRSA.

Keywords: nitrogen heterocycles, thiazoles, pyrazoles, antimicrobial activity

Introduction

Bacterial and fungal diseases affect millions of human worldwide every day. Several million doses of antibiotics are prescribed annually for these infections, contributing to the current problem of increased antibiotic resistance. The global charge to society of this is a very high annual financial cost to treat antibiotic-resistant infections in addition to the lives lost due to inefficient therapies.[1,2] Thiazole compounds have been described as antimicrobial agents.[3,4] This heterocycle is a prevalent scaffold in a number of naturally occurring and synthetic compounds with attractive pharmacological activities such as anti-inflammatory, analgesic, anticonvulsant, antitumor, antiviral, antifungal, and antibacterial, including antitubercular.[5,6] Niridazole and levamisole, thiazole derivatives, are used as antihelmintic drugs;[7] ritonavir is an antiretroviral drug, from the protease inhibitor class, used to treat HIV (human immunodeficiency virus) infections and AIDS (acquired immunodeficiency syndrome).[8] Therefore, a great deal of interest has been focused on the design and biological activity of thiazole derivatives. On the other hand, CF_3-containing 1*H*-pyrazoles have emerged as a group of compounds possessing a broad spectrum of useful biological properties such as herbicides, fungicides, and analgesic activities, and because of this, their synthesis has also received intense attention.[9,10]

The 4-alkoxy-1,1,1-trihalo-3-alken-2-ones have been proven to be key intermediates in the heterocycle synthesis protocols.[11,12] They represent a range of precursors containing a 1,3-dielectrophilic system with differentiated reactivity carbons applied in the development of heterocycle

*e-mail: alexflores@furg.br

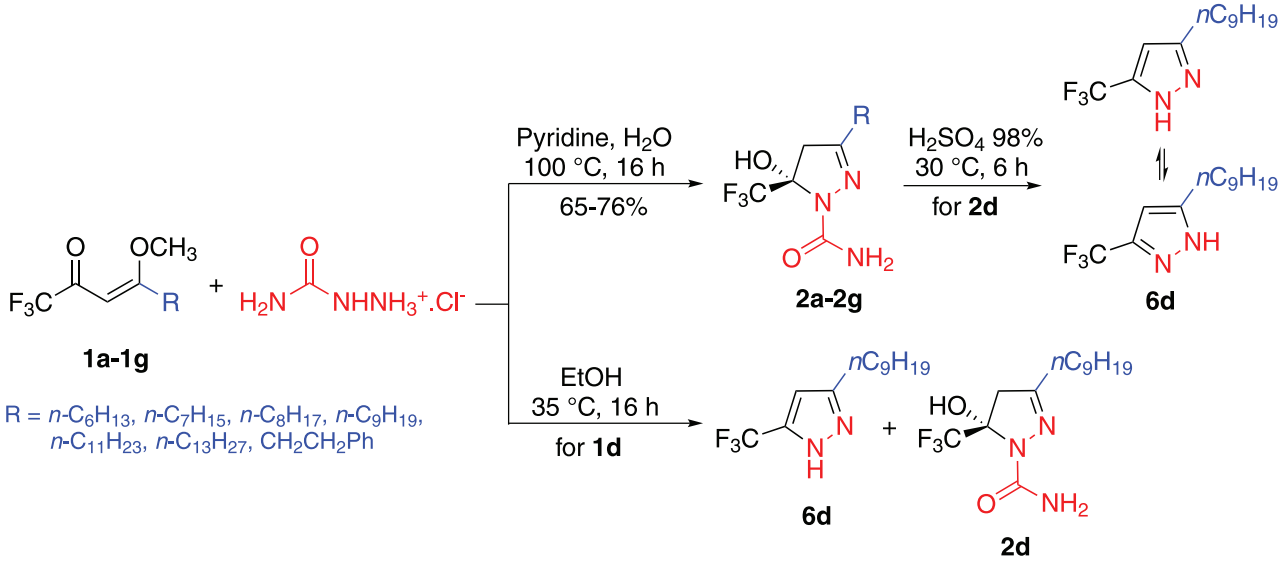
libraries.[13,14] Based on the above observations, and as a part of our research program on understanding the biological activities and the synthesis of novel trifluoromethyl heterocyclic systems, the objective was to obtain a series of 3-(long unbranched)alkyl-5-trifluoromethyl-1*H*-pyrazol-1-(thio)carboxamides and 5-trifluoromethyl-1*H*-pyrazol-1-yl-thiazoles derivatives from sequential [3 + 2] cyclocondensations between 1,1,1-trifluoro-4-methoxy-3-alken-2-ones (**3a**-**3g**), semicarbazide and thiosemicarbazide, and a [3 + 2] cyclocondensation between 5-trifluoromethyl-1*H*-pyrazol-1-thiocarboxamides and 2-bromoacetophenone.

Results and Discussion

The 1,1,1-trifluoro-4-methoxy-3-alken-2-ones were obtained as described earlier.[13] Following the conventional route to CX_3-containing 1*H*-pyrazole,[15] the synthesis of the 3-alkyl-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazole-1-carboxamides was performed by reacting semicarbazide hydrochloride, pyridine, and 1,1,1-trifluoro-4-methoxy-3-tridecen-2-one (**1d**) in H_2O at reflux for 16 h to give 65-76% yields, as outlined in Scheme 1. It was found that semicarbazide does not react with 1,1,1-trifluoro-4-methoxy-3-tridecen-2-one (**1d**) in water without pyridine. On the other hand, when the reaction was conducted in ethanol without pyridine, the product was a mixture of 3(5)-nonyl-5(3)-trifluoromethyl-1*H*-pyrazol (**6d**) and the respective 5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**2d**). As reported earlier for 3-alkyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazole-1-carboxamide and 1-thiocarboxamide derivatives,[15,16] attempts at the aromatization of **2d** with sulfuric acid led to the formation of only a tautomeric compound (**6d**), which was obtained as a yellowish wax at an 80% yield, demonstrating that in this medium the elimination of carbamic acid also occurs for these trifluoromethyl derivatives (Scheme 1).

The 3-alkyl-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazole-1-thiocarboxamides (**3a**-**3g**) were prepared by [CCC + NN] cyclocondensations between the 1,1,1-trifluoro-4-methoxy-3-alken-2-ones (**1a**-**1g**) and thiosemicarbazide. The satisfactory reaction conditions applicable to the whole series of trifluoromethyl-1,3-dielectrophile precursors (**1a**-**1g**) were determined by monitoring the consumption of these precursors using TLC (thin layer chromatography). To find the best reaction conditions, considering the yield and purity of the product (**3**), different reaction conditions were tested, varying the stoichiometric ratio between the precursor (**1d**), thiosemicarbazide, the solvent, temperature, and reaction time (Table 1).

