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 additions 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 thiocarbamoyl 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]. Plaunotol 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 substitutant, the compounds were also evaluated for their antibacterial and antifungal activities [4], and the biological results were satisfactory and these laters proved to be good antibacterial and antifungal products.

Very recently, fluoro-methyl, or metoxy substituents on the 3-position benzene ring also improve antimicrobial potency [45,46], however, and to the best our knowledge, the reaction of isothiocyanates with an amine having an F-alkylgroup 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 (70-230 mesh).

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**

\[C_{13}H_{10}F_{5}N_{2}OS \text{(1)}\]

White solid m.p. = 131°C; IR (KBr); ν (cm\(^{-1}\)) 3242 (NH), 3242 (NH), 1086 (C=S), RMN 1\(^H\) (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, CF, CF). RMN 13\(^C\) (75 MHz, CDCl\(_3\)+DMSO) δ (ppm): 12.14; 12.31; 125.38; 125.61; 138.18; 142.83; 143.64 (8s, C\(_{arom}\)); 180.16 (C=S). SMHR (ESI), m/z: cald for C\(_{13}\)H\(_{10}\)F\(_5\)N\(_2\)OSNa\(^+\) 381.0491 [M+H\(^+\)]; found 381.0491. SMHR (ESI), m/z: cald for C\(_{13}\)H\(_{10}\)F\(_5\)N\(_2\)OSNa\(^+\) 381.0475 [M+H\(^+\)]; found 381.0475.

- **N-nitrophenyl-N’-trifluorourethane**

\[C_{10}H_{10}F_2N_3O_2S \text{(2)}\]

White solid; m.p. = 144°C; IR (KBr); ν (cm\(^{-1}\)) 3242 (NH), 3242 (NH), 1086 (C=S), 1\(^H\) 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). 13\(^C\) NMR (75 MHz, CDCl\(_3\)+DMSO) δ (ppm): 121.96; 123.40; 124.16; 143.30; 143.07; 145.55; 145.75 (C\(_{arom}\)); 179.41 (C=S). HRMS (ESI), m/z: cald for C\(_{10}\)H\(_{10}\)F\(_2\)N\(_3\)O\(_2\)SNa\(^+\) 342.0519 [M+H\(^+\)]; found 342.0500. HRMS (ESI), m/z: cald for C\(_{10}\)H\(_{10}\)F\(_2\)N\(_3\)O\(_2\)SNa\(^+\) 340.0324 [M+Na\(^+\)]; found 340.0338.
White solid; m.p.= 138 °C; IR (KBr); ν (cm⁻¹) 3223 (NH), 3046 (NH), 1596 (C=S). NMR ¹H (300 MHz, CDCl₃ + DMSO) δ (ppm): 7.19-7.33 (m, 1H); 7.56-7.77 (syst AB, 4H, H aren); 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 ¹³F (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 aren); 180.49 (C=S). HRMS (ESI), m/z: cald for C₁₅H₁₁F₃N₂S₃H⁺ = 298.0620 [M+H]⁺; found 298.067. HRMS (ESI), m/z: cald for C₁₃H₁₀F₃N₃SNa⁺ = 320.0440 [M+Na⁺]; found 320.0420.

N-ethyl-N'-trifluoromethylphenylthiourea C₁₅H₁₃F₃N₂S (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, J_H-H = 6 Hz); 3.68 (q, 2H, CH₂); 6.23 (broad signal (NH)); 7.36-7.68 (syst AB, 4H, H aren); 8.83 (broad signal (NH)). RMN ¹³F (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 aren); 128.27 (q, CF, J CF = 20 Hz); 180.16 (C=S). HRMS (ESI), m/z: cald for C₁₅H₁₃F₃N₂SNa⁺ = 249.0668 [M+Na⁺]; found 249.0665. HRMS (ESI), m/z: cald for C₁₅H₁₃F₃N₂SNa⁺ = 271.0487 [M+Na⁺]; found 271.0478.

