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Convergent synthesis and cruzain inhibitory activity of novel 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines

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ABSTRACT

To search for new cruzain inhibitors, the synthesis of a series of novel 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines in a convergent manner is presented. The cruzain inhibitory activity of some of these compounds was evaluated and a binding model was proposed. All derivatives tested were active and the most significant inhibitory effect (80% at 100 μM) and IC_{50} value (85 μM) were obtained from the 2-(*N*′-4-chloro-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine. Although further structural optimization to improve solubility is necessary, the molecular docking studies suggest that these inhibitors occupy the S2 pocket without irreversible enzyme inactivation, through hydrophobic interactions, thus, indicating a desirable mode of interaction for the design of novel inhibitors.

1. Introduction

The development of new strategies for synthesizing trifluoromethylated heterocycles has received much attention recently, because the presence of halogenated groups is often associated with improvements in pharmacological properties of organic molecules.[1–3] 4-Alkoxyvinyl trifluoromethyl ketones are appealing building blocks for the synthesis of trifluoromethyl containing heterocycles, since they are versatile reagents readily available from the acylation of enol ethers or acetals.[4]

Among the great variety of existing heterocycles, the pyrimidine core stands out due to its broad spectrum of interesting biological activities. These compounds have been widely used in the fields of agriculture[5], microbiology[6–8] and medicine[9–11] mainly because of their marked growth-regulating proprieties.[12–14]

More specifically, *N*-benzylidenehydrazino pyrimidines are extremely interesting since the presence of azo groups (diazenes) in organic compounds is often associated with a wide range of technological and medicinal applications.[15] They have already been reported as coronary vasodilators,[16] xantine oxidase inhibitors[17] and have been proven to possess analgesic, anti-inflammatory,[18] antimicrobial, anti-HIV, antitumor[19] and antimalarial[20] activities.

N-Benzylidenehydrazino pyrimidines are structurally related to classes of compounds that have been reported to inhibit the growth of *Trypanosoma cruzi*,[21] and have a close connection with their potent inhibitory effects upon cruzain activity.[22–24] Cruzain, the major cysteine protease of *T. cruzi*, is an essential enzyme for this parasite and an attractive target for antitrypanosomal chemotherapy.[21] Chagas' disease is a tropical infectious disease widely distributed throughout Latin America, with devastating consequences in terms of human morbidity and mortality. In spite of the alarming health, economic, and social consequences of these parasitic infections, the limited existing drug therapy (which consists of two nitro-heterocyclic drugs—nifurtimox and benznidazole) suffers from a combination of drawbacks including poor efficacy, high toxicity, and serious side effects. Hence, there is an urgent need for new drugs for chemotherapy of the disease.[22] In this context, the search for novel potent inhibitors of cruzain, which can effectively cause the death of the parasite, is of great importance.[25] Thiosemicarbazide (**A**), chalcone (**B**) and acylhydrazide derivatives (**C**) (Fig. 1) are among the most studied classes of cruzain inhibitors. As part of our research program aimed at discovering novel *T. cruzi* cruzain inhibitors, we synthesized a series of novel 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines in a convergent method. The cruzain inhibitory activity of some of these compounds was evaluated and a binding model was proposed.

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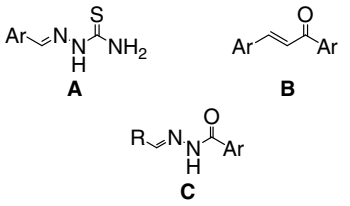


Figure 1. General structures of thiosemicarbazide (A), chalcone (B) and acylhydrazide (C) derivatives as potent inhibitors of *T. cruzi* cruzain.

The synthesis of 2-(*N*′-benzylidenehydrazino)pyrimidines usually requires the condensation of 2-hydrazino pyrimidines with different aldehydes or ketones[26–28] or the direct cyclocondensation between *N*-guanidinobenzylimines and cumalinaldehyde.[29]

In a previous publication of our research group, the synthesis of new 2-hydrazino-4-methyl-6-trifluoro(chloro)methylpyrimidines used as precursors to a series of trifluoro(chloro)methylated 2-(5-hydroxy-5-trifluoro(chloro)methyl-4,5-dihydro-1*H*-pyrazol-1-yl]pyrimidines was reported.[30] This method relied on the preparation of 2-methylsulfanyl-pyrimidines, followed by oxidation of the 2-methylthio group to 2-methylsulfone, which underwent nucleophilic displacement with hydrazine hydrate to furnish the respective 2-hydrazino-pyrimidine. Although this strategy was successful, it required several reaction steps, presented moderate global yield, and promoted the evolution of methyl mercaptans and other sulfurous gases in the atmosphere. Furthermore, in order to access the desired 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines, an additional step involving the condensation of the 2-hydrazino-pyrimidines with an aldehyde or ketone would be required. Thus, the possibility of obtaining some novel 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines by a convergent method employing a simple, efficient, and an *one–pot* procedure is highly desired.

2. Chemistry

Scheme 1 outlines the synthesis of a series of novel 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines (**5–7a–i**), in good to excellent yields from the cyclocondensation reaction between *N*-guanidinobenzylimines **1a–i** and enones **2–4**. All reaction products were fully characterized by ^{1}H and ^{13}C NMR, mass spectra, and elemental analysis. Additionally, the crystal structure of compounds **5a** and **6b** were determined by single-crystal X-ray diffraction.[31]

