

Synthesis, *In vitro* Antibacterial and Antifungal Activities of Trifluoroalkyl-N, N'-Disubstituted Thioureas



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Submission: May 09, 2020; Published: July 06, 2020

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Abstract

A series of N-(4-trifluoromethylphenyl) thiourea derivatives of biological interest has been prepared by the condensation of various isothiocyanates with primary 4-trifluorophenylamine under uncatalyzed conditions. The chemical structures of all the reported compounds were confirmed by FT-IR, multinuclear NMR (1H, 13C, 19F) and HRMS spectrometry. Some of these compounds were screened for their *in vitro* antibacterial activity against ten pathogenic strains representing different types of gram-positive and gram-negative bacteria, two pathogenic fungi (*Penicillium sp*, *Aspergillus flavus*) and two yeasts (*Candida albicans* and *Candida glabrata*). More than four of the synthesized compounds showed promising inhibition activities against the tested strains *Enterococcus faecium* ATCC 19436 with reference to standard vancomycin. Best antimicrobial activity was founded for the 3-pyridylthiourea derivative against *Enterococcus faecium* ATCC 25923 with CMI= 3.9 mg/mL. The results indicated also that all the new screened compounds have promising antifungal activity comparable to the activity observed for the reference compounds.

Keywords: Isothiocyanates; Thioureas; Antibacterial activity; Antifungal activity

Introduction

This work is a continuation of our research on the study of sulfur having compounds in search of lead molecules [1-4]. In particular isothiocyanates, which are attractive synthons in organic chemistry due to their availability and their tendency to undergo nucleophilic addition and cycloadditions [5,6]. The strong electrophilicity of the NCS group of isothiocyanates enables these heterocumulenes to undergo nucleophilic addition and cycloaddition reactions, making them extremely useful in the synthesis of thiocarbonyl derivatives [7]. Particularly isothiocyanates have been used for the synthesis of thioureas of synthetic, biological, agricultural and pharmaceutical interest [8-17]. Recently, thioureas have attracted considerable attention for their potential use as binding units for artificial receptors in supramolecular chemistry because of their characteristic behavior based on Lewis acids and strong hydrogen-bond donors [18-24].

Furthermore, in the field of advanced material chemistry, thioureas can serve as a useful scaffold by connecting them to

electroluminescent organic dyes [25,26]. Thiourea group are also present in many drugs such as antithyroid, and anaesthetic [27]. Monothiourea derivatives, which are obtained by the condensation of isothiocyanate with esteramines, have shown strong antifungal activities, especially against *Candida* and *Aspergillus* in several studies [28-30]. Plautonol and its thioureas derivatives presented antibacterial activities against *Helicobacter pylori* as urease inhibitors [31,32]. Anti-HIV activity of thioureas were also reported in recent studies [33,34]. The literature survey reveals also that incorporation of halogen atom(s) within the molecule is one of the most effective strategies to enhance its biopotency, bioavailability and lipophilicity [35,36].

Importantly, in recent studies fluorinated thioureas were also reported as novel class of potent influenza virus neuraminidase inhibitors [37,38]. Their enormous potential has led to the development of several methods for their preparation [27,39-41]. The most common of these methods involves the condensation of isothiocyanates with amino derivatives; Suresha et al. [42]

proved that fluoro-containing arylthiourea compounds show better activity as compared to other analogues [42]. According to other authors findings [27,38,43], the antibacterial and antifungal efficiency depends on the presence of such electron-withdrawing substituent at C-2 and C-4 position of the phenyl ring. On the other hand, modification of isoxyl, the symmetrical diphenylthiourea derivative, produced the library of compounds with antimycobacterial activity [44].

To sum up, and as a part of the continuing research in our laboratory toward the development of the chemistry of new bioactive compounds containing fluoro groups under mild condition; we have previously disclosed the preparations of fluoroalkyl thiocarbamates and fluoroalkyl dithiocarbonates by the action of isothiocyanates and alcohols and thiols respectively [1,2], and to investigate the role of the F-alkyl substituent, the compounds were also evaluated for their antibacterial and antifungal activities [4], and the biological results were satisfactory and these later proved to be good antibacterial and antifungal products.