N-butyl-N'-trifluoromethylphenylthiourea C₁₃H₁₅F₃N₂S (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, J_H-H = 6 Hz); 1.35 (m, 2H, CH₂); 1.59 (m, 2H, CH₂); 3.63 (q, 2H, CH₂, J_H-H = 6 Hz); 6.30 (broad signal (NH)); 7.37-7.67 (syst AB, 4H, H aren); 8.78 (broad signal (NH)). ¹³F NMR (282 MHz, CDCl₃) δ (ppm): -62.45 (s, 3F, CF₃). ¹³C NMR (75 MHz, CDCl₃) δ (ppm): 13.76 (s, CH₂); 20.16 (s, CH₃); 30.93 (s, CH₂); 45.32 (s, CH₂); 121.96; 123.93; 127.03; 140.07 (4s, C aren); 128.15 (q, CF, J CF = 20 Hz);
The microdilution broth method was used. Stock solutions of tested compounds in DMSO were twofold serially diluted to final concentrations ranging from 500 to 3.90 µg/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 µL 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 µL of 0.1%) was incorporated in each well. Wells containing inoculum alone was used as negative 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 (BiologOmnilog® 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 µg/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 µL 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 µL of 0.1%) was incorporated in each well. Wells containing inoculum alone was used as negative control. The minimum inhibitory concentrations (MICs) were analyzed 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 (BiologOmnilog® 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].

**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.
Ines Chniti, A Thebti, M A K Sanhoury, H I Ouzari Cherif, I Chehidi. Synthesis, in vitro Antibacterial and Antifungal Activities of Trifluoroalkyl-N, N'-Disubstituted Thioureas. Organic & Medicinal Chem IJ. 2020; 9(4): 555770. DOI: 10.19080/OMCIJ.2020.09.555770.

**Table 1: N-alkyl/aryl-N’-trifluoromethylphenylthiourea 1-9**

| Compound | R           | Yield |
|----------|-------------|-------|
| 1        | 4-CF<sub>3</sub>O-Ph | 93    |
| 2        | 4-NO<sub>2</sub>-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 <sup>1</sup>H NMR spectrum exhibited singlets at δ 9.80-6.30 ppm, which were assigned to the N-H protons. <sup>13</sup>C NMR revealed peaks, in the range of 178-181 ppm, for the typical signals for the thiocarbonylic carbons (C=S thiourea). Other <sup>13</sup>C NMR signals were considered a singlet if the multiplicity was not assigned. The very strong broad peak between 3200 and 3300 cm<sup>−1</sup> 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 | E.f<sup>a</sup> | E.f<sup>b</sup> | S.a<sup>a</sup> | S.a<sup>b</sup> | B.c |
|------------------------|----------|----------------|----------------|---------------|---------------|-----|
| 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          |

**Gram Negative Bacteria**

| Compound | E.c<sup>a</sup> | E.c<sup>b</sup> | E.c<sup>c</sup> | E.c<sup>d</sup> | S.sp | P.a |
|----------|----------------|----------------|----------------|---------------|------|-----|
| 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<sup>a</sup>: Escherichia coli DH5α, E.c<sup>b</sup>: Escherichia coli ATCC 8739, E.c<sup>c</sup>: Escherichia coli BLSE Aq 3, E.c<sup>d</sup>: Escherichia coli BLSE Aq 10, S.sp: Salmonella sp, P.a: Pseudomonas aeruginosa**
Thioureas (1-9) (Table 1) were assayed in vitro against eleven bacterial strains: six are Gram-negative (Escherichia coli DH5α, Escherichia coli ATCC 8739, Escherichia coli BSE Aq3, Escherichia coli BSE 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: 500-3.90μg/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 DH5α and Escherichia coli ATCC 8739. Thiourea compounds do not show the effect on the growth of Escherichia coli BSE 3 with MIC over than 500μg/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 allyl 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 7.81-62.5μg/mL.

![image]

Table 3: Antifungal activity of N-(4-(trifluoromethyl)-phenylthiourea derivatives 1-9 MIC (μg/mL).

| Compound | Aspergillus flavus | Penicillium expansum | Candida Albicans | Candida Glabrata |
|----------|-------------------|---------------------|------------------|------------------|
| 3        | 31.25             | 31.25               | ≥500             | ≥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              | 7.81                | ≥500             | ≥500             |
| Fluconazole | 62.5            | 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 (31.25-7.81μg/mL) or equal (62.5μg/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 31.25μg/mL against Aspergillus flavus and from 15.62 to 31.25μg/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 μg/mL against Aspergillus flavus and of 15.62μg/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 μg/mL compared to the reference drug Fluconazole. However, the synthesized compounds have weak effects on Penicillium expansum with MICs of 62.5μg/mL and did not show the effect on the growth of Candida albicans ATCC 10231 and Candida glabrata with MIC over than 500μg/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. In fact, 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.
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References

1. Chniti I, Sanhoury MAK, Chehidi I (2013) O-perfluoroalkyl thiocarbamates: Synthesis and perfluoroalkyl effects on the barrier to NCS rotation. J Fluorine Chem 156: 101-105.