After the solvent tests, the cyclocondensation reactions in water were chosen, the conditions described in entry 2 in Table 1 were extended to all series. In the aqueous reactional medium products precipitate and could be isolated just by filtration (Scheme 2). These 3-alkyl-trifluoromethyl-1*H*-pyrazol-1-(thio)carboxamides (**3a**-**3g**) were reacted with 2-bromoacetophenone in EtOH to afford two series of thiazole derivatives, depending on the reaction temperature. When the reaction of **3d** with 2-bromoacetophenone was conducted at 30 °C for 16 h, 3-nonyl-1-(4-phenylthiazol-2-yl)-5-hydroxy-5-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazole (**4d**) was obtained at a good yield of 86%, without any indication of another product being formed. However, when



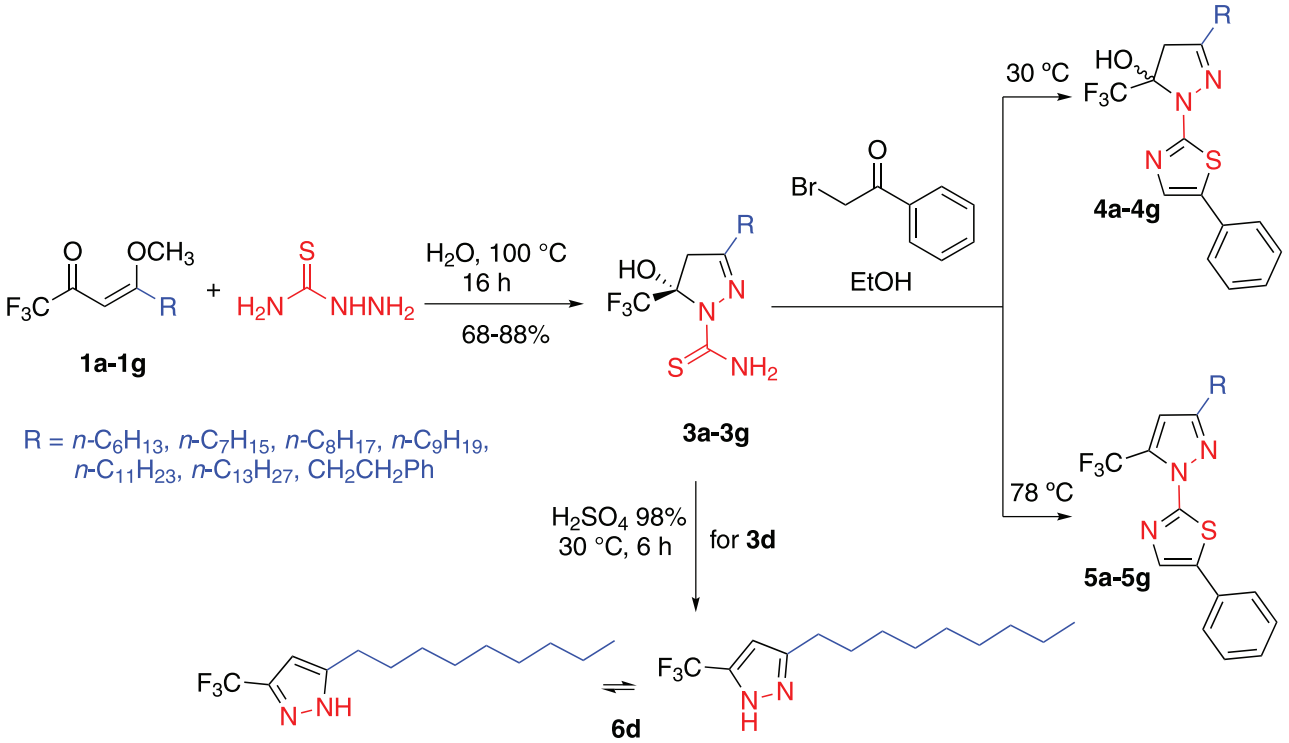
Scheme 1. Synthesis of the 3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-carboxamides **2a**-**2g**.

Table 1. Reactional condition optimization for [3 + 2] cyclocondensation between **1d** and thiosemicarbazide

entry	Solvent (10 mL)	Ratio **1d**:thiosemicarbazide	Temperature / °C	time / h	Yield / %
1	water	1:1	100	24	60
2	water	1:1.2	100	16	88
3	water	1:1.5	100	16	85
4	methanol	1:1	65	24	65
5	methanol	1:1.2	65	16	80
6	ethanol	1:1	78	24	66
7	ethanol	1:1.2	78	16	82
8	ethanol	1:1.5	78	16	85

the reaction was conducted under EtOH reflux, the pure 2-(3-nonyl-5-(trifluoromethyl)-1*H*-pyrazol-1-yl)-4-phenylthiazole (**5d**) was obtained at an 80% yield. Thus, it was concluded that under EtOH reflux the hydrobromic acid released during [NCS + CC] cyclocondensation, assists in the water elimination/aromatization of the 4,5-dihydro-1*H*-pyrazole cycle (Scheme 2). A slight increase in reaction time from 16 to 20 h was required for the complete conversion of derivative **3f** to **5f**. Attempts to use sulfuric acid for the dehydration of derivatives **2d**, **3d**, and **4d** led to 1*H*-pyrazol (**6d**), as reported by other researchers.[15,16]

The data used for the characterization of all the synthesized compounds is shown in the Experimental section. All the newly synthesized compounds gave satisfactory analyses for the proposed structures, which were confirmed based on their 1D / 2D nuclear magnetic resonance (NMR) and Fourier transform mass spectrometry with probe electrospray ionization (FTMS-pESI) spectral data. For three series containing a 4,5-dihydro-1*H*-pyrazole moiety, **2**, **3**, and **4a-4g**, a remarkable doublet due to diastereotopics H4a and H4b from the pyrazole ring between 3.0 and 3.5 ppm was observed in the ^{1}H NMR spectra,[14-16] in addition to the straight saturated chain (fatty chain) characteristic signals set, including a terminal methyl, the overlapping methylenes and, α and β methylene to the pyrazole ring at consistent chemical shifts (see Supplementary Information).[17] Each series has spectral peculiarities such as the signal due to NH_2 groups that appear as a broad singlet at 5.75 ppm



Scheme 2. Synthesis of the 3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-thiocarboxamides **3a**-**3g** and 2-(3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)-thiazoles **4**, **5a**-**5g**.

for the **2a**-**2g** series; however, the signals due to NH_2 for **3a**-**3g** appear as two broad singlets at 6.22 and 7.14 ppm. The signal for H4 from the aromatic 1*H*-pyrazol ring of product **5** was at δ 6.6-6.7 ppm, while that for H5 of the 4-phenylthiazole ring of products **4** and **5** had a signal at δ 6.90-7.25 ppm. In the ^{13}C NMR spectra are similar for the 4,5-dihydro-1*H*-pyrazole series, the quartet signals due to carbons coupling to fluorine atoms, the C5 at 90-92 ppm with $^2J_{CF}$ 34 Hz, and CF_3 group were observed at about 123 ppm with J_{CF} 284-286 Hz. The quartet signal related to C5-CF_3 from the aromatic 1*H*-pyrazole ring was at about δ 132 ppm with J_{CF} 42 Hz. Besides the signal set related to the straight saturated chain substituent, including the terminal methyl, the overlapping methylenes and, α and β methylene to pyrazole ring at consistent chemical shifts (see Supplementary Information).

Biological studies

All the novel trifluoromethyl-1*H*-pyrazol-1-(thio) carboxamides (**2**, **3**) and trifluoromethyl-1*H*-pyrazol-1-yl-thiazoles (**4**, **5**) were evaluated *in vitro* for antibacterial activity against a representative human pathogenic Gram-positive bacteria, such as *S. aureus* American Type Culture Collection (ATCC) 6538, clinically isolated methicillin-resistant *S. aureus* (MRSA), and clinically isolated *Streptococcus* sp.; and Gram-negative bacteria, such as *S. enteritidis* ATCC 13076, *E. coli* ATCC 35218, and *P. aeruginosa* ATCC 15692. The antifungal profile of the compounds was evaluated against *C. albicans* ATCC 14053 and *C. krusei* ATCC 6258. An agar-diffusion method was utilized for the initial rating of antimicrobial activities. Chloramphenicol and ketoconazole were used as standard antibacterial and antifungal agents. The antimicrobial activities of 1*H*-pyrazoles (**2**-**5**) are summarized in Tables 2 and 3. The measurements were recorded for each new compound as the average diameter of the inhibition zones of bacterial or fungal growth around the disks in mm, for the triplicate experiment. The minimum inhibitory concentration (MIC) in μg mL^{-1} was measured for all compounds for a representative microorganism, which showed large growth inhibition zones, using the twofold

Table 2. Inhibition zones formed after the exposure of strains to compounds **2**, **4**, **5**