3. Biology

3.1. Inhibition assays

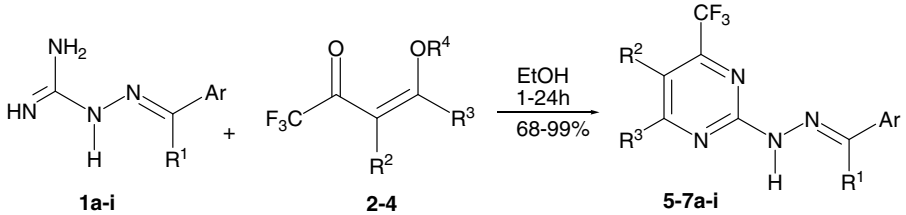
The cruzain inhibitory activity of some of the 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines **5–7a–i** was evaluated. The activity of the enzyme was quantified through active-site titration with the irreversible inhibitor E-64, as described previously.[32] Cruzain (0.64 nM) was incubated in 50 mM sodium phosphate, 100 mM sodium chloride, 5 mM EDTA, pH 6.5, containing 5 mM DTT (buffer A), for 5 min at room temperature. Then 100 μM inhibitor was added and after 5 min the buffer A containing a fluorogenic substrate *Z*-Phenyl-Arginine 7-amido-4-methylcoumarin hydrochloride (*Z*-Phe-Arg-AMC) (K_M = 1.8 μM) was added to give 10 μM substrate, and the increase in fluorescence (excitation at 380 nM and emission at 460 nM) was carried out in a 96 well-microplate spectrofluorometer, monitored for 5 min at 30 °C in a Wallac 1420-042 PerkinElmer spectrofluorometer. The final assay volume was 200 μL and the final DMSO concentration was 2.5%. At this concentration, DMSO did not significantly affect the activity of cruzain. Inhibitor stock solutions were prepared at 10 mM in DMSO. Controls were performed using enzyme alone and enzyme with DMSO. IC_{50} values were independently determined by making rate measurements for at least six inhibitor concentrations. The values represent means of at least three individual experiments and were estimated from the data collected employing the SigmaPlot enzyme kinetics module.

4. Results and discussion

4.1. Chemistry

Although the synthetic methodology employed by us has previously been described by Kvita et al.,[29] the 4-alcoxyvinyl-trifluoromethyl ketones **2–4** have never been used as precursors to these compounds before.

Reaction times were between 1 and 24 h depending on the 4-alkoxyvinyl ketone employed. All reactions were performed at room temperature, except when the *N*-guanidinobenzylimine derived from acetophenone (**1i**) was used. These reactions were carried out under reflux for 24 h to give compounds **5i**, **6i**, and **7i**, in excellent yields (Table 1).



Compd.	Ar	R^1
1,5-7a	Ph	H
1,5-7b	2-Me-Ph	H
1,5-7c	2-OH-Ph	H
1,5-7d	2-OMe-Ph	H
1,5-7e	4-Me-Ph	H
1,5-7f	4-Cl-Ph	H
1,5-7g	4-OMe-Ph	H
1,5-7h	4-NO_2-Ph	H
1,5-7i	Ph	Me

Compd.	R^2	R^3	R^4
2	H	H	Et
3	Me	H	Et
4	H	Me	Me

Compd.	R^2	R^3
5	H	H
6	Me	H
7	H	Me

Scheme 1.

NMR (^{1}H and ^{13}C) and GC-MS data showed that most pyrimidines (**5**–**7a**–**i**) were isolated as one diastereoisomer with very similar spectral profiles. In order to unequivocally assign the stereochemistry of the compounds, the crystal structures of derivatives **5a** and **6b** were determined by single-crystal X-ray analysis.[31] Crystals suitable for X-ray diffraction were obtained by slow evaporation of dichloromethane/methanol solutions of compounds **5a** and **6b**. For both hydrazones (**5a** and **6b**), the crystallographic structures exhibited an *E* configuration. Thus, all compounds presenting the same spectral pattern as pyrimidines **5a** and **6b** were assigned as *E* isomers by analogy. Some 2-(*N*′-benzylidenehydrazino)-pyrimidines, however, were not isolated as single diastereoisomers (compounds **5d**–**e**, **6d**–**e**, **7c**, and **7f**—Table 1). In order to isolate only the major isomer, successive recrystallizations in a dichloromethane/methanol mixture were carried out. An equally efficient, but faster purification method used, was flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant.

4.2. Cruzain inhibition

The ability of nine *N*-benzylidenehydrazino pyrimidines of the series of compounds **5** and **7** to inhibit the *T. cruzi* cruzain was evaluated at a concentration of 100 μM and the results are shown in Table 2.

From the results obtained, one can see that all the new derivatives tested were active against *T. cruzi* cruzain (20–80% inhibition at 100 μM). The most active compound was **5f** which was able to produce the most significant inhibitory effect (80%) and presented an IC_{50} value of 85 μM. The IC_{50} values correspond to the concentration of the compound required to provide a 50% inhibition of cruzain, and were determined from the collected data by nonlinear regression analysis. Compounds **5h** and **7f** presented IC_{50} values of about 100 μM. The IC_{50} values of the other pyrimidines were not determined due to solubility limitations (IC_{50} values >100 μM for compounds **5b**, **5e**, **5g** and **7b** and >150 μM for compounds **5a** and **7d**).

Table 2
Inhibitory effects and IC_{50} values of a series of synthetic *N*-benzylidenehydrazino pyrimidines derivatives against *T. cruzi* cruzain.

Compound	% Inhibition (100 μM)	IC_{50} (μM)
5a	30	>150
5b	45	>100
5e	40	>100
5f	80	85 ± 7
5g	40	>100
5h	50	≈100
7b	45	>100
7d	20	>150
7f	60	≈100

4.3. Molecular modeling

Structure-based approaches have become a vital part on modern drug design.[33,34] The understanding of protein–ligand interactions is essential for the design of new inhibitors with improved potency.[35] The inhibitory effects of the series of *N*-benzylidenehydrazino pyrimidines on *T. cruzi* cruzain shown in Table 2 allowed us to elucidate the possible binding mode of these inhibitors. Docking protocols as implemented in GOLD[36] 2.1 (Cambridge Crystallographic Data Centre, Cambridge, UK), a genetic algorithm-based software, were employed using inhibitors **5**–**7a**–**i** and the X-ray crystallographic data for *T. cruzi* cruzain retrieved from the Protein Data Bank (PDB, ID code: 1ME4). Figure 2 shows the structure of cruzain in complex with the inhibitor **5f** (colored in yellow), highlighting the amino acid residue of the active site cysteine (Cys25, colored in blue) and the hydrophobic residues of the pocket S2 (colored in violet).