Very recently, fluoro-methyl, or methoxy substituents on the 3-position benzene ring also improve antimicrobial potency [45,46], however, and to the best of our knowledge, the reaction of isothiocyanates with an amine having an F-alkyl group at the para-position still remains much less explored. Here, we report the synthesis of new thioureas derivatives by condensation of isothiocyanates with 4-trifluoromethylphenyl, and testing for antifungal, antibacterial activities of these compounds. By using such amine, we want to combine antimicrobial effect of the F-alkyl group with the biological activity of thiourea group, together with the different R isothiocyanates groups (aliphatic, aromatic carbocyclic and heterocyclic groups, possessing electron withdrawing or electron-releasing groups).

Materials and Methods

General

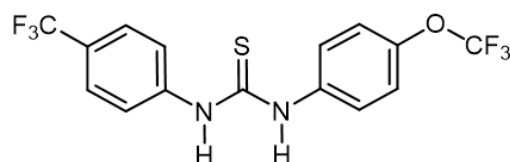
Melting points (m.p.) were determined by using an Electrothermal 9100 apparatus and are uncorrected. Mass spectra were recorded on a high resolution Micromass micrOTOF-Q II 10027 spectrometer by electron spray ionization method. FT-IR spectra were recorded on Nicolet IR200 spectrometer within the wave number range of 4000-400 cm^{-1} at 25 °C. Yields are of purified products. NMR spectra were recorded in the given solvent with Bruker AC300 spectrometer. Chemical shifts are reported as (values in parts per million) relative to TMS. The splitting pattern abbreviations are as follows: m (multiple), s (singlet), d (doublet), t (triplet). All reagents, solvents, and starting materials were obtained from commercial suppliers and used without further purification. Evaporations were conducted under reduced pressure. Reactions were monitored by thin layer chromatography (TLC) using Kieselgel 60 F254 (E. Merck) plates and UV detector for visualization. Flash column chromatography was performed on Silica Gel (70-230mesh).

General experimental procedure for the synthesis of target compounds

To a solution of the 4-trifluorophenylamine (10mmol) in 10mL of THF, was added triethylamine (15mmol). Then the isothiocyanate (10mmol) was added. The mixture was Heat at the least for 2 hours. After vacuum evaporation of the solvent, the crude product was filter and purified either by chromatography (petroleum ether/diethyl ether (8:2) or by recrystallize from hexane or petroleum ether to give the corresponding pure compounds (1-9). We should note that the compound 7 has already been prepared by another experimental protocol in an earlier work [47] and the compounds 1, 3 and 8 are commercially available.

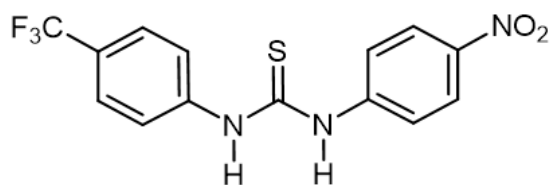
3-3- Spectroscopic data

N-trifluoromethoxy-N'-trifluoromethylphenylthiourea
 $\text{C}_{15}\text{H}_{10}\text{F}_6\text{N}_2\text{OS}$ (1)

