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Article

Synthesis, Structure Elucidation, Antioxidant and Antimicrobial Activity of Novel 2-(5-Trifluoromethyl-1*H*-pyrazol-1-yl)-5-(5-trihalomethyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridines

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This paper describes an efficient approach for the synthesis of a novel series of sixteen 2-(5-trifluoromethyl-1*H*-pyrazol-1-yl)-5-(5-trihalomethyl-1*H*-pyrazol-1-yl-1-carbonyl) pyridines, for the first time with non-identical substituents in both pyrazole rings, through the cyclocondensation reaction of 4-methoxy-4-alkyl(aryl/heteroaryl-1,1,1-trihaloalk-3-en-2-ones [$CX_3C(O)CH=CR^1OCH_3$, in which $R^1 = CH_3$, C_6H_5, 4-$CH_3C_6H_4$, 4-$OCH_3C_6H_4$, 2-furyl and X = F, Cl] or acetylacetone with some 6-[3-alkyl(aryl)-5-trifluoromethyl-1*H*-pyrazol-1-yl]nicotinohydrazides. Optimized yields of 67-91% were obtained when the reactions were performed in ethanol (green solvent) at reflux for 16 h. Subsequent antioxidant and antimicrobial evaluation revealed promising 1,1-diphenyl-2-picrylhydrazyl (DPPH) inhibition percentage and exhibited fungiostatic and fungicidal activities against yeasts, dermatophytes and filamentous, especially for the pyridine systems, when the both pyrazole rings attached to a pyridine ring contain CX_3 groups (X = H, F, Cl) of different kinds. It is also observed the trichloromethyl substituted compounds presented higher antioxidant activity in comparison to their fluorinated analogous.

Keywords: pyrazole, pyridine, hydrazide, antimicrobial activity, DPPH

Introduction

Free radicals are related to a large number of diseases affecting humans; for example, cardiovascular problems, cancer, immune system decline, and brain dysfunctions like the neurodegenerative Alzheimer and Parkinson disease.[1-4] The balance between the formation and removal of different types of radicals in the body must be regulated so that there is physiological cell maintenance. For this reason, the use of antioxidant agents in medicine has been a great field to explore, and more than this, these agents have been shown to have many industrial uses; for example, as food preservatives, cosmetic products, and gasoline additives.[5]

Consequently, there is great interest in the discovery of new antioxidant agents for preventing the presumably harmful effect that free radicals have on the human body. Antioxidant agents are capable of stabilizing or deactivating free radicals before they attack the biological targets in the cell system.[6]

Methods for the analysis of antioxidant activity have become relevant, because they help in the search for bioactive substances. Due to various types of radicals and different sites of action, several methods (with their peculiarities) are available and have been used to assess: the antioxidant potential of extracts, isolated or synthesized substances, peroxyl capture methods, metal reduction ability, hydroxyl radical capture, organic radical capture, etc.[7-10] It is important to select and employ a stable and rapid method to assess antioxidant activity.

On the other hand, antimicrobial and antibiotic substances have undergone major advance in the last five decades, with unlimited progress in drug therapy. As the advent of antibiotics and chemotherapeutics allowed the control and cure of infectious diseases, there was a marked change in the natural history of these diseases. However, the indiscriminate use of antibiotics in the 1970s resulted in the acceleration of the emergence of resistant bacterial

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strains.[11,12] The search for new antimicrobial agents capable of inhibiting bacteria and fungi growth in a safer effective way that causes fewer side effects, has been the study object for many researchers. Infectious diseases caused by bacteria and fungi affect millions of people around the globe.[13] Although invasive fungal infections can affect healthy people, most of these diseases occur in the context of a compromised host.

Despite the latest technology for antifungal therapy, mortality rates for invasive infections with the three most common species of human fungal pathogens remain at: 20-40% for *Candida albicans*; 50-90% for *Aspergillus fumigatus*; and 20-70% for *Cryptococcus neoformans*. The variety of antifungal agents is limited, particularly when compared to the number of agents available for bacterial infections. In fact, it took around 30 years for the development of the newest class of antifungal drugs.[14]

The great interest in novel drugs for the treatment of infectious diseases is still a challenge, due to a combination of factors, such as emerging infectious diseases and the increasing number of multidrug-resistant pathogens.[15] Therefore, the pyrazole nucleus represents a very attractive scaffold for applications in the agrochemical and pharmaceutical industries, due to it being capable of exhibiting a wide range of bioactivities; for example, antimicrobial,[16] anticancer,[17] anticonvulsant,[18] antitubercular,[19] antipyretic[20] and selective enzyme inhibitory activities.[21]

Specifically, *bis*-pyrazoles have been reported to be effective components in capturing active oxygen and free radicals *in vivo*,[22,23] and other derivatives have been synthesized and used as promising agents with antifungal,[24] herbicidal,[25] and central nervous system activities.[26]

A brief review of the literature shows only a few reports on the synthesis of *bis*-pyrazole systems up until now. Soliman *et al.*[27] published the synthesis of *bis*-3,5-dimethylpyrazoles bridged by carbonyl groups with considerable antidiabetic activity, from the substitution reactions between ethyl chloroformate and 3,5-dimethylpyrazole. Two decades later, Hayter *et al.*[28] described the synthesis of *bis*-3-alkyl(aryl)-pyrazoles bridged by a benzene ring, from the cyclocondensation reaction of two equivalents of hydrazine and the appropriated *bis*-(β-diketones).

Kanagarajan *et al.*[29] reported improvements in the synthesis of carbocycle-bridged 5,5'-(1,4-phenylene)-*bis*-(3-aryl-1*H*-pyrazole) derivatives through the reaction of *bis*-chalcones and hydrazine, which was performed under ultrasonic irradiation. In the field of luminescent materials, Bao *et al.*[30] reported a novel ligand containing *bis*-pyrazolone pyridine and with this they synthesized complexes with Eu^{III} and Tb^{III}. However, these approaches only explore simple hydrazine derivatives, most of them with carbocyclic bridges and leading to a limited scope and identical substituents in both pyrazole rings.

Our research group reported a one-pot regioselective synthesis of a series of *bis*-(3-aryl-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl) methanones from the reactions of 4-alkoxy-4-aryl(heteroaryl)-1,1,1-trifluoroalk-3-en-2-ones with carbohydrazides, at yields of 73-89%.[31] In the same year, our research group started to explore the reactivity of 6-hydrazinonicotinic hydrazide in cyclocondensation reactions with trifluoromethyl vinyl ketones, at a 1:2 molar ratio, respectively. The first work resulted in the synthesis of a series of 2-(1*H*-pyrazol-1-yl)-5-(1*H*-pyrazol-1-yl-1-carbonyl)pyridines with yields of 62-97%.[32] It is also known that the 6-hydrazinonicotinic hydrazide has two distinct nucleophilic centers in its structure: a hydrazine and a hydrazide function, which would give this building block differentiated reactivity with electrophiles. However, in a recent study performed by us with 6-hydrazinonicotinic hydrazide, it was observed that no chemoselectivity is achieved when cyclocondensation reactions are promoted with the trifluoromethyl vinyl ketones at a 1:1 molar ratio, resulting in a mixture of products and partial recovery of the hydrazide.[33] Following our continuous studies of the reactivity of 6-hydrazinonicotinic hydrazide, in 2014, we reported a convenient access by a green procedure in ethanol as solvent to a series of (*E*)-6-[2-ferrocenylalkylidenehydrazino] nicotinic hydrazides from the quimioselective reactions of 6-hydrazinonicotinc hydrazide with some acylferrocenes. Subsequently, cyclocondensation reactions of ferrocenylalkylidene hydrazones with 4-alkoxy-1,1,1-trifluoroalk-3-en-2-ones to obtain (*E*)-pyrazolyl-pyridinohydrazones, were also reported.[34] In the same year, a synthetic route for the selective "deprotection" reaction (amide hydrolysis reaction) for 2-(1*H*-pyrazol-1-yl)-5-(1*H*-pyrazol-1-yl-1-carbonyl)pyridines, which furnished the corresponding methyl trifluoromethylpyrazolyl nicotinates, preserving only the pyrazole ring bonded to C-2 pyridine, and which originated from the hydrazine moiety, was reported by us.[35] In a subsequent reaction with hydrazine hydrate, the ester function at the C-5 pyridine was restored to the initial hydrazide moiety, which led to the possibility of new cyclocondensation reactions and the construction of various heterocycles from this new nucleophilic precursor.

In view of this consistent literature review, and based on the results of our previous work,[32-35] the aim of this work is to present the results of an efficient methodology in order to construct *bis*-trihalomethylated pyrazolyl-pyridine systems containing two non-identical substituted pyrazole rings, from the [3 + 2] cyclocondensation reactions

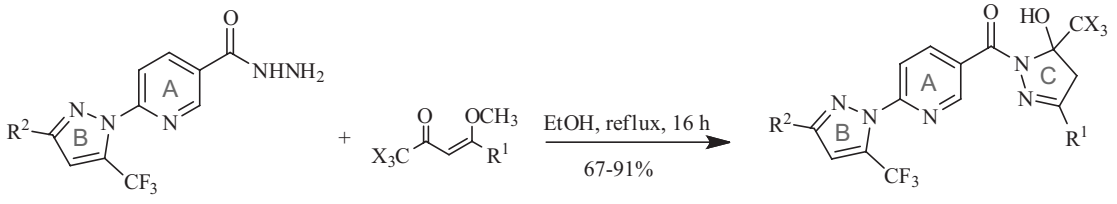
of pyrazol-1-yl-nicotinohydrazides with 4-alkoxy-4-alkyl(aryl/heteroaryl-1,1,1-trifluoro(chloro)alk-3-en-2-ones or acetylacetone, and then elucidating their chemical structures by nuclear magnetic resonance (NMR) and X-ray diffraction, as well as evaluating their antimicrobial and antioxidant activity *in vitro*.

Results and discussion

Synthesis and structure

Firstly, the 4-methoxy-4-(alkyl/aryl/heteroaryl)-1,1,1-trihaloalk-3-en-2-ones (**4a**, **5a**),[36] (**4b**, **5b, 5c**, **5d**),[37] and (**5e**)[38] were obtained from the acylation reaction of 2-methoxypropenyl ether or acetals derived from acetophenones or 2-acetylfuran with trifluoroacetic anhydride or trichloroacetyl chloride, in accordance with the methodology developed in our laboratory. Subsequently, we reacted ketones **4** and **5** with substituted pyrazolyl-nicotinohydrazides (**1**-**3**)[35] in ethanol (green solvent), obtaining novel *bis*-pyrazolyl-pyridines (**6**-**10**) in a one-step reaction (Scheme 1, Table 1), which were then dehydrated and furnished, for example, the corresponding *bis*-pyrazolyl-pyridine systems **11e** and **12c** (Scheme 2, Table 1). The synthesis of all compounds was monitored by thin-layer chromatography (TLC).