Table 1
Reaction conditions and yield for the synthesis of 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines **(5–7a–i).**

Entry	Reagents[a]	Yield (%)[b]	Product	Reaction conditions	Diasterioisomeric ratio (*E*:*Z*)[c]
1	**1a** + **2**	68	**5a**	EtOH, rt, 1 h	100:0
2	**1b** + **2**	99	**5b**	EtOH, rt, 1 h	100:0
3	**1c** + **2**	98	**5c**	EtOH, rt, 1 h	100:0
4	**1d** + **2**	98	**5d**	EtOH, rt, 1 h	90:10
5	**1e** + **2**	96	**5e**	EtOH, rt, 1 h	90:10
6	**1f** + **2**	91	**5f**	EtOH, rt, 1 h	100:0
7	**1g** + **2**	96	**5g**	EtOH, rt, 1 h	100:0
8	**1h** + **2**	87	**5h**	EtOH, rt, 1 h	100:0
9	**1i** + **2**	98	**5i**	EtOH, reflux, 24 h	100:0
10	**1a** + **3**	98	**6a**	EtOH, rt, 24 h	100:0
11	**1b** + **3**	97	**6b**	EtOH, rt, 24 h	100:0
12	**1c** + **3**	95	**6c**	EtOH, rt, 24 h	100:0
13	**1d** + **3**	95	**6d**	EtOH, rt, 24 h	60:40
14	**1e** + **3**	96	**6e**	EtOH, rt, 24 h	60:40
15	**1f** + **3**	94	**6f**	EtOH, rt, 24 h	100:0
16	**1g** + **3**	94	**6g**	EtOH, rt, 24 h	100:0
17	**1h** + **3**	97	**6h**	EtOH, rt, 24 h	100:0
18	**1i** + **3**	93	**6i**	EtOH, reflux, 24 h	100:0
19	**1a** + **4**	86	**7a**	EtOH, rt, 4 h	100:0
20	**1b** + **4**	88	**7b**	EtOH, rt, 4 h	100:0
21	**1c** + **4**	88	**7c**	EtOH, rt, 4 h	85:15
22	**1d** + **4**	94	**7d**	EtOH, rt, 4 h	100:0
23	**1e** + **4**	92	**7e**	EtOH, rt, 4 h	100:0
24	**1f** + **4**	90	**7f**	EtOH, rt, 4 h	100:0
25	**1g** + **4**	94	**7g**	EtOH, rt, 4 h	85:15
26	**1h** + **4**	78	**7h**	EtOH, rt, 4 h	100:0
27	**1i** + **4**	94	**7i**	EtOH, reflux, 24 h	100:0

[a] Molar ratio 1:1.
[b] Yields of isolated products.
[c] Obtained by 1H NMR integrals.

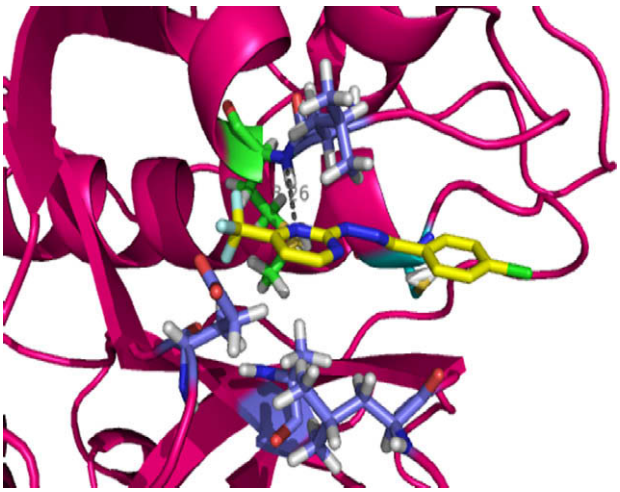


Figure 2. Structure of *T. cruzi* cruzain in complex with inhibitor **5f** (colored in yellow), highlighting the amino acid residues Cys25 (colored in blue), Met68 (colored in green) and the hydrophobic residues of pocket S2 (colored in violet).

The cruzain has binding pockets that can be occupied by non-covalent inhibitors, decreasing its activity.[37] As the S2 pocket is notably large, hydrophobic and electronegative substituents, such as amino, would be preferred.[36] The molecular docking studies indicate that the inhibitors occupy the S2 pocket through hydrophobic interactions with the side chains of Leu67, Ala133, Leu157 and Glu205. (Fig. 3). In addition, favored hydrogen bond interactions were observed between the pyrimidine nitrogen and the amino acid residue Met68 (Fig. 2).

5. Conclusion

This work described the convergent synthesis a novel series of 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidines in a simple and efficient method. The 2-(*N*′-benzylidenehydrazino)-pyrimidines were obtained, in a single step, from the cyclocondensation reaction between *N*-guanidinobenzylimines and 4-alkoxyvinyl-trifluoromethyl ketones in good to excellent yields. Most heterocycles were isolated as a single diastereoisomer (*E* isomers). Some pyrimidines, however, presented a small portion of the *Z* isomer after isolation. This isomer could be separated from the mixture after several recrystallizations or flash chromatography in silica gel. This methodology is extremely simple and allows the construction of a great scope of hydrazones since there are a large number of aldehydes and ketones commercially available. In addition, a significant inhibitory effect was shown by a series of *N*′-benzylidenehydrazino pyrimidines on *T cruzi* cruzain, an important target for the development of new drug candidates against Chagas' disease. Further optimization of these compounds to improve solubility is currently underway, considering the structural requirements for potency. The molecular modeling studies showed the occupation of the S2 pocket, without the event of irreversible enzyme inactivation (that is, covalent bond formation with Cys25). This is a desirable mode of interaction for the design of novel inhibitors having promising use in clinical medicine.

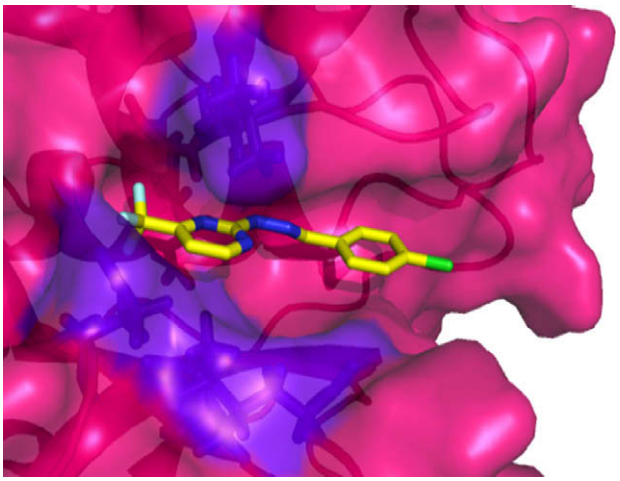


Figure 3. Surface of cruzain in complex with inhibitor **5f** (colored in yellow), highlighting the hydrophobic residues of pocket S2 (colored in violet).

