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New trifluoromethyl-containing (*E*)-*N*′-arylidene-[3-alkyl(aryl/heteroaryl)-4,5-dihydro-1*H*-pyrazol-1-yl]carbohydrazides: Synthesis, crystal structure and antimicrobial/antioxidant activity

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ABSTRACT

A new series of twenty-one trifluoromethyl-containing (*E*)-*N*′-arylidene-1*H*-pyrazole-1-carbohydrazides (**4**) was synthesized by the cyclocondensation reactions of six 3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazole-1-carbohydrazides (**2**) with eleven aryl/heteroaryl aldehydes and acetophenone (**3**) in 52–97% yields and six new carbohydrazides **2** were easily obtained from the reaction of 1,1,1-trifluoro-4-alkyl(aryl/heteroaryl)-4-methoxy-3-alken-2-ones (**1**) with carbohydrazide. Subsequently, four examples of carbohydrazides **4** were fully studied by X-ray diffraction and the twenty-seven pyrazolyl-carbohydrazides **2** and **4** screened for their antioxidant and antimicrobial proprieties and evaluated by DPPH and MIC methods, respectively. Both series **2** and **4** showed ability to capture DPPH free radical for IC_{50} from 47.57 to 487 μg/mL. Most of the compounds presented fungistatic and bacteriostatic activity only for high MIC levels ranging from 0.25 mg/mL to 0.5 mg/mL.

1. Introduction

Convenient synthetic routes to aromatic heterocycles have been of ongoing interest. Especially desirable are methods of placing fluorine selectively on heterocycle moieties since these derivatives often exhibit bioactivity [1]. Fluorocarbon systems, with minor exceptions, do not occur in nature and, in general, present no peculiar handling difficulties; e.g. the familiar and powerful techniques of isolation, purification, and identification are applicable in similar ways to those for corresponding hydrocarbon systems. However, because fluorine is unique in that it can replace hydrogen in a wide range of hydrocarbon systems without gross distortion of the geometry of the system and because the difference between hydrogen and fluorine creates entirely different electronic environments for functional groups in the fluorocarbon system, new fluorinated compounds become available with reactions and properties quite different from their hydrocarbon analogues [2]. Moreover, many trifluoromethylated 1*H*-pyrazoles and derivatives are known to exhibit important biological activities in medicinal and agricultural scientific fields [3]. Therefore, much attention has been paid to the development of new methods for the synthesis of fluorine containing pyrazoles.

Another class of relevance is the semicarbazones, which are of considerable pharmacological interest since a number of derivatives has shown a broad spectrum of chemotherapeutic properties [4]. Also, they are associated with diverse pharmacological activities, such as antibacterial and antifungal, antihypertensive, hypolipidemic, antineoplasic, hypnotic and anticonvulsant [5].

Thus, in an effort to synthesize new fluorinated 1*H*-pyrazoles containing a fluorinated fragment as well as the semicarbazone moiety in their structures, the aim of this work is to synthesize, to study the new structures via X-ray diffraction and to evaluate the antioxidant and antimicrobial activity of a series of six pyrazolylcarbohydrazides and twenty-one new semicarbazones (Scheme 1). These new heterocyclic systems are of interest principally for antioxidant properties due to the presence of a conjugated π-system. Some similar molecules might be important in preventing the treatment of diseases related to the imbalance between formation and detoxification [6].

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2. Results and discussion

2.1. Synthesis and structure

A review of the literature [7,8] shows that the methodologies that have been used until now, though efficient for the synthesis of similar compounds such **2** and **4**, require drastic conditions and long reaction times, which vary from 12 h to 4 days, involving several reactions steps with yields from 20% to 50%. Therefore, it was developed a simple method, where it is possible to obtain new trifluoromethyl-containing semicarbazones (**4**), in good yields. The present method in general allows the convenient synthesis of a series of ligands in which the substituents at the position 3 (R^1) of the 5-trifluoromethyl-5-hydroxy-2-pyrazoline ring and two substituents (R^2, R^3) of the semicarbazone moiety can be varied.

Based on the literature data previously described by us [9], the reactions of 1,1,1-trifluoro-4-alkyl(aryl/heteroaryl)-4-methoxy-3-alken-2-ones (**1a–f**) [10] with carbohydrazide to obtain the pyrazolyl-carbohydrazide system (**2**) were carried out in a 1:1 molar ratio, in ethanol as solvent at 0–50 °C, for 4 h with yields of 44–86%. Herein, in a reinvestigation we found that better results were obtained when the reactions were performed under room temperature for 18–20 h with chromatographic accompaniment (TLC), followed by a simple filtration and evaporation of the residual solvent under reduced pressure after the reaction time. The known products **2a–b**, **2d–e** and the new carbohydrazides **2c** (R^1 = *p*-tolyl) and **2f** (R^1 = 1-naphthyl) were obtained as pure and stable solids in better yields (62–92%).

Subsequently, the reactions of 3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazolyl-1-carbohydrazides (**2a–f**), with the respective aldehydes **3a–j** or acetophenone **3k** were carried out in 1:1 molar ratio in ethanol and the reactions were also monitored by TLC. The first results for aldehyde derivative **3a** were obtained when the reactions were carried out at 60 °C for 7 h using 1–2 drops of 37% concentrated hydrochloric acid as catalyst (82% yield). The optimal condition to isolate the **4aa** (93% yield) was accessed when the reaction was performed by the same time, solvent and temperature but without the concentrated HCl catalyst.

In order to test a possible solvent-free reaction condition, we carried out reactions using pure liquid aldehydes as solvent. However, a lot of starting material (**2**) was observed by ^{1}H NMR spectrum probably due to the difficulty of homogenization of the reagents. When the amount of aldehydes was added to the reactions, there was a remain of aldehyde which could not be removed by extraction or distillation.

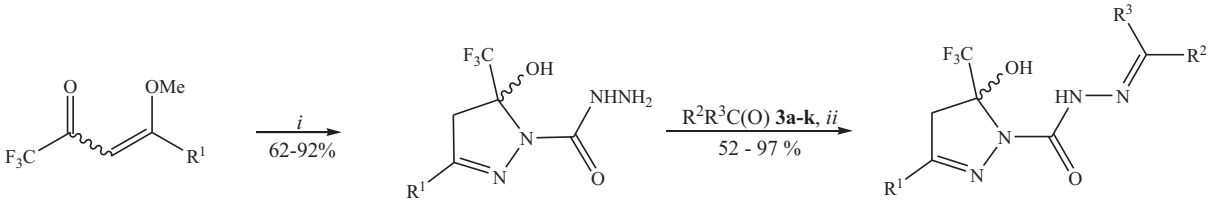
The structures of all compounds **2** were determined by ^{1}H, ^{13}C NMR and by comparison with data of other compounds previously synthesized [9]. The structures of new semicarbazones (**4**) were deduced from NMR experiments, X-ray diffraction studies and by comparison based on the interpretation of literature data [8].

Compounds **4** showed the ^{1}H NMR chemical shifts of the diastereotopic methylene protons (H4) as a characteristic AB system with a doublet in average at δ 3.78 and the other doublet at δ 3.41 ppm, respectively with a *geminal* coupling constant in average at 19 Hz. The hydroxy protons are shown in the ^{1}H spectra in average at δ 7.82 ppm. Moreover, NH shows signs in the range of δ 10.39 ppm and the protons for the arylidene moiety R^2C<u>H</u>=N′N in average of 8.64 ppm. Compounds **4** present the typical ^{13}C chemical shifts of the pyrazoline ring at δ 148.1 ppm (C3) and δ 45.85 ppm (C4). C5 presents a characteristic quartet at δ 91.25 ppm with $^2J_{CF}$ = 34 Hz due to the attached CF_3 group. The CF_3 group shows a typical quartet at δ 122.9 ppm with $^1J_{CF}$ = 285 Hz. The carbonyl carbon showed signal around of δ 151 ppm. The arylidene substituents show signs for the non-aromatic carbon (R^2HC=N′NH–) in average of 146.7 ppm and (R^2CH_3C=N′NH–) of 149.4 ppm.

In parallel, for a full structural elucidation, the X-ray diffraction measurements were carried out for the compounds **4aa**, **4ae**, **4af** and **4df**.

These four compounds are constituted by the 4,5-dihydro-1*H*-pyrazole ring with a hydroxy and a trifluorometyl group in position-5 and still a phenylsemicarbazone group present in position-1 (Fig. 1). Also, these 2-pyrazolines crystallized in monoclinic system and space group $P2_1/n$ for **4aa**, $P2_1/c$ (**4af** and **4ae**) and P(1) for **4df**. The overall view and labeling of the molecule are displayed in Figs. 2 and 3. The lengths and angles of the compounds are showed in Table 1.

For each molecule, the five-membered ring showed r.m.s. deviations from the plane of 0.0278, 0.0122, 0.0946 and 0.1006 Å and the torsion angle of N(2)–N(1)–C(11)–O(12)– are 159.67(18), 158.3(2), 179.7(3) and −179.09(16)° for compounds **4aa**, **4af**, **4df** and **4ae**, respectively. The geometry of the 1-carbonyl-4,5-dihydro-1*H*-pyrazole system is similar to that reported in the literature [11]. Also, the fragment [O(12)=C(11)–N(12)–N(13)=C(14)] showed a r.m.s. deviations from the plane of 0.0422, 0.0484, 0.0430 and 0.0189 Å for compounds **4aa**, **4af**, **4df** and **4ae**, respectively and the geometry of the phenylsemicarbazone system is similar to that



i = $(NH_2NH)_2CO$, EtOH, 18 - 20 h, r.t.; *ii* = EtOH, 7 h, 60ºC

1,2,4	a	b	c	d	e	f
R^1	Me	Ph	4-MePh	2-Furyl	2-Thienyl	1-Naphthyl

3,4	a	b	c	d	e	f	g	h	i	j	k
R^2	Ph	3-NO_2Ph	4-NO_2Ph	2-BrPh	4-ClPh	4-Tolyl	4-OMePh	1,3-Benzodioxol-5-yl	1-Naphthyl	2-Furyl	Ph
R^3	H	H	H	H	H	H	H	H	H	H	Me

Scheme 1.