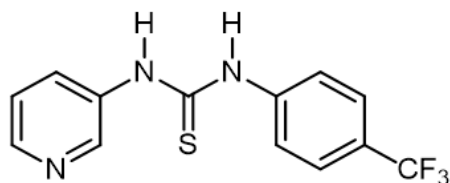


White solid m.p.= 131°C; IR (KBr); ν (cm^{-1}) 3242 (NH), 3242 (NH), 1086 (C=S), RMN ^1H (300 MHz, CDCl_3 +DMSO) δ (ppm): 7.13-7.73 (syst AB, 8H, H_{arom}); 9.77 (broad signal (2 NH)). RMN ^{19}F (282 MHz, CDCl_3 +DMSO) δ (ppm): - 57.66 (s, 3F, O- CF_3); -61.57 (s, 3F, CF). RMN ^{13}C (75 MHz, CDCl_3 +DMSO) δ (ppm): 121.14; 123.11; 125.38; 125.61; 138.18; 142.83; 145.64 (8s, C_{arom}); 180.16 (C=S). SMHR (ESI), m/z: cald for $\text{C}_{15}\text{H}_{10}\text{F}_6\text{N}_2\text{OSH}^+$ 381.0475 [M+H] $^+$; found 381.0491. SMHR (ESI), m/z: cald for $\text{C}_{15}\text{H}_{10}\text{F}_6\text{N}_2\text{OSNa}^+$ 381.0475 [M+H] $^+$; found 403.0310.

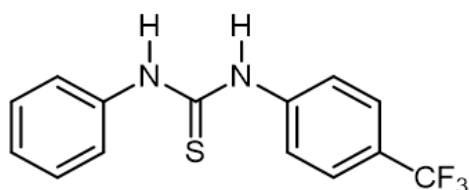
N-nitrophenyl-N'-trifluoromethylphenylthiourea
 $\text{C}_{13}\text{H}_{10}\text{F}_3\text{N}_3\text{O}_2\text{S}$ (2)



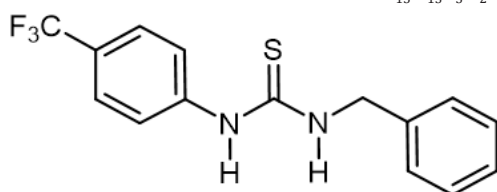
White solid; m.p.= 144°C; IR (KBr); ν (cm^{-1}) 3242 (NH), 3242 (NH), 1086 (C=S). ^1H NMR (300 MHz, CDCl_3 +DMSO) δ (ppm): 7.56-8.17 (m, 8H, H_{arom}). ^{19}F NMR (282 MHz, CDCl_3 +DMSO) δ (ppm): -61.81 (s, 3F, CF_3). ^{13}C NMR (75 MHz, CDCl_3 +DMSO) δ (ppm): 121.96; 123.40; 124.16; 142.30; 143.07; 143.22; 145.55; 145.75 (C_{arom}); 179.41 (C=S). HRMS (ESI), m/z: cald for $\text{C}_{14}\text{H}_{10}\text{F}_3\text{N}_3\text{O}_2\text{SH}^+$ 342.0519 [M+H] $^+$; found 342.0500. HRMS (ESI), m/z: cald for $\text{C}_{14}\text{H}_{10}\text{F}_3\text{N}_3\text{O}_2\text{SNa}^+$ 364.0324 [M+Na] $^+$; found 364.0338.

N-pyridyl-N'-trifluoromethylphenylthiourea C₁₃H₁₀F₃N₃S (3)


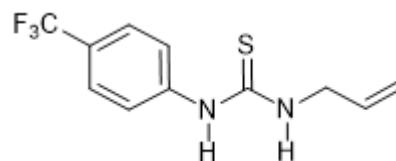
White solid; m.p.= 146°C; IR (KBr); ν (cm⁻¹) 3223 (NH), 3046 (NH), 1596 (C=S). RMN ¹H (300 MHz, CDCl₃+DMSO) δ (ppm): 7.29-7.33 (m, ¹H); 7.56-7.77 (syst AB, 4H, H_{arom.}); 8.07-8.10 (m, ¹H); 8.31-8.35 (m, ¹H); 8.60-8.64 (m, ¹H); 9.80 (broad signal (NH)); 9.97 (broad signal (NH)). RMN ¹⁹F (282 MHz, CDCl₃+DMSO) δ (ppm): -61.65 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃+DMSO) δ (ppm): 123.08; 123.27; 125.50; 125.55; 131.82; 136.01; 145.37; 145.60 (9s, C_{arom.}); 180.49 (C=S). HRMS (ESI), m/z: calcd for C₁₃H₁₀F₃N₃SH+ 298.0620 [M+H]⁺; found 298.0607. HRMS (ESI), m/z: calcd for C₁₃H₁₀F₃N₃SNa+ 320.0440 [M+Na]⁺; found 320.0420.