6. Experimental

6.1. Chemistry

Unless otherwise indicated all common reagents and solvents were used as obtained from commercial suppliers without further purification. The synthesis of compounds **1a**–**i**[38] and **2**–**4**[39] are reported in the literature. All melting points were determined on a MQAPF-301 apparatus and are uncorrected. The CHN microanalyses were performed on a Perkin-Elmer 2400 elemental analyzer from the Chemistry Department of the Universidade de São Paulo (USP), São Paulo, SP, Brazil. Mass spectra were registered in a HP 5973 MSD connected to a HP 6890 GC. The GC was equipped with a split–splitless injector, auto-sampler, cross-linked HP-5 capillary column (30 m, 0.32 mm of internal diameter), and helium was used as the carrier gas. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker DPX 200 or DPX 400 spectrometers, 5 mm sample tubes, 298 K, digital resolution ±0.01 ppm, in $CDCl_3$ or DMSO-d_6 using TMS as internal reference. X-ray data were collected on a Bruker SMART CCD diffractometer.[40] The crystallographic structures of the pyrimidines were solved by direct methods (SHELXS-97).[41] Refinements were carried out with the SHELXL-97[42] package.

6.2. Synthesis of 2-(*N*′-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine hydrate (5–7a–i); General procedure

An ethanolic solution of compound **1a** (0.243 g, 1.5 mmol) and **2** (0.252 g, 1.5 mmol) was stirred at room temperature for 1 h (24 h for compound **5**–**7i**). The solvent was evaporated under reduced pressure and the crude solid was dried in desiccator under vacuum. The crude solid was recrystallized from a 10% chloroform/methanol mixture.

6.2.1. 2-(*N*′-Benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5a)

Yield: 68%; mp: 117–120 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.86 (1H, br s), 8.83 (1H, d, J = 4.8 Hz), 8.22 (1H, s), 7.71 (1H, d, J = 7.0 Hz), 7.47–7.40 (3H, m), 7.28 (1H, d, J = 4.8 Hz); ^{13}C NMR (DMSO-d_6/TMS): δ 161.9, 160.0, 154.9 (q, $^{2}J_{C-F}$ = 34.5 Hz), 143.7, 134.60, 129.4, 128.7, 126.6, 124.7, 120.6 (q, $^{1}J_{C-F}$ = 275.3 Hz), 107.7; CG-MS (EI, 70 Ev): m/z(%) = 266 (M^+, 11), 189 (8), 163 (100), 136 (22), 94 (32), 77 (13); Anal. Calcd for $C_{12}H_9F_3N_4.H_2O$ (284.24): C, 50.71; H, 3.90; N, 19.71. Found: C, 50.74; H, 3.56; N 19.90.

6.2.2. 2-(*N*′-2-Methyl-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5b)

Yield: 99%; mp: 120–125 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 8.72 (1H, d, J = 4.9 Hz); 8.25 (1H, s); 7.98 (1H,

d, J = 8.8 Hz); 7.26–7.16 (4H, m); 7.04 (1H, d, J = 4.9 Hz); ^{13}C NMR (DMSO-d_6/TMS): δ 161.9, 160.0, 154.9 (q, $^2J_{C-F}$ = 34.6 Hz), 142.4, 136.6, 132.6, 130.7, 129.1, 126.1, 125.6, 120.6 (q, $^1J_{C-F}$ = 274.8 Hz), 107.6; CG-MS (EI, 70 Ev): m/z(%) = 280 (M^+, 15); 189 (8); 163 (100), 136 (35); 94 (53); Anal. Calcd for $C_{13}H_{11}F_3N_4.H_2O$ (298.27): C, 52.35; H, 4.39; N, 18.78. Found: C, 52.70; H, 4.01; N, 19.12.

6.2.3. 2-(*N*′-2-Hydroxy-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5c)

Yield: 98%; mp: 172–176 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 12.09 (1H, br s), 11.42 (1H, br s), 8.86 (1H, d, J = 4.8 Hz), 8.41 (1H, s), 7.48 (1H, dd, J_1 = 7.6 Hz, J_2 = 1.8 Hz), 7.48 (1H, d, J = 4.8 Hz), 7.30–7.26 (2H, m), 6.96–6.90 (2H, m); ^{13}C NMR (DMSO-d_6/TMS): δ 162.1, 159.5, 154.9 (q, $^2J_{C-F}$ = 35.3 Hz), 144.7, 130.6, 129.4, 120.5 (q, $^1J_{C-F}$ = 274.8 Hz), 119.2, 118.8, 116.3, 108.0; CG-MS (EI, 70 Ev): m/z(%) = 282 (M^+, 37), 265 (15), 189 (7), 163 (100), 136 (35), 94 (59); Anal. Calcd for $C_{12}H_9F_3N_4O$ (282.22): C, 51.07; H, 3.21; N, 19.85. Found: C, 51.03; H, 3.09; N, 19.81.

6.2.4. 2-(*N*′-2-Methoxy-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5d)

The isolation of the major isomer was accomplished after successive crystallizations in a dichloromethane/methanol mixture or by flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant. Yield: 98%; mp: 171–174 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.83 (1H, br s), 8.81 (1H, d, J = 4.9), 8.56 (1H, s), 7.87 (1H, dd, J_1 = 7.5 Hz, J_2 = 1.3 Hz, Ar), 7.39 (1H, td, J_1 = 7.2 Hz, J_2 = 1.5 Hz), 7.25 (1H, d, J = 4.9 Hz), 7.11–6.98 (2H, m), 3.86 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 161.8, 160.0, 154.9 (q, $^2J_{C-F}$ = 35.0 Hz), 139.2, 130.7, 125.3, 122.7, 120.5 (q, $^1J_{C-F}$ = 275.2 Hz), 120.4, 107.7; CG-MS (EI, 70 Ev): m/z(%) = 296 (M^+, 19), 189 (11), 163 (100), 94 (90); Anal. Calcd for $C_{13}H_{11}F_3N_4O$ (296.25): C, 52.71; H, 3.74; N, 18.91. Found: C, 52.30; H, 3.41; N, 19.10.