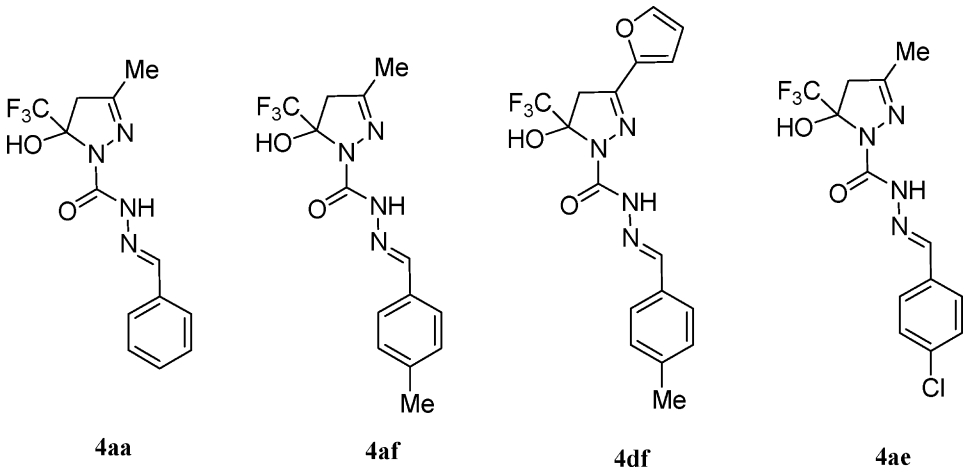


Fig. 1. Planar structures of (*E*)-*N*′-Benzylidene-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl)carbohydrazide (**4aa**), (*E*)-*N*′-(4-Methylbenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl)carbohydrazide (**4af**), (*E*)-*N*′-(4-Methylbenzylidene)-[5-trifluoromethyl-3-(2-furyl)-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl]carbohydrazide (**4df**) and (*E*)-*N*′-(4-Chlorobenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl)carbohydrazide (**4ae**).

reported in the literature [12]. The least square plane angle between the fragment [O(12)═C(11)–N(12)–N(13)═C(14)] and the pyrazole ring showed values of 18.71(5), 24.27(14), 9.26(26) and 13.56(12)° for **4aa**, **4af**, **4df** and **4ae** respectively. Moreover, the least square plane angle between the fragment [O(12)═C(11)–N(12)–N(13)═C(14)] and the phenyl ring showed values of 8.28(9), 39.88(9), 21.77(24) and 9.61(11)° for **4aa**, **4af**, **4df** and **4ae** respectively. The values referred to **4aa**, **4af**, **4df** and **4ae** release that the pyrazole ring, the fragment O(12)═C(11)–N(12)–N(13)═C(14) and phenyl ring are close to the same plane. All this planarity may be associated to a small electronic resonance involving the pyrazole ring, the fragment O(12)═C(11)–N(12)–N(13)═C(14) and phenyl ring.

The presence of intramolecular hydrogen bonds O(51)–H(51)···O(61) were shown to stabilize the position of the carbonyl group in molecules **4aa** and **4af** with interatomic distance of 2.729(2) and 2.697(3) Å for O(51)···O(61), respectively (Table 2). These patterns generated pseudo six membered rings geminated to pyrazole ring (Fig. 4), similar to other 4,5-dihydro-1*H*-pyrazole derivatives already reported in the literature [13]. Still in compound **4aa** was found an intramolecular hydrogen bond O(101)–H(101)···O(62) with interatomic distance of 2.666(2) Å for O(101)···O(62) with a second independent molecule presents in the asymmetric unit of these compound.

The crystalline structures of compounds are stabilized by intermolecular hydrogen bond (Table 2) determining the crystalline packing type. Hydrogen bonds containing highly electronegative atoms in both donor and acceptor groups in systems such as N–H···O and O–H···O showed dominant electrostatic character and have been considered strong interactions [14], which will be discussed in this work. On the other hand, in C–H···O or C–H···N gradually decreases the electrostatic character while the dispersion character increases, making the weak hydrogen bonding [14], which are also present in the crystal packing of compounds, but it

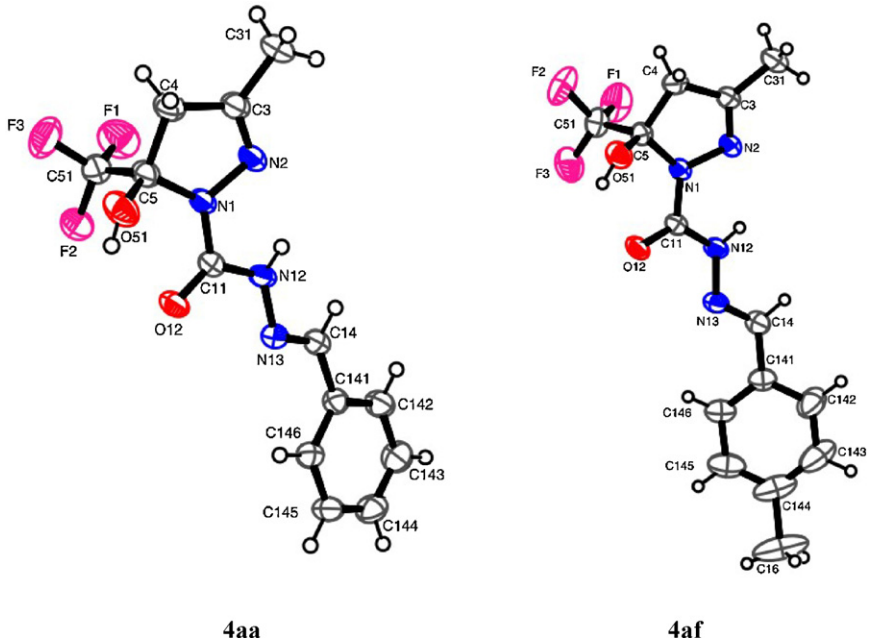
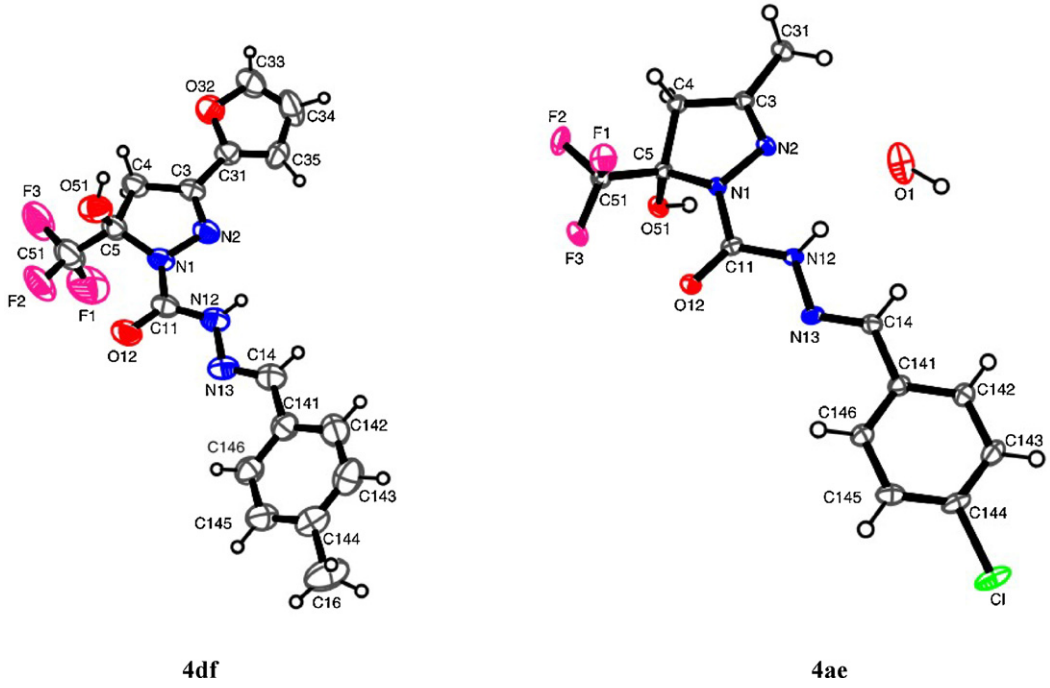


Fig. 2. View of the asymmetric unit of the compounds **4aa** and **4af**, showing the atom labeling scheme. Displacement ellipsoids are drawn at the 50% probability level. H atoms are represented by circles of arbitrary radii.



4df 4ae

Fig. 3. View of the asymmetric unit of the compounds **4df** and **4ae**, showing the atom labeling scheme. Displacement ellipsoids are drawn at the 50% probability level. H atoms are represented by circles of arbitrary radii.

will not be discussed in detail. These interactions occur when donor groups or acceptor protons have low electronegativity.

For the four compounds the crystal packing occurred in the form of infinite chains along of the plane. For compound **4aa** the infinites chains were formed (Fig. 4) through the intermolecular hydrogen bonds N(62)–H(62)···O(12) and N(12)–H(12)···O(62) with interatomic distances of the 2.883(2) and 2.966(2) Å, respectively, involving two independent molecules. For compound **4af** (Fig. 4), appears only the intermolecular hydrogen bond N(12)–H(12)···O(12) with interatomic distances of 2.943(3) Å for N(12)···O(12) (x, $-y+1/2$, $z+1/2$). Although compound **4aa** has two independent molecules in the asymmetric unit of the crystal packing, it occurred in the same way that the two compounds involving the same atoms in hydrogen bonding. This observation shows that the presence of the methyl group in the benzene ring does not cause a molecular change in **4af**, which would be able to alter the proton acceptor or donor in a strong hydrogen bond N(12)H(12)···O(12).

Table 1
Bond lengths [Å] and angles [°] for compounds **4**.

Compounds	4aa	4af	4df	4ae
N(1)–N(2)	1.407(2)	1.399(3)	1.401(4)	1.409(2)
C(3)–N(2)	1.273(3)	1.276(3)	1.285(4)	1.272(2)
C(4)–C(3)	1.499(3)	1.492(4)	1.490(5)	1.493(2)
C(4)–C(5)	1.530(3)	1.523(3)	1.535(4)	1.534(2)
C(5)–N(1)	1.463(3)	1.462(3)	1.462(4)	1.476(2)
C(5)–O(51)	1.388(2)	1.395(3)	1.412(4)	1.395(2)
C(51)–C(5)	1.517(3)	1.523(4)	1.518(6)	1.530(2)
C(31)–C(3)	1.490(3)	1.484(3)	1.441(5)	1.486(3)
N(1)–C(11)	1.368(2)	1.383(3)	1.377(4)	1.379(2)
O(12)–C(11)	1.232(2)	1.230(3)	1.218(4)	1.222(2)
N(12)–C(11)	1.348(3)	1.336(3)	1.341(5)	1.353(2)
N(12)–N(13)	1.372(2)	1.378(3)	1.379(3)	1.377(2)
N(13)–C(14)	1.272(3)	1.281(3)	1.267(5)	1.272(2)
C(141)–C(14)	1.457(3)	1.458(4)	1.456(5)	1.461(2)
C(3)–N(2)–N(1)	107.28(16)	107.9(2)	107.1(3)	107.94(14)
N(2)–C(3)–C(4)	114.81(17)	114.3(2)	114.1(3)	113.63(15)
C(3)–C(4)–C(5)	102.72(17)	103.0(2)	101.7(2)	102.23(14)
N(1)–C(5)–C(4)	101.57(15)	101.97(18)	100.5(3)	99.91(13)
N(2)–N(1)–C(5)	113.21(15)	112.77(18)	111.8(2)	110.92(13)
N(2)–C(3)–C(31)	121.6(2)	121.9(2)	122.3(3)	122.05(17)
N(1)–C(5)–C(51)	108.32(17)	108.5(2)	114.2(3)	113.90(14)
O(51)–C(5)–N(1)	113.90(17)	114.3(2)	111.6(3)	113.39(14)
O(51)–C(5)–C(51)	109.60(16)	109.2(2)	106.4(4)	105.16(14)
C(11)–N(1)–N(2)	121.07(16)	119.5(2)	118.7(3)	117.87(14)
O(12)–C(11)–N(1)	120.79(18)	120.9(2)	121.5(3)	120.56(16)
N(12)–C(11)–N(1)	114.30(16)	114.1(2)	114.7(3)	114.64(15)
O(12)–C(11)–N(12)	124.91(18)	125.0(2)	123.7(3)	124.78(16)
C(11)–N(12)–N(13)	118.69(16)	118.3(2)	118.2(3)	118.13(15)
C(14)–N(13)–N(12)	115.06(17)	115.5(2)	116.5(3)	115.66(15)
N(13)–C(14)–C(141)	121.86(18)	120.2(3)	121.2(4)	121.35(17)