2. Chniti I, Sanhoury MAK, Merlet D, Chehidi I (2014) Synthesis and conformational study of new S-perfluoroalkyl dithiocarbamates. J Fluorine Chem 168: 223-229.

3. Chniti I, Maoouti H, Sanhoury MAK, Merlet D, Chehidi I (2017) Selective S-methylation of highly fluorinated thiocarbamates. Synth Commun 47: 15-21.

4. Thebti A, Chniti I, Sanhoury MAK, Chehidi I, Ouzari H, et al. (2019) Antimicrobial activity of highly fluorinated thiocarbamates and dithiocarbamates. Curr Chem Biol 13 (2): 120-128.

5. Mukerjee AK, Ashare R (1991) Isothiocyanates in the chemistry of heterocycles. Chem Rev 91: 1-24.

6. Al Masoudi N, Hassan NA, Al Soud YA, Schmidt P, Gaafar ADM, et al. (1998) Syntheses of C- and N-nucleosides from 1-aza-2-azoniaallene and 1,3-diaza-2-azoniaallene salts. J Chem Soc Perkin Trans 1: 947-954.

7. Maya I, Lopez O, Fernandez Bolanos JG, Robina I, Fuentes J (2001) A practical one-pot synthesis of O-protected glycosyl thioureas. Tetrahedron Letters 42: 5413-5416.

8. G Singh, A Saroa, SRani, Promila, Shally Girdhar, et al. (2016) Substituted phenyl urea and thiourea silaranes: Synthesis, characterization and anion recognition properties by photophysical and theoretical studies. Polyhedron 112: 51-60.

9. Garcia Fernandez JM, Mellet CO (2000) Chemistry and applications of N-thiocarbonyl carbodihydrate derivatives: Sugar isothiocyanates, thioamides, thioureas, thiacarbamates, and their conjugates. Adv Carbohydr Chem Biochem 55: 135-135.

10. Gasch C, Pradera MA, Salameh BA, Molina JL, J Fuentes (2001) Isothiocyanate derivatives of sugars in the stereoselective synthesis of spironucleosides and spiro-C-glycosides. Tetrahedron: Asymmetry 12: 1267-1277.

11. Fernandez Bolanos JG, Zafra E, Lopez O, Robina I, Fuentes J (1999) Stereoselective synthesis of imidazoline, imidazoline and imidazole C- and N-pseudonucleosides. Tetrahedron: Asymmetry 10: 3011–3023.

12. Avacos M, Babiano R, Cintas P, MM Chavero, FJ Higes, et al. (2000) Reactions of 2-Amino-2-thiazolines with Isocyanates and Isothiocyanates: Chemical and Computational Studies on the Regioselectivity, Adduct Rearrangement, and Mechanistic Pathways. J Org Chem 65: 8882-8892.

13. Herr RJ, Kuhler JL, Meckler H, CJ Opalka (2000) Convenient Method for the Preparation of Primary and Symmetrical N, N'-Disubstituted Thioureas. J Synthesis 2000 (11): 1569-1574.

14. Venkataramak TK, Sudbeck EA, Uckon FM (2001) Regiospecific synthesis, X-ray crystal structure and biological activities of 5-bromothiophenethyl thioureas. Tetrahedron Lett 42: 6629-6632.

15. Tsoukou OG, Papadaki Valiraki AE, Filipatos EC, Ikeda S, De Clercq E (1994) Synthesis and anti-myxovirus activity of some novel N, N'-disubstituted thioureas. Eur J Med Chem 29: 127-131.

16. Roy R (1996) Syntheses and some applications of chemically defined multivalent glycoconjugates. Curr Opin Struct Biol 6: 692-702.

17. Asghar E, Lal B, Badshah A, Butler IS, Tahir MN (2019) Synthesis and computational study of new meta- and para-substituted ferrocenyl thioureas as potent protein kinase inhibitors and cytotoxic agents. Inorganic Chimica Acta 488: 8-18.

18. Snellink Ruel BHM, Antonisse MMG, Engbersen JF, Timmerman P, Reinholds NT (2000) Neutral Anion Receptors with Murel-Urea-Binding Sites. Eur J Org Chem 2000: 165-170.