Taking into consideration the results of the previous reactions involving halogenated ketones **4** and **5**, we performed the synthesis of **13** with the hydrazide **2** and acetylacetone. The initial methodology tested employed ethanol as the solvent, at reflux temperature for 16 h. However, a mixture of pyrazoline-pyridine derivative **13** and the pyrazole-pyridine product **14** was obtained, being clearly observed by TLC and ^{1}H NMR analysis. Since it is well known that basic conditions favor the obtainment of pyrazolines, and that acidic catalysis can improve the formation of pyrazole products by dehydration reaction, we first tested a basic catalysis with triethylamine (Et_3N) and ethanol reflux for 16 h. After the reaction time, the formation of pyrazoline (cycle C), confirmed as a single product **13**, was characterized and proven by ^{1}H NMR spectra. In the following step, compound **13** was easily dehydrated with $SOCl_2$, leading to the corresponding pyridine **14** (Scheme 3) which contains all three aromatic rings (cycles A, B and C) and according to the procedure reported in the literature for similar compounds.[39] In previous works, researchers have demonstrated efficient and mild methodologies for intramolecular dehydration reaction for

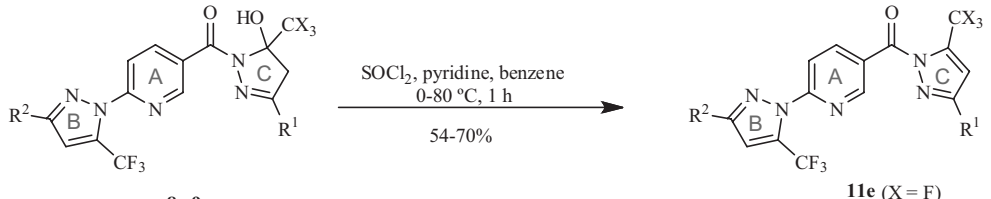


Compound	1	2	3
R^2	CH_3	C_6H_5	2-furyl

4-5	a	b	c	d	e
R^1	CH_3	C_6H_5	4-$CH_3C_6H_4$	4-$OCH_3C_6H_4$	2-furyl

6-10	a	b	c	d	e	f	g	h
R^1	CH_3	C_6H_5	CH_3	C_6H_5	4-$CH_3C_6H_4$	4-$OCH_3C_6H_4$	2-furyl	C_6H_5
R^2	CH_3	CH_3	C_6H_5	C_6H_5	C_6H_5	C_6H_5	C_6H_5	2-furyl

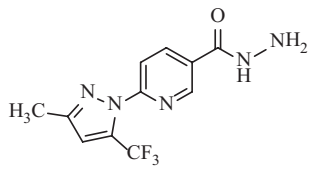
Scheme 1. Synthetic route for the synthesis of *bis*-pyrazolyl-pyridines **6**-**10**.



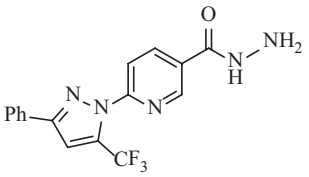
8-12	c	e
R^1	CH_3	4-$CH_3C_6H_4$
R^2	C_6H_5	C_6H_5

Scheme 2. Dehydration reactions for *bis*-pyrazolyl-pyridines **8c**, **9e**.

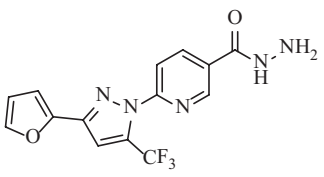
Table 1. Scope and yields for the isolated compounds **1**-**12**



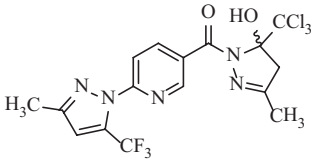
1, 73% (70%)[35]



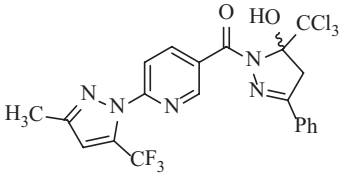
2, 62% (62%)[35]



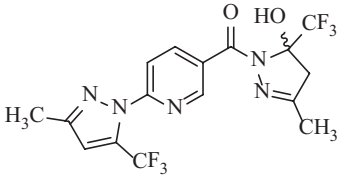
3, 57%



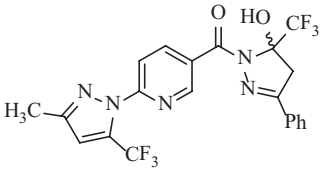
6a, 71%



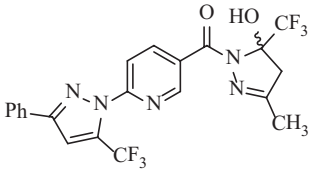
6b, 80%



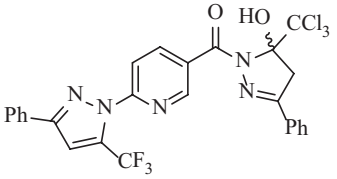
7a, 91%



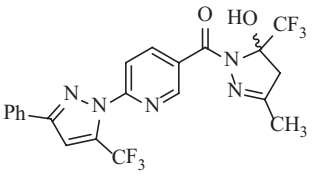
7b, 81%



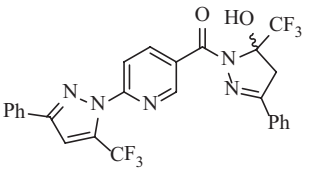
8c, 83%



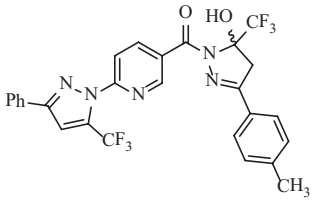
8d, 79%



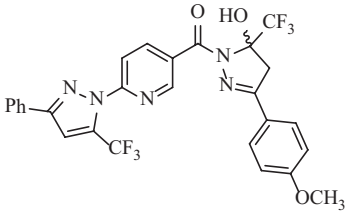
9c, 87%



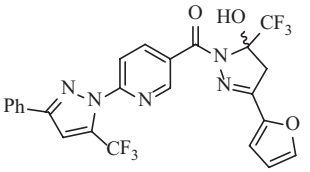
9d, 75%



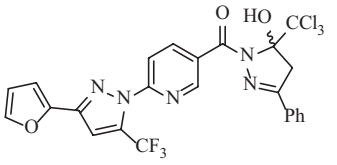
9e, 67%



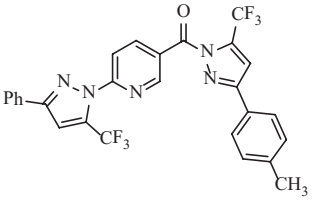
9f, 80%



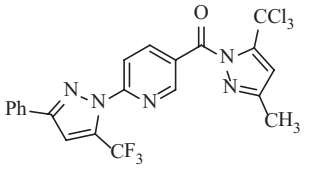
9g, 69%



10h, 88%



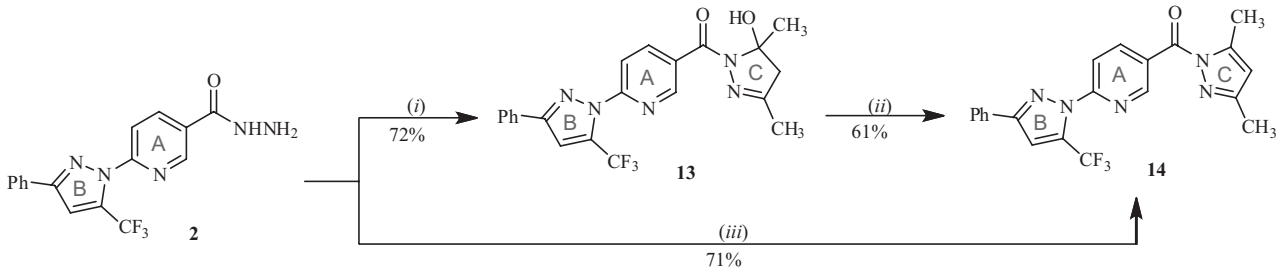
11e, 70%



12c, 54%

non-halogenated 5-hydroxy-4,5-dihydro-pyrazoles, under acidic conditions.[40,41] Thus, the cyclocondensation reaction was then performed by mild acidic conditions (ethanol, acetic acid mixture), under reflux for 16 h in ethanol. As a result, the pyridine **14** was isolated directly from **2**, in a one-step, one-pot reaction, as a single product-identified by ^{1}H NMR analysis-with a much more satisfactory yield of 71% (Scheme 3).

The structures of the new pyrazoline-pyridine derivatives **6**-**10** were deduced from ^{1}H, ^{13}C, and ^{19}F NMR spectra, and by comparison with the NMR data of other pyrazole derivatives previously synthesized in our laboratory.[32-35,41] The ^{1}H NMR analysis in dimethylsulfoxide, DMSO-d_6, revealed a chemical shift for the H-4 as a sharp singlet in the range of 7.04 ppm (R^2 = CH_3), 7.84 ppm (R^2 = Ph), and 7.59 ppm (R^2 = 2-furyl) at the pyrazole ring (cycle B). For



(*i*) $H_3CC(O)CH{=}C(OH)CH_3$, EtOH, Et_3N, reflux, 16 h
(*ii*) $SOCl_2$, pyridine, benzene, 0-80 °C, 1 h
(*iii*) $H_3CC(O)CH{=}C(OH)CH_3$, EtOH, AcOH, reflux, 16 h

Scheme 3. Synthesis of *bis*-pyrazolyl-pyridine **13** and its dehydration reaction to **14**.