On the other hand, for **4df** and **4ae** (Fig. 5) the crystalline packing occurred through intermolecular hydrogen bond O(51)–H(51)···O(12) with interatomic distance of 2.882(4) Å for O(51)···O(12) ($x+1$, y, z) in **4df** and of 2.6475(18) Å for O(51)···O(12) ($-x+3$, $y-1/2$, $-z-1/2$) in **4ae**. We have described the interaction between the same hydroxyl and carbonyl groups in 1-carbonyl-5-hydroxy-4,5-dihydropyrazoles, but leading to formation of supramolecular dimers [13]. Moreover, it was observed in **4ae** a hydrogen bond [O(1)–H(1B)···O(51)] involving a water molecule with interatomic distance of 2.908(2) Å for O(1)···O(51) ($-x+3$, $y-1/2$, $-z-1/2$). Now, the presence of a bulky group (furan) in position-3 of the pyrazole ring in compound **4df** and the presence of a water molecule in the asymmetric unit in the structure **4ae** were able to alter the proton donor in the strong hydrogen bonding in the crystal packing of these structures, being the hydroxyl O(51) interacting with carbonyl proton acceptor O(12). Whole interatomic distance showed to be less than the sum of Van der Waals radii of the acceptors and donors (D···A) atoms of proton involved in the interaction [15].

A notable difference observed was that the compounds **4aa** and **4af**, which containing the intramolecular hydrogen bond O(51)–H(51)···O(12) and the intermolecular hydrogen bond N(12)–H(12)···O(12), showed the pyrazole rings more plane when compared with **4df** and **4ae**. On the other hand, compounds **4df** and **4ae** that contained the intermolecular hydrogen bond O(51)–H(51)···O(12) showed the fragment N(2)–N(1)–C(11)═O(12) more

Table 2
Hydrogen bonding geometry in structures [Å],°.

Compounds	D–H···A	D–H	H···A	D···A	D–H···A	Symmetry codes
4aa	O(51)–H(51)···O(12)	0.82	2.19	2.729(2)	123.6	
	O(101)–H(101)···O(62)	0.82	2.13	2.666(2)	122.8	
	N(62)–H(62)···O(12)	0.92(2)	2.04(2)	2.883(2)	151(2)	
	N(12)–H(12)···O(62)	0.83(2)	2.20(2)	2.966(2)	153(2)	$x+1, y, z$
4af	O(51)–H(51)···O(12)	0.82	2.16	2.697(3)	123.1	
	N(12)–H(12)···O(12)	0.91(3)	2.14(3)	2.943(3)	147(2)	$x, -y+1/2, z+1/2$
4df	O(51)–H(51)···O(12)	0.82	2.10	2.882(4)	161.0	$x+1, y, z$
4ae	O(51)–H(51)···O(12)	0.82	1.83	2.6475(18)	173.6	$-x+3, y-1/2, -z-1/2$
	O(1)–H(1B)···O(51)	0.9143(17)	2.0072(13)	2.908(2)	168.29(14)	$-x+3, y-1/2, -z-1/2$

plane when compared to **4aa** and **4af**. These data demonstrate the effect of the interactions present in the crystal packing affecting the molecular structure.

2.2. Antioxidant activity

Antioxidants are considered important nutraceuticals on account of many health benefits [6]. Antioxidants may be classified according to their mode of action as free radical scavengers, chelators of metal ions involved in catalyzing lipid oxidation, or oxygen scavengers that react with oxygen in closed systems [6,16]. Several different methods are available and have been used to assess the total antioxidant capacity of numerous molecules. The commonly used antioxidant evaluation method, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging was applied to determine the antioxidant capacity. The target carbohydrazides **2** and **4** were compared with BHT (Butylated hydroxytoluene) and Quercetin, as reference compounds.

The DPPH antioxidant assay measures the hydrogen donating capacity of the molecules in the sample. When the free radical DPPH is reduced by the sample its color changes from violet to yellow. This absorbance decline is measured and the antioxidant capacity can be determined.

Compounds **2** and **4** were active as free radical DPPH trapping for screening. By determining the IC_{50}, it was found that the compounds of series **2** demonstrated value in the range from 77.47 to 109.42 μg/mL and the new series of semicarbazones **4** in the range from 47.57 to 487 μg/mL (Table 3). Compound **4bf** was active at the concentration of 47.57 μg/mL, being the lowest value able to inhibit 50% of DPPH for the tested samples.

In this work, we also obtained the percentages of inhibition of compounds **2** and **4** to evaluate free radical scavenging activity DDPH (Table 2). The percentage of inhibition at various concentrations measured through the absorbance of the samples shows that the compounds **2a**–**f** reached relevant data on the concentrations of 250 μg/mL, which approached the standard BHT (Table 4).

Samples of the new series of semicarbazones (**4bf**, **4bg** and **4bj**) from the precursor **2b** showed a greater ability to capture DPPH free radical when compared with the other compounds, especially the **4bf**, which was capable of inhibiting a higher percentage of 97.7% at a concentration of 250 μg/mL (Table 4).

2.3. Antimicrobial activity

Numerous recent publications have documented the fact that despite growing problems with resistance to antimicrobial agents amongst important bacterial pathogens, the number of new antibiotics being brought to the market has shown a precipitous decline over the past several decades, with only five new antibacterial agents approved for clinical use in the USA between 2003 and 2007 [17]. The use of antimicrobial drugs to treat public health is growing and it is possible to see an emergence of bacterial

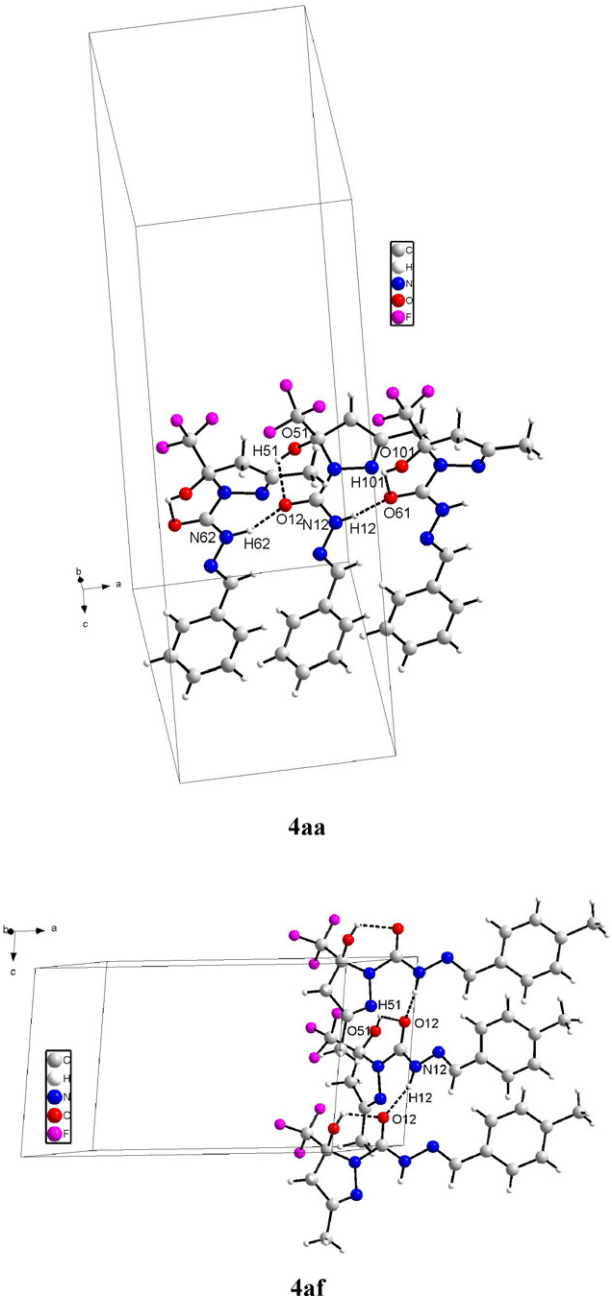
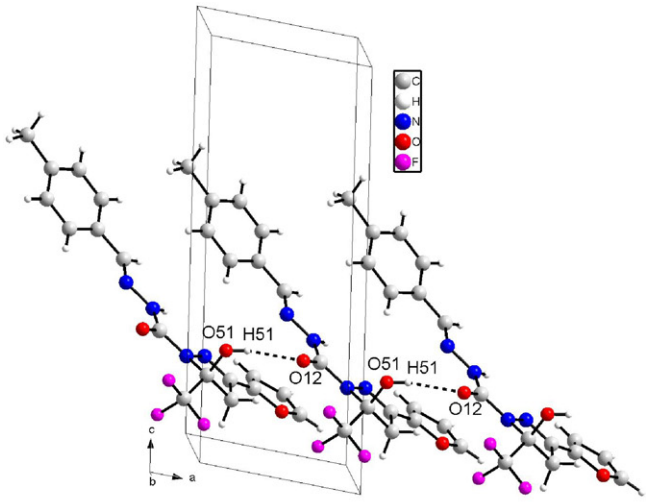
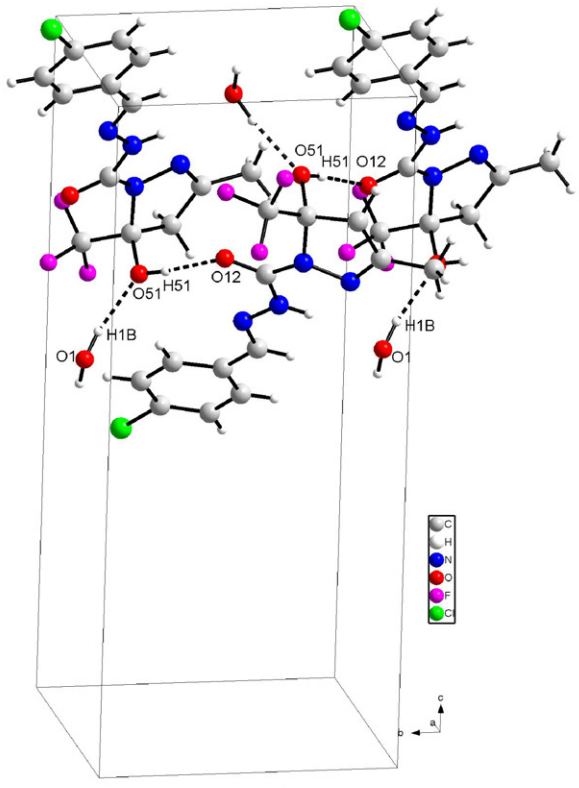


Fig. 4. A stereoview of part of the crystal structure of **4aa** and **4af** showing the formation of a hydrogen bonded.