6.2.5. 2-(*N*′-4-Methyl-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5e)

The isolation of the major isomer was accomplished after successive crystallizations in a dichloromethane/methanol mixture or by flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant. Yield: 96%; mp: 155–160 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.76 (1H, br s), 8.81 (1H, d, J = 4.9 Hz), 8.18 (1H, s), 7.59 (1H, d, J = 7.9 Hz), 7.27–7.23 (3H, m), 2.34 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 161.9, 160.0, 154.9 (q, $^2J_{C-F}$ = 35.4 Hz), 143.8, 139.1, 131.9, 129.3, 126.6, 120.6 (q, $^1J_{C-F}$ = 273.9 Hz), 120.4, 107.5; CG-MS (EI, 70 Ev): m/z(%) = 280 (M^+, 13), 189 (13), 163 (100), 136 (24), 94 (53); Anal. Calcd for $C_{13}H_{11}F_3N_4.H_2O$ (298.27): C, 52.35; H, 4.39; N, 18.78. Found: C, 52.54; H, 4.17; N, 18.74.

6.2.6. 2-(*N*′-4-chloro-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5f)

Yield: 91%, mp: 162–165 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.91 (1H, br s), 8.83 (1H, d, J = 4.9 Hz), 8.19 (1H, s), 7.72 (2H, d, J = 8.5 Hz), 7.50 (2H, d, J = 8.5 Hz), 7.29 (1H, d, J = 4.9 Hz); ^{13}C NMR (DMSO-d_6/TMS): δ 161.9, 159.9, 154.9 (q, $^2J_{C-F}$ = 35.3 Hz), 142.2, 133.7, 133.5, 128.8, 128.1, 120.5 (q, $^1J_{C-F}$ = 275.5 Hz), 107.9; CG-MS (EI, 70 Ev): m/z(%) = 300 (M^+, 18), 189 (6), 163 (100), 136 (26), 94 (52); Anal. Calcd for $C_{12}H_8ClF_3N_4.H_2O$ (318.69): C, 45.23; H, 3.16; N, 17.58. Found: C, 45.23; H, 2.85; N, 17.70.

6.2.7. 2-(*N*′-4-methoxy-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5g)

Yield: 96%; mp: 141–145 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.72 (1H, br s), 8.81 (1H, d, J = 4.9 Hz), 8.18 (1H, s), 7.66 (2H, d, J = 8.8 Hz), 7.23 (1H, d, J = 4.9 Hz), 7.02 (2H, d, J = 8.8 Hz), 3.82 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 161.9, 160.4, 160.1, 154.9 (q, $^2J_{C-F}$ = 35.0 Hz), 143.8, 128.2, 127.5, 120.6 (q, $^1J_{C-F}$ = 275.2 Hz), 107.4, 55.2; CG-MS (EI, 70 Ev): m/z(%) = 296 (M^+, 27), 189 (4), 163 (100), 133 (91), 94 (52); Anal. Calcd for $C_{13}H_{11}F_3N_4O.H_2O$ (314.27): C, 49.68; H, 4.14; N, 17.83. Found: C, 50.08; H, 3.74; N 17.85.

6.2.8. 2-(*N*′-4-Nitro-benzylidenehydrazino)-4-trifluoromethyl-pyrimidine (5h)

Yield: 87%; mp: 203–208 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 12.17 (1H, br s), 8.88 (1H, d, J = 4.8 Hz), 8.29–8.27 (3H, m), 7.94 (2H, d, J = 8.8 Hz), 7.36 (1H, d, J = 4.8 Hz); ^{13}C NMR (DMSO-d_6/TMS): δ 162.1, 159.8, 154.9 (q, $^2J_{C-F}$ = 35.3 Hz), 147.2, 141.0, 140.9, 127.3, 124.0, 120.5 (q, $^1J_{C-F}$ = 275.3 Hz), 108.5, 107.5; CG-MS (EI, 70 Ev): m/z(%) = 311 (M^+, 9), 189 (15), 163 (100), 136 (11), 94 (64); Anal. Calcd for $C_{12}H_8F_3N_5O_2.H_2O$ (329.24): C, 43.78; H, 3.06; N, 21.27. Found: C, 44.18; H, 2.64; N, 21.31.

6.2.9. 2-(*N*′-1-phenyl-ethylidenehydrazino)-4-trifluoromethyl-pyrimidine) (5i)

Yield: 98%; mp: 157–159 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 10.71 (1H, br s), 8.84 (1H, d, J = 4.6 Hz), 7.86–7.82 (2H, m), 7.46–7.40 (3H, m), 7.28 (2H, d, J = 4.6 Hz), 2.38 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 161.7, 160.8, 154.8 (q, $^2J_{C-F}$ = 35.1 Hz), 149.4, 138.4, 128.7, 128.2, 126.0, 120.6 (q, $^1J_{C-F}$ = 275.2 Hz), 107.8, 13.9; CG-MS (EI, 70 Ev): m/z(%) = 280 (M^+, 43), 263 (100), 136 (14), 94 (18); Anal. Calcd for $C_{13}H_{11}F_3N_4$ (280.25): C, 55.72; H, 3.96; N, 19.99. Found: C, 55.85; H, 4.46; N, 19.78.

6.2.10. 2-(*N*′-Benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6a)

Yield: 98%; mp: 132–136 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.62 (1H, br s), 8.65 (1H, s), 8.18 (1H, s), 7.69–7.67 (2H, m), 7.45–7.38 (3H, m), 2.29 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.4, 158.3, 152.2 (q, $^2J_{C-F}$ = 33.7 Hz), 142.8, 134.9, 129.2, 128.7, 126.5, 121.4 (q, $^1J_{C-F}$ = 276.6 Hz), 117.9, 13.5; CG-MS (EI, 70 Ev): m/z(%) = 280 (M^+, 15), 203 (15), 177 (100), 108 (25), 81 (28); Anal. Calcd for $C_{13}H_{11}F_3N_4.H_2O$ (298.27): C, 52.35; H, 4.39; N, 18.78. Found: C, 52.75; H, 4.25; N, 18.84.