 N-phenyl-N'-trifluoromethylphenylthiourea C₁₄H₁₁F₃N₂S (4)


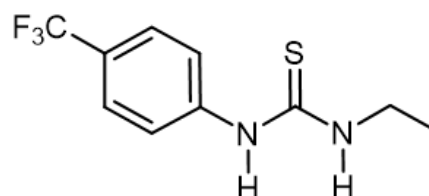
White solid; m.p.= 152 °C; IR (KBr); ν (cm⁻¹) 3203 (NH), 3030 (NH), 1551 (C=S). NMR ¹H (300 MHz, CDCl₃+DMSO) δ (ppm): 7.17-7.77 (m, 9H, H_{arom.}); 9.47 (broad signal (2 NH)). RMN ¹⁹F (282 MHz, CDCl₃+DMSO) δ (ppm): -61.84 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃+DMSO) δ (ppm): 123.30; 123.56; 125.60; 125.64; 128.81; 138.52; 142.60 (8s, C_{arom.}); 180.01 (C=S). HRMS (ESI), m/z: calcd for C₁₄H₁₁F₃N₂SH+ 297.0668 [M+H]⁺; found 297.0659. HRMS (ESI), m/z: calcd for C₁₄H₁₁F₃N₂SNa+ 319.0487 [M+H]⁺; found 319.0473.

 N-benzyl-N'-trifluoromethylphenylthiourea C₁₅H₁₃F₃N₂S (5)


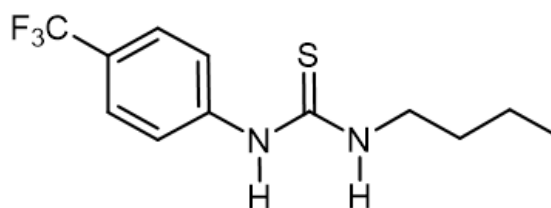
White solid; m.p.= 138°C; IR (KBr); ν (cm⁻¹) 3299 (NH), 3047 (NH), 1527 (C=S). NMR ¹H (300 MHz, CDCl₃) δ (ppm): 4.86 (d, 2H, CH₂, ³J_{H-H} = 6.0 Hz); 6.46 (broad signal (NH)); 7.29-7.64 (m, 5H, H_{arom.}); 8.55 (broad signal (NH)). NMR ¹⁹F (282 MHz, CDCl₃) δ (ppm): -62.50 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 49.54 (s, CH₂); 124.10; 127.81; 128.07; 128.96; 136.70; 139.66 (C_{arom.}); 180.50 (C=S). HRMS (ESI), m/z: calcd for C₁₅H₁₃F₃N₂SH+ 311.0830 [M+H]⁺; found 311.0821.

 N-allyl-N'-trifluoromethylphenylthiourea C₁₃H₁₀F₃N₂O₂S (6)


White solid; m.p.= 134°C; IR (KBr); ν (cm⁻¹) 3265 (NH), 3077 (NH), 1561 (C=S). ¹H NMR (300 MHz, CDCl₃) δ (ppm): 4.30 (t, 2H, CH₂, ³J_{H-H} = 6 Hz); 5.23 (m, 2H, CH₂); 5.90 (m, 2H, =CH); 6.23 (broad signal (NH)); 7.36-7.70 (syst AB, 4H, H_{arom.}); 8.44 (broad signal (NH)). ¹⁹F NMR (282 MHz, CDCl₃) δ (ppm): -62.50 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 47.97 (s, CH₂); 117.92 (s, CH₂); 124.28; 127.33; 127.37; 139.68 (4s, C_{arom.}); 132.78 (=CH); 180.61 (C=S). HRMS (ESI), m/z: calcd for C₉H₁₃F₃N₂SNa+ 261.0649 [M+Na]⁺; found 261.0660.