the pyrazoline ring (cycle C), the methylene protons of H-4 showed a characteristic doublet on average at δ 3.83 ppm, and the other doublet at δ 3.53 ppm, respectively, with a germinal coupling constant on average at 19.0 Hz. The hydroxyl proton was shown at an average of δ 8.30 ppm. All compounds present the typical ^{13}C NMR chemical shifts of pyrazole rings (cycle B) at an average of 149.3 ppm (C-3), and the C-4 exhibit signals at 111.0 ppm with a characteristic quartet of ^3J 3 Hz. The C-5 exhibit signals at around 132.8 ppm with a characteristic quartet of ^2J 40 Hz, because they are attached to the CF_3 group. The CF_3 shows a typical quartet at an average of 120.5 ppm with ^1J 268 Hz. As for the pyrazoline rings (cycle C), values were at an average of 154.5 ppm (C-3) and 47.1 ppm (C-4). The compounds **7** and **9** (X = F) at C-5 exhibit signals around 92.0 ppm and a characteristic quartet of ^2J 34 Hz, because they are attached to the CF_3 group. The CF_3 shows a typical quartet at an average of 123.1 ppm, with ^1J 285 Hz. For compounds **6**, **8**, and **10**, in which X = Cl, the C-5 exhibit signals around 102.5 ppm. The carbonyl carbon displayed a signal in the range of 166.6 ppm. The ^{19}F NMR showed a typical singlet at an average of δ −56.6 ppm for the CF_3 pyrazole (cycle B) and δ −76.2 ppm for the CF_3 pyrazoline (cycle C). After dehydration, compound **11e** presented the ^{19}F singlet signal in δ −58.54 ppm of pyrazole (cycle C). The structures of **13**-**14**, for which X = H, were then deduced from NMR spectra (^1H, ^{13}C, and ^{19}F) and also by comparison with the NMR data of other formerly synthesized pyrazoles **6**-**10** (in which X = Cl, F). Compound **13** showed: the hydroxyl at δ 6.51 ppm; the doublet (H-4) with *J* 18.0 Hz at 3.02 and 2.91 ppm; and singlets each one of three hydrogen methyls (CH_3) at 1.98 and 1.92 ppm. Vinylic hydrogens from the pyrazoline ring (cycle C) of compound **14** were seen at δ 6.33 ppm as a singlet (H-4) and at 2.60 and 2.20 ppm for the two methyls. Additionally, to confirm the structures, we performed X-ray diffraction measurements for a monocrystal of structures **6a** (Figure 1), **7a** (Figure 2) and **14** (Figure 3).

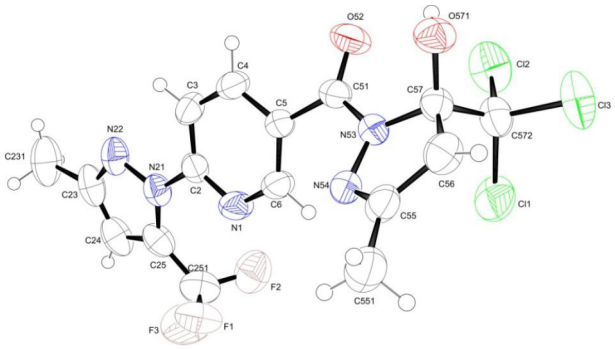


Figure 1. A perspective view of molecule 2-(3-methyl-5-trifluoromethyl-1*H*-pyrazol-1-yl)-5-(3-methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**6a**) with atoms labeled (CCDC 1040652). Displacement ellipsoids are drawn at the 50% probability level.

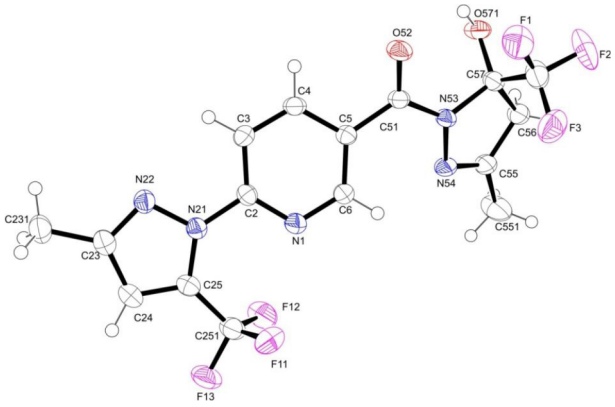


Figure 2. A perspective view of molecule 2-(5-trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-methyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**7a**) with atoms labeled (CCDC 1040653). Displacement ellipsoids are drawn at the 50% probability level.

Biological activity

In the present work we evaluate the *in vitro* antimicrobial activity of nine representative compounds (**6a**, **8d**, **9c**, **9d**, **9f**, **9g**, **10h**, **11e** and **14**) against a panel of microorganisms including bacteria and fungi (yeasts, filaments and dermatophytes). These selected compounds present structural scaffolds that allowed to verify the influence of the CX_3, R^1 and R^2 substituents on this

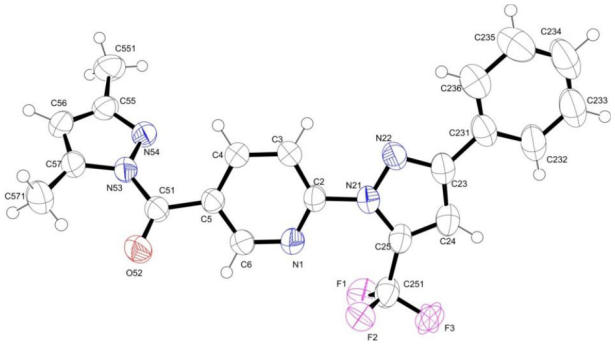


Figure 3. A perspective view of molecule 2-(5-trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**14**) with atoms labeled (CCDC 1040654). Displacement ellipsoids are drawn at the 50% probability level.

biological study. The assays were performed by broth microdilution techniques, in accordance with Clinical Laboratory Standard Institute (CLSI) guidelines, using Muller-Hinton broth (bacteria) and RPMI 1640 broth (fungi). The minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) were determined. Consequently, for these tests it was found that none of the tested compounds showed inhibition to Gram-positive and Gram-negative bacteria at concentrations ranging from 1000 to 1.95 μg mL^{-1}. As for the fungi, the concentrations used ranged from 500 to 0.98 μg mL^{-1}. We found that the fungiostatic and fungicidal activities depends not only of the CX_3 group but also of the R^1 and R^2 substituents. For example: a comparison between compounds **8d** ($CX_3 = CCl_3$) and **9d** ($CX_3 = CF_3$), both containing R^1 and R^2 = Ph as substituents, showed none activity. However, a comparison between compounds **8d** and **6a** ($CX_3 = CCl_3$, R^1 and R^2 = Me), showed activity only for **6a**. Moreover, we found none biological activity for compounds where $CX_3 = CF_3$ or CCl_3 and $R^1 \neq R^2$ (**9c**, **9f**, **9g**, **10h** and **11e**), but good results for CX_3 = Me (**14**). Thus, we found that compounds **6a** and **14** were active against the various fungi tested and their results are expressed in μM for a better comparison (Table 2). The best inhibition results were obtained with compound **14** (R^1 = Me, R^2 = Ph; CX_3 = Me) for the yeasts *C. dubliniensis*, *C. parapsilosis*, and *C. neoformans* in 303 μM; and for the dermatophytes *M. canis* in 151 μM, and *T. mentagrophytes* and *T. rubrum* in 76 μM (31.25 μg mL^{-1}), resulting in fungiostatic activity for **14**. The following concentrations were lethal: 607 μM for *C. parapsilosis* and *C. neoformans*; 303 μM for *C. dubliniensis* and *M. canis*; 151 μM for *T. rubrum*; and 76 μM for *T. mentagrophytes*, but they also granted fungicidal activity for compound **14**. However, compound **6a** (R^1 and $R^2 = CH_3$; $CX_3 = CCl_3$) resulted in fungicidal activity for four micro-organisms, for which compound **14** obtained no results. Compound **6a** inhibited the growth at a concentration of 265 μM for *C. krusei, C. glabrata*, and *S. schenckii*; and at a concentration of 531 μM for *R. oryzae*. It also presented fungicidal activity at a concentration of 531 μM for *C. krusei, C. glabrata*, and *S. schenckii*; and at 1062 μM for *R. oryzae* (Table 2). The other tested substances did not show antimicrobial activity for the micro-organisms and concentrations employed.

Antioxidant activity

In this work, two different chemical methods were used for the *in vitro* evaluation of antioxidant activity for compounds: the free radical capture method 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the method used for measuring the total antioxidant capacity based on the

Table 2. Antifungal activity of compounds **6a** and **14**

Characteristic	Microorganism[a]	Sample **6a**		Sample **14**		Standard[d]
		MIC[b] / μM	MLC[c] / μM	MIC[b] / μM	MLC[c] / μM	MIC[b] / μM
Yeast	*Candida krusei*	265	531	> 1215	–	52
	Candida glabrata	265	531	> 1215	–	26
	Candida dubliniensis	> 1062	–	303	303	0.81
	Candida parapsilosis	> 1062	–	303	607	6.5
	Cryptococcus neoformans	1062	1062	303	607	13
Dermatophyte	*Microsporum canis*	1062	1062	151	303	0.20
	Trichophyton mentagrophytes	265	265	76	76	0.013
	Trichophyton rubrum	265	265	76	151	0.10
Filamentous	*Sporothrix schenckii*	265	531	> 1215	–	0.54
	Rhizopus oryzae	531	1062	–	–	1.08

[a]ATCC (American Type Culture Collection); [b]minimal inhibitory concentration (MIC); [c]minimal lethal concentration (MLC); [d]Fluconazole for yeasts and Terbinafine for dermatophytes (*M. canis, T. mentagrophytes, T. rubrum*) and Amphotericin B for filamentous fungi (*S. schenckii* and *R. oryzae*).

reduction of Mo^{VI} to Mo^{V} by antioxidants, and subsequent formation of phosphate/Mo^{V} complex. Both methods are photometric, using wavelengths of 518 nm (DPPH) and 695 nm (phosphomolybdenum).

Free radical scavenging activity by DPPH

One of the most widely employed methods is DPPH because it is simple, efficient and inexpensive. It consists of assessing the performance of the compound as a radical (oxidant) or as a hydrogen donor (antioxidant). As the method works equally well with methanol or ethanol, the latter one was chosen because of its lower toxicity. The pyrazole pyridine targets **1**, **2**, **6b**, **8c**, **8d**, **9c**, **10h**, **11e**, **12c**, **13** and **14** were compared with ascorbic acid as the reference compound (Table 3). These selected compounds allowed to verify the influence of the CX_3, R^1 and R^2 substituents on the antioxidant property. The DPPH antioxidant assay measures the hydrogen-donating capacity of the molecules in the sample. When the stable free-radical DPPH is reduced by the sample, its color changes from violet/purple to pale yellow. This absorbance decline is measured and the scavenging or inhibitory capacity can be determined.