Compound	Inhibition zone / mm						
	C. albicans	*C. krusei*	*S. aureus*	MRSA	*Streptococcus* sp.	*E. coli*	*S. enteritidis*
2a	26 ± 4	10 ± 0	–	14 ± 0	11 ± 1	7 ± 0	–
2b	15 ± 1	10 ± 1	–	12 ± 2	–	–	–
2c	–	9 ± 0	–	–	10 ± 1	–	9 ± 1
2d	23 ± 3	11 ± 1	9 ± 0	15 ± 0	12 ± 2	8 ± 0	8 ± 0
2e	–	–	–	13 ± 2	–	–	8 ± 0
2f	17 ± 0	–	–	–	–	9 ± 0	8 ± 0
2g	18 ± 2	–	–	10 ± 1	10 ± 1	9 ± 0	–
4a	–	–	–	–	9 ± 1	7 ± 0	–
4b	–	–	–	–	–	–	–
4c	10 ± 1	9 ± 2	–	–	12 ± 1	–	–
4d	–	–	8 ± 0	13 ± 1	12 ± 1	8 ± 0	–
4e	–	–	–	–	9 ± 2	–	–
4f	9 ± 1	9 ± 2	–	10 ± 2	10 ± 1	–	–
4g	10 ± 1	10 ± 1	–	–	8 ± 1	8 ± 0	–
5a	–	–	8 ± 1	10 ± 1	–	9 ± 1	–
5b	7 ± 1	–	10 ± 1	10 ± 2	10 ± 2	–	–
5c	8 ± 2	–	9 ± 1	–	–	8 ± 1	7 ± 0
5d	–	–	–	–	–	9 ± 1	–
5e	13 ± 1	–	10 ± 2	9 ± 1	10 ± 1	8 ± 1	10 ± 1
5f	8 ± 1	–	10 ± 1	9 ± 1	–	8 ± 0	–
5g	8 ± 1	–	10 ± 1	9 ± 2	9 ± 1	8 ± 0	8 ± 1
CLO[a]			22 ± 2			26 ± 2	

[a]Chloramphenicol 30 μg mL^{-1}. MRSA: methicillin resistant *Staphylococcus aureus*.

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration/minimal fungicidal concentration (MBC/MFC) to compounds **2**, **3**, **4**, **5**

Compound	MIC / (μg mL^{-1})			MFC or MBC / (μg mL^{-1})		
	C. albicans	*S. aureus*	*E. coli*	*C. albicans*	*S. aureus*	*E. coli*
2a	780	780	780	3125	3125	3125
2b	780	780	780	3125	3125	3125
2c	780	780	780	3125	3125	3125
2d	–	–	–	–	–	–
2e	1560	780	780	6250	3125	3125
2f	780	780	780	3125	3125	3125
2g	780	780	780	3125	3125	3125
3a	–	–	1560	–	–	6250
3b	–	780	–	–	3125	–
3c	1560	780	1560	6250	3125	6250
3d	1560	780	1560	6250	3125	6250
3e	1560	780	1560	6250	3125	6250
3f	1560	–	1560	6250	–	6250
3g	1560	780	1560	6250	3125	6250
4a	–	150	2500	–	600	–
4b	–	150	–	–	600	–
4c	1250	150	5000	–	600	–
4d	–	150	5000	–	600	–
4e	–	–	–	–	–	–
4f	1250	150	–	–	600	–
4g	1250	150	–	–	600	–
5a	1560	–	1560	6250	–	625
5b	1560	780	1560	6250	3125	625
5c	1560	780	1560	6250	3125	625
5d	1560	780	1560	6250	3125	625
5e	1560	780	1560	6250	3125	625
5f	–	780	–	–	3125	625
5g	–	–	1560	–	–	–
CLO[a]	–	125	62.5	–	125	62.5
KET[b]	62.5	–	–	62.5	–	–

[a]Chloramphenicol standard for antibacterial activity; [b]ketoconazole standard for antifungal activity.

serial dilution method. The inhibition zone diameter values shown in Table 2 refer to the tested concentration of 2500 μg mL^{-1} and the MICs (μg mL^{-1}) for selected microorganisms are shown in Table 3. From the results, it has been observed that all the tested compounds possess moderate to poor antimicrobial activity against selected bacteria strains. On the basis of the inhibition zone test against *S. aureus*, compounds **2d** (R = *n*-C_9H_{19}), **4d** (R = *n*-C_9H_{19}) and those in series **5** were found to have inhibitory activity when compared with the standard drug chloramphenicol. The selective activity on Gram-positive bacteria of the studied compounds may indicate good permeability by the outer layer of the membrane of these monoderms bacteria, a thick peptidoglycan layer crossed by teichoic and lipoteichoic acids. But they do not cross the more complex outer membrane of Gram-negative bacteria with the same ease.[18]

In vitro MIC and minimal bactericidal concentrations (MBC) determined by means of a standard twofold dilution method are reported in Table 3. In this study, both Gram-positive cocci and Gram-negative bacteria showed poor susceptibilities to the tested

trifluoromethyl-1*H*-pyrazol-1-(thio)carboxamides (**2**, **3**) and trifluoromethyl-1*H*-pyrazol-1-yl-thiazoles (**4**, **5**) when compared to standard chloramphenicol. The results showed that the unbranched C6-C13 alkyl chain in trifluoromethyl-1*H*-pyrazol-1-(thio)carboxamides (**2**, **3**) and trifluoromethyl-1*H*-pyrazol-1-yl-thiazoles (**4**, **5**) was not very relevant to antimicrobial activity. The investigation of the antibacterial screening data revealed that the tested compounds exhibit poor to moderate bacterial inhibitions. Compounds **4a**-**4g** showed good inhibition against *S. aureus* with an MIC 150 μg mL^{-1}; however, they were not efficient against *E. coli*. The 1*H*-pyrazoles (**2**, **3**), and pyrazolyl-thiazole (**5**) showed poor inhibition against tested bacteria. The newly prepared compounds (**2**-**5**) were screened for their antifungal activity against *C. albicans* and *C. krusei*; the inhibition zone tests had some relevance for both, however, when the minimal fungicidal concentration (MFC) for the *C. albicans* was determined, the values obtained for the series (**2**-**5**) show very low activity in relation to the standard ketoconazole.

Conclusions

In conclusion, we have successfully developed an easy practical way to approach to the synthesis of new 3-alkyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-(thio)carboxamides (**2**, **3**) and 2-(3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)-4-phenylthiazoles (**4**, **5**) derivatives. [CCC + NN] cyclocondensation of 1,1,1-trifluoro-4-methoxy-3-alken-2-ones with semicarbazide and thiosemicarbazide was regioselective, leading to 3-alkyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-(thio)carboxamides. It was then possible to modulate the reaction temperature to obtain the 1*H*-pyrazole cycle as 4,5-dihydro-1*H*-pyrazole or aromatic 1*H*-pyrazole during [SCN + CC] cyclocondensations. All products were obtained in good to excellent yields, 65-88%, the newly synthesized compounds (**2**-**5**) displayed some antibacterial and antifungal activities, demonstrating some selectivity for Gram-positive bacteria.