 N-ethyl-N'-trifluoromethylphenylthiourea C₁₀H₁₁F₃N₂S (7)


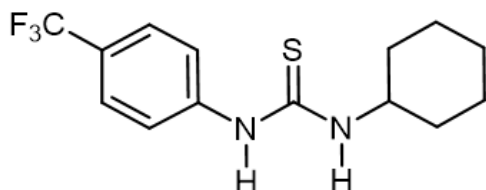
White solid; m.p.= 140°C; IR (KBr); ν (cm⁻¹) 3268 (NH), 3088 (NH), 1558 (C=S). NMR ¹H (300 MHz, CDCl₃) δ (ppm): 1.23 (t, 3H, CH₃, ³J_{H-H} = 6 Hz); 3.68 (q, 2H, CH₂); 6.23 (broad signal (NH)); 7.36-7.68 (syst AB, 4H, H_{arom.}); 8.83 (broad signal (NH)). NMR ¹⁹F (282 MHz, CDCl₃) δ (ppm): -62.46 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 14.16 (s, CH₃); 40.41 (s, CH₂); 124.07; 127.28; 127.44; 139.92 (4s, C_{arom.}); 128.27 (q, CF₃, ¹J_{C-F} = 20 Hz); 180.16 (C=S). HRMS (ESI), m/z: calcd for C₁₀H₁₁F₃N₂SH+ 249.0668 [M+H]⁺; found 249.0665. HRMS (ESI), m/z: calcd for C₁₀H₁₁F₃N₂SNa+ 271.0487 [M+Na]⁺; found 271.0478.

 N-butyl-N'-trifluoromethylphenylthiourea C₁₂H₁₅F₃N₂S (8)


White solid; m.p.= 143°C; IR (KBr); ν (cm⁻¹) 3268 (NH), 3088 (NH), 1558 (C=S). ¹H NMR (300 MHz, CDCl₃) δ (ppm): 0.39 (t, 3H, CH₃, ³J_{H-H} = 7 Hz); 1.35 (m, 2H, CH₂); 1.59 (m, 2H, CH₂); 3.63 (q, 2H, CH₂, ³J_{H-H} = 6 Hz); 6.30 (broad signal (NH)); 7.37-7.67 (syst AB, 4H, H_{arom.}); 8.78 (broad signal (NH)). ¹⁹F NMR (282 MHz, CDCl₃) δ (ppm): -62.45 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 13.76 (s, CH₃); 20.16 (s, CH₂); 30.93 (s, CH₂); 45.32 (s, CH₂); 121.96; 123.93; 127.03; 140.07 (4s, C_{arom.}); 128.15 (q, CF₃, ¹J_{C-F} = 20 Hz);

180.17 (C=S). HRMS (ESI), m/z: calcd for $C_{12}H_{15}F_3N_2SH^+$ 277.0981 [M+H]⁺; found 277.0976. HRMS (ESI), m/z: calcd for $C_{12}H_{15}F_3N_2SNa^+$ 299.0800 [M+Na]⁺; found 299.0783.

N-cyclohexyl-N'-trifluoromethylphenylthiourea
C₁₃H₁₀F₃N₂O₂S (9)



White solid; m.p.= 147°C; IR (KBr); ν (cm⁻¹) 3269 (NH), 3046 (NH), 1543 (C=S), RNM ¹H (300 MHz, CDCl₃) δ (ppm): 1.20-2.11 (m, 1¹H); 6.15 (d, NH); 7.35-7.67 (syst AB, 4H, H_{arom.}); 8.49 (broad signal (NH)). NMR ¹⁹F (282 MHz, CDCl₃) δ (ppm): 62.42 (s, 3F, CF₃). NMR ¹³C (75 MHz, CDCl₃) δ (ppm): 24.72 (CH₂); 25.36 (CH₂); 32.50 (CH₂); 54.15 (CH); 123.75; 127.20; 127.24; 140.00 (8s, C_{arom.}); 128.05 (q, CF₃); 178.78 (C=S). HRMS (ESI), m/z: calcd for $C_{14}H_{17}F_3N_2SH^+$ 303.1137 [M+H]⁺; found 303.1126. SMHR (ESI), m/z: calcd for $C_{14}H_{17}F_3N_2SNa^+$ 325.0957 [M+Na]⁺; found 325.0947.