19. Sasaki S, Mizuno M, Naemura K, Tobe Y (2000) Synthesis and Anion-Selective Complexation of Cyclophane-Based Cyclic Thioureas. J Org Chem 65: 275-283.

20. Tozawa T, Misawa Y, Tokita S, Kubo Y (2000) A regioselectively bis(thioura)-substituted dibenzo-diaza-30-crown-10: a new strategy for the development of multi-site receptors. Tetrahedron Lett 41: 5219-5223.

21. Lee KH, Hong JJ (2000) C3-Symmetric metacyclophane-based anion receptors with three thiourea groups as linkers between aromatic groups. Tetrahedron Lett 41: 6093-6097.

22. Lee DH, Lee KH, Hong JJ (2001) An Azophenol-Based Chromogenic Anion Sensor. Org Lett 3: 5-8.

23. Benito JM, Gomez Garcia M, Jimenez Blanco JL, Gomez Garcia M, Mellet CO (2001) Carbohydrate-Based Receptors with Multiple Thiourea Binding Sites. Multipoint Hydrogen Bond Recognition of Dicarboxylates and Monosaccharides. J Org Chem 66: 1366-1372.

24. Boas U, Karlsson AJ, de Waal BFM, EW Meijer (2001) Synthesis and Properties of New Thiourea-Functionalized Poly(propylene imine) Dendrimers and Their Role as Hosts for Urea Functionalized Guests. J Org Chem 66: 2136-2145.

25. Werts MHV, Woudenberg RH, Emmerink PG, van Gassel, R, Hofstraat JW, et al. (2000) A Near-Infrared Luminescent Label Based on YbIII Ions and Its Application in a Fluorimunoassay. Angew Chem Int Ed Engl 39: 4542-4544.

26. Ueda M, Sawai Y, Yamamura S (2000) Syntheses of fluorescence-labeled artificial leaf-opening substances, fluorescent probe compounds useful for bioorganic studies of nictinactin. Tetrahedron Lett 41: 3433-3436.

27. Satchell DPN, Satchell RS (1993) The Chemistry of Sulfur-Containing Functional Groups. In: Patai S, Rappoport Z (Eds.), Wiley: New York, Suppl. S, Chapter 12, pp. 599-631.

28. Caujolle R, Amarouch H, Payard M, Leoiseau PM, Bories C, et al. (1995) Synthesis, antifungal and nematocidal activities of thioureas with an aminoester sequence. Eur J Med Chem 30: 801-807.

29. Truong P, Ngo DTH (1999) J Vietnamese Pharm 12. Chem Abstr 2000 135: 263-752.

30. Truong P, Tran PY (2000) J Vietnamese Pharm 9. Chem Abstr 2001 135: 73923.

31. Hiroshi K, Kato K, Masami A, Minami E, Masuda K, et al. (1999) A highly stereoselective synthesis of pluunotol and its thiourea derivatives as potent antibiotic agents against Helicobacter pylori. Bioorg Med Chem Lett 9: 1347-1350.

32. Demuth Jr, Thomas P, White RE (1997) United States patent US 5 631: 256.

33. Uckon FM, Mao C, Pendergrass S, Maher D, Zhu D et al. (1999) N-[2-(1-cyclohexenyl)ethyl]-N2-[5-(5-bromopyridyl)]-thioura and N-[2- (1-cyclohexenyl)ethyl]-N2-[5-(5-chloropyridyl)]-thioura as potent inhibitors of multidrug-resistant human immunodeficiency virus-1. Bioorg Med Chem Lett 9: 2721-2726.

34. Dong Y, Venkataramak TK, Narla RK, Trien VN, Sudbeck EA, et al. (2000) Antioxidant function of phenethyl-5-bromo-pyridyl thiourea compounds with potent anti-HIV activity. Bioorg Med Chem Lett 10: 87-90.
35. Ehrihard AA, Jäger S, Malm C, Basaran S, Hunger J (2019) CF3-groups critically enhance the catalytic binding of thiourea catalysts to ketones – a NMR and FT-IR critically studied. Journal of Molecular Liquids 296:111829.

36. Rainer W, Zimmermann MO, Lange A, Joergler AG, Boedeker FM (2012) Principles and Applications of Halogen Bonding in Medicinal Chemistry and Chemical Biology. J Med Chem 1363-1388.