6.2.11. 2-(*N*′-2-Methyl-benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6b)

Yield: 97%; mp: 158–161 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.56 (1H, br s), 8.65 (1H, s), 8.49 (1H, s), 7,82 (1H, d, J = 7.4 Hz), 7.27–7.21 (3H, m), 2.43 (3H, s), 2.29 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.33, 158.2, 152.0 (q, $^2J_{C-F}$ = 33.2 Hz), 141.4, 135.9–122.5, 121.3 (q, $^1J_{C-F}$ = 276.2 Hz), 117.7, 19.0, 13.4; CG-MS (EI, 70 Ev): m/z(%) = 294 (M^+, 10), 203 (5), 177 (100), 108 (15), 81 (17); Anal. Calcd for $C_{14}H_{13}F_3N4$ (294.28): C, 57.14; H, 4.45; N, 19.04. Found: C, 56.83; H, 4.25; N, 18.69.

6.2.12. 2-(*N*′-2-Hydroxy-benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6c)

Yield: 95%; mp: 185–190 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.90 (1H, br s), 11.47 (1H, s), 8.69 (1H, s), 8.34 (1H, s), 7.43 (1H, dd, J_1 = 7,6 Hz, J_2 = 1,6 Hz), 7.22 (1H, td, J_1 = 7,6 Hz, J_2 = 1,6 Hz), 6.91 (2H, m), 2.30 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.5, 157.7, 157.1, 152.2 (q, $^2J_{C-F}$ = 33.2 Hz), 143.9, 130.3, 129.4, 121.3 (q, $^1J_{C-F}$ = 276.5 Hz), 119.1, 118.8, 118.3, 116.3, 13.4; CG-MS (EI, 70 Ev): m/z(%) = 296 (M^+, 33), 279 (15), 203 (5), 177 (100), 108 (20), 81 (23); Anal. Calcd for $C_{13}H_{11}F_3N_4O$ (296.25): C, 52.71; H, 3.74; N, 18.91. Found: C, 52.32; H, 3.47; N, 18.78.

6.2.13. 2-(*N'*-2-Methoxy-benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6d)

The isolation of the major isomer was accomplished after successive crystallizations in a dichloromethane/methanol mixture or by flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant. Yield: 95%; mp: 183–186 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.62 (1H, br s), 8.64 (1H, s), 8.34 (1H, s), 7.86 (1H, dd, J_1 = 7.8 Hz, J_2 = 1.7 Hz), 7.37 (1H, td, J_1 = 7.4 Hz, J_2 = 1.7 Hz), 7.10–6.89 (2H, m), 3.85 (3H, s), 2.28 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.3, 158.6, 157.1, 152.1 (q, $^2J_{C-F}$ = 32.9 Hz), 138.2, 130.5, 125.4, 122.9, 121.3 (q, $^1J_{C-F}$ = 276.9 Hz),120.5, 117.6, 111.6, 55.5, 13.4; CG-MS (EI, 70 Ev): *m/z*(%) = 310 (M^+, 20), 203 (14), 177 (100), 108 (35); Anal. Calcd for $C_{14}H_{13}F_3N_4O$ (310.28): C, 54.19; H, 4.22; N, 18.06. Found: C, 53.78; H, 4.23; N, 17.75.

6.2.14. 2-(*N'*-4-Methyl-benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6e)

The isolation of the major isomer was accomplished after successive crystallizations in a dichloromethane/methanol mixture or by flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant. Yield: 96%; mp: 183–188 °C (from $CHCl_3$/MeOH); ^1H NMR ($CDCl_3$/TMS): δ 8.70 (1H, br s), 8.51 (1H, s), 7.88 (1H, s), 7.63 (2H, d, J = 8.0 Hz), 7.19 (2H, d, J = 8.0 Hz), 2.37 (3H, s), 2.33 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.3, 159.8, 152.1 (q, $^2J_{C-F}$ = 33.2 Hz), 142.8, 138.8, 132.1, 129.3, 126.4, 121.3 (q, $^1J_{C-F}$ = 274.8 Hz), 117.6, 20.9, 13.5; CG-MS (EI, 70 Ev): *m/z*(%) = 294 (M^+, 16), 203 (31), 177 (100), 108 (31), 81 (29).

6.2.15. 2-(*N'*-4-chloro-benzylidenehydrazino)-5-methyl-4-trifluoro-methyl-pyrimidine (6f)

Yield: 94%; mp: 199–200 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.75 (1H, br s), 8.67 (1H, s), 8.17 (1H, s), 7.71 (2H, d, J = 8.0 Hz), 7.49 (2H, d, J = 8.0 Hz), 2.29 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.3, 158.2, 152.1 (q, $^2J_{C-F}$ = 33.1 Hz), 144.2, 134.1, 133.7, 133.2, 120.7, 127.9, 121.3 (q, $^1J_{C-F}$ = 275.4 Hz), 118.0, 13.4; CG-MS (EI, 70 Ev): *m/z*(%) = 314 (M^+, 29), 203 (13), 177 (100), 108 (38), 81 (40); Anal. Calcd for $C_{13}H_{10}ClF_3N_4$ (314.70): C, 49.62; H, 3.20; N, 17.80. Found: C, 49.86; H, 3.49; N, 17.95.

6.2.16. 2-(*N'*-4-methoxy-benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6g)

Yield: 94%; mp: 155–160 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.48 (1H, br s), 8.62 (1H, s), 8.13 (1H, s), 7.62 (2H, d, J = 8.8 Hz, Ar), 8.00 (2H, d, J = 8.8 Hz), 3.80 (3H, s), 2.28 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.3, 160.2, 158.3, 152.1 (q, $^2J_{C-F}$ = 33.6 Hz), 142.7, 128.0, 127.4, 121.4 (q, $^1J_{C-F}$ = 275.2 Hz), 117.2, 114.4, 114.2, 55.1, 13.4; CG-MS (EI, 70 Ev): *m/z*(%) = 310 (M^+, 100), 203 (11), 177 (98), 108 (77), 81 (83); Anal. Calcd for $C_{14}H_{13}F_3N_4O$ (310.28): C, 54.19; H, 4.22; N, 18.06. Found: C, 53.79; H, 4.05; N, 18.25.