Experimental

The starting 1,1,1-trifluoro-4-methoxy-3-alken-2-ones (**3**) were synthesized by the acylation of the respective fatty alkyl methyl ketones with trifluoroacetic anhydride in pyridine and dichloromethane or chloroform, which resulted in good yields of 90%. ^{1}H, ^{13}C, ^{19}F NMR spectra were collected at 300 K using a Bruker 5 mm dual-probe on a Bruker DPX 400 spectrometer (^{1}H at 400.13 MHz, ^{19}F at 376.4 MHz, ^{13}C at 100.62 MHz). Chemical shifts (δ) for ^{1}H are given in parts *per* million (ppm) from tetramethylsilane (TMS), for ^{19}F (δ) Cl_3CF was the standard, setting the signal at 0.0 ppm, and coupling constants (*J*) are given in Hz. Melting points were determined using open capillaries on an Electrothermal Mel-Temp 3.0 apparatus and are uncorrected. Electrospray ionization (ESI) high-resolution mass spectra (HRMS) were determined using an Agilent 6460 Triple Quadrupole connected to a 1200 series LC and equipped with a solvent degasser, binary pump, column oven, auto-sampler, and an ESI source. The Agilent QQQ 6460 tandem mass spectrometer was operated in the positive jet stream ESI mode. Nitrogen was used as a nebulizer, turbo (heater) gas, curtain gas, and collision-activated dissociation gas. The capillary voltage was set to +3500 V, and the nozzle voltage was set to +500 V. The ion source gas temperature was 300 °C with a flow rate of 5 L min^{-1}. The jet stream sheath gas temperature was 250 °C with a flow rate of 11 L min^{-1}. All samples were infused into the ESI source at a flow rate of 5 μL min^{-1}. Data was acquired in positive MS total ion scan mode (mass scan range *m/z* 50-650) and in positive MS/MS product ion scan mode. The mass spectra recorded were evaluated using the Qualitative Analysis software from Agilent Technologies.

The antimicrobial susceptibility assay was performed using the disc diffusion method described by Bauer *et al.*[19] in Mueller-Hinton agar. The suspensions containing different strains were seeded on a plate containing Mueller-Hinton agar. 10 μL of the compounds at a concentration of 100 mg mL^{-1} was added to the discs which in turn were deposited on a plate. The plate, with the discs, was incubated for 24 h at 37 °C, then the inhibition zones were measured with a calliper ruler. For the determination of the MIC and MBC values of the substances against the test microorganisms, the broth dilution method was used according to the Clinical Laboratory Standards Institute (CLSI).[20] Stock solutions of the substances were prepared in 20% dimethyl sulfoxide (DMSO) and a twofold dilution series from 50.0-0.39 mg mL^{-1} was prepared using sterile distilled water. 100 μL from each of the dilutions was added to 96-well microtiter plates. Bacterial and yeast cultures were standardized to 10^8 colony forming unit (CFU) mL^{-1} using a 0.5 McFarland standard solution. The fungal spore suspensions were prepared using sterile 0.1% Tween 80 and adjusted to about 10^6 spores mL^{-1}. 100 μL of each microorganism suspension was then added into the wells and incubated at 37 °C for 24 h in aerobic conditions. The culture medium alone and medium containing bacteria without testing compounds were considered as controls. Triplicate wells were applied for each concentration of the individual test

material. Chloramphenicol and ketoconazole were used as standard antibacterial and antifungal drugs, respectively. MIC and MBC/MFC values were detected using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromine] method.[21] A concentration of 5 mg mL^{-1} MTT was prepared in phosphate-buffered saline (PBS pH 7.2) of Mueller-Hinton broth. It was used in suspensions containing *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. After dilution, 10 μL of the bacterial suspension was added in all wells; the plate was then incubated for 24 h at 37 °C. After incubation, 100 μL of 2,3,5-triphenyltetrazolium chloride was used to indicate microbial growth.

General procedure for the synthesis of 3-alkyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamides (**2a**-**2g**)

A solution of the semicarbazide hydrochloride (1.2 mmol), pyridine (1.2 mmol), and a 1,1,1-trifluoro-4-methoxyalk-3-en-2-one (1.2 mmol) in EtOH/H_2O (3:1, 8 mL) was kept under stirring at 79 °C until the starting semicarbazide was fully consumed, monitored using TLC, for 16 h. The mixture solvent was evaporated under reduced pressure. The crude product (**2a**-**2g**) was solubilized in chloroform (10 mL), washed with water (2 × 10 mL), and dried over $MgSO_4$ to give the following:

3-Hexyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2a**)

It was obtained (66%) as a slightly yellow wax; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.33 (m, 6H, CH_2), 1.57 (m, 2H, CH_2), 2.32 (t, 2H, J_{HH} 7.8 Hz, CH_2), 3.08 (d, 1H, J_{HH} 18.9 Hz, H4), 3.23 (d, 1H, J_{HH} 18.9 Hz, H4), 5.78 (broad s, 2H, NH_2), 6.32 (broad s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.4, 25.9, 28.7, 29.7, 31.3 (–CH_2–), 46.1 (C4), 90.5 (C5, q, J_{CF} 37 Hz), 123.4 (CF_3, q, J_{CF} 287 Hz), 156.5 (C3), 156.7 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –81.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{11}H_{19}F_3N_3O_2$ $[M + H]^+$: 282.1429, found: 282.1436.

3-Heptyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2b**)

It was obtained (69%) as a yellowish solid, m.p. 56-57 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.33 (m, 8H, CH_2), 1.58 (m, 2H, CH_2), 2.33 (t, 2H, J_{HH} 7.7 Hz, CH_2), 3.09 (d, 1H, J_{HH} 18.9 Hz, H4), 3.23 (d, 1H, J_{HH} 18.9 Hz, H4), 5.67 (broad s, 2H, NH_2), 6.29 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.5, 26.0, 28.8, 29.0, 29.7, 31.5 (–CH_2–), 46.0 (C4), 90.6 (C5, q, J_{CF} 34 Hz), 123.3 (CF_3, q, J_{CF} 287 Hz), 156.5 (C3), 156.6 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –81.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{12}H_{21}F_3N_3O_2$ $[M + H]^+$: 296.1586, found: 296.1662.

5-Hydroxy-3-octyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2c**)

It was obtained (65%) as a yellowish wax; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.31 (m, 10H, CH_2), 1.58 (m, 2H, CH_2), 2.32 (t, 2H, J_{HH} 7.7 Hz, CH_2), 3.08 (d, 1H, J_{HH} 18.9 Hz, H4), 3.22 (d, 1H, J_{HH} 18.9 Hz, H4), 5.67 (broad s, 2H, NH_2), 6.35 (broad s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.8 (CH_3), 22.4, 25.9, 28.9, 29.0, 29.6, 31.6 (–CH_2–), 46.0 (C4), 90.5 (C5, q, J_{CF} 34 Hz), 123.3 (CF_3, q, J_{CF} 286 Hz), 156.5 (C3), 156.7 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –82.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{13}H_{23}F_3N_3O_2$ $[M + H]^+$: 310.1742, found: 310.1793.

5-Hydroxy-3-nonyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2d**)

It was obtained (68%) as a yellowish wax; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.27 (m, 12H, CH_2), 1.57 (m, 2H, CH_2), 2.33 (t, 2H, J_{HH} 7.8 Hz, CH_2), 3.08 (d, 1H, J_{HH} 18.7 Hz, H4), 3.24 (d, 1H, J_{HH} 18.9 Hz, H4), 5.66 (broad s, 2H, NH_2), 6.31 (broad s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 26.0, 29.0, 29.1, 29.3, 29.7, 31.8 (–CH_2–), 46.0 (C4), 90.5 (C5, q, J_{CF} 34 Hz), 123.2 (CF_3, q, J_{CF} 287 Hz), 156.6 (C3), 156.6 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –81.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{14}H_{25}F_3N_3O_2$ $[M + H]^+$: 324.1899, found: 324.1911.

5-Hydroxy-3-undecyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2e**)

It was obtained (72%) as a yellowish wax; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.89 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.26 (m, 16H, CH_2), 1.57 (m, 2H, CH_2), 2.32 (t, 2H, J_{HH} 7.8 Hz, CH_2), 3.08 (d, 1H, J_{HH} 18.7 Hz, H4), 3.22 (d, 1H, J_{HH} 18.9 Hz, H4), 5.93 (broad s, 2H, NH_2), 6.30 (broad s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 26.0, 29.0, 29.1, 29.3, 29.7, 31.8 (–CH_2–), 46.1 (C4), 90.5 (C5, q, J_{CF} 34 Hz), 123.3 (CF_3, q, J_{CF} 287 Hz), 156.6 (C3), 156.9 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –81.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{16}H_{29}F_3N_3O_2$ $[M + H]^+$: 352.2212, found: 352.2239.