37. Ghorab MM, Alsaid MS, El Gaby MSA, Elaesser MM, Nissan YM (2017) Antimicrobial and anticancer activity of some novel fluorinated thiourea derivatives carrying sulfonamide moieties: synthesis, biological evaluation and molecular docking. Chemistry Central Journal 11: 32-46.

38. Maalik A, Rahim H, Saleem M, Fatima N, Rauf A, et al. (2019) Synthesis, antimicrobial, antioxidant, cytotoxic, antiurease and molecular docking studies of N-(3 trifluoromethyl) benzoyl-N′-aryl thiourea derivatives. Bioorganic Chemistry 88: 102946-102955.

39. Wahid S, Jahangir S, Versiani MA, Khan KM, Salar U, et al. (2020) Atenolol Thiourea Hybrid as Potent Urease Inhibitors: Design, Biology-Oriented Drug Synthesis, Inhibitory Activity Screening, and Molecular Docking Studies. Bioorganic Chemistry 94: 103355-103367.

40. Al Harbi RAK, El Sharief MAM Sh, Abbas SY (2019) Synthesis and anticancer activity of bis-benz[d][1,3]dioxol-5-yl thiourea derivatives with molecular docking study. Bioorganic Chemistry 90:103088.

41. Sreed A, Shaheen U, Hameed A, Haider Naqvi SZ (2009) Synthesis, characterization and antimicrobial activity of some new 1-(fluorobenzoyl)-3-(fluorophenyl)thioureas. J Fluorine Chem 130: 1028-1034.

42. Suresha GP, Suhas R, Kapfo W, D Channe Gowda (2011) Urea/thiourea derivatives of quinoxaline–lysine conjugates: Synthesis and structure–activity relationships of some new series of antimalarials. Eur J Med Chem 46: 2530-2540.

43. Chkhalia KH, Patel MJ (2009) Design, synthesis and evaluation of some 1,3,5-triazinyl urea and thiourea derivatives as antimicrobial agents. J Enzyme Inhib 24: 960-966.

44. Sreed A, Rachid N, Jones PG, Ali M, Hussain R (2010) Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. Eur J Med Chem 45: 1323-1331.

45. A Bielenica K Stępień, A Napórkowska, E Augustynowicz Kopeć, S Krukowski, et al. (2016) Synthesis and Antimicrobial Activity of 4-Chloro-3-Nitrophenylthiourea Derivatives Targeting Bacterial Type II Topoisomerases. Chem Biol Drug Des 87: 905-917.

46. Bielenica A, Stefaniška J, Stępień K, Napórkowska A, Augustynowicz Kopeć E (2015) Synthesis, cytotoxicity and antimicrobial activity of thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety. Eur J Med Chem 101: 111-125.

47. Duke RM, Gunnlaugsson T (2017) Selective fluorescent PET sensing of fluoride (F⁻) using naphthalimide–thiourea and –urea conjugates. Tetrahedron Letters 48: 8043-8047.

48. National Committee for Clinical Laboratory Standards (2000) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania.

49. Bochner BR, Gazdzinski P, Panomitros E (2001) Phenotype microarrays for high-throughput phenotypic testing and assay of gene function. Genome Res 11: 1246-1255.

50. Bochner BR (2003) New technologies to assess genotype-phenotype relationships. Nat Rev Genet 4 (4): 309-314.

51. Clinical Laboratory Standards Institute (2008) Reference method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard (3rd edn), M27-A3, Wayne, Pennsylvania.

52. Kumamoto K, Misawa Y, Tokita S, Kubo Y, Kotsuki H (2002) High-pressure-promoted condensation of isothiocyanates with aminopyridines: efficient synthesis of pyridine–thiourea conjugates as building blocks for hydrogen-bonding receptors. Tetrahedron Lett 43: 1035-1038.

53. Bhowruth V, Brown AK, Reynolds RC, Coxon GD, Mackay SP, et al. (2006) Symmetrical and unsymmetrical analogues of isoxyl; active building blocks for hydrogen-bonding receptors. Tetrahedron Letters 48: 8043-8047.

54. Faidallah HM, Rostom SA, Basaif SA, Makki MS, Khan KA (2013) Synthesis and biological evaluation of some novel urea and thiourea derivatives of isoxazolo[4,5-d]-pyridazine and structurally related thiazolo[4,5-d]-pyridazines as antimicrobial agents. Arch Pharm Res 36: 1354-1368.