4df



4ae

Fig. 5. A stereoview of part of the crystal structure of **4df** and **4ae** showing the formation of a hydrogen bonded.

resistance. To maintain the efficiency of the treatment of diseases caused by bacteria, new active compounds are always being searched for [18].

The compounds **2a**, **2c**, **2d** and **2e** forward to microorganisms and tested concentrations were active in the yeast *Candida albicans* MIC = 0.25 mg/mL. Compounds **4aa**, **4ab**, **4ad**, **4ae**, **4af**, **4ag**, **4ah**, **4aj**, **4ak**, **4bf**, **4bg**, **4bj**, **4ce**, **4df**, **4dh**, **4ea**, **4ef** and **4fe** presented fungistatic activity, whose minimal inhibitory concentration (MIC) level ranged from 0.25 to 0.5 mg/mL (Table 5).

Table 3
Antioxidant activity IC_{50} for 4,5-dihydro-1*H*-pyrazolyl-1-carbohydrazides (**2**) and (*E*)-*N*′-arylidene-[4,5-dihydro-1*H*-pyrazol-1-yl]-carbohydrazides (**4**).

Compounds	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**4aa**
IC_{50}(μg/mL)	96.28	101.89	109.42	96.4	102.92	77.47	238.46
Compounds	**4ab**	**4ac**	**4ad**	**4ae**	**4af**	**4ag**	**4ah**
IC_{50}(μg/mL)	487.0	259.55	145.83	235.71	219.69	256.39	363.63
Compounds	**4ai**	**4aj**	**4ak**	**4bf**	**4bg**	**4bj**	**4ce**
IC_{50}(μg/mL)	254.21	206.58	72.44	47.57	84.28	82.09	270.59
Compounds	**4df**	**4dh**	**4ea**	**4ef**	**4fb**	**4fe**	**BHT**
IC_{50}(μg/mL)	270.37	216.67	194.26	451.11	220.79	167.03	21.55

The best results, but not satisfactory, refer to the compound **2f**, which presented bacteriostatic for gram-positive bacterium *S. pyogenes* and for gram-negative bacterium *E. coli*, MIC = 0.125 mg/mL. This compound was also bactericidal against the bacterium *E. coli* at the concentration of 0.25 mg/mL (Table 5).

3. Experimental

3.1. Synthesis and structure

3.1.1. General procedures

Unless otherwise indicated all common reagents and solvents were used from commercial suppliers without further purification. All melting points were determined using open capillaries on an Electrothermal Mel-Temp 3.0 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker DPX 200 MHz or DPX 400 MHz spectrometers, 5 mm sample tubes, 298 K, digital resolution ± 0.01 ppm, in DMSO-d_6 (**2**, **4**) and $CDCl_3$ (**1**) using TMS as internal reference. The CHN elemental analyses were performed on a Perkin-Elmer 2400 CHN elemental analyzer (Universidade de São Paulo, USP/Brazil).

Table 4
Antioxidant activity for 4,5-dihydro-1*H*-pyrazolyl-1-carbohydrazides (**2**) and (*E*)-*N*′-arylidene-[4,5-dihydro-1*H*-pyrazol-1-yl]carbohydrazides (**4**).

Compounds	% Inhibition					
	250[a]	125[a]	62.5[a]	31.25[a]	15.62[a]	7.81[a]
2a	80.2	51.2	22.6	11.6	6.8	4.9
2b	84	61.1	31.3	20.4	10.1	6.8
2c	65.8	54.6	26.7	16.8	10	8.2
2d	87.2	64.4	33.1	14.6	9.4	6.4
2e	83.6	59.1	33.4	18.5	11.4	7
2f	90.4	78.9	40.9	20.8	13	10.3
4aa	52.4	26.4	10.1	5.4	2.1	0.26
4ab	26.3	13.8	9.3	7.8	3.6	1.4
4ac	40.8	18.5	11.1	9.2	7.3	3
4ad	64	28	10.4	4.3	2.2	1.4
4ae	31.8	10.7	2.2	1.9	–	–
4af	56	31.8	20.8	16.7	15.4	14
4ag	78.8	40.5	23.7	17.1	11.3	5.7
4ah	40	29	19.8	19.2	18	16.7
4ai	49.3	25.5	17.2	15.5	11.8	10
4aj	54.3	25.7	13	9.9	7.5	5
4ak	92.5	67	46.8	34	18.2	10.6
4bf	97.7	70	54.3	45.3	36.1	25.9
4bg	86	64.3	42.4	23.5	18	9.6
4bj	90.8	73	39.5	17.8	10.8	4.3
4ce	38.6	17.3	10.7	5.5	3.2	1.1
4df	48	34.4	30.8	29.5	26.6	24
4dh	57.8	29.2	16.5	12.3	10.1	9.7
4ea	63.6	33.1	15	5.9	4.7	4.5
4ef	32	20.7	15.8	14.4	13.8	12.3
4fb	56	30.7	15.3	9	6.9	4.1
4fe	64	29.8	14.6	4.6	2.6	1.7
BHT	91.2	89.2	71.2	56.9	45.8	33.2

[a] Concentration in μg/mL.

Table 5
Antimicrobial activity for 4,5-dihydro-1*H*-pyrazolyl-1-carbohydrazides (**2**) and (*E*)-*N*′-arylidene-[4,5-dihydro-1*H*-pyrazol-1-yl]carbohydrazides (**4**).

Sample	MIC[a]/MLC[a] Microorganism[c]							
	E. coli	*K. pneumonia*	*S. pyogenes*	*S. aureus*	*S. epidermidis*	*B. subtilis*	*S. cerevisiae*	*C. Albicans*
2a	0.25/>0.5	0.25/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.25/>0.5	>0.5	0.25/>0.5
2b	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
2c	>0.5	>0.5	0.25/>0.5	>0.5	>0.5	>0.5	>0.5	0.25/>0.5
2d	0.25/>0.5	0.25/>0.5	0.5/>0.5	0.25/>0.5	0.25/>0.5	0.25/>0.5	>0.5	0.25/>0.5
2e	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.25/>0.5
2f	0.125/0.25	>0.5	0.125/>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
4aa	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4ab	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4ac	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
4ad	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.25/>0.5	0.25/>0.5
4ae	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.25/>0.5	0.25/>0.5
4af	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4ag	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4ah	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4ai	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	>0.5	>0.5
4aj	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4ak	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.25/>0.5	0.25/>0.5
4bf	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.25/>0.5	0.25/>0.5
4bg	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.25/>0.5	0.25/>0.5
4bj	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.5/>0.5
4ce	>0.5	>0.5	0.5/>0.5	>0.5	0.5/>0.5	>0.5	0.25/>0.5	0.25/>0.5
4df	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.5/>0.5	0.25/>0.5
4dh	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5
4ea	0.25/>0.5	0.25/>0.5	0.25/>0.5	0.25/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5
4ef	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.5/>0.5	0.25/>0.5
4fb	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
4fe	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	0.25/>0.5	0.25/>0.5
Standard[b]	3.12×10^{-3}	3.12×10^{-3}	6.25×10^{-3}	3.12×10^{-3}	3.12×10^{-3}	3.12×10^{-3}	10.3×10^{-3}	10.3×10^{-3}

[a] mg/mL.
[b] Chloramphenicol for bacteria and Nistatin for yeast.
[c] ATCC (American Type Culture Collection).

4-Alkyl(aryl/heteroaryl)-4-methoxy 1,1,1-trifluoroalk-3-en-2-ones (**1a–f**) were prepared according to the previous publications [10] from the trifluoroacetylation reactions of the respective enolether (**1a**) or acetals (**1b–f**) with trifluoroacetic anhydride in the presence of pyridine. The pure compounds **1** were obtained by distillation under reduced pressure.

*General procedure for synthesis of 3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1H-pyrazolyl-1-carbohydrazides (**2a-f**).*

General procedure: To the corresponding and pure 4-alkyl(aryl/heteroaryl)-1,1,1-trifluoro-4-methoxy-3-alken-2-ones (**1a–f**) (10 mmol) at room temperature, a solution of carbohydrazide (0.9009 g, 10 mmol) in ethanol (10 mL) was added. The mixtures were stirred for an additional 18–20 h at room temperature. Then, the crude products were filtered, washed with cold ethanol and recrystallized from ethanol. Subsequently, the pure products **2a–f** were submitted to reduced pressure to eliminate any trace of the residual solvents, and isolated as white or yellow solids (yields 62–92%).

*5-Trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazolyl-1-carbohydrazide (**2a**). White solid; yield 62%; mp 161–162 °C. Lit.* [9]*: yield 49%; mp 161–162 °C.*

*5-Trifluoromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1H-pyrazolyl-1-carbohydrazide(**2b**). White solid; yield 88%; mp 234–236 °C. Lit.* [9]*: yield 86%; mp 234–235 °C.*

*5-Trifluoromethyl-5-hydroxy-3-(4-methylphenyl)-4,5-dihydro-1H-pyrazolyl-1-carbohydrazide (**2c**). White solid; yield 67%; mp 240–242 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 8.34 (s, 1H, NH), 7.76 (s, 1H, OH), 7.75 (d, 2H, Ar, J = 8), 7.26 (d, 2H, Ar, J = 8), 4.14 (s, 2H, NH_2), 3.74 (d, 1H, H-4, ^2J = 19), 3.44 (d, 1H, H-4, ^2J = 19).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 154.7 (C=O), 149.5 (C-3), 139.9, 137.9, 129.0, 126.5, (6 C, Ar), 123.2 (q, ^1J = 285, CF_3), 91.1 (q, ^2J = 33, C-5), 44.1 (C-4), 20.8 (Me).