5-Hydroxy-3-tridecyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2f**)

It was obtained (74%) as a yellowish solid, m.p. 68-70 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.88

(t, 3H, J_{HH} 7.0 Hz, CH_3), 1.31 (m, 20H, CH_2), 1.57 (m, 2H, CH_2), 2.32 (t, 2H, J_{HH} 7.7 Hz, CH_2), 3.08 (d, 1H, J_{HH} 18.9 Hz, H4), 3.22 (d, 1H, J_{HH} 18.9 Hz, H4), 5.75 (broad s, 2H, NH_2), 6.31 (broad s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 26.0, 29.0, 29.1, 29.3, 29.5, 29.6, 29.7, 31.8 (–CH_2–), 46.1 (C4), 90.5 (C5, q, J_{CF} 34 Hz), 123.3 (CF_3, q, J_{CF} 287 Hz), 156.5 (C3), 156.7 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –82.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{33}F_3N_3O_2$ $[M + H]^+$: 380.2525, found: 380.2548.

5-Hydroxy-3-phenethyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-carboxamide (**2g**)

It was obtained (60%) as a yellowish solid, m.p. 66-67 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 2.63 (m, 2H, CH_2), 2.89 (m, 2H, CH_2), 3.05 (d, 1H, J_{HH} 19 Hz, H4), 3.18 (d, 1H, J_{HH} 19 Hz, H4), 5.83 (broad s, 2H, NH_2), 6.35 (broad s, 1H, OH), 7.21 (m, 3H, Ph), 7.29 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 31.3 (–CH_2–), 32.4 (–CH_2–), 46.5 (C4), 90.5 (C5, q, J_{CF} 34 Hz), 123.2 (CF_3, q, J_{CF} 287 Hz),127.5, 128.1, 128.5, 139.9 (Ph), 156.6 (C3), 156.8 (CO); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –82.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{13}H_{15}F_3N_3O_2$ $[M + H]^+$: 302.1116, found: 302.1153.

General procedure for the synthesis of 3-alkyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamides (**3a-3g**)

A solution of the thiosemicarbazide (1.2 mmol) and a 1,1,1-trifluoro-4-methoxyalk-3-en-2-one (1 mmol) in EtOH (6 mL) was kept under stirring at 78 °C until the starting dielectrophile was fully consumed, monitored using TLC, for 16 h. After cooling the solution, the precipitated product (**3a**-**3g**) was filtered off, washed with cooled EtOH (2 × 10 mL), and dried in air to give the following:

3-Hexyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3a**)

It was obtained (70%) as a yellow solid, p.f. 60-62 ºC. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 6.8 Hz, CH_3), 1.33 (m, 6H, CH_2), 1.59 (m, 2H, CH_2), 2.37 (t, 2H, J_{HH} 7.5 Hz, CH_2), 3.24 (d, 1H, J_{HH} 18.9 Hz, H4), 3.34 (d, 1H, J_{HH} 18.9 Hz, H4), 6.42 (broad s, 1H, NH_2), 7.14 (broad s, 1H, NH_2), 7.96 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.2, 25.7, 28.6, 29.7, 31.2 (–CH_2–), 46.6 (C4), 92.4 (C5, q, J_{CF} 34 Hz), 123.2 (CF_3, q, J_{CF} 290 Hz), 158.0 (C3), 177.1 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{11}H_{19}F_3N_3OS$ $[M + H]^+$: 298.1201, found: 298.1287.

3-Heptyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3b**)

It was obtained (69%) as a yellowish solid, m.p. 56-57 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.32 (m, 8H, CH_2), 1.59 (m, 2H, CH_2), 2.37 (t, 2H, J_{HH} 7.6 Hz), 3.24 (d, 1H, J_{HH} 18.9 Hz, H4), 3.34 (d, 1H, J_{HH} 18.9 Hz, H4), 6.31 (broad s, 1H, NH_2), 7.13 (broad s, 1H, NH_2), 7.95 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.4, 25.8, 28.7, 28.9, 29.8, 31.5 (–CH_2–), 46.6 (C4), 92.4 (C5, q, J_{CF} 34 Hz), 123.4 (CF_3, q, J_{CF} 290 Hz), 158.9 (C3), 177.2 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{12}H_{21}F_3N_3OS$ $[M + H]^+$: 312.1357, found: 312.1395.

5-Hydroxy-3-octyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3c**)

It was obtained (73%) as a yellowish wax; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.31 (m, 10H, CH_2), 1.58 (m, 2H, CH_2), 2.38 (t, 2H, J_{HH} 7.7 Hz, CH_2), 3.08 (d, 1H, J_{HH} 18.9 Hz), 3.22 (d, 1H, J_{HH} 18.9 Hz), 6.25 (broad s, 1H, NH_2), 7.25 (broad s, 1H, NH_2), 7.94 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.5, 25.8, 28.9, 29.0, 29.8, 31.6 (–CH_2–), 46.7 (C4), 92.5 (C5, q, J_{CF} 34 Hz), 123.4 (CF_3, q, J_{CF} 290 Hz), 158.9 (C3), 177.4 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –79.9; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{13}H_{23}F_3N_3OS$ $[M + H]^+$: 326.1514, found: 326.1591.

5-Hydroxy-3-nonyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3d**)

It was obtained (80%) as a yellowish solid, m.p. 59-60 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.1 Hz, CH_3), 1.31 (m, 12H, CH_2), 1.60 (m, 2H, CH_2), 2.38 (t, 2H, J_{HH} 7.8 Hz, CH_2), 3.24 (d, 1H, J_{HH} 18.9 Hz, H4), 3.35 (d, 1H, J_{HH} 18.9 Hz, H4), 6.23 (broad s, H, NH_2), 7.14 (broad s, H, NH_2), 7.97 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 26.0, 29.0, 29.1, 29.3, 29.7, 31.8 (–CH_2–), 46.6 (C4), 92.4 (C5, q, J_{CF} 34 Hz), 123.4 (CF_3, q, J_{CF} 287 Hz), 158.9 (C3), 177.6 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{14}H_{25}F_3N_3OS$ $[M + H]^+$: 340.1670, found: 340.1745.

5-Hydroxy-3-undecyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3e**)

It was obtained (77%) as a yellowish solid, m.p. 61-62 °C; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.31 (m, 16H, CH_2), 1.58 (m, 2H, CH_2), 2.36 (t, 2H, J_{HH} 7.7 Hz, CH_2), 3.23 (d, 1H, J_{HH} 18.7 Hz, H4), 3.33 (d, 1H, J_{HH} 18.9 Hz, H4), 6.27 (broad s, 1H,

NH_2), 7.11 (broad s, 1H, NH_2), 7.96 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 25.8, 28.9, 29.1, 29.2, 29.3, 29.4, 29.5, 29.8, 31.8 (–CH_2–), 46.7 (C4), 92.4 (C5, q, J_{CF} 34 Hz), 123.4 (CF_3, q, J_{CF} 290 Hz), 158.9 (C3), 177.6 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{16}H_{29}F_3N_3OS$ $[M + H]^+$: 368.1983, found: 367.2037.

5-Hydroxy-3-tridecyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3f**)

It was obtained (82%) as a yellowish oil; ^1H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.31 (m, 20H, CH_2), 1.58 (m, 2H, CH_2), 2.36 (t, 2H, J_{HH} 7.6 Hz, CH_2), 3.23 (d, 1H, J_{HH} 19.0 Hz, H4), 3.33 (d, 1H, J_{HH} 18.9 Hz, H4), 6.32 (broad s, 1H, NH_2), 7.14 (broad s, 1H, NH_2), 7.99 (s, 1H, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 25.8, 28.9, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.8, 31.8 (–CH_2–), 46.7 (C4), 92.3 (C5, q, J_{CF} 34 Hz), 123.3 (CF_3, q, J_{CF} 290 Hz), 158.9 (C3), 177.2 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.0; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{18}H_{33}F_3N_3OS$ $[M + H]^+$: 396.2296, found: 396.2376.