The results indicate the potential of compounds as antioxidants or pro-oxidants. In the calibration curve (ascorbic acid) the inhibition values obtained were: 10.60, 19.40, 51.20 and 93.50% for concentrations of 1, 5, 10 and 100 μL mL^{-1}, respectively, and with a value of IC_{50} = 11.88 ± 0.82 μg mL^{-1}. Compounds **1**, **2**, **8c**, **9c**, **10h**, and **14** were active as DPPH free radicals, trapping at concentrations > 100 μg mL^{-1}; however, when compared to ascorbic acid, compound **8c** presented relevant data at various concentrations. Compounds **8d**, **11e** and **12c** were not able to inhibit DPPH at the concentrations tested, but were shown to be molecules with pro-oxidant activity.

Compounds **6b** and **13** can act either as pro-oxidants or as antioxidants, depending on the concentration. It can be seen that, for precursor compound **2** (R^2 = Ph), antioxidant activity was 29.57% at a concentration of 1 mg mL^{-1}. When evaluated, for compound **8c** (R^1 = CH_3; R^2 = Ph; CX_3 = CCl_3) free radical inhibition reached 85.74%; compound **8d** (R^1 and R^2 = Ph; CX_3 = CCl_3) obtained prooxidant activity (–41.67%); in substance **9c** (R^1 = CH_3; R^2 = Ph; CX_3 = CF_3) there was a decrease in the antioxidant activity (20.67%); and for compound **12c**, a product of the dehydration reaction of **8c** (R^1 = CH_3; R^2 = Ph; CX_3 = CCl_3), the assay value was –42.05% (prooxidant). Derivative compounds of pentane-2,4-dione **13**-**14** (R^1 = CH_3; R^2 = Ph; CX_3 = CH_3) presented activity results of 3.65% and 17.07%, respectively, at a concentration of 1 mg mL^{-1} (Table 3). According to Herbert,[42] some ingredients may serve both as antioxidants and pro-oxidants, depending on conditions, including the concentration of these chemicals and if transition metals or oxygen are present.

Antioxidant capacity by phosphomolybdenum method

Our results demonstrated that all compounds presented a total antioxidant capacity (TAC, %) that is concentration dependent (Table 4). A standard butylhydroxytoluene (BHT) curve was used with positive control, and for estimating the TAC we used the absorbance of BHT (50 μg mL^{-1}) as positive control (100%). This way, it was possible to affirm that compounds **1** and **2** (50 μg mL^{-1}) presented a similar effect to BHT (50 μg mL^{-1}). Additionally, compounds **8c** and **9c** showed similar antioxidant capacity to BHT (50 μg mL^{-1}) at concentrations of 100 and 500 μg mL^{-1},

Table 3. Antioxidant activity of some synthesized compounds, by DPPH method

Compound	Inhibition / %				
	10 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	500 μg mL^{-1}	1000 μg mL^{-1}
1	6.54 ± 2.7	–1.52 ± 1.9	2.77 ± 2.3	12.5 ± 0.6	25.8 ± 1.1
2	5.55 ± 3.1	–1.46 ± 1.0	1.15 ± 3.6	17.16 ± 0.6	29.57 ± 1.8
6b	–2.68 ± 8.4	0.01 ± 5.4	3.21 ± 6.9	–20.87 ± 0.4	–43.96 ± 8.6
8c	–0.61 ± 4.8	21.38 ± 1.9	43.34 ± 2.4	77.25 ± 6.5	85.74 ± 2.9
8d	–9.17 ± 8.8	–4.97 ± 13.9	–9.77 ± 5.4	–8.94 ± 10.1	–41.67 ± 7.8
9c	–7.80 ± 9.6	6.37 ± 5.2	–9.5 ± 4.5	4.4 ± 7.6	20.67 ± 2.4
10h	–9.40 ± 6.9	–3.93 ± 12.4	–2.2 ± 6.5	16 ± 2.4	21.67 ± 3.1
11e	–2.73 ±9.4	–4.60 ± 10.7	–4.93 ± 4.2	–12.2 ± 17.6	–19.8 ± 10.35
12c	–9.21 ± 5.3	–2.93 ± 3.0	–4.02 ± 6.5	–23.84 ± 1.1	–42.05 ± 6.5
13	–10.89 ± 6.0	–11.21 ± 2.9	–11.04 ± 5.5	–5.77 ± 1.9	3.65 ± 2.4
14	–8.73 ± 8.8	14.2 ± 7.9	–6.57 ± 3.6	11.1 ± 12.8	17.07 ± 2.9

Table 4. Total antioxidant capacity of the compounds by phosphomolybdenum method, presented as mean ± standard derivation

Compound	Total antioxidant capacity / %				
	10 μg mL^{-1}	50 μg mL^{-1}	100 μg mL^{-1}	500 μg mL^{-1}	1000 μg mL^{-1}
1	25.28 ± 0.3	97.54 ±1.9	137.62 ± 4.3	> 275	> 275
2	22.53 ± 0.9	87.92 ± 2.2	138.27 ± 2.6	> 275	> 275
8c	8.58 ± 1.3	42.63 ± 1.6	73.95 ± 2.2	158.44 ± 8.8	167.10 ± 10.1
8d	10.55 ± 0.5	25.54 ± 1.0	30.01 ± 0.4	37.24 ± 1.1	37.61 ± 1.5
9c	7.55 ± 0.6	13.88 ± 2.8	23.74 ± 2.1	72.87 ± 6.9	87.35 ± 6.7
9d	15.66 ± 1.2	24.02 ± 1.9	29.85 ± 1.7	38.08 ± 2.3	48.52 ± 3.1

respectively. On the contrary, compounds **8d** and **9d** presented antioxidant activity lower than that found for the BHT (50 μg mL^{-1}). The phosphomolybdenum method was used to characterize the antioxidant properties in natural and chemical compounds.[43,44] This method comprehends if the compounds have the ability to promote the reduction of the Mo^{VI} to Mo^{V} due to an electron donation. The initial test solution has a yellow color, becoming green as the molybdenum phosphate solution decreases. This method has the advantage of assessing the antioxidant activity of both lipophilic components as hydrophilic.[8]

Our results demonstrate that the chemical structures of compounds **1** and **2** changed only in the R^2 position (CH_3 and C_6H_5, respectively) and thus probably did not interfere in the ability to donate an electron to the Mo^{VI}, because these compounds showed a similar antioxidant effect, which was also higher when compared with the other compounds. Additionally, it was possible to observe that the compounds with X = Cl in the structure presented higher antioxidant activity than with X = F. Moreover, the presence of the CH_3 in the R^1 for compound **8c** promoted higher antioxidant activity than found for compound **9c** with C_6H_5 in the R^1 position. Therefore, the antioxidant capacity of the compounds was found to decrease in the following order: **1** ca. **2** > **8c** > **9c** > **9d** > **8d**.

Conclusions

In summary, we have described a useful approach for building *bis*-pyrazoles with the insertion of different non-halogenated, trifluoromethyl, and trichloromethyl groups, in both pyrazole derivative rings, with good yields when the structures are determined by NMR (^1H, ^{13}C, ^{19}F) and X-ray diffraction. When evaluated, compound **8c** presented the highest antioxidant capacity, similar to BHT with 158.44% and 167.10% at a concentration of 100 and 500 μg mL^{-1}, respectively, and DPPH free radical inhibition reached 85.74%. However, when compared to ascorbic acid, **8c** presented relevant data at various concentrations. Given all these considerations, it may contribute as a new antioxidant for preventing or reducing the development of pathologies associated with oxidative stress.

The preliminary *in vitro* antimicrobial screening results of novel 2-(5-trifluoromethyl-1*H*-pyrazol-1-yl)-5-(5-trihalomethyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridines reported here revealed that the compounds are not good candidates for antibiotics, due to there being no inhibition of the bacteria tested. However, it is known that the azole heterocycle has good antifungal activity, and we envision compounds **6a** and **14** as being potential agents against dermatophytes, because they showed promising results.

Furthermore, additional studies regarding the action mechanism are necessary for a complete understanding of the antifungal activity of these compounds and the development of new agents based on structures containing pyrazole derivatives, in the hope of generating new bioactive molecules that could be useful as potent agents. We also performed X-ray diffraction measurements for a monocrystal of representative compounds.

Experimental

General

Unless otherwise indicated, all common reagents and solvents were used as obtained from commercial suppliers, without further purification. The melting points were determined using coverslips on a Microquímica MQAPF-302 apparatus and are uncorrected. ^1H and ^{13}C spectra were acquired on a Bruker DPX 400 (^1H at 400.13 MHz and ^{13}C at 100.61 MHz) and a Bruker Avance III DPX 600 spectrometer (^1H at 600 MHz and ^{13}C at 150 MHz), with 5 mm sample tubes, 298 K, digital resolution of ± 0.01 ppm, in $CDCl_3$ or DMSO-d_6, and using TMS as an internal reference.

The ^{19}F spectra were acquired on the same Bruker Avance III DPX 600, at 564.68 MHz, with 5 mm sample tubes, 0.3 mol L^{-1} solutions at 298 K, digital resolution

of ± 0.01 ppm, in $CDCl_3$ or DMSO-d_6, and using $CFCl_3$ (δ 0.0 ppm) as the external reference. Mass spectra were registered in a HP 5973 MSD connected to a HP 6890 GC and interfaced by a Pentium PC. The CHN elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer (University of São Paulo, SP, Brazil), and the high resolution mass spectrometry was performed using an Agilent-QTOF 6530 spectrometer (UFSM, RS, Brazil) and a Micro TOF Bruker Daltonic spectrometer (University of São Paulo, SP, Brazil). Diffraction measurements were done by graphite-monochromatized Mo Kα radiation, with λ 0.71073 Å, on a Bruker SMART CCD diffractometer.[45,46] The structures of **6a**, **7a** and **14** were solved with direct methods using the SHELXS-97 program, and refined on F^2 by full-matrix least-squares using the SHELXL-97 package.[39]

The absorption correction was performed by Gaussian methods.[47] Anisotropic displacement parameters for non-hydrogen atoms were applied. The hydrogen atoms were placed at calculated positions with 0.96 Å (methyl CH_3) and 0.93 Å (aromatic CH) using a riding model. The hydrogen isotropic thermal parameters were kept equal to $U_{iso}(H) = \chi U_{eq}$ (carrier C atom), with χ 1.5 for methyl groups and χ 1.2 for all others. The valence angles C–C–H and H–C–H of the methyl groups were set to 109.5º, and the H atoms were allowed to rotate around the C–C bond. The molecular graph was prepared using ORTEP-3 for Windows.[47]

General procedure for the synthesis of 6-[3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-1*H*-pyrazol-1-yl]nicotinohydrazides (**1**-**3**)

Methyl nicotinate hydrochlorides[35,48] (1 mmol) were added to a stirred solution of ethanol (5 mL) and hydrazine hydrate 24% (0.5 mL). After stirring the reaction mixture at reflux temperature for 20 h, the solids **1**-**3** (Figure 4) were isolated by filtration, washed with cold ethanol/H_2O, and dried under reduced pressure in a desiccator containing P_2O_5.