Anal. Calc. for $C_{12}H_{13}F_3N_4O_2$ (302.25): C, 47.68; H, 4.34; N, 18.54%.

Found: C, 47.82; H, 4.31; N, 18.75%.

*5-Trifluoromethyl-3-(2-furyl)-5-hydroxy-4,5-dihydro-1H-pyrazolyl-1-carbohydrazide (**2d**). Yellow solid; yield 85%; mp 217–219 °C. Lit.* [9]*: yield 44%; mp 216–217 °C.*

*5-Trifluoromethyl-5-hydroxy-3-(2-thienyl)-4,5-dihydro-1H-pyrazolyl-1-carbohydrazide (**2e**). White solid; yield 92%; mp 229–231 °C. Lit.* [9]*: yield 80%; mp 230–231 °C.*

*5-Trifluoromethyl-5-hydroxy-3-(1-naphthyl)-4,5-dihydro-1H-pyrazolyl-1-carbohydrazide (**2f**). Yellow solid; yield 65%; mp 214–215 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 9.16 (d, 1H, Ar, J = 8), 8.30 (s, 1H, NH), 8.04 (d, 1H, Ar, J = 8), 8.00 (d, 1H, Ar, J = 8), 7.75 (s, 1H, OH), 7.70–7.66 (m, 1H, Ar), 7.63–7.55 (m, 2H, Ar), 4.27 (s, 2H, NH_2), 4.02 (d, 1H, H-4a, ^2J = 18), 3.71 (d, 1H, H-4b, ^2J = 18).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 154.9 (C=O), 150.6 (C-3), 133.5, 130.8, 129.6, 128.8, 128.4, 127.7, 126.5, 126.2, 124.9 (10C, Ar), 123.4 (q, ^1J = 285, CF_3), 90.1 (q, ^2J = 33, C-5), 46.7 (C-4).

Anal. Calc. for $C_{15}H_{13}F_3N_4O_2$ (338.28): C, 53.26; H, 3.87; N, 16.56%.

Found: C, 53.31; H, 3.84; N, 16.69%.

*General procedure for the synthesis of new (E)-N′-arylidene-[3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazides (**4**).*

General procedure: To a stirred solution of the respective 3-alkyl(aryl/heteroaryl)-5-trifluoromethyl-5-hydroxy-4,5-dihydro-1*H*-pyrazol-1-yl-carbohydrazides **2a–f** (5 mmol) in ethanol (5 mL)

was added the aldehydes **3a–j** or desired ketone **3k** (5 mmol). The mixtures were subjected to magnetic stirring for 7 h at 60 °C. After the reaction time, the mixtures were stored for 48 h at room temperature. The precipitates were filtered under reduced pressure and washed with cold ethanol. The solids **4** were recrystallized from a mixture of ethanol:acetone:di-*iso*-propyl ether (2:1:2) and isolated as white to yellow solids (yields 52–97%).

*(E)-N′-Benzylidene-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4aa**). White solid; yield 93%; mp 164–165 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.73 (s, 1H, NH), 8.38 (s, 1H, CH), 7.62–7.60 (m, 2H, Ar), 7.61 (s, 1H, OH), 7.43–7.41 (m, 3H, Ar), 3.43 (d, 1H, H-4, J = 19), 3.06 (d, 1H, H-4, J = 19), 2.05 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.3 (C=O), 149.8 (C-3), 145.4 (C=N), 134.6, 129.2, 128.5, 126.4 (6C, Ar), 123.2 (q, ^1J = 285, CF_3), 90.6 (q, ^2J = 33, C-5), 47.7 (C-4), 14.9 (Me).

Anal. Calc. for $C_{13}H_{13}F_3N_4O_2$ (314.26): C, 49.68; H, 4.17; N, 17.83%.

Found: C, 49.74; H, 4.03; N, 17.99%.

*(E)-N′-(3-Nitrobenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ab**). Yellow solid; yield 66%; mp 191–192 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 11.04 (s, 1H, NH), 8.49 (s, 1H, CH), 8.43 (s, 1H, Ar), 8.22 (d, 1H, Ar, J = 8), 8.02 (d, 1H, Ar, J = 8), 7.76–7.72 (m, 1H, Ar), 7.69 (s, 1H, OH), 3.45 (d, 1H, H-4, J = 19), 3.07 (d, 1H, H-4, J = 19), 2.06 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.8 (C=O), 149.6 (C-3), 148.2 (C=N), 142.8, 136.6, 132.8, 130.3, 123.58, 120.2 (6C, Ar), 123.3 (q, ^1J = 285, CF_3), 90.6 (q, ^2J = 33, C-5), 47.9 (C-4), 15.1 (Me).

Anal. Calc. for $C_{13}H_{12}F_3N_5O_4$ (359.26): C, 43.46; H, 3.37; N, 19.49%.

Found: C, 43.21; H, 3.71; N, 19.45%.

*(E)-N′-(4-Nitrobenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ac**). Yellow solid; yield 82%; mp 252–253 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 11.09 (s, 1H, NH), 8.50 (s, 1H, CH), 8.28 (d, 2H, Ar, J = 8), 7.87 (d, 2H, Ar, J = 8), 7.70 (s, 1H, OH), 3.46 (d, 1H, H-4, J = 19), 3.08 (d, 1H, H-4, J = 19), 2.07 (s, 3H, Me).

^{13}C NMR (50.32 MHz, DMSO-d_6): δ = 152.9 (C=O), 149.5 (C-3), 147.4 (C=N), 142.8, 141.2, 127.4, 124.0, (6C, Ar), 123.3 (q, ^1J = 285, CF_3), 90.7 (q, ^2J = 33, C-5), 48.0 (C-4), 15.2 (Me).

Anal. Calc. for $C_{13}H_{12}F_3N_5O_4$ (359.26): C, 43.46; H, 3.37; N, 19.49%.

Found: C, 43.48; H, 3.39; N, 19.39%.

*(E)-N′-(2-Bromobenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ad**).White solid; yield 54%; mp 172–173 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 11.20 (s, 1H, NH), 8.75 (s, 1H, CH), 7.90 (d, 1H, Ar, J = 8), 7.68 (s, 1H, Ar), 7.65 (s, 1H, OH), 7.43 (t, 1H, Ar, J = 7), 7.35–7.28 (m, 1H, Ar), 3.44 (d, 1H, H-4, J = 19), 3.06 (d, 1H, H-4, J = 19), 2.06 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.4 (C=O), 149.7 (C-3), 143.6 (C=N), 133.6, 133.0, 131.0, 127.8, 126.8, 123.0 (6C, Ar), 123.3 (q, ^1J = 284, CF_3), 90.6 (q, ^2J = 33, C-5), 47.9 (C-4), 15.1 (Me).

Anal. Calc. for $C_{13}H_{12}BrF_3N_4O_2$ (393.16): C, 39.71; H, 3.08; N, 14.25%.

Found: C, 39.72; H, 2.92; N, 14.41%.

*(E)-N′-(4-Chlorobenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ae**). White solid; yield 74%; mp 133–134 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.80 (s, 1H, NH), 8.36 (s, 1H, CH), 7.63 (d, 2H, Ar, J = 8), 7.62 (s, 1H, OH), 7.48 (d, 2H, Ar, J = 8), 3.43 (d, 1H, H-4, J = 19), 3.06 (d, 1H, H-4, J = 19), 2.05 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.4 (C=O), 149.8 (C-3), 144.1 (C=N), 133.8, 133.5, 128.6, 128.0 (6C, Ar), 123.2 (q, ^1J = 284, CF_3), 90.6 (q, ^2J = 34, C-5), 47.7 (C-4), 14.8 (Me).

Anal. Calc. for $C_{13}H_{12}ClF_3N_4O_2{\cdot}H_2O$ (366.72): C, 42.58; H, 3.85; N, 15.28%.

Found: C, 42.57; H, 3.95; N, 15.57%.

*(E)-N′-(4-Methylbenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4af**). White solid; yield 75%; mp 155–156 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.65 (s, 1H, NH), 8.33 (s, 1H, CH), 7.57 (s, 1H, OH), 7.50 (d, 2H, Ar, J = 8), 7.24 (d, 2H, Ar, J = 8), 3.42 (d, 1H, H-4, J = 19), 3.05 (d, 1H, H-4, J = 19), 2.33 (s, 3H, Me–Ar), 2.05 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.4 (C=O), 149.8 (C-3), 145.4 (C=N), 139.1, 132.0, 129.3, 124.7 (6C, Ar), 123.3 (q, ^1J = 284, CF_3), 90.6 (q, ^2J = 33, C-5), 47.9 (C-4), 20.9 (Me–Ar), 15.1 (Me).

Anal. Calc. for $C_{14}H_{15}F_3N_4O_2$ (328.29): C, 51.22; H, 4.61; N, 17.07%.

Found: C, 51.27; H, 4.11; N, 17.29%.

*(E)-N′-(4-Methoxybenzylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ag**). White solid; yield 97%; mp 152–153 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.57 (s, 1H, NH), 8.31 (s, 1H, CH), 7.56 (s, 1H, OH), 7.55 (d, 2H, Ar, J = 9), 6.99 (d, 2H, Ar, J = 9), 3.79 (s, 3H, OMe), 3.42 (d, 1H, H-4, J = 19), 3.06 (d, 1H, H-4, J = 19), 2.05 (s, 3H, Me).

^{13}C NMR (50.32 MHz, DMSO-d_6): δ = 152.4 (C=O), 150.0 (C-3), 145.3 (C=N), 160.4, 128.1, 127.4, 114.2 (6C, Ar), 123.4 (q, ^1J = 285, CF_3), 90.6 (q, ^2J = 33, C-5), 55.2 (OMe), 47.9 (C-4), 15.2 (Me).

Anal. Calc. for $C_{14}H_{15}F_3N_4O_3$ (344.29): C, 48.84; H, 4.39; N, 16.27%.

Found: C, 49.07; H, 4.01; N, 16.31%.

*(E)-N′-(1,3-Dioxobenzyliden-5-yl)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ah**). White solid; yield 83%; mp 167–168 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 10.66 (s, 1H, NH), 8.28 (s, 1H, CH), 7.61 (s, 1H, OH), 7.20 (s, 1H, Ar), 7.01–6.94 (m, 2H, Ar), 6.07 (s, 2H, dioxol), 3.44 (d, 1H, H-4, J = 19), 3.05 (d, 1H, H-4, J = 19), 2.05 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.3 (C=O), 149.8 (C-3), 148.5 (C=N), 147.8, 145.1, 129.15, 122.5, 108.3, 104.6, 101.3 (7C, Ar), 123.3 (q, ^1J = 283, CF_3), 90.6 (q, ^2J = 33, C-5), 47.8 (C-4), 15.1 (Me).