5-Hydroxy-3-phenethyl-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3g**)

It was obtained (65%) as a yellowish wax; ^1H NMR (400.1 MHz, $CDCl_3$) δ 2.73 (m, 2H, CH_2), 2.95 (m, 2H, CH_2), 3.23 (d, 1H, J_{HH} 19 Hz, H4), 3.32 (d, 1H, J_{HH} 19 Hz, H4), 6.44 (broad s, 2H, NH_2), 6.35 (broad s, 1H, OH), 7.25 (m, 3H, Ph), 7.36 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 31.3 (–CH_2–), 32.4 (–CH_2–), 46.9 (C4), 90.5 (C5, q, J_{CF} 34 Hz), 123.2 (CF_3, q, J_{CF} 287 Hz), 124.6, 128.1, 128.6, 139.8 (Ph), 158.2 (C3), 177.1 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.2; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{13}H_{15}F_3N_3OS$ $[M + H]^+$: 318.0888, found: 318.0951.

General procedure for the synthesis of 2-(3-alkyl-5-hydroxy-5-trifluoromethyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles (**4a**-**4g**)

A solution of a 3-alkyl-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide (**3a**-**3g**) (1.0 mmol) and 2-bromoacetophenone (1 mmol, 0.2 g) in EtOH (6 mL) was kept under stirring at 30 °C until the starting 2-bromoacetophenone was fully consumed, monitored using TLC, 8 h for **3a**-**3e**, **3g** and 24 h for **3f**. Then EtOH was evaporated under reduced pressure, and the crude product (**4a**-**4g**) was solubilized in chloroform (6 mL), washed with water (2 × 6 mL), and dried over $MgSO_4$ to give the following:

2-(5-Trifluoromethyl-3-hexyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**4a**)

It was obtained (80%) as a brown oil; ^1H NMR (400.1 MHz, $CDCl_3$) δ 0.89 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.33 (m, 6H, CH_2), 1.60 (m, 2H, CH_2), 2.38 (t, 2H, J_{HH} 7.8 Hz, CH_2), 3.17 (d, 1H, J_{HH} 18.8 Hz, H4), 3.36 (d, 1H, J_{HH} 18.8 Hz, H4), 6.91 (s, 1H, H5, thz), 7.28 (m, 1H, Ph), 7.37 (m, 2H, Ph), 7.75 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.4, 26.1, 28.7, 29.6, 31.3 (–CH_2–), 46.4 (C4), 92.2 (C5, q, J_{CF} 34 Hz), 104.6 (C5, thz), 123.4 (CF_3, q, J_{CF} 287 Hz), 124.8, 128.0, 128.6, 134.0 (Ph), 150.7 (C4, thz), 157.4 (C2, thz), 165.3 (C3), 177.1 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{23}F_3N_3OS$ $[M + H]^+$: 398.1514, found: 398.1591.

2-(5-Trifluoromethyl-3-heptyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**4b**)

It was obtained (82%) as a yellowish oil; ^1H NMR (400.1 MHz, $CDCl_3$) δ 0.89 (t, 3H, J_{HH} 7.1 Hz, CH_3), 1.32 (m, 8H, CH_2), 1.59 (m, 2H, CH_2), 2.38 (t, 2H, J_{HH} 7.8 Hz), 3.16 (d, 1H, J_{HH} 18.8 Hz, H4), 3.35 (d, 1H, J_{HH} 18.8 Hz, H4), 6.91 (s, 1H, H5, thz), 7.28 (m, 1H, Ph), 7.36 (m, 2H, Ph), 7.75 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.5, 26.2, 28.8, 29.0, 29.6, 31.6 (–CH_2–), 46.4 (C4), 92.0 (C5, q, J_{CF} 34 Hz), 104.6 (C5, thz), 123.0 (CF_3, q, J_{CF} 286 Hz), 125.8, 127.9, 128.6, 134.0 (Ph), 150.9 (C4, thz), 157.4 (C2, thz), 165.3 (C3); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.4; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{20}H_{25}F_3N_3OS$ $[M + H]^+$: 412.1670, found: 412.1723.

2-(5-Trifluoromethyl-5-hydroxy-3-octyl-4,5-dihydro-1*H*-pyrazol-1-yl)4-phenylthiazole (**4c**)

It was obtained (84%) as a yellowish oil; ^1H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.1 Hz, CH_3), 1.34 (m, 10H, CH_2), 1.60 (m, 2H, CH_2), 2.39 (t, 2H, J_{HH} 7.8 Hz, CH_2), 3.16 (d, 1H, J_{HH} 18.8 Hz, H4), 3.35 (d, 1H, J_{HH} 18.8 Hz, H4), 6.90 (s, 1H, C5, thz), 7.28 (m, 1H, Ph), 7.37 (m, 2H, Ph), 7.75 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.5, 26.2, 29.0, 29.1, 29.6, 31.7 (–CH_2–), 46.5 (C4), 92.0 (C5, q, J_{CF} 34 Hz), 104.7 (C5, thz), 123.4 (CF_3, q, J_{CF} 287 Hz), 125.9, 127.8, 128.6, 134.1 (Ph), 150.8 (C4, thz), 157.4 (C2, thz), 165.4 (C3), 177.4 (CS); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –79.8; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{21}H_{27}F_3N_3OS$ $[M + H]^+$: 426.1827, found: 426.1920.

2-(5-Trifluoromethyl-5-hydroxy-3-nonyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**4d**)

It was obtained (78%) as a yellowish oil; ^1H NMR (400.1 MHz, $CDCl_3$) δ 0.86 (t, 3H, J_{HH} 7.0 Hz, CH_3),

1.30 (m, 12H, CH_2), 1.59 (m, 2H, CH_2), 2.36 (t, 2H, J_{HH} 7.6 Hz, CH_2), 3.16 (d, 1H, J_{HH} 18.7 Hz, H4), 3.34 (d, 1H, J_{HH} 18.7 Hz, H4), 6.90 (s, 1H, H5, thz), 7.27 (m, 1H, Ph), 7.35 (m, 2H, Ph), 7.74 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6, 26.1, 29.0, 29.1, 29.3, 29.5, 32.1 (–CH_2–), 46.5 (C4), 92.1 (C5, q, J_{CF} 34 Hz), 104.6 (C5, thz), 123.4 (CF_3, q, J_{CF} 287 Hz), 125.7, 127.9, 128.8, 134.0 (Ph), 150.7 (C4, thz), 157.4 (C2, thz), 165.6 (C3); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –79.8; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{22}H_{29}F_3N_3OS$ $[M + H]^+$: 440.1983, found: 440.2053.

2-(5-Trifluoromethyl-5-hydroxy-3-undecyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**4e**)

It was obtained (78%) as a yellowish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.89 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.32 (m, 16H, CH_2), 1.60 (m, 2H, CH_2), 2.38 (t, 2H, J_{HH} 7.7 Hz, CH_2), 3.17 (d, 1H, J_{HH} 18.8 Hz, H4), 3.36 (d, 1H, J_{HH} 18.8 Hz, H4), 6.91 (s, 1H, H5, thz), 7.29 (m, 1H, Ph), 7.37 (m, 2H, Ph), 7.75 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.7, 26.3, 29.0, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9 (–CH_2–), 46.4 (C4), 92.0 (C5, q, J_{CF} 34 Hz), 104.7 (C5, thz), 123.4 (CF_3, q, J_{CF} 287 Hz), 125.9, 127.7, 128.7, 134.2 (Ph), 151.0 (C4, thz), 157.7 (C2, thz), 158.9 (C3), 165.5 (C3); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –79.8; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{24}H_{33}F_3N_3OS$ $[M + H]^+$: 468.2296, found: 468.2337.