6-(5-Trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)nicotinohydrazide (**1**)[35]

White solid; yield 73%; mp 156-157 °C (70%, mp 154-156 °C);[35] ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 9.98 (s, 1H, NH), 8.87 (s, 1H, H-2), 8.39 (dd, 1H, *J* 2.0, 8.0, H-4), 7.92 (d, 1H, *J* 9.0, H-5), 4.58 (s, 2H, NH_2), (Pyr-B): δ 7.04 (s, 1H, H-4), 2.34 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 163.3 (CO), 151.7 (C-6), 115.1 (C-5), 138.1 (C-4), 127.9 (C-3), 146.5 (C-2), (Pyr-B): δ 131.5 (q, *J* 40.0, C-5), 112.3 (q, *J* 3.0, C-4), 119.7 (q, *J* 268.0, CF_3), 150.2 (C-3), 12.9 (CH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6) Pyrazole B: δ –56.61 (CF_3); HRMS (FTMS + pESI) *m/z,* calcd. for $C_{11}H_{10}F_3N_5O$ $[M]^+$: 286.0915; found: 286.0987.

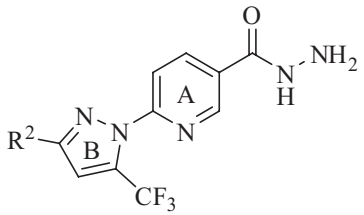


Figure 4. General chemical structure of compounds **1**-**3**.

6-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)nicotinohydrazide (**2**)[35]

White solid; yield 62%; mp 217-219 °C (62%, mp 218-219 °C);[35] ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 10.06 (s, 1H, NH), 8.93 (s, 1H, H-2), 8.47 (dd, 1H, *J* 2.0, 8.0, H-4), 8.11 (d, 1H, *J* 9.0, H-5), 4.60 (s, 2H, NH_2), (Pyr-B): δ 8.02-8.04 (m, 2H, Ph), 7.82 (s, 1H, H-4), 7.44-7.54 (m, 3H, Ph); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 163.1 (CO), 151.8 (C-6), 115.5 (C-5), 138.2 (C-4), 128.3 (C-3), 146.5 (C-2), (Pyr-B): δ 132.5 (q, *J* 40.0, C-5), 109.8 (q, *J* 3.0, C-4), 151.7 (C-3), 130.5 (Ph), 129.1 (Ph), 128.8 (Ph), 125.7 (Ph), 119.6 (q, *J* 267.0, CF_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): Pyr-B: δ –56.58 (CF_3); HRMS (FTMS + pESI) *m/z,* calcd. for $C_{16}H_{12}F_3N_5O$ $[M]^+$: 348.1072; found: 348.1064.

6-(5-Trifluoromethyl-3-(fur-2-yl)-1*H*-pyrazol-1-yl)nicotinohydrazide (**3**)

Pale brown solid; yield 57%; mp 204-206 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.02 (d, 1H, *J* 8.0, H-5), 8.46 (dd, 1H, *J* 2.0, 8.0, H-4), 8.92 (d, 1H, *J* 2.0, H-2), 10.04 (s, 1H, NH), 4.61 (s, 2H, NH_2), (Pyr-B): δ 7.84 (s, 1H, 2-furyl), 7.56 (s, 1H, H-4), 7.08 (d, 1H, *J* 3.0, 2-furyl), 6.67 (dd, 1H, *J* 3.0, 2-furyl); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 163.1 (CO), 151.5 (C-6), 115.4 (C-5), 138.3 (C-4), 128.3 (C-3), 145.6 (C-2), (Pyr-B): δ 132.2 (q, *J* 41.0, C-5_B), 109.4 (q, *J* 3.0, C-4_B), 119.4 (q, *J* 269, CF_3), 146.5 (C-3), 144.2 (2-furyl), 143.8 (2-furyl), 111.7 (2-furyl), 109.2 (2-furyl); ^{19}F NMR (564.68 MHz, DMSO-d_6): Pyr-B: δ –56.73 (CF_3); HRMS (FTMS + pESI) *m/z,* calcd. for $C_{16}H_{13}Cl_3F_3N_5O_2$ $[M]^+$: 338.0866; found: 338.0832.

General procedure for the synthesis of 2-[3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-1*H*-pyrazol-1-yl]-5-[3-alkyl(aryl/heteroaryl)-5-trihalomethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl-1-carbonyl]-pyridines (**6**-**10**)

To a solution of absolute ethanol (10 mL) and 4-alkyl(aryl/heteroaryl)-4-methoxy-1,1,1-trihaloalk-3-en-

2-ones (**4**,**5**)[36-38] (1 mmol) at room temperature and under magnetic stirring, pyrazolyl nicotinohydrazide (**1**-**3**)[35] (1 mmol) was added. For precursor **1**, the reaction needed a small quantity of acetic acid (0.2 mL). The reaction stayed for 16 h at reflux temperature (78 ºC) under magnetic agitation. After the reaction time, solids **6**-**10** (Figure 5) were isolated either by cold filtration (if precipitation occurred when cooled) or the solvent was partly reduced in a rotary evaporator and only then isolated by cold filtration in a Büchner funnel, washed in ethanol and water (4:1), cooled and recrystallized from ethanol/acetone (2:1), washed in ethanol, and then dried in a low pressurized desiccator containing P_2O_5.

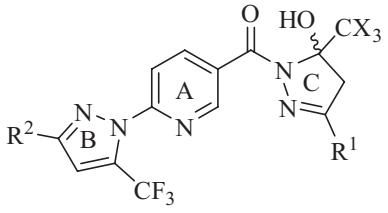


Figure 5. General chemical structure of compounds **6**-**10**.

2-(5-Trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)-5-(5-trichloromethyl-4,5-dihydro-5-hydroxy-3-methyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**6a**)

Yellow crystalline solid; yield 71%; mp 109-111 °C; ^{1}H NMR (400.13 MHz, $CDCl_3$): (Py-A): δ 8.98 (d, 1H, *J* 2.0, H-6), 8.29 (dd, 1H, *J* 2.0, 8.0, H-4), 7.91 (d, 1H, *J* 8.0, H-3), (Pyr-B): δ 6.70 (s, 1H, H-4), 2.37 (s, 3H, CH_3), (Pyr-C): δ 3.58 (d, 1H, *J* 19.0, H-4), 3.32 (d, 1H, *J* 19.0, H-4), 2.04 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, $CDCl_3$): (Py-A): δ 152.7 (C-2), 114.1 (C-3), 140.2 (C-4), 128.0 (C-5), 150.0 (C-6), (Pyr-B): δ 150.3 (C-3), 112.2 (q, *J* 3.0, C-4), 133.2 (q, *J* 40.0, C-5), 119.8 (q, *J* 268.0, CF_3), 13.4 (CH_3), (Pyr-C): δ 169.8 (CO), 157.0 (C-3), 50.1 (C-4), 102.5 (C-5), 103.6 (CCl_3), 15.6 (CH_3); ^{19}F NMR (564.68 MHz, $CDCl_3$): (Pyr-B): δ –56.60 (CF_3); anal. calcd. for $C_{16}H_{13}Cl_3F_3N_5O_2$ (470.66): C, 40.83, H, 2.78; N, 14.88; found: C, 40.93, H, 2.82, N, 15.09%.

2-(5-Trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)-5-(5-trichloromethyl-4,5-dihydro-5-hydroxy-3-phenyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**6b**)

Pale orange solid; yield 80%; mp 127-128 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.82 (s, 1H, H-6), 8.40 (d, 1H, *J* 10.0, H-4), 8.00 (d, 1H, *J* 8.0, H-3), (Pyr-B): δ 7.07 (s, 1H, H-4), 2.37 (s, 3H, CH_3), (Pyr-C): δ 8.30 (s, OH), 7.71 (d, 2H, *J* 7.0, Ph), 7.5-7.44 (m, 3H, Ph), 4.11 (d, 1H, *J* 19.0, H-4), 3.88 (d, 1H, *J* 19.0, H-4); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.2 (C-6), 130.7 (C-5), 140.0 (C-4), 114.5 (C-3), 151.4 (C-2), (Pyr-B): δ 131.6 (q, *J* 40.0, C-5), 112.4 (q, *J* 3.0, C-4), 150.3 (C-3), 119.6 (q, *J* 268.0, CF_3), 12.8 (CH_3), (Pyr-C): δ 166.1 (CO), 102.4 (C-5), 46.8 (C-4), 154.0 (C-3), 129.9 (Ph), 129.5 (Ph), 128.6 (Ph), 126.4 (Ph), 103.2 (CCl_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –56.56 (CF_3); anal. calcd. for $C_{21}H_{15}Cl_3F_3N_5O$ (532.73): C 47.35, H, 2.84, N, 13.15; found: C, 47.71, H, 3.11, N, 13.42%.

2-(5-Trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-methyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**7a**)

Yellow crystalline solid; yield 91%; mp 135-137 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.75 (d, 1H, *J* 2.0, H-6), 8.29 (dd, 1H, *J* 2.0, 8.0, H-4), 7.92 (d, 1H, *J* 8.0, H-3), (Pyr-B): δ 7.05 (s, 1H, H-4), 2.36 (s, 3H, CH_3), (Pyr-C): δ 7.97 (s, 1H, OH), 3.53 (d, 1H, H-4, *J* 19.0), 3.16 (d, 1H, *J* 19.0, H-4), 2.01 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.2 (C-6), 129.9 (C-5), 140.1 (C-4), 114.5 (C-3), 151.3 (C-2), (Pyr-B): δ 131.5 (q, C-5, *J* 40.0), 112.5 (q, C-4, *J* 3.0); 150.3 (C-3), 119.7 (q, CF_3, *J* 267.0), 12.9 (CH_3), (Pyr-C): δ 163.6 (CO), 91.3 (q, C-5, *J* 34.0), 123.2 (q, CF_3, *J* 286.0), 47.6 (C-4), 155.4 (C-3), 15.2 (CH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –56.63 (CF_3), (Pyr-C): δ –76.28 (CF_3); anal. calcd. for $C_{16}H_{13}F_6N_5O_2$ (421.29): C, 45.61, H, 3.11, N, 16.62; found: C, 45.29, H, 3.11, N, 16.04%.