Anal. Calc. for $C_{14}H_{13}F_3N_4O_4$ (358.27): C, 46.93; H, 3.66; N, 15.64%.

Found: C, 46.91; H, 3.64; N, 15.61%.

*(E)-N′-(1-Naphythylidene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ai**). White solid; yield 85%; mp 182–183 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.86 (s, 1H, NH), 9.10 (s, 1H, CH), 8.85 (d, 1H, Ar, J = 8), 8.00–7.97 (m, 2H, Ar), 7.86 (d, 1H, Ar, J = 8), 7.68 (s, 1H, OH), 7.66–7.57 (m, 3H, Ar), 3.47 (d, 1H, H-4, J = 19), 3.10 (d, 1H, H-4, J = 19), 2.09 (s, 3H, Me).

^{13}C NMR (50.32 MHz, DMSO-d_6): δ = 152.6 (C=O), 149.9 (C-3), 145.2 (C=N), 133.5, 130.2, 130.1, 129.9, 128.7, 127.0, 126.7, 126.1, 125.5, 124.3 (10C, Ar), 122.4 (q, ^1J = 285, CF_3), 90.7 (q, ^2J = 33, C-5), 48.0 (C-4), 15.2 (Me).

Anal. Calc. for $C_{17}H_{15}F_3N_4O_2$ (364.32): C, 56.04; H, 4.15; N, 15.38%.

Found: C, 56.13; H, 4.15; N, 15.30%.

*(E)-N′-(Furan-2-ylmethylene)-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4aj**). White solid; yield 61%; mp 166–167 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 10.76 (s, 1H, NH), 8.28 (s, 1H, CH), 7.78 (s, 1H, Fur), 7.60 (s, 1H, OH), 6.74 (s, 1H, Fur), 6.59 (s, 1H, Fur), 3.42 (d, 1H, H-4, J = 19), 3.06 (d, 1H, H-4, J = 19), 2.05 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 152.6 (C=O), 149.9 (C-3), 149.8 (C=N), 144.4, 135.5, 112.0, 111.7, (4C, Fur), 123.4 (q, ^1J = 285, CF_3), 90.6 (q, ^2J = 33, C-5), 47.9 (C-4), 15.1 (Me).

Anal. Calc. for $C_{11}H_{11}F_3N_4O_3$ (304.22): C, 43.43; H, 3.64; N, 18.42%.

Found: C, 43.41; H, 3.39; N, 18.39%.

*(E)-N′-[(1-Phenyl)ethyliden-1-yl]-(5-trifluoromethyl-5-hydroxy-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4ak**). White solid; yield 62%; mp 130–132 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 9.39 (s, 1H, NH), 7.81–7.78 (m, 2H, Ar), 7.77 (s, 1H, OH), 7.40 (s, 3H, Ar), 3.48 (d, 1H, H-4, J = 19), 3.09 (d, 1H, H-4, J = 19), 2.23 (s, 3H, Me), 2.05 (s, 3H, Me).

^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 152.7 (C=O), 149.8 (C-3), 149.4 (C=N), 137.9, 128.8, 128.1, 125.9 (6C, Ar), 123.1 (q, ^1J = 285, CF_3), 90.8 (q, ^2J = 34, C-5); 47.8 (C-4); 14.7 (Me); 12.8 (Me).

Anal. Calc. for $C_{14}H_{15}F_3N_4O_2$ (328.29): C, 51.22; H, 4.61; N, 17.07%.

Found: C, 51.20; H, 4.62; N, 17.01%.

*(E)-N′-(4-Methylbenzylidene)-(5-trifluoromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4bf**). Yellow solid; yield 52%; mp 173–175 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 10.80 (s, 1H, NH), 8.43 (s, 1H, CH), 7.95 (s, 1H, OH), 7.97–7.92 (m, 2H, Ar), 7.57 (d, 2H, ^3J = 8, Ar), 7.52–7.49 (m, 3H, Ar), 7.26 (d, 2H, Ar, J = 8), 3.89 (d, 1H, H-4, J = 19), 3.56 (d, 1H, H-4, J = 19), 2.34 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 150.1 (C=O), 149.5 (C-3), 145.9 (C=N), 139.3, 132.0, 130.5, 130.2, 128.6, 126.9, 126.6 (12 C, Ar), 123.3 (q, ^1J = 285, CF_3), 91.4 (q, ^2J = 34, C-5), 44.4 (C-4), 20.9 (Me).

Anal. Calc. for $C_{19}H_{17}F_3N_4O_2$ (390.36): C, 58.46; H, 4.39; N, 14.35%.

Found: C, 58.61; H, 4.38; N, 14.19%.

*(E)-N′-(4-Methoxybenzylidene)-(5-trifluoromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4bg**). Yellow solid; yield 53%; mp 185–186 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.71 (s, 1H, NH), 8.40 (s, 1H, CH), 7.96–7.93 (m, 2H, Ar), 7.91 (s, 1H, OH), 7.62 (d, 2H, Ar, J = 8), 7.51–7.49 (m, 3H, Ar), 7.02 (d, 2H, Ar, J = 8), 3.87 (d, 1H, H-4, J = 19), 3.81 (s, 3H, OMe), 3.56 (d, 1H, H-4, J = 19).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 151.7 (C=O), 149.9 (C-3), 149.4 (C=N), 160.2, 130.4, 130.1, 128.6, 128.2, 127.2, 126.2, 114.0 (12 C, Ar), 123.2 (q, ^1J = 285, CF_3), 92.1 (q, ^2J = 33, C-5), 55.1 (OMe), 43.7 (C-4).

Anal. Calc. for $C_{19}H_{17}F_3N_4O_3{\cdot}H_2O$ (424.37): C, 53.77; H, 4.51; N, 13.20%.

Found: C, 53.56; H, 4.54; N, 13.11%.

*(E)-N′-(Furan-2-ylmethylene)-(5-trifluoromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)carbohydrazide (**4bj**). White solid; yield 67%; mp 142–143 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 11.39 (s, 1H, NH), 8.18 (s, 1H, CH), 8.13 (d, 1H, Fur, J = 2), 7.88–7.83 (m, 2H, Ar), 7.60–7.56 (m, 3H, Ar), 7.46 (s, 1H, OH), 7.06 (d, 1H, Fur, J = 3), 6.83 (q, 1H, Fur, J = 2), 3.98 (d, 1H, H-4, J = 19), 3.64 (d, 1H, H-4, J = 19).

^{13}C NMR (50.32 MHz, DMSO-d_6): δ = 150.9 (C=O), 148.9 (C-3), 148.2 (C=N), 145.2, 130.7, 116.0, 112.5, (4C, Fur), 129.8, 129.4, 129.1, 126.4 (6C, Ar), 123.1 (q, ^1J = 285, CF_3), 91.2 (q, ^2J = 34, C-5), 44.7 (C-4).

Anal. Calc. for $C_{16}H_{13}F_3N_4O_3$ (366.29): C, 52.46; H, 3.58; N, 15.30%.

Found: C, 52.24; H, 3.31; N, 15.73%.

*(E)-N′-(4-Chlorobenzylidene)-[5-trifluoromethyl-5-hydroxy-3-(methylphenyl)-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (**4ce**). White solid; yield 61%; mp 185–186 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 10.93 (s, 1H, NH), 8.48 (s, 1H, CH), 7.98 (s, 1H, OH), 7.85 (d, 2H, Ar, J = 8), 7.71 (d, 2H, Ar, J = 8), 7.52 (d, 2H, Ar, J = 8), 7.32 (d, 2H, Ar, J = 8), 3.87 (d, 1H, H-4, J = 19), 3.56 (d, 1H, H-4, J = 19), 2.38 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 151.9 (C=O), 150.2 (C-3), 149.4 (C=N), 144.4, 140.4, 133.9, 129.2, 128.8, 128.7, 127.5, 126.8, (12 C, Ar), 123.4 (q, ^1J = 285, CF_3), 91.2 (q, ^2J = 33, C-5), 44.4 (C-4), 21.0 (Me).

Anal. Calc. for $C_{19}H_{16}ClF_3N_4O_2$ (424.80): C, 53.72; H, 3.80; N, 13.19%.

Found: C, 53.73; H, 3.75; N, 13.26%.

*(E)-N′-(4-Methylbenzylidene)-[5-trifluoromethyl-3-(2-furyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (**4df**). Yellow solid; yield 70%; mp 180–181 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.69 (s, 1H, NH), 8.41 (s, 1H, CH), 7.95 (s, 1H, OH), 7.93 (q, 1H, Fur, J = 2), 7.54 (d, 2H, Ar, J = 8), 7.25 (d, 2H, Ar, J = 8), 7.08 (dd, 1H, Fur, J = 1), 6.70 (q, 1H, Fur, J = 2), 3.79 (d, 1H, H-4, J = 19), 3.45 (d, 1H, H-4, J = 19), 2.34 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 149.5 (C=O), 146.0 (C-3), 145.5 (C=N), 145.3, 131.9, 114.1, 112.2 (4 C, Fur), 141.7, 139.2, 129.3, 126.6 (6C, Ar), 123.2 (q, ^1J = 285, CF_3), 90.65 (q, ^2J = 33, C-5), 44.2 (C-4), 20.9 (Me).

Anal. Calc. for $C_{17}H_{15}F_3N_4O_3$ (380.32): C, 53.69; H, 3.98; N, 14.73%.

Found: C, 53.61; H, 3.34; N, 14.90%.

*(E)-N′-(1,3-Dioxobenzyliden-5-yl)-[5-trifluoromethyl-3-(2-furyl)-5-hydroxy-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (**4dh**). Yellow solid; yield 65%; mp 177–178 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 10.71 (s, 1H, NH), 8.36 (s, 1H, CH), 8.00 (s, 1H, Ar), 7.94 (d, 1H, Fur, J = 1), 7.23 (s, 1H, OH), 7.09 (d, 1H, Fur, J = 3), 7.05 (s, 1H, Ar), 7.00 (s, 1H, Ar), 6.71 (q, 1H, Fur, J = 2), 6.09 (s, 2H, dioxol), 3.80 (d, 1H, H-4, J = 19), 3.49 (d, 1H, H-4, J = 19).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 149.5 (C=O), 148.6 (C-3), 147.9 (C=N), 145.8, 145.5, 145.3, 141.7, 129.1, 122.7, 114.1, 112.2, 108.3, 104.7, 101.4 (11C, Fur, Ar), 123.2 (q, ^1J = 285, CF_3), 90.9 (q, ^2J = 33, C-5), 44.2 (C-4).

Anal. Calc. for $C_{17}H_{13}F_3N_4O_5$ (410.30): C, 49.76; H, 3.19; N, 13.65%.