6.2.17. 2-(*N'*-4-Nitro-benzylidenehydrazino)-5-methyl-4-trifluoromethyl-pyrimidine (6h)

Yield: 97%; mp: 200–205 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.98 (1H, br s), 8.27 (1H, s), 8.25 (1H, d, J = 9.1 Hz), 7.92 (2H, d, J = 9.1 Hz), 2.31 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 163.5, 157.9, 152.1 (q, $^2J_{C-F}$ = 33.1 Hz), 147.0, 141.2, 139.9, 127.1, 123.9, 121.2 (q, $^1J_{C-F}$ = 274.9 Hz), 118.1, 13.5; CG-MS (EI, 70 Ev): *m/z*(%) = 325 (M^+, 11), 203 (24), 177 (100), 108 (30), 81 (27); Anal. Calcd for $C_{13}H_{10}F_3N_5O_2.H_2O$ (343.27): C, 45.49; H, 3.52; N, 20.40. Found: C, 45.91; H, 3.69; N, 21.02.

6.2.18. 2-(*N'*-1-phenyl-ethylidenehydrazino)-5-methyl-4-trifluoro-methyl-pyrimidine (6i)

Yield: 93%; mp: 167–169 °C (from $CHCl_3$/MeOH); ^1H NMR ($CDCl_3$/TMS): δ 8.52 (1H, s), 8.45 (1H, s), 7.81 (1H, d, J = 7.6 Hz), 7.40–7.32 (3H, m), 2.34 (3H, s), 2.32 (3H, s); ^{13}C NMR ($CDCl_3$/TMS): δ 163.0, 158.2, 153.9 (q, $^2J_{C-F}$ = 34.1 Hz), 147.8, 129.0, 128.3, 126.3, 121.1 (q, $^1J_{C-F}$ = 276.7 Hz), 119.2, 14.0, 13.0; CG-MS (EI, 70 Ev): *m/z*(%) = 293 (M-1, 38), 279 (54), 217 (24), 104 (26), 77 (100); Anal. Calcd for $C_{14}H_{13}F_3N_4$ (294.28): C, 57.14; H, 4.45; N, 19.04. Found: C, 57.31; H, 4.55; N, 19.06.

6.2.19. 2-(*N'*-Benzylidenehydrazino)-6-methyl-4-trifluoromethyl-pyrimidine (7a)

Yield: 86%; mp: 150–155 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.74 (1H, br s), 8.21 (1H, s), 7.72–7.69 (2H, m), 7.48–7.39 (3H, m), 7.19 (1H, s), 2.51 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 171.9, 159.9, 154.7 (q, $^2J_{C-F}$ = 34.3 Hz), 143.2, 134.7, 129.3, 128.7, 126.5, 124.9, 120.8 (q, $^1J_{C-F}$ = 272.8 Hz), 107.4, 24.0; CG-MS (EI, 70 Ev): *m/z*(%) = 280 (M^+, 12), 203 (14), 177 (100), 108 (12), 77 (13); Anal. Calcd for $C_{13}H_{11}F_3N_4$ (280.25): C, 55.72; H, 3.96; N, 19.99. Found: C, 55.32; H, 3.85; N, 20.43.

6.2.20. 2-(*N'*-2-Methyl-benzylidenehydrazino)-6-methyl-4-trifluoromethyl-pyrimidine (7b)

Yield: 88%; mp: 150–154 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.68 (1H, br s), 8.50 (1H, s), 7.84–7.81 (2H, m), 7.30–7.23 (3H, m), 7.18 (1H, s), 2.49 (3H, s), 2.42 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 171.8, 160.0, 154.7 (q, $^2J_{C-F}$ = 34.5 Hz), 142.0, 136.1, 132.7, 130.7, 129.0, 126.0, 125.6, 120.8 (q, $^1J_{C-F}$ = 274.6 Hz), 107.3, 24.0, 18.9; CG-MS (EI, 70 Ev): *m/z*(%) = 294 (M^+, 34), 203 (15), 177 (100), 108 (35); Anal. Calcd for $C_{14}H_{13}F_3N_4$ (294.28): C, 57.14; H, 4.45; N, 19.04. Found: C, 56.74; H, 4.34; N, 19.50.

6.2.21. 2-(*N'*-2-Hydroxy-benzylidenehydrazino)-6-methyl-4-trifluoromethyl-pyrimidine (7c)

The isolation of the major isomer was accomplished after successive crystallizations in a dichloromethane/methanol mixture or by flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant. Yield: 88%; mp: 143–146 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.99 (1H, br s), 11.52 (1H, br s), 8.36 (1H, s), 7.45 (1H, d, J = 7.7 Hz), 7.32–7.23 (2H, m), 6.96–6.88 (2H, m), 2.50 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 172.1, 159.4, 157.1, 154.7 (q, $^2J_{C-F}$ = 34.4 Hz), 144.3, 130.5, 129.4, 120.7 (q, $^1J_{C-F}$ = 274.4 Hz), 119.19, 118.8, 116.3, 107.8, 24.1; CG-MS (EI, 70 Ev): *m/z*(%) = 296 (M^+, 32), 203 (8), 177 (100), 108 (21).

6.2.22. 2-(*N'*-2-Methoxy-benzylidenehydrazino)-6-methyl-4-trifluoromethyl-pyri-midine (7d)

Yield: 94%; mp: 158–162 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 11.70 (1H, br s), 8.56 (1H, s), 7.90 (1H, dd, J_1 = 7.6 Hz, J_2 = 1.8 Hz), 7.38 (1H, td, J_1 = 7.5 Hz, J_2 = 1.8 Hz), 7.15 (1H, s), 7.08 (1H, d, J = 8.3 Hz), 7.02 (1H, t, J = 7.5 Hz), 3.87 (3H, s), 2.50 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 171.7, 159.9, 157.2, 154.7 (q, $^2J_{C-F}$ = 34.6 Hz), 138.8, 130.6, 125.3, 122.8, 120.8 (q, $^1J_{C-F}$ = 275.5 Hz), 111.5, 107.2, 55.5, 24.0; CG-MS (EI, 70 Ev): *m/z*(%) = 310 (M^+, 86), 203 (25), 177 (100), 108 (66); Anal. Calcd for $C_{14}H_{13}F_3N_4O$ (310.28): C, 54.19; H, 4.22; N, 18.06. Found: C, 53.83; H, 4.09; N, 17.90.