2-(5-Trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-phenyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**7b**)

Yellow solid; yield 81%; mp 114-116 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.83 (d, 1H, *J* 2.0, H-6), 8.42 (dd, 1H, *J* 2.0, 8.0, H-4), 8.0 (d, 1H, *J* 8.0, H-3), (Pyr-B): δ 7.01 (s, 1H, H-4), 2.36 (s, 3H, CH_3), (Pyr-C): δ 8.33 (s, OH), 7.73 (d, 2H, *J* 8.0, Ph), 7.49-7.45 (m, 3H, Ph), 4.01 (d, 1H, *J* 19.0, H-4), 3.68 (d, 1H, *J* 19.0, H-4); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.4 (C-6), 130.9 (C-5), 140.4 (C-4), 114.6 (C-3), 151.4 (C-2), (Pyr-B): δ 131.6 (q, *J* 40.0, C-5), 112.6 (q, *J* 2, C-4), 119.7 (q, *J* 268.0, CF_3), 150.5 (C-3), 13.0 (CH_3), (Pyr-C): δ 163.8 (CO), 92.1 (q, *J* 34, C-5), 44.2 (C-4), 153.0 (C-3), 129.8 (Ph), 129.7 (Ph), 128.8 (Ph), 126.6 (Ph), 123.2 (q, *J* 285.0, CF_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –56.56 (CF_3), (Pyr-C): δ –76.03 (CF_3); anal. calcd. for $C_{21}H_{15}F_6N_5O_2$ (483.36): C, 52.18, H, 3.13, N, 14.49; found: C, 51.64, H, 3.12, N, 14.25%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trichloromethyl-4,5-dihydro-5-hydroxy-3-methyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**8c**)

Yellow solid; yield 83%; mp 140-142 °C; ^{1}H NMR

(400.13 MHz, DMSO-d_6): (Py-A): δ 8.79 (d, 1H, *J* 2.0, H-6), 8.11 (d, 1H, H-3, *J* 8 Hz), 8.34 (dd, *J* 2.0, 8.0, H-4), (Pyr-B): δ 8.02 (d, 2H, Ph), 7.80 (s, 1H, H-4), 7.5-7.46 (m, 3H, Ph), (Pyr-C): δ 7.52 (s, OH), 3.66 (d, 1H, *J* 19.0, H-4), 3.43 (d, 1H, *J* 19.0, H-4), 2.01 (s, 3H, CH_3); ^{13}C {^1H} NMR (100.61 MHz, $CDCl_3$): (Py-A): δ 150.1 (C-6); 131.3 (C-5); 140.3 (C-4); 114.4 (C-3); 152.9 (C-2), (Pyr-B): δ 134.2 (q, *J* 41.0, C-5), 109.6 (q, *J* 3.0, C-4), 119.9 (q, CF_3, *J* 268.0), 152.5 (C-3), 129.2 (Ph), 128.9 (Ph); 128.4 (Ph); (Pyr-C): δ 169.8 (CO); 102.6 (C-5), 50.3 (C-4), 157.0 (C-3); 126.0 (Ph),103.7 (CCl_3) 15.6 (CH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –57.89 (CF_3); anal. calcd. for $C_{21}H_{15}Cl_3F_3N_5O$ (532.73): C, 47.35, H, 2.84, N, 13.15; found: C, 47.33, H, 2.86, N, 13.01%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trichloromethyl-4,5-dihydro-5-hydroxy-3-phenyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**8d**)

Yellow solid; yield 79%; mp 157-159 °C; ^1H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.86 (s, 1H, H-6), 8.46 (d, 1H, *J* 8.0, H-4), 8.18 (d, 1H, *J* 8.0, H-3), (Pyr-B): δ 7.85 (s, 1H, H-4), 8.05 (d, 2H, *J* 7.0, Ph), (Pyr-C): δ 8.33 (s, OH), 7.73 (d, 2H, *J* 7.0, Ph), 7.55-7.46 (m, 6H, Ph), 4.12 (d, 1H, *J* 19.0, H-4), 3.89 (d, 1H, *J* 19.0, H-4); ^{13}C {^1H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.3 (C-6), 130.7 (C-5), 140.2 (C-4), 114.8 (C-3); 151.9 (C-2), (Pyr-B): δ 132.5 (q, *J* 40.0, C-5), 109.9 (C-4), 151.4 (C-3), 119.5 (q, *J* 268.0, CF_3), 130.3 (Ph), 129.0 (Ph), 128.6 (Ph), 125.7 (Ph); (Pyr-C): δ 165.9 (CO), 102.4 (C-5), 46.8 (C-4), 154.0 (C-3), 130.4 (Ph), 129.5 (Ph), 128.6 (Ph), 126.4 (Ph), 103.2 (CCl_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –56.56 (CF_3); anal. calcd. for $C_{26}H_{17}F_3Cl_3N_5O_2$ (594.79): C, 52.50, H, 2.88, N, 11.77; found: C, 52.49, H, 2.98, N, 11.99%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-methyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**9c**)

Yellow solid; yield 87%; mp 153-155 °C; ^1H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.80 (d, 1H, *J* 2.0, H-6), 8.36 (dd, 1H, *J* 2.0, 8.0, H-4), 8.12 (s, 1H, H-3), (Pyr-B): δ 8.04 (d, 2H, *J* 7.0, Ph), 7.84 (s, 1H, H-4), 7.54-7.46 (m, 3H, Ph), (Pyr-C): δ 8.10 (s, 1H, OH), 3.55 (d, 1H, *J* 19.0, H-4), 3.18 (d, 1H, *J* 19.0, H-4), 2.02 (s, 3H, CH_3); ^{13}C {^1H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.2 (C-6); 130.5 (C-5), 140.2 (C-4), 114.9 (C-3); 151.9 (C-2); (Pyr-B): δ 132.5 (q, *J* 40.0, C-5), 110.0 (q, *J* 2.0, C-4), 151.3 (C-3), 130.3 (Ph), 129.2 (Ph), 128.8 (Ph), 125.7 (Ph), 119.6 (q, *J* 268.0, CF_3), (Pyr-C): δ 163.5 (CO), 91.4 (q, *J* 34.0, C-5), 47.6 (C-4), 155.5 (C-3), 123.2 (q, *J* 285.0, CF_3), 15.2 (CH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –56.60 (CF_3), (Pyr-C): δ –76.23 (CF_3); anal. calcd. for $C_{21}H_{15}F_6N_5O_2$ (483.36): C, 52.18, H, 3.13, N, 14.49; found: C, 52.09, H, 3.33, N, 14.41%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-phenyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**9d**)

Pale beige solid; yield 75%; mp 192-194 °C; ^1H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.88 (dd, 1H, *J* 2.0, 8.0, H-6), 8.48 (dd, 1H, *J* 2.0, 8.0, H-4), 8.17 (d, 1H, *J* 8.0, H-3), (Pyr-B): δ 8.05 (d, 2H, Ph, *J* 8.0), 7.85 (s, 1H, H-4), (Pyr-C): δ 8.35 (s, OH), 7.74 (d, 2H, Ph, *J* 2.0), 7.55-7.47 (m, 6H, Ph), 4.02 (d, 1H, *J* 19.0, H-4), 3.68 (d, 1H, *J* 19.0, H-4); ^{13}C {^1H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 149.1 (C-6), 131.4 (C-5), 141.1 (C-4), 115.5 (C-3); 152.5 (C-2), (Pyr-B): δ 133.1 (q, *J* 40.0, C-5), 110.7 (q, *J* 2.0, C-4), 152.0 (C-3), 120.2 (q, CF_3, *J* 268.0), 130.3 (Ph), 129.8 (Ph), 129.3 (Ph), 127.2 (Ph), (Pyr-C): δ 164.3 (CO), 92.7 (q, C-5, *J* 34.0), 44.7 (C-4), 153.6 (C-3), 131.6 (Ph), 130.7 (Ph), 129.4 (Ph), 126.4 (Ph), 123.7 (q, CF_3, *J* 285.0); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ –51.80 (CF_3), (Pyr-C): δ –71.24 (CF_3); anal. calcd. for $C_{26}H_{17}F_6N_5O_2$ (545.44): C, 57.25, H, 3.14, N, 12.84; found: C, 57.32, H, 3.11, N, 12.62%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-(4-methylphenyl)-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**9e**)

Pale yellow solid; yield 67%; mp 206-207 °C; ^1H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.87 (d, 1H, H-6, *J* 2.0), 8.48 (dd, 1H, H-4, *J* 2.0, 8.0), 8.16 (d, 1H, H-3, *J* 9.0), (Pyr-B): δ 8.06 (d, 2H, Ph, *J* 8.0), 7.86 (s, 1H, H-4), 7.55-7.47 (m, 3H, Ph), (Pyr-C): δ 8.35 (s, OH), 7.62 (d, 2H, Ph, *J* 8.0), 7.28 (d, 2H, Ph, *J* 8.0), 3.99 (d, 1H, H-4, *J* 19.0), 3.66 (d, 1H, H-4, *J* 19.0), 2.35 (s, 3H, CH_3); ^{13}C {^1H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.3 (C-6), 130.5 (C-5),140.2 (C-4), 114.8 (C-3), 151.8 (C-2), (Pyr-B): δ 132.6 (q, C-5, *J* 40.0), 109.8 (q, C-4, *J* 2.0), 151.3 (C-3), 119.5 (q, CF_3, *J* 268.0), 130.1 (Ph), 129.1 (Ph), 126.9 (Ph), 125.7 (Ph), (Pyr-C): δ 163.5 (CO), 91.9 (q, C-5, *J* 34.0), 44.0 (C-4), 152.8 (C-3), 140.7 (Ph), 128.9 (Ph), 128.6 (Ph), 126.4 (Ph), 123.0 (q, CF_3, *J* 285.0), 20.7 (CH_3); anal. calcd. for $C_{27}H_{19}F_6N_5O_2$ (559.46): C, 57.96, H, 3.42, N, 12.52; found: C, 57.76, H, 3.58, N, 12.86%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-4,5-dihydro-5-hydroxy-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**9f**)