Found: C, 49.71; H, 3.18; N, 13.73%.

*(E)-N′-(Benzylidene)-[5-trifluoromethyl-5-hydroxy-3-(2-thienyl)-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (**4ea**). White solid; yield 62%; mp 113–115 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.60 (s, 1H, NH), 8.46 (s, 1H, CH), 7.87 (s, 1H, OH), 7.77 (d, 1H, Thienyl, J = 5), 7.44–7.39 (m, 3H, Ar), 7.67 (d, 2H, Ar, J = 7), 7.57 (d, 1H, Thienyl, J = 3), 7.19–7.17 (m, 1H, Thienyl), 3.88 (d, 1H, H-4, J = 19), 3.57 (d, 1H, H-4, J = 19).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 149.3 (C=O), 146.1 (C-3), 145.9 (C=N), 134.5, 130.3, 128.5, 126.5 (6C, Ar), 132.9, 129.6, 129.3, 127.7 (4C, Thienyl), 123.0 (q, ^1J = 284, CF_3), 91.2 (q, ^2J = 33, C-5), 44.9 (C-4).

Anal. Calc. for $C_{16}H_{13}F_3N_4O_2S$ (382.36): C, 50.26; H, 3.43; N, 14.65%.

Found: C, 50.28; H, 3.01; N, 14.56%.

*(E)-N′-(4-Methylbenzylidene)-[5-trifluoromethyl-5-hydroxy-3-(2-thienyl)-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (**4ef**). White solid; yield 73%; mp 182–183 °C.*

^{1}H NMR (400.13 MHz, DMSO-d_6): δ = 10.63 (s, 1H, NH), 8.41 (s, 1H, CH), 7.95 (s, 1H, OH), 7.93 (dd, 1H, Thienyl, J = 1), 7.54 (d, 2H, Ar, J = 8), 7.25 (d, 2H, Ar, J = 8), 7.09 (d, 1H, Thienyl, J = 3,4), 6.71 (q, 1H, Thienyl, J = 1), 3.88 (d, 1H, H-4, J = 19), 3.56 (d, 1H, H-4, J = 19), 2.34 (s, 3H, Me).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 149.4 (C=O), 146.2 (C-3), 145.9 (C=N), 139.2, 131.9, 130.6, 126.6 (6 C, Ar), 133.0, 129.9, 129.3, 127.9, (4 C, Thienyl), 123.2 (q, ^1J = 286, CF_3), 91.3 (q, ^2J = 33, C-5), 45.1 (C-4), 20.9 (Me).

Anal. Calc. for $C_{17}H_{15}F_3N_4O_2S$ (396.39): C, 51.51; H, 3.81; N, 14.13%.

Found: C, 51.42; H, 3.74; N, 14.09%.

*(E)-N′-(3-Nitrobenzylidene)-[5-trifluoromethyl-5-hydroxy-3-(1-naphthyl)-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (**4fb**). Yellow solid; yield 96%; mp 194–196 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 11.15 (s, 1H, NH), 9.03 (d, 1H, Ar, J = 8), 8.59 (s, 1H, CH), 8.52 (s, 1H, Ar), 8.27–8.22 (m, 2H, Ar), 8.12 (s, 1H, Ar), 8.08 (s, 1H, OH), 7.91 (d, 1H, Ar, J = 7), 7.79 (s, 1H, Ar), 7.75 (s, 1H, Ar), 7.71–7.59 (m, 3H, Ar), 4.15 (d, 1H, H-4, J = 19), 3.78 (d, 1H, H-4, J = 19).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 151.2 (C=O), 149.5 (C-3), 148.1 (C=N), 143.6, 136.4, 133.3, 132.7, 130.7, 130.2, 129.7, 128.5, 127.5, 126.7, 126.1, 124.8, 123.5, 120.3 (16C, Ar), 122.9 (q, ^1J = 285, CF_3), 90.4 (q, ^2J = 33, C-5), 47.0 (C-4).

Anal. Calc. for $C_{22}H_{16}F_3N_5O_2$ (471.39): C, 56.05; H, 3.42; N, 14.86%.

Found: C, 56.01; H, 3.41; N, 14.94%.

*(E)-N′-(4-Chlorobenzylidene)-[5-trifluoromethyl-5-hydroxy-3-(1-naphthyl)-4,5-dihydro-1H-pyrazol-1-yl]carbohydrazide (***4fe***). Yellow solid; yield 75%; mp 171–173 °C.*

^{1}H NMR (200.13 MHz, DMSO-d_6): δ = 11.30 (s, 1H, NH), 8.98 (d, 1H, Ar, J = 8), 8.88 (s, 1H, CH), 8.08 (s, 1H, OH), 8.04–7.99 (m, 2H, Ar), 7.88 (s, 1H, Ar), 7.73–7.62 (m, 3H, Ar), 7.54–7.40 (m, 4H, Ar), 4.13 (d, 1H, H-4, J = 19), 3.76 (d, 1H, H-4, J = 19).

^{13}C NMR (100.61 MHz, DMSO-d_6): δ = 151.0 (C=O), 149.7 (C-3), 142.0 (C=N), 133.3, 132.6, 131.9, 130.6, 129.7, 129.5, 128.3, 128.2, 127.4, 127.2, 126.9, 126.5, 126.1, 124.8 (16C, Ar), 123.1 (q, ^1J = 285, CF_3), 90.4 (q, ^2J = 33, C-5), 47.1 (C-4).

Anal. Calc. for $C_{22}H_{16}ClF_3N_4O_2$ (460.83): C, 57.34; H, 3.50; N, 12.16%.

Found: C, 57.31; H, 3.48; N, 12.07%.

3.2. X-ray crystallography/X-ray diffraction data

X-ray data were collected on a Bruker SMART CCD diffractometer [19]. Data were collected using graphite-monochromatized Mo Kα radiation with λ = 0.71073 Å. The crystallographic structures of the 4,5-dihydropyrazoles (**4aa**, **4ae**, **4af**, **4df**) were solved by direct methods (SHELXS-97) [20]. Refinements were carried out with the SHELXL97 package [21]. The absorption correction was performed by Gaussian methods [22]. All refinements were made by full-matrix least-squares on F2 with anisotropic displacement parameters for all non-hydrogen atoms. Hydrogen atoms were included in the refinement in calculated positions with C–H distances of 0.93 Å (aromatic CH), 0.97 Å (methylene CH2), 0.98 Å (methine CH), 0.86 (NH) and 0.82 Å (OH) using a riding model. The hydrogen isotropic thermal parameters were kept equal to Uiso(H) c 1.5 Ueq for Csp3, and 1.2 for Csp2. Molecular graphics were prepared using ORTEP3 for Windows [23]. The crystal data and details concerning data collection and structure refinement are given in Table 6. Crystallographic data for the structure of **4aa**, **4ae**, **4af** and **4df** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre and allocated the deposition number CCDC **762876**, **787517**, **762875** and **762877**, 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 deposit@ccdc.com.ac.uk).

3.3. Antioxidant activity

3.3.1. DPPH assay on TLC (screening)

The compound was measured from the bleaching of the purple-colored methanol solution of 2,2-diphenylpicrylhydrazyl (DPPH). Samples at the concentrations of 10, 25, 50, 100 and 200 μg/mL were applied to the TLC plates (aluminum sheets covered with silica gel 60 F_{254}, Merck). The plate was sprayed with a 0.2% DPPH reagent in methanol and left at room temperature for 30 min using

Table 6
General and crystal data and summary of intensity data collection and structure refinement for compounds.

Compound	**4aa**	**4af**	**4df**	**4ae**
Formula	$C_{26}H_{26}F_6N_8O_4$	$C_{14}H_{15}F_3N_4O_2$	$C_{17}H_{15}F_3N_4O_3$	$C_{13}H_{14}ClF_3N_4O_3$
Mr	628.55	328.30	380.33	366.73
CCDC				
Temperature (K)	293(2)	293(2)	293(2)	296(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$	$P2(1)$	$P2_1/c$
Unit cell parameters				
a (Å)	8.1603(2)	13.6533(4)	6.057(5)	9.6176(15)
b (Å)	15.1323(5)	14.1863(5)	9.272(5)	8.2904(13)
c (Å)	23.7584(9)	8.1226(2)	15.708(5)	19.471(3)
α (°)	90	90	90	90
β (°)	90	95.623(2)	99.941(5)	94.365(9)
γ (°)	90	90	90	90
V (Å^3)	2933.65(16)	1565.69(8)	868.9(9)	1548.0(4)
Z	4	4	2	4
Density (calculated) (g cm^{-3})	1.423	1.393	1.454	1.574
Absorption coefficient (mm^{-1})	0.124	0.120	0.123	0.301
F(000)	1296	680	392	752
Crystal size (mm)	0.585 × 0.190 × 0.088	0.331 × 0.156 × 0.061	0.220 × 0.210 × 0.034	0.20 × 0.16 × 0.15
θ range for data collection (°)	1.60–28.33	1.50–26.08	3.41–27.18	2.10–26.82
h, k, l range	$-10 \le h \le 10$	$-16 \le h \le 16$	$-7 \le h \le 7$	$-12 \le h \le 12$
	$-20 \le k \le 20$	$-17 \le k \le 17$	$-11 \le k \le 11$	$-8 \le k \le 10$
	$-31 \le l \le 31$	$-10 \le l \le 10$	$-20 \le l \le 20$	$-22 \le l \le 24$
Reflections collected	30,226	13,349	7820	12,912
Independent reflections	7294 [R(int) = 0.0515]	3089 [R(int) = 0.0539]	3798 [R(int) = 0.0369]	3302 [R(int) = 0.0303]
Data/restraints/parameters	7294/0/405	3089/0/217	3798/1/246	3302/1/217
Absorption correction	Gaussian	Gaussian	Gaussian	Gaussian
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0486, wR2 = 0.1222	R1 = 0.0556, wR2 = 0.1425	R1 = 0.0513, wR2 = 0.1097	R1 = 0.0394, wR2 = 0.1050
R indices (all data)	R1 = 0.1017, wR2 = 0.1567	R1 = 0.1071, wR2 = 0.1826	R1 = 0.1151, wR2 = 0.1352	R1 = 0.0478, wR2 = 0.1115
Goodness of fit on F^2	1.007	0.976	0.963	1.036
Largest diff. peak and hole (eÅ^{-3})	0.379 and −0.420	0.366 and −0.317	0.163 and −0.173	0.436 and −0.492

as standard antioxidant, quercetin, at the concentration of 2 μg/mL. Yellow spots formed from bleaching of the purple color of DPPH reagent were evaluated as positive antioxidant activity [24].