Biological Essay

In vitro antibacterial evaluation

The *in vitro* antibacterial activity was tested against five Gram positive strains (*Enterococcus faecium* ATCC 19436, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6539 and *Bacillus cereus* 49) and six Gram negative bacteria (*Escherichia coli* BLSE 3, *Escherichia coli* BLSE 10, *Escherichia coli* DH5 α , *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* and *Salmonella sp*). The microdilution broth method according to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) [48] was used. Stock solutions of tested compounds in DMSO were twofold serially diluted to final concentrations ranging from 500 to 3.90 μ g/mL in sterile 96 well microtiter plates which contained Mueller–Hinton Broth (MHB, Biolife Italiana S.r.l. viale Monza, 272-20128 Milano, Italy). The bacterial cell density was kept uniformly throughout the experimentation at 1 \times 10⁸ CFU/mL by comparing with 0.5 McFrand turbidity standards and 100 μ L of test organisms was added in each well.

All procedures were performed in duplicate and microplates were incubated at optimal temperature and time (37°C for 24h, 30 °C for *Bacillus cereus* 49). Growth indicator (Dye A for Gram negative strains and Dye G for positive ones (100 μ L of 0.1 %) were incorporated in each well to assess the bacterial inhibition. Well containing inoculum alone was used as negative control. Vancomycin was used as positive control. The minimum inhibitory

concentrations (MICs) were assayed as a reduction in growth of at least 90% (IC90) as compared to the control. The MICs were determined and checked by a Biolog technology which enables high throughput automated kinetic cell assays. Biolog's Omnilog (Biolog Omnilog® Phenotype MicroArray™ USA), Cell response in each assay well is determined by the amount of color development produced by the reduction of a tetrazolium compound (a redox Dye Mix) during cell respiration [49,50].

In vitro antifungal evaluation

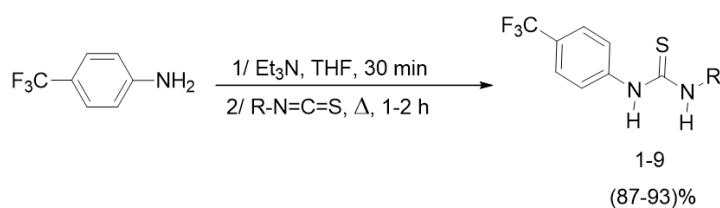
The antifungal properties were evaluated *in vitro* against four fungal strains: *Aspergillus flavus*, *Penicillium expansum*, *Candida albicans* and *Candida glabrata*. The microdilution broth method was used according to NCCLS guidelines [51]. Stock solutions of tested compounds in DMSO were twofold serially diluted to final concentrations ranging from 500 to 3.90 μ g/mL in sterile 96 well microtiter plates which contained Malt extracts (2%). The fungal suspension turbidity was adjusted to 65 % (104 conidia/mL) and 100 μ L was added to each well. All procedures were performed in duplicate and microplates were incubated at 28°C for 72h. Growth indicator (Dye E, 100 μ L of 0.1 %) was incorporated in each well. Wells containing inoculum alone was used as negative control. The minimum inhibitory concentrations (MICs) were analyzed visually and determined as the lowest concentrations for which there is no fungal growth then checked by the Biolog tool.

Results and Discussion

Synthesis

Our aim was to prepare a small library of new F-alkylated thiourea derivatives through a short synthetic method [52]. By choosing the appropriate precursors, the primary amine and the alkyl or aryl isothiocyanate, we can generate the chemical diversity of compounds. In the present study nine unsymmetrically N, N-disubstituted thioureas 1-9 were synthesized from commercially available 4-trifluoromethylphenylamine and a variety of substituted isothiocyanates. The reaction took place in one-step at refluxing THF and was completed in 1-2 hours in high yield (83-92%) as shown on Scheme 1.

Different alkyl and aryl substituents of isothiocyanates were introduced to evaluate their effects on the biological activity of the compounds. Depending on these substituent group, a longer reaction period was needed in some cases to obtain the desired adducts and the results were summarized in Table 1. That gives us a pool of compounds that, after screening process, will provide information about the structure-activity relationship. Table 1 shows the thiourea derivatives grouped by the type of lipophilic substituent. The structures were determined using different spectroscopic methods like multinuclear NMR, IR and HRMS spectra.