6.2.23. 2-(*N'*-4-Methyl-benzylidenehydrazino)-6-methyl-4-trifluoromethyl-pyrimi-dine (7e)

Yield: 92%; mp: 139–143 °C (from $CHCl_3$/MeOH); ^1H NMR (DMSO-d_6/TMS): δ 8.64 (1H, br s), 7.89 (1H, s), 7.65 (1H, d, J = 8.0 Hz), 7.20 (1H, d, J = 8.0 Hz), 6.93 (1H, s), 2.57 (3H, s), 2.37 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 171.7, 159.9, 154.7 (q, $^2J_{C-F}$ = 34.6 Hz), 143.4, 138.9, 131.9, 129.2, 126.5, 120.7 (q, $^1J_{C-F}$ = 274.5 Hz), 107.1, 24.0, 20.9; CG-MS (EI, 70 Ev): *m/z*(%) = 294 (M^+, 11), 203 (9), 177 (100), 108 (18), 91 (21); Anal. Calcd for $C_{14}H_{13}F_3N_4$ (294.28): C, 57.14; H, 4.45; N, 19.04. Found: C, 57.54; H, 4.39; N, 19.05.

6.2.24. 2-(*N*′-4-chloro-benzylidenehydrazino)-6-methyl-4-trifluoro-methyl-pyrimidine (7f)

The isolation of the major isomer was accomplished after successive crystallizations in a dichloromethane/methanol mixture or by flash column chromatography in silica gel using a 10% dichloromethane/methanol mixture as the eluant. Yield: 90%; mp: 165–170 °C (from $CHCl_3$/MeOH); ^{1}H NMR ($CDCl_3$/TMS): δ 8.85 (1H, br s), 7.89 (1H, s), 7.69 (2H, d, J = 8.5 Hz), 7.36 (2H, d, J = 8.5 Hz), 6.96 (1H, s), 2.58 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 171.8, 159.8, 154.6 (q, $^2J_{C-F}$ = 34.7 Hz), 141.8, 133.6, 128.7, 128.1, 120.7 (q, $^1J_{C-F}$ = 275.2 Hz), 107.5, 24.0; CG-MS (EI, 70 Ev): m/z(%) = 314 (M^+, 20), 203 (14), 177 (100), 108 (29); Anal. Calcd for $C_{13}H_{10}ClF_3N_4$ (314.70): C, 49.62; H, 3.20; N, 17.80. Found: C, 52.22; H, 3.37; N, 18.73.

6.2.25. 2-(*N*′-4-methoxy-benzylidenehydrazino)-6-methyl-4-trifluoro-methyl-pyrimidine (7g)

Yield: 94%; mp: 120–125 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 11.56 (1H, br s), 8.13 (1H, s), 7.62 (2H, d, J = 8.8 Hz), 7.14 (1H, s), 7.00 (2H, d, J = 8.8 Hz), 3.80 (3H, s), 2.48 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 171.7, 160.2, 159.9, 154.6 (q, $^2J_{C-F}$ = 34.5 Hz), 143.2, 128.3, 127.2, 120.7 (q, $^1J_{C-F}$ = 275.3), 114.2, 106.9, 55.1, 24.0; CG-MS (EI, 70 Ev): m/z(%) = 310 (M^+, 20), 203 (6), 177 (100), 108 (19); Anal. Calcd for $C_{14}H_{13}F_3N_4O$ (310.28): C, 54.19; H, 4.22; N, 18.06. Found: C, 53.79; H, 4.07; N, 18.47.

6.2.26. 2-(*N*′-4-nitro-benzylidenehydrazino)-6-methyl-4-trifluoro-methyl-pyrimidine (7h)

Yield: 78%; mp: 226–229 °C (from $CHCl_3$/MeOH); ^{1}H NMR (DMSO-d_6/TMS): δ 12.05 (1H, br s), 8.28–8.26 (3H, m), 7.92 (2H, d, J = 8.8 Hz Ar), 2.53 (3H, s); ^{13}C NMR (DMSO-d_6/TMS): δ 172.1, 159.7, 154.7 (q, $^2J_{C-F}$ = 34.7), 147.1, 141.8, 140.6, 128.9, 124.0, 120.7 (q, $^1J_{C-F}$ = 275.5 Hz), 108.3, 24.1; CG-MS (EI, 70 Ev): m/z(%) = 325 (M^+, 11), 203 (31), 177 (100), 108 (30); Anal. Calcd for $C_{13}H_{10}F_3N_5O_2$ (325.25): C, 48.01; H, 3.10; N, 21.53. Found: C, 47.61; H, 3.01; N, 21.48.

6.2.27. 2-(*N*′-1-phenyl-ethylidenehydrazino)-6-methyl-4-trifluoro-methyl-pyrimidine) (7i)

Yield: 94%; mp: 135–138 °C (from $CHCl_3$/MeOH); ^{1}H NMR ($CDCl_3$/TMS): δ 8.45 (1H, br s), 7.85–7.83 (2H, m), 7.40–7.36 (3H, m), 6.96 (1H, s), 2.58 (3H, s), 2.34 (3H, s); ^{13}C NMR ($CDCl_3$/TMS): δ 172.1, 160.2, 156.4 (q, $^2J_{C-F}$ = 35.1 Hz), 148.2, 138.2, 129.1, 128.4, 126.3, 120.6 (q, $^1J_{C-F}$ = 275.2 Hz), 24.8, 13.0; CG-MS (EI, 70 Ev): m/z(%) = 293 (M^+, 100), 279 (27), 217 (15), 77 (26); Anal. Calcd for $C_{14}H_{13}F_3N_4$ (294.28): C, 57.14; H, 4.45; N, 19.04. Found: C, 56.87; H, 4.62; N, 19.11.

6.3. Biology

6.3.1. Materials

Cruzain truncated in the C-terminal extension was obtained from *Escherichia coli* (strain DH5α containing the expression plasmid—kindly supplied by Prof. Ana Paula C. de A. Lima) following the previously reported procedure.[43] All reagents for buffer preparation, the standard inhibitor E-64 and the substrate *Z*-Phe-Arg-AMC were purchased from Sigma–Aldrich. Substrate and inhibitors candidates at 10 mM stock solutions in neat DMSO were stored at −20 °C and at −4 °C, respectively.

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