3.3.2. Spectrophotometric DPPH assay

The DPPH radical scavenging model is extensively used to evaluate antioxidant activities faster than other methods. Radical scavenging activity of solutions at several concentrations of the compounds **2** and **4** were evaluated against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) spectrophotometrically according to Yen and Chen [6,25], with some modifications. The absorbance decrease was measured at 517 nm on a UV-Visible spectrophotometer.

For the quantitative evaluation of antioxidant capacity, samples and standard BHT (Butylatedhydroxytoluene) were dissolved in ethanol (3 mg/mL). To the 2 mL of ethanol was added 1 mL of diluted sample. A serial dilution was performed resulting in concentrations ranging from 250 μg/mL to 7.81 μg/mL. An amount of 0.5 mL of methanol solution of DPPH 0.004% was added to each sample. After 30 min of incubation at room temperature away from light, the reduction of the DPPH free radical was obtained by reading the absorbance against a blank in each specific assess met, including samples in their respective dilutions. As a control (0% inhibition), a solution of 2 mL of ethanol and 0.5 mL of methanol of DPPH 0.004% solution was used. BHT was used as a positive control. The experiment was carried out in duplicate. Radical scavenging activity was calculated as follows:

$$\%Inhibition = \left[\frac{Absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control}\right] \times 100$$

The determination of the IC_{50} or concentration of sample or pattern that causes 50% inhibition of DPPH was obtained by linear regression from the points plotted by the average percentages obtained.

3.3.3. Antimicrobial testing (MIC)

The in vitro antimicrobial activity of the compounds **2** and **4** was assessed against a collection of microrganisms including two yeasts *C. albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC 2601 and six bacteria *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Klebsiellapneumoniae* ATCC 13883 and *Streptococcus pyogenes* ATCC 19615. Standard microorganism strains were obtained from American Type Culture Collection (ATCC), and standard antibiotics chloramphenicol and nistatine were used in order to control microbial test sensitivity [26,27]. MIC as well as minimal fungicidal and bactericidal concentrations were determined by broth microdilution methods according to modified Hammer et al. [26] and NCCLS standards [28–30].

Compounds from the series **2** and **4** were dissolved in DMSO/EtOH (2.5 mg/mL) and the solutions were diluted with medium. By further progressive dilutions with test medium the required concentrations (0.5, 0.25, 0.125, 0.062, 0.031, 0.015 mg/mL) were obtained.

The MIC was determined on 96 well culture plates by a micro dilution method using a microorganism suspension with a density of 10^5 CFU/mL in Casein Soy Broth (CSB) incubated for 24 h at 35 °C ± 1 °C for bacteria, and Sabouraud Broth (SB) incubated for 48 h at 25 °C ± 1 °C for yeasts. The cultures that did not present growth were used to inoculate solid medium plates (Muller Hinton Agar and Sabouraud Agar) in order to determine the Minimal Lethal Concentration (MLC). Proper blanks were assayed simultaneously and samples were tested in duplicate.

4. Conclusions

In summary, we have demonstrated the facile and convenient synthesis and a crystal structure study of a new series of twenty-one trifluoromethyl-containing (*E*)-*N*′-arylidene-1*H*-pyrazole-1-carbohydrazides (**4**) by the cyclocondensation reactions of carbohydrazides (**2**) with aryl/heteroaryl aldehydes and acetophenone in 52–97% yields. The carbohydrazides **2** were easily obtained from the reaction of 1,1,1-trifluoro-4-alkyl(aryl/heteroaryl)-4-methoxy-3-alken-2-ones (**1**) with carbohydrazide. Subsequently, the twenty-seven pyrazolyl-carbohydrazides **2** and **4** were screened for their antioxidant and antimicrobial proprieties and evaluated by DPPH and MIC methods. Both series 2 and 4 showed ability to capture DPPH free radical for IC_{50} from 47.57 to 487 μg/mL but presented weak fungistatic and bacteriostatic activity for high MIC levels ranging from 0.25 mg/mL to 0.5 mg/mL.

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References

[1] J.C. Sloopa, C.L. Bumgardnerb, W.D. Loehlea, J. Fluorine Chem. 118 (2002) 135.
[2] H.G. Bonacorso, A.P. Wentz, R.V. Lourega, C.A. Cechinel, T.S. Moraes, H.S. Coellho, N. Zanatta, M.A.P. Martins, M. Höerner, S.H. Alves, J. Fluorine Chem. 127 (2006) 1006.
[3] H.G. Bonacorso, A.D. Wastowski, N. Zanatta, M.A.P. Martins, J.A. Naue, J. Fluorine Chem. 92 (1998) 23.
[4] R.B. Oliveira, E.M.S. Fagundes, R.P.P. Soares, A.A. Andrade, A.U. Krettli, C.L. Zani, Eur. J. Med. Chem. 43 (2008) 1983.
[5] (a) L.T.S. Rocha, K.A. Costa, C.P. Oliveira, E.B. Nascimento Jr., C.M. Bertollo, F. Araújo, L.R. Teixeira, S.P. Andrade, H. Beraldo, M.M. Coelho, Life Sci. 79 (2006) 499; (b) T. Hemalatha, P.K.M. Imran, A. Gnanamani, S. Nagarajan, Nitric Oxide 19 (2008) 303.
[6] V. Gressler, S. Moura, A.F.C. Flores, D.C. Flores, P. Colepicolo, E. Pinto, J. Braz. Chem. Soc. 21 (2010) 1477.
[7] X. Du, G. Chum, E. Hansell, P.S. Doyle, C.R. Caffrey, T.P. Holler, J.H. McKerrow, F. Cohen, Eur. J. Med. Chem. 45 (2002) 2695.
[8] A. Shafiee, A. Rineh, A. Kebriaeezadeh, A. Foroumadi, V. Sheibani, M.R. Afarinesh, Med. Chem. Res. 18 (2009) 758.
[9] H.G. Bonacorso, C.A. Cechinel, E.D. Deon, R.C. Sehnem, F.M. Luz, M.A.P. Martins, N. Zanatta, N. ARKIVOC ii, 2009, p. 174.
[10] (a) F. Effenberger, R. Maier, K.H. Schonwalder, T. Ziegler, Chem. Ber. 115 (1982) 2766;
(b) F. Effenberger, K.H. Schonwalder, Chem. Ber. 117 (1984) 3270;
(c) M. Hojo, R. Masuda, E. Okada, Synthesis (1986) 1013;
(d) M. Hojo, R. Masuda, S. Sakaguchi, M. Takagawa, Synthesis (1986) 1016;
(e) G.M. Siqueira, A.F.C. Flores, G. Clar, N. Zanatta, M.A.P. Martins, Quim. Nova 17 (1994) 24;
(f) A.F.C. Flores, S. Brondani, N. Zanatta, A. Rosa, A.M.P. Martins, Tetrahedron Lett. 43 (2002) 8701;
(g) H.G. Bonacorso, M.B. Costa, S. Moura, L. Pizzuti, M.A.P. Martins, N. Zanatta, A.F.C. Flores, J. Fluorine Chem. 126 (2005) 1396;
(h) H.G. Bonacorso, C.A. Cechinel, M.B. Costa, M.R. Oliveira, M.A.P. Martins, N. Zanatta, A.F.C. Flores, J. Heterocycl. Chem. 42 (2005) 1055;
(i) M.A.P. Martins, E. Guarda, C.P. Frizzo, E. Scapin, P. Beck, A.C. Costa, N. Zanatta, H.G. Bonacorso, J. Mol. Catal. A: Chem. 266 (2007) 100.
[11] M.A.P. Martins, D.N. Moreira, C.P. Frizzo, P.T. Campos, K. Longhi, M.R.B. Marzari, N. Zanatta, H.G. Bonacorso, J. Mol. Struct. 969 (2010) 111.
[12] S.M.S.V. Wardell, M.V.N. de Souza, J.L. Wardell, J.N. Low, C. Glidewell, Acta Crystallogr., Sect. B: Struct. Sci. 63 (2007) 879.
[13] P. Machado, P.T. Campos, G.R. Lima, F.A. Rosa, A.F.C. Flores, H.G. Bonacorso, N. Zanatta, M.A.P. Martins, J. Mol. Struct. 917 (2009) 176.
[14] (a) G.R. Desiraju, Acc. Chem. Res. 35 (2002) 565;
(b) T. Steiner, Angew. Chem. Int. Ed. 41 (2002) 48.
[15] P. Pyykko, Chem. Rev. 97 (1997) 597.
[16] I.A. Vladimirov, Vestn. Ross. Akad. Med. Nauk. 7 (1998) 43.
[17] (a) H.W. Boucher, G.H. Talbot, J.S. Bradley, J.E. Edwards, D. Gilbert, L.B. Rice, M. Sched, B. Spellberg, J. Bartlett, Clin. Infect. Dis. 48 (2009) 1;
(b) R.C. Moellering, Int. J. Antimicrob. Agents 37 (2011) 2.

[18] T. Kelesidis, I. Kelesidis, P.I. Rafailidis, M.E. Falagas, Antimicrob. J. Chemother. 60 (2007) 214.
[19] Bruker, APEX2 (Version 2.1), COSMO (Version 1.56), BIS (Version 2.0.1.9), SAINT (Version 7.3A), SADABS (Version 2004/1) and XPREP (Version 2005/4), Bruker AXS Inc., Madison, WI, USA, 2006.
[20] (a) G.M. Sheldrick, SHELXS-97, Program for Crystal Structure Solution, University of Göttingen, Germany, 1997.
(b) M. Ozgen, R.N. Reese, A.Z. Tulio Jr., J.C. Scheerens, A.R. Miller, Agric. J. Food. Chem. 54 (2006) 1151.
[21] G.M. Sheldrick, SHELXL-97, Program for Crystal Structure Refinement, University of Göttingen, Germany, 1997.
[22] P. Coppens, L. Leiserowitz, D. Rabinovich, Acta Crystallogr. 18 (1965) 1035.
[23] L.J. Farrugia, J. Appl. Crystallogr. 30 (1997) 565.
[24] (a) C. Bicchi, A. D'Amato, P. Rubiolo, J. Chromatogr. 99 (1999) 843;
(b) E. Simionatto, Z.C. Stüker, J. Braz. Chem. Soc. 18 (2007) 879.
[25] G.C. Yen, H.Y. Chen, J. Agric. Food Chem. 43 (1995) 27.
[26] K.A. Hammer, C.F. Carson, T.V. Riley, J. Appl. Microbiol. 86 (1999) 985.
[27] E. Simionatto, C. Porto, Z.C. Stüker, Quim. Nova 30 (2007) 1923.
[28] NCCLS, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, NCCLS Document M27-A2, National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 2002.
[29] NCCLS, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard, 5th ed., NCCLS Document M7-A5, National Committee for Clinical Laboratory Standards, Wayne, PA, USA, 2002.
[30] S. Burt, Int. J. Food Microbiol. 94 (2004) 223.