Table 1: N-alkyl/aryl-N'-trifluoromethylphenylthiourea 1-9


Compound	R	Yield
1	4-CF ₃ O-Ph	93
2	4-NO ₂ -Ph	86
3	3-pyridyl	87
4	Phenyl	89
5	Benzyl	85
6	Allyl	83
7	Ethyl	90
8	Butyl	88
9	Cyclohexyl	87

Characterization

Spectral data (NMR, HRMS, IR) of all compounds were in full agreement with the proposed structures. The ¹H NMR spectrum exhibited singlets at δ 9.80-6.30ppm, which were assigned to the N-H protons. ¹³C NMR revealed peaks, in the range of 178-

181ppm, for the typical signals for the thiocarbonylic carbons (C=S thiourea). Other ¹³C NMR signals were considered a singlet if the multiplicity was not assigned. The very strong broad peak between 3200 and 3300cm⁻¹ on the FTIR spectrum, should be assigned to the extension vibration of the N-H groups.

Biological evaluation

Antibacterial activity

Table 2: Antibacterial activity of thioureas, MIC (μg/mL).

Gram Positive Bacteria					
Compound	<i>E.f^a</i>	<i>E.f^b</i>	<i>S.a^a</i>	<i>S.a^b</i>	<i>B.c</i>
3	7.8	250	3.9	3.9	500
4	7.8	250	≥500	≥500	125
5	7.8	125	≥500	≥500	62.5
6	15.62	250	≥500	250	500
7	250	500	≥500	≥ 500	250
Vancomycin	125	62.5	3.9	31.25	0.39

E.f^a: *Enterococcus faecium* ATCC 19436, *E.f^b*: *Enterococcus faecalis* ATCC 29212, *B.c*: *Bacillus cereus* 49, *S.a^a*: *Staphylococcus aureus* ATCC 6538, *S.a^b*: *Staphylococcus aureus* ATCC 25923.

Gram Negative Bacteria						
Compound	<i>E.c^a</i>	<i>E.c^b</i>	<i>E.c^c</i>	<i>E.c^d</i>	<i>S.sp</i>	<i>Pa</i>
3	250	125	250	500	≥500	250
4	≥500	500	≥500	500	≥ 500	≥500
5	≥500	≥500	250	250	≥500	≥500
6	500	≥500	500	≥ 500	7.81	≥ 500
7	≥500	≥500	≥ 500	≥ 500	≥ 500	≥500
Vancomycin	125	62.5	250	250	125	250

E.c^a: *Escherichia coli* DH5α, *E.c^b*: *Escherichia coli* ATCC 8739, *E.c^c*: *Escherichia coli* BLSE Aq 3, *E.c^d*: *Escherichia coli* BLSE Aq 10, *S.sp*: *Salmonella sp*, *Pa*: *Pseudomonas aeruginosa*

Thioureas (1-9) (Table 1) were assayed *in vitro* against eleven bacterial strains: six are Gram-negative (*Escherichia coli* DH5 α , *Escherichia coli* ATCC 8739, *Escherichia coli* BLSE Aq3, *Escherichia coli* BLSE Aq10, *Pseudomonas aeruginosa* and *Salmonella sp*) and five are Gram-positive (*Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 19436, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC6538 and *Bacillus cereus* 49) bacteria (Table 2). Gentamycin was used as reference drug for comparison purposes. The results showed that most of the compounds expressed moderate to excellent antibacterial activity (MIC values: 500-3.90 μ g/mL) not only towards typical Gram-positive bacteria, but also towards Gram-negative bacteria.