Orange solid; yield 80%; mp 200-201 °C; ^1H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.87 (d, 1H, H-6, *J* 2.0), 8.48 (dd, 1H, H-4, *J* 2.0, 8.0), 8.16 (d, 1H, H-3,

J 8.0), (Pyr-B): δ 8.05 (d, 2H, Ph, *J* 7.0), 7.85 (s, 1H, H-4), 7.55-7.47 (m, 3H, Ph), (Pyr-C): δ 8.28 (s, 1H, OH), 7.68 (d, 2H, Ph, *J* 8.0), 7.03 (d, 2H, Ph, *J* 8.0), 3.97 (d, 1H, H-4, *J* 19.0), 3.81 (s, 3H, OCH_3), 3.64 (d, 1H, H-4, *J* 19.0); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.6 (C-6), 130.6 (C-5), 140.6 (C-4), 114.9 (C-3), 152.0 (C-2), (Pyr-B): δ 132.6 (q, C-5, *J* 40.0), 110.2 (d, C-4, *J* 3.0), 151.4 (C-3), 119.7 (q, CF_3, *J* 268.0), 130.3 (Ph), 129.3 (Ph), 128.9 (Ph), 122.3 (Ph), (Pyr-C): δ 163.6 (CO), 92.0 (q, C-5, *J* 34.0), 44.3 (C-4), 152.8 (C-3), 161.4 (Ph), 128.5 (Ph), 125.9 (Ph), 114.3 (Ph), 123.2 (q, CF_3, *J* 285.0), 55.3(OCH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ −56.55 (CF_3), (Pyr-C): δ −75.99 (CF_3); anal. calcd. for $C_{27}H_{19}F_6N_5O_3$ (575.46): C, 56.35, H, 3.33, N, 12.17; found: C, 56.38, H, 3.29, N, 12.12%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(3-(fur-2-yl)-5-trifluoromethyl-4,5-dihydro-5-hydroxy-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**9g**)

Pale brown solid; yield 69%; mp 146-148 °C; ^{1}H NMR (600 MHz, DMSO-d_6): (Py-A): δ 8.86 (d, 1H, H-6, *J* 2.0), 8.42 (dd, 1H, H-4, *J* 2.0, 8.0), 8.16 (d, 1H, H-3, *J* 8.0), (Pyr-B): δ 8.05 (d, 2H, Ph, *J* 7.0), 7.87 (s, 1H, H-4), 7.53 (t, 2H, Ph, *J* 7.0), 7.48 (t, 1H, Ph, *J* 7.0), (Pyr-C): δ 8.49 (s, 1H, OH), 7.91 (s, 1H, 2-furyl), 7.13 (d, 1H, 2-furyl, *J* 3.0), 6.69 (dd, 1H, 2-furyl, *J* 2.0, 2.0), 3.44 (d, 1H, H-4, *J* 19.0), 3.33 (d, 1H, H-4, *J* 19.0); ^{13}C {^{1}H} NMR (150 MHz, DMSO-d_6): (Py-A): δ 148.5 (C-6); 130.6 (C-5), 140.6 (C-4), 115.2 (C-3), 152.0 (C-2), (Pyr-B): δ 132.6 (q, C-5, *J* 40.0), 110.3 (s, C-4), 146.2 (C-3), 121.5 (q, CF_3, *J* 268.0), 130.2 (Ph); 129.4 (Ph), 129.0 (Ph), 125.9 (Ph), (Pyr-C): δ 163.8 (CO), 91.7 (q, C-5, *J* 34.0), 43.9 (C-4), 151.5 (C-3), 123.1 (q, CF_3, *J* 285.0), 144.8 (2-furyl), 144.3 (2-furyl), 115.6 (2-furyl); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ −56.57 (CF_3), (Pyr-C): δ −76.05 (CF_3); anal. calcd. for $C_{24}H_{15}F_6N_5O_3$ (535,39): C, 53.84, H, 2.82, N, 13.08; found: C, 54.19, H, 3.23, N, 12.78%.

2-(3-(Fur-2-yl)-5-trifluoromethyl-1*H*-pyrazol-1-yl)-5-(5-trichloromethyl-4,5-dihydro-5-hydroxy-3-phenyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**10h**)

Pale brown solid; yield 88%; mp 144-145 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.87 (d, H-6, *J* 2.0), 8.47 (dd, H-4, *J* 2.0, 8.0), 8.11 (d, H-3, *J* 8.0), (Pyr-B): δ 7.62 (s, 1H, H-4), 7.88 (s, 1H, 2-furyl), 7.14 (d, 1H, 2-furyl, *J* 3.0), 6.70 (dd, 1H, 2-furyl, *J* 3.0, 2.0), (Pyr-C): δ 7.74 (d, 2H, Ph, *J* 8.0), 7.51-7.46 (m, 3H, Ph), 4.13 (d, 1H, H-4, *J* 19.0), 3.91 (d, 1H, H-4, *J* 19.0); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 145.6 (C-6), 130.7 (C-5), 140.2 (C-4), 144.9 (C-3), 151.2 (C-2), (Pyr-B): δ 132.4 (q, *J* 40.0, C-5), 109.4 (d, *J* 3.0, C-4), 148.3 (C-3), 144.2 (2-furyl); 143.7 (2-furyl), 116.3 (2-furyl), 119.3 (q, *J* 268.0, CF_3), (Pyr-C): δ 165.9 (CO), 102.4 (C-5), 46.8 (C-4), 154.1 (C-3), 130.4 (Ph), 129.5 (Ph), 128.6 (Ph), 126.4 (Ph), 103.2 (CCl_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ −56.71 (CF_3); anal. calcd. for $C_{24}H_{15}F_3Cl_3N_5O_3$ (583.76): C, 49.29, H, 2.59, N, 11.98; found: C, 49.46, H, 2.89, N, 11.56%.

General procedure for the synthesis of 2-(3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-1*H*-pyrazol-1-yl)-5-(3-alkyl(aryl/heteroaryl)-5-trihalomethyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**11e**, **12c**, **14**)[49]

A solution of **8c**, **9e**, **13** (2.8 mmol) and pyridine (3 mL, 37.1 mmol) in 50 mL of benzene was cooled to 0 ºC, and thionyl chloride (1.22 mL, 16.8 mmol) diluted in benzene (25 mL) was added dropwise over a period of 10 min. The solution was stirred for an additional 30 min, during which time the temperature was allowed to rise to 20 ºC. The mixture was then heated under reflux (bath temperature 80 ºC) for 1 h and filtered to remove the pyridine hydrochloride at room temperature. The solution was washed twice with water and dried over sodium sulfate. Evaporation of the solvent left a solid (Figure 6) which was recrystallized from ethanol.

Compound **12c** was purified by column chromatography in a mixture of solvents (hexane and ethyl acetate at 6:4 ratio).

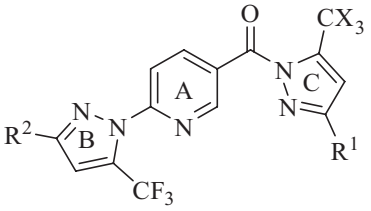


Figure 6. General chemical structure of compounds **11e**, **12c** and **14**.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trifluoromethyl-3-(4-methylphenyl)-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**11e**)

Beige solid; yield 70%; mp 153-155°C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 9.13 (s, 1H, H-6), 8.26 (d, 1H, H-4, *J* 8.0), 8.73 (d, 1H, H-3, *J* 9.0), (Pyr-B): δ 7.87 (s, 1H, H-4), 8.06 (d, 2H, Ph, *J* 7.0), 7.55-7.49 (m, 3H, Ph), (Pyr-C): δ 8.00 (s, 1H, H-4), 7.84 (s, 2H, Ph), 7.31 (d, 2H, Ph, *J* 7.0), 2.35 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, $CDCl_3$): (Py-A): δ 151.6 (C-6), 131.0 (C-5), 140.3 (C-4), 114.3 (C-3), 153.6 (C-2), (Pyr-B): δ 134.3 (q, C-5, *J* 41.0), 110.1 (q, C-4, *J* 3.0), 152.7 (C-3), 119.8 (q, CF_3, *J* 268.0), 129.7 (Ph), 129.3 (Ph), 127.2 (Ph), 126.0 (Ph), (Pyr-C): δ 163.0 (CO), 136.3 (q, C-5, *J* 42.0), 111.4 (q, C-4, *J* 3.0),

153.9 (C-3), 141.9 (Ph), 129.3 (Ph), 128.2 (Ph), 126.2 (Ph), 119.3 (q, CF_3, *J* 285.0), 21.3 (CH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ −56.58 (CF_3), (Pyr-C): δ −58.54 (CF_3); anal. calcd. for $C_{27}H_{17}F_6N_5O$ (541.45): C, 59.89, H, 3.16, N, 12.93; found: C, 60.29, H, 3.91, N, 12.34%.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(5-trichloromethyl-3-methyl-1*H*-pyrazol-1-yl-1-carbonyl) pyridine (**12c**)

Yellow solid; yield 54%; mp 160-162 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 9.00 (s, 1H, H-6), 8.55 (d, 1H, H-4, *J* 8.0), 8.18 (d, 1H, H-3, *J* 8.0), (Pyr-B): δ 7.82 (s, 1H, H-4), 8.03 (d, 2H, Ph, *J* 7.0), 7.52-7.47 (m, 3H, Ph), (Pyr-C): δ 7.22 (s, 1H, H-4), 2.29 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 149.1 (C-6), 126.1 (C-5), 140.4 (C-4), 115.6 (C-3), 152.9 (C-2), (Pyr-B): δ 132.8 (q, C-5, *J* 41.0), 110.3 (q, C-4, *J* 3.0), 119.8 (q, CF_3, *J* 268.0), 152.2 (C-3), (Pyr-C): δ 165.4 (CO), 142.2 (C-5), 141.3 (C-4), 162.5 (C-3), 130.5 (Ph), 129.3 (Ph), 128.9 (Ph), 125.9 (Ph), 106.8 (CCl_3) 10.9 (CH_3); ^{19}F NMR (564.68 MHz, $CDCl_3$): (Pyr-B): δ −57.94 (CF_3); HRMS (FTMS + pESI): *m/z*, calcd. for $C_{21}H_{13}F_3Cl_3N_5O$ $[M]^+$: 514.0216; found: 514.0302.