2-(5-Trifluoromethyl-5-hydroxy-3-tridecyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**4f**)

It was obtained (87%) as a yellowish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.85 (t, 3H, J_{HH} 6.9 Hz, CH_3), 1.31 (m, 20H, CH_2), 1.60 (m, 2H, CH_2), 2.39 (t, 2H, J_{HH} 7.6 Hz, CH_2), 3.17 (d, 1H, J_{HH} 18.7 Hz, H4), 3.36 (d, 1H, J_{HH} 18.7 Hz, H4), 6.92 (s, 1H, H5, thz), 7.29 (m, 1H, Ph), 7.37 (m, 2H, Ph), 7.75 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.5 (CH_3), 23.0, 26.3, 29.1, 29.2, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9 (–CH_2–), 46.5 (C4), 92.1 (C5, q, J_{CF} 34 Hz), 104.7 (C5, thz), 123.4 (CF_3, q, J_{CF} 286 Hz), 125.9, 128.0, 128.7, 134.2 (Ph), 150.9 (C4, thz), 157.8 (C2, thz), 165.8 (C3); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –80.2; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{26}H_{37}F_3N_3OS$ $[M + H]^+$: 496.2609, found: 496.2661.

2-(5-Trifluoromethyl-5-hydroxy-3-(2-phenylethyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**4g**)

It was obtained (71%) as a yellowish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 2.67 (m, 2H, CH_2), 2.93 (m, 2H, CH_2), 3.10 (d, 1H, J_{HH} 18.8 Hz, H4), 3.27 (d, 1H, J_{HH} 18.8 Hz, H4), 6.89 (s, 1H, H5, thz), 7.20 (m, 4H, Ph), 7.29 (m, 4H, Ph), 7.36 (m, 2H, Ph), 7.74 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 31.3 (–CH_2–), 32.3 (–CH_2–), 46.8 (C4), 92.1 (C5, q, J_{CF} 34 Hz), 104.8 (C5, thz), 123.3 (CF_3, q, J_{CF} 287 Hz), 125.8, 127.6, 128.1, 128.2, 128.5, 128.6, 133.9, 140.1 (Ph), 150.7 (C4, thz), 156.5 (C2, thz), 165.3 (C3); ^{19}F NMR ($CDCl_3$) δ –80.4; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{21}H_{19}F_3N_3OS$ $[M + H]^+$: 418.1201, found: 418.1264.

General procedure for the synthesis of 2-(3-alkyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)-4-phenylthiazoles (**5a-5g**)

A solution of a 3-alkyl-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-thiocarboxamide **3a**-**3g** (1.0 mmol) and 2-bromoacetophenone (1 mmol, 0.2 g) in EtOH (6 mL) was kept under stirring at 80 °C until the starting 2-bromoacetophenone was fully consumed, monitored using TLC, 8 h for **3a**-**3e**, **3g** and 24 h for **3f**. Then EtOH was evaporated under reduced pressure, and the crude product (**4a**-**4g**) was solubilized in chloroform (6 mL), washed with water (2 × 6 mL), and dried over $MgSO_4$ to give the following:

2-(5-Trifluoromethyl-3-hexyl-1*H*-pyrazol-1-yl)-4-phenylthiazole (**5a**)

It was obtained (82%) as a brown oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.89 (t, 3H, J_{HH} 7.1 Hz, CH_3), 1.34 (m, 16H, CH_2), 1.68 (m, 2H, CH_2), 2.66 (t, 2H, J_{HH} 7.9 Hz, CH_2), 6.67 (s, 1H, H4), 7.25 (s, 1H, H5, thz), 7.32 (m, 1H, Ph), 7.39 (m, 2H, Ph), 7.90 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 13.9 (CH_3), 22.5, 27.9, 28.8, 29.0, 29.1, 31.9 (–CH_2–), 109.9 (C5, thz), 110.9 (C5, q, J_{CF} 3.0 Hz), 119.4 (CF_3, q, J_{CF} 270 Hz), 126.0, 128.3, 128.7 (Ph), 132.3 (C5, q, J_{CF} 42 Hz), 133.8 (Ph), 152.6 (C3), 155.4 (C4, thz), 158.9 (C2, thz); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –59.1; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{19}H_{21}F_3N_3S$ $[M + H]^+$: 380.1408, found: 380.1484.

2-(5-Trifluoromethyl-3-heptyl-1*H*-pyrazol-1-yl)-4-phenylthiazole (**5b**)

It was obtained (78%) as a yellow brownish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.89 (t, 3H, J_{HH} 7.1 Hz, CH_3), 1.34 (m, 16H, CH_2), 1.68 (m, 2H, CH_2), 2.66 (t, 2H, J_{HH} 7.9 Hz, CH_2), 6.67 (s, 1H, H4), 7.25 (s, 1H, H5, thz), 7.32 (m, 1H, Ph), 7.39 (m, 2H, Ph), 7.90 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.6, 27.8, 28.8, 29.0, 31.5 (–CH_2–), 109.9 (C5, thz), 110.9 (C5, q, J_{CF} 3.0 Hz), 119.4 (CF_3, q, J_{CF} 270 Hz), 126.0, 128.3, 128.7 (Ph), 132.3 (C5, q, J_{CF} 42 Hz), 133.8 (Ph), 152.6 (C3), 155.4 (C4, thz), 158.9 (C2, thz); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ –59.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{20}H_{23}F_3N_3S$ $[M + H]^+$: 394.1565, found: 394.1618.

2-(5-Trifluoromethyl-3-octyl-1*H*-pyrazol-1-yl)4-phenyl-thiazole (**5c**)

It was obtained (81%) as a yellow brownish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.90 (t, 3H, J_{HH} 7.1 Hz, CH_3), 1.34 (m, 16H, CH_2), 1.72 (m, 2H, CH_2), 2.71 (t, 2H, J_{HH} 7.9 Hz, CH_2), 6.71 (s, 1H, H4), 7.32 (s, 1H, H5, thz), 7.38 (m, 1H, Ph), 7.47 (m, 2H, Ph), 7.93 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.7, 27.9, 28.8, 29.1, 29.2, 29.3, 31.8 (–CH_2–), 109.9 (C5, thz), 110.9 (C5, q, J_{CF} 3.0 Hz), 119.4 (CF_3, q, J_{CF} 270 Hz), 126.0, 128.3, 128.7 (Ph), 132.4 (C5, q, J_{CF} 42 Hz), 133.8 (Ph), 152.6 (C3), 155.4 (C4, thz), 158.9 (C2, thz); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ −59.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{21}H_{25}F_3N_3S$ [M + H]$^+$: 408.1721, found: 408.1804.

2-(5-Trifluoromethyl-3-nonyl-1*H*-pyrazol-1-yl)-4-phenyl-thiazole (**5d**)

It was obtained (79%) as a yellow brownish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.34 (m, 16H, CH_2), 1.68 (m, 2H, CH_2), 2.66 (t, 2H, J_{HH} 7.9 Hz, CH_2), 6.67 (s, 1H, H4), 7.25 (s, 1H, H5, thz), 7.32 (m, 1H, Ph), 7.39 (m, 2H, Ph), 7.89 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.7, 27.8, 28.8, 29.2, 29.3, 29.4, 31.9 (–CH_2–), 109.9 (C5, thz), 110.9 (C5, q, J_{CF} 3.0 Hz), 119.6 (CF_3, q, J_{CF} 270 Hz), 126.0, 128.3, 128.7 (Ph), 132.3 (C5, q, J_{CF} 42 Hz), 133.8 (Ph), 152.7 (C3), 155.4 (C4, thz), 158.9 (C2, thz); ^{19}F NMR ($CDCl_3$) δ −59.7; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{22}H_{27}F_3N_3S$ [M + H]$^+$: 422.1878, found: 422.1962.