Interestingly, all compounds were selectively more potent against *Enterococcus faecium* ATCC 19436 than the reference drug vancomycin. The most substantial antibacterial profile was found for derivative 3 bearing electron withdrawing halogen atom on the phenyl ring, since they produced stronger electronegativity effect than it is observed for monosubstituted derivatives. These results are in accordance with our observations made for non-F-alkyl

thiourea [45,46,53,54]. The presence of a nitrogen atom, at the aromatic ring is essential for a noticeable antimicrobial activity. However, the synthesized compounds have very weak effects on *Escherichia coli* DH5 α and *Escherichia coli* ATCC 8739. Thiourea compounds do not show the effect on the growth of *Escherichia coli* BLSE 3 with MIC over than 500 μ g/mL.

Compound 3 show a light effect on *Salmonella sp*, *Staphylococcus aureus* 6539 and *Staphylococcus aureus* ATCC 25923 with MIC ranging between 3.9 and 7.8mg/mL; therefore, and in terms of structure-activity relationships (SARs), the potent antifungal activities are descending from the aryl groups and the alkyl moiety showed less effect on their antibacterial potency. The MICs of the antifungal activity of trifluoro phenyl thiourea derivatives against *Aspergillus flavus* and *Penicillium expansum* is shown in Table 3. All derivatives showed significant *in vitro* antifungal activities against tested fungi. These compounds exhibited to have strong antifungal activities with low MICs values included in the range of 7.81-62.5 μ g/mL.

Table 3: Antifungal activity of N-(4-(trifluoromethyl)-phenyl)thiourea derivatives 1-9 MIC (μ g/mL).

Compound	Fungi		Yeast	
	<i>Aspergillus Flavus</i>	<i>Penicillium Expansum</i>	<i>Candida Albicans</i>	<i>Candida Glabrata</i>
3	31.25	31.25	\geq 500	\geq 500
4	31.25	31.25	500	500
5	62.5	15.62	125	500
6	31.25	15.62	125	500
7	7.81	15.62	\geq 500	\geq 500
Fluconazole	62.5	7.81	128	62.5

a Values are the average of three reading.

In terms of structure-activity relationships (SARs), the potent antifungal activities are descending from the thiourea function and the R group. In general, Compounds 3, 4, 5, 6 and 7 exhibited stronger (31.25-7.81 μ g/mL) or equal (62.5 μ g/mL) antifungal activities than standard agent (Fluconazole). The results show that all the derivatives have effective activity against the tested fungi, compared with the standard Fluconazole, with MIC values ranging from 7.81 to 31.25 μ g/mL against *Aspergillus flavus* and from 15.62 to 31.25 μ g/mL against *Penicillium expansum* according to the type of the derivative (Table 1). The results also showed that the substituent R (from the isothiocyanates employed in the reaction) plays a key role in varying the efficacy of antimicrobial activity.

Notably, the highest antifungal activity was observed for compound 7 (R=Ethyl) with MIC of 7.81 μ g/mL against *Aspergillus flavus* and of 15.62 μ g/mL against *Penicillium expansum*. The compounds 3-6 exhibited to have strong antifungal activities with low MICs values included in the range of 31.25-62.5 μ g/mL compared to the reference drug Fluconazole. However, the synthesized compounds have weak effects on *Penicillium*

expansum with MICs of 62.5 μ g/mL and did not show the effect on the growth of *Candida albicans* ATCC 10231 and *Candida glabrata* with MIC over than 500 μ g/mL.

Conclusion

This paper reports the synthesis and the characterization of small libraries of trifluorophenyl-bisubstituted thioureas through an easy and high yielded reaction. We have also evaluated there *in vitro* antibacterial and antifungal properties. As can be seen from our results, most of these compounds found to be potent antibacterial and antifungal agent exhibited comparable to or even higher antibacterial and antifungal activity than the standard. Biological data revealed that the substituent R (from the isothiocyanates employed in the reaction) plays a key role in varying the efficacy of antimicrobial activity. Infact, the presence of an electron withdrawing group in the aromatic rings enhanced the antimicrobial activity of the synthesized compounds, showing in most cases more activity than that of the controls. The results presented in this work encourage us to continue in this line of research.

Funding

This research was funded by the Tunisian Ministry of High Education and Scientific Research.

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DOI: [10.19080/OMCIJ.2020.09.555770](https://doi.org/10.19080/OMCIJ.2020.09.555770)

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