General procedure for the synthesis of 2-(5-trifluoromethyl-3-methyl-1*H*-pyrazol-1-yl)-5-(3,5-dimethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**13**)

Acetylacetone (0.2 mL, 0.196 g, 2 mmol) and drops (0.3 mL) of triethylamine (Et_3N) were added under magnetic stirring to a solution of treated absolute ethanol (10 mL) and hydrazide **2** (0.347 g, 1 mmol). The reaction stayed under reflux for 16 h. After the reaction time, the solvent was evaporated in a rotatory evaporator, the solid **13** (Figure 7) was isolated directly by filtration, washed with cold ethanol to remove the excess of acetylacetone, and the residual solvent was removed under reduced pressure.

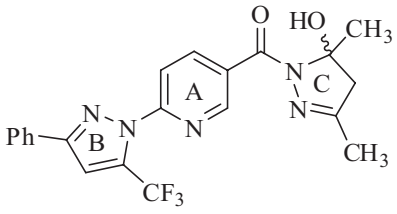


Figure 7. Chemical structure of compound **13**.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(4,5-dihydro-5-hydroxy-3,5-dimethyl-1*H*-pyrazol-1-yl-1-carbonyl) pyridine (**13**)

Beige solid; yield 72%; mp 131-133 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 8.80 (s, 1H, H-6), 8.34 (d, 1H, H-4, *J* 8.0), 8.06 (d, 3H, H-3, *J* 8.0), (Pyr-B): δ 8.02 (d, 2H, Ph, *J* 7.0), 7.78 (s, 1H, H-4), 7.53-7.46 (m, 3H, Ph), (Pyr-C): δ 6.51 (s, 1H, OH), 3.02 (d, 1H, H-4, *J* 18.0), 2.91 (d, 1H, H-4, *J* 18.0), 1.98 (s, 3H, CH_3), 1.92 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 148.1 (C-6), 130.6 (C-5), 140.0 (C-4), 114.9 (C-3), 151.7 (C-2), (Pyr-B): δ 132.5 (q, C-5, *J* 40.0), 109.7 (q, C-4, *J* 3.0), 150.9 (C-3), 119.6 (q, *J* 268.0, CF_3), 131.3 (Ph), 129.1 (Ph), 128.8 (Ph), 125.7 (Ph), (Pyr-C): δ 162.9 (CO), 91.5 (C-5), 51.7 (C-4), 156.1 (C-3), 25.7 (CH_3), 15.7 (CH_3); anal. calcd. for $C_{21}H_{18}F_3N_5O_2$ (429.39): C, 58.74, H, 4.23, N, 16.31; found: C, 58.41, H, 4.59, N, 16.04%.

General procedure for the synthesis of 2-(5-trifluoromethyl-3-phenyl-1H-pyrazol-1-yl)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**14**)

Acetylacetone (0.2 mL, 0.200 g, 2 mmol) and drops (0.3 mL) of acetic acid (AcOH) were added under magnetic stirring to a solution of treated absolute ethanol (10 mL) and hydrazide **2** (0.347 g, 1 mmol). The reaction stayed under reflux for 16 h. After the reaction time, the solvent was evaporated in a rotatory evaporator, and the isolated solid **14** (Figure 8) was washed with cold ethanol and dried in a vacuum apparatus.

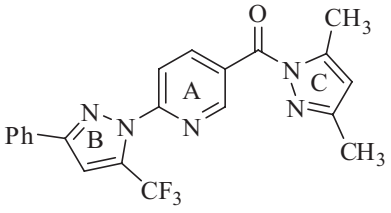


Figure 8. Chemical structure of compound **14**.

2-(5-Trifluoromethyl-3-phenyl-1*H*-pyrazol-1-yl)-5-(3,5-dimethyl-1*H*-pyrazol-1-yl-1-carbonyl)pyridine (**14**)

Pale yellow solid; yield 71%; mp 128-130 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): (Py-A): δ 9.00 (s, 1H, H-6), 8.55 (d, 1H, H-4, *J* 8.0), 8.16 (d, 1H, H-3, *J* 8.0), (Pyr-B): δ 8.03 (d, 2H, Ph, *J* 7.0), 7.82 (s, 1H, H-4), 7.53-7.48 (m, 3H, Ph), (Pyr-C): δ 6.33 (s, 1H, H-4), 2.60 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); ^{13}C {^{1}H} NMR (100.61 MHz, DMSO-d_6): (Py-A): δ 149.0 (C-6), 114.6 (C-5), 130.4 (C-4), 141.7 (C-3), 151.8 (C-2), (Pyr-B): δ 132.6 (q, C-5, *J* 40.0), 110.2 (q, *J* 3.0, C-4), 152.0 (C-3), 129.1 (Ph), 128.7 (Ph), 128.5 (Ph), 125.7 (Ph), 119.5 (q, CF_3, *J* 268.0), (Pyr-C): δ 164.9 (CO), 144.5 (C-5), 111.6 (C-4), 152.3 (C-3), 13.6 (CH_3), 13.3 (CH_3); ^{19}F NMR (564.68 MHz, DMSO-d_6): (Pyr-B): δ −57.88 (CF_3); anal. calcd. for $C_{21}H_{16}F_3N_5O$ (411.38): C, 61.31, H, 3.92, N, 17.02; found: C, 61.18, H, 4.12, N, 16.95%.

Antimicrobial testing

Among the synthesized compounds, those representatives within the series (**6a**, **8d**, **9c**, **9d**, **9f**, **9g**, **10h**, **11e** and **14**) were screened *in vitro* for their antimicrobial activities. They were individually evaluated using either Gram-positive bacteria: *Staphylococcus aureus* ATCC 29213*, Staphylococcus epidermidis* ATCC 1228*, Staphylococcus saprophyticus*, *Enterococcus faecalis* ATCC 51299*, Listeria monocytogenes*, and *Bacillus cereus* ATCC 14579; or Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603*, Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei*, and *Salmonella enterica (typhi).* These compounds were also evaluated for yeasts: *Candida albicans* ATCC 14057, *Candida dubliniensis*, *Candida glabrata* ATCC 2301, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22018, and *Cryptococcus neoformans*; and for filamentous fungi: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus, Fusarium solani* ATCC 36031*, Rhizopus oryzae*, *Microsporum canis*, *Trichophyton mentagrophytes* ATCC 9533*, Trichophyton rubrum,* and *Sporothrix schenckii.* The MIC and MLC of each fraction of the tested microorganisms were determined by the broth microdilution methods in Muller-Hinton broth (bacteria) and in RPMI 1640 broth (fungi), following protocols M07-A9 (2012), M27-A3 (2008), and M38-A2 (2008) as approved by CLSI. For bacteria, the tested concentrations were 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 μg mL^{-1}. As for the fungi, the concentrations were 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, and 0.975 μg mL^{-1}. The MIC end point was taken as the lowest concentration of the compound or fraction inhibiting the total growth of microorganisms, and it was detected by lack of visual turbidity. To obtain the MLC, 10 μL was subcultured from each well that showed complete inhibition, from the last positive well onto Muller-Hinton agar (bacteria) or Sabouraud Dextrose agar plates (fungi). Plates were incubated at 35 °C for 24 h (bacteria) or at 30 °C for 48 h (fungi). The MLC was defined as the lowest concentration of the compound or fraction that showed either no growth or less than three colonies to obtain approximately 99-99.5% killing activity.[50]

Antioxidant activity

Radical scavenging activity using DPPH method

The DPPH assay was conducted at the NEUROTOX Research Laboratory, in accordance with the lab's adapted protocol[7,51] in which 10 mg of each compound analyzed (**1**, **2**, **6b**, **8c**, **8d**, **9c**, **10h**, **11e**, **12c**, **13** and **14**) as a free radical scavenger was individually solubilized in ethanol/acetone 1:1 (1 mL), in which stock solution A [ ]i = 10000 μg mL^{-1} was used to prepare stock solution B [ ]i = 1000 μg mL^{-1} (100 μL of stock solution A plus 900 μL of solubilizing mean). To each of the 96 wells in a microplate, 20 μL of ascorbic acid was added at concentrations of 1, 5, 10 and 100 μL mL^{-1}, as well as 80 μL of ethanol/acetone (1:1), for a final volume of 100 μL for each well. Subsequently, 20 and 10 μL of stock solution A and 20, 10, and 2 μL of stock solution B was added separately to other wells for each analyzed compound, and then 80, 90 and 98 μL of ethanol/acetone (1:1), thus totaling a volume of 100 μL in each well. Then, 100 μL of ethanol of DPPH solution 0.3 mmol L^{-1} was added to each well, for a final volume of 200 μL. The samples' final concentrations in the wells were: [ ]f = 1000, 500, 100, 50 and 10 μL mL^{-1}. Proper blanks were assayed simultaneously and samples were tested in duplicate in three plates. After 30 min of incubation at room temperature away from light, the 96 well culture plates were analyzed using a TP-THERMOPLATE® at 518 nm wavelength. The percentage of antioxidant activity (%, AA) is the amount of DDPH consumed by antioxidants. Radical scavenging activity was calculated as equation 1.[52]

$$\%AA = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100] / Abs_{control}\} \quad (1)$$

Data was processed using the GraphPad Prism software and the results are presented as mean ± standard derivation (S.E.M.) of each concentration tested.

Determination of the TAC by the phosphomolybdenum method

The TAC of the compounds was evaluated by the phosphomolybdenum assay, as previously described by Prieto *et al.,*[8] with a few modifications. Each compound was tested at 10, 50, 100, 500 and 1000 μg mL^{-1} in 10% DMSO. Seven different concentrations of butylhydroxytoluene (BHT), 10, 50, 100, 500 and 1000 μg mL^{-1} were used as positive controls. An aliquot of sample solution was combined with reagent solution (600 mmol L^{-1} sulfuric acid, 28 mmol L^{-1} sodium phosphate, and 4 mmol L^{-1} ammonium molybdate). The tubes were capped and incubated in a water bath at 95 ºC for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695 nm against a blank (reagent and 0.05 mL of water). The TAC was expressed in relation to the BHT 50 μg mL^{-1} absorbance (control 100%) and calculated by the equation 2.

$$\%TAC = \{[(Abs_{sample} - Abs_{blank}) \times 100] / (Abs_{control} - Abs_{blank})\} \quad (2)$$

Supplementary Information

Crystallographic data reported in this paper for compounds **6a**, **7a**, and **14** have been deposited at the Cambridge Crystallographic Data Center (CCDC), No. 1040652, 1040653 and 1040654, respectively. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK (Fax +44-1223-336033 or e-mail deposit@ccdc.cam.ac.uk).

Supplementary information (experimental details and analytical data for all new compounds, as well as the copies of ^{1}H and ^{13}C NMR spectra) is available free of charge at http://jbcs.sbq.org.br as PDF file.

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