2-(5-Trifluoromethyl-3-undecyl-1*H*-pyrazol-1-yl)-4-phenyl-thiazole (**5e**)

It was obtained (75%) as a yellowish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.32 (m, 16H, CH_2), 1.67 (m, 2H, CH_2), 2.66 (t, 2H, J_{HH} 7.9 Hz, CH_2), 6.67 (s, 1H, H4), 7.24 (s, 1H, H5, thz), 7.30 (m, 1H, Ph), 7.39 (m, 2H, Ph), 7.90 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.6, 27.8, 28.8, 29.2, 29.3, 29.5, 29.6, 31.9 (–CH_2–), 109.8 (C5, thz), 110.9 (C5, q, J_{CF} 3.0 Hz), 119.4 (CF_3, q, J_{CF} 270 Hz), 126.0, 128.3, 128.7 (Ph), 132.3 (C5, q, J_{CF} 42 Hz), 133.8 (Ph), 152.8 (C3), 155.4 (C4, thz), 159.1 (C2, thz); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ −59.3; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{24}H_{31}F_3N_3S$ [M + H]$^+$: 450.2191, found: 450.2289.

2-(5-Trifluoromethyl-3-tridecyl-1*H*-pyrazol-1-yl)-4-phenyl-thiazole (**5f**)

It was obtained (89%) as a yellow oil. ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 0.88 (t, 3H, J_{HH} 7.0 Hz, CH_3), 1.32 (m, 20H, CH_2), 1.68 (m, 2H, CH_2), 2.67 (t, 2H, J_{HH} 7.9 Hz, CH_2), 6.67 (s, 1H, H4), 7.26 (s, 1H, H5, thz), 7.30 (m, 1H, Ph), 7.39 (m, 2H, Ph), 7.90 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.0 (CH_3), 22.7, 27.9, 28.8, 29.2, 29.3, 29.4, 29.5, 29.6, 31.9 (–CH_2–), 109.9 (C5, thz), 110.9 (C4, q, J_{CF} 3.0 Hz), 119.6 (CF_3, q, J_{CF} 267 Hz), 126.0, 128.3, 128.7 (Ph), 132.4 (C5, q, J_{CF} 42 Hz), 133.8 (Ph), 152.6 (C3), 155.4 (C4, thz), 158.9 (C2, thz); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ −59.6; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{26}H_{35}F_3N_3S$ [M + H]$^+$: 478.6422, found: 478.6514.

2-(5-Trifluoromethyl-3-phenethyl-1*H*-pyrazol-1-yl)-4-phenyl-thiazole (**5g**)

It was obtained (70%) as a yellowish oil; ^{1}H NMR (400.1 MHz, $CDCl_3$) δ 2.67 (m, 2H, CH_2), 2.93 (m, 2H, CH_2), 6.89 (s, 1H, H5, thz), 7.20 (m, 4H, Ph), 7.29 (m, 4H, Ph), 7.35 (m, 2H, Ph), 7.36 (s, 1H, H4), 7.74 (m, 2H, Ph); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 29.7 (–CH_2–), 34.9 (–CH_2–), 110.1 (C5, thz), 111.2 (C4, q, J_{CF} 3.0 Hz), 119.5 (CF_3, q, J_{CF} 270 Hz), 126.1, 126.3, 128.4 (Ph), 132.4 (C5, q, J_{CF} 42 Hz), 133.9 (Ph), 152.8 (C3), 154.4 (C2, thz), 158.9 (C2, thz); ^{19}F NMR (376.4 MHz, $CDCl_3$) δ −60.2; HRMS (FTMS + pESI) *m/z*, calcd. for $C_{21}H_{17}F_3N_3S$ [M + H]$^+$: 400.1095, found: 400.1193.

Supplementary Information

Spectroscopic ^{1}H, ^{13}C, ^{19}F NMR and mass spectra of the title compounds are provided in the Supplementary Information, available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

The authors are grateful for the financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Universal grant 6577818477962764-01), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, PqG grant 1016236). Fellowships from CNPq (J. L. M., D. L. M.) and CAPES (D. C. F.) are also acknowledged.

Author Contributions

Debora L. de Mello was responsible for the data curation, methodology and writing original draft; Juliana L. Malavolta for the data curation, investigation, project administration, supervision, visualization and writing original draft; Roberto C. V. Santos for the formal analysis and supervision; Leonardo Q. Lopes for the investigation and methodology; Sidnei Moura for the data curation, formal analysis, funding acquisition, validation and visualization; Darlene C. Flores for the data curation,

formal analysis, investigation, methodology, supervision and writing original draft; Alex F. C. Flores for the conceptualization, funding acquisition, methodology, supervision, writing original draft, review and editing.

References

1. Chandler, C. I. R.; *Palgrave Commun.* **2019**, *5*, 53.
2. Rather, I. A.; Kim, B.-C.; Bajpai, V. K.; Park, Y.-H.; *Saudi J. Biol. Sci.* **2017**, *24*, 808.
3. Bondock, S.; Founda, A. M.; *Synth. Commun.* **2018**, *48*, 561.
4. Grybaitè, B.; Vaickelionierè, R.; Stasevych, M.; Komarovska-Porokhnyavets, O.; Kantminienè, K.; Novikov, V.; Mickevicius, V.; *ChemistrySelect* **2019**, *4*, 6965.
5. Kashyap, S. J.; Garg, V. K.; Sharma, P. K.; Kumar, N.; Dudhe, R.; Gupta, J. K.; *Med. Chem. Res.* **2012**, *21*, 2123.
6. Kryshchyshyn, A.; Kaminskyy, D.; Karpenko, O.; Gzella, A.; Grellier, P.; Lesyk, R.; *Eur. J. Med. Chem.* **2019**, *174*, 292.
7. O'shea, I. P.; Shahed, M.; Aguilera-Venegas, B.; Wilkinson, S. R.; *Antimicrob. Agents Chemother.* **2016**, *60*, 1137.
8. Qiao, J.; Zhao, C.; Liu, J.; Du, Y.; *Bioorg. Med. Chem. Lett.* **2018**, *28*, 2379.
9. Abdellatif, K. R. A.; Moawad, A.; Knaus, E. E.; *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5015.
10. Kaur, K.; Kumar, V.; Gupta, G. K.; *J. Fluorine Chem.* **2015**, *178*, 306.
11. Bareño, V. D. O.; Santos, D. S.; Frigo, L. M.; de Mello, D. L.; Malavolta, J. L.; Blanco, R. F.; Pizzuti, L.; Flores, D. C.; Flores, A. F. C.; *J. Braz. Chem. Soc.* **2020**, *31*, 244.
12. Nenajdenko, V. G.; Balenkova, E. S.; *Arkivoc* **2011**, 246.
13. Flores, A. F. C.; Piovesan, L. A.; Pizzuti, L.; Flores, D. C.; Malavolta, J. L.; Martins, M. A. P.; *J. Heterocycl. Chem.* **2014**, *51*, 733.
14. Flores, A. F. C.; Malavolta, J. L.; Frigo, L. M.; Doneda, M.; Flores, D. C.; *Synth. Commun.* **2015**, *45*, 1198.
15. Bonacorso, H. G.; Oliveira, M.; Wentz, A. P.; Wastowski, A. D.; Oliveira, A. B.; Hörner, M.; Zanatta, N.; Martins, M. A. P.; *Tetrahedron* **1999**, *55*, 345.
16. Bonacorso, H. G.; Wastowski, A. D.; Zanatta, N.; Martins, M. A. P.; Naue, J. A.; *J. Fluorine Chem.* **1998**, *92*, 23.
17. Knothe, G.; Kenar, J. A.; *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 88.
18. Jones, S.; *Nat. Biotechnol.* **2017**, *35*, 639.
19. Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turck, M.; *Am. J. Clin. Pathol.* **1966**, *45*, 493.
20. Clinical and Laboratory Standards Institute (CLSI); *M07-A9: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard*, 9th ed.; CLSI: Wayne, PA, USA, 2012.
21. Moellering Jr., R. C.; *Int. J. Antimicrob. Agents* **2011**, *37*, 2.

Submitted: October 8, 2020
Published online: December 8, 2020