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The synthesis and antimicrobial activity of some new methyl *N*-arylthiocarbamates, dimethyl *N*-aryldithiocarbonimidates and 2-arylamino-2-imidazolines

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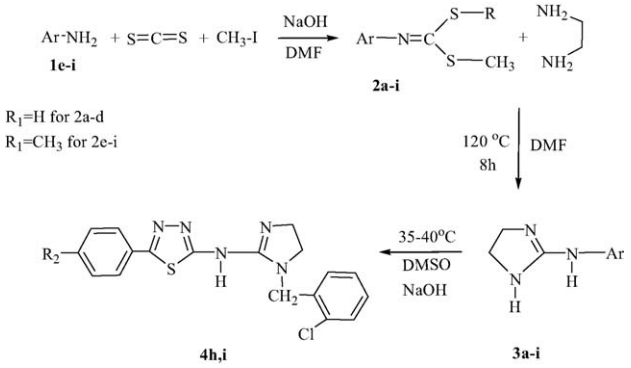
Abstract

Methyl *N*-arylthiocarbamates (**2a–d**) and dimethyl *N*-aryldithiocarbonimidates (**2e–i**) were synthesized from the reaction of aromatic amines with carbon disulfide and methyl iodide and NaOH in various quantitative amounts. 2-Arylamino-2-imidazolines (**3a–i**) were prepared by heating both methyl *N*-arylthiocarbamates (**2a–d**) and dimethyl *N*-aryldithiocarbonimidates (**2e–i**) with 1,2-diaminoethane under reflux. *o*-chlorobenzyl derivatives of [1,3,4]-thiadiazole-2-yl substituted aminoimidazoline compounds were synthesized by treatment of [1,3,4]-thiadiazole-2-yl substituted aminoimidazolines (**3h–i**) with 2-benzyl chloride in basic medium and DMSO. Some of the synthesized compounds were tested in vitro for their antimicrobial activity. All of the selected compounds showed some antimicrobial activity against test microorganisms. Compounds **2f** and **3f** which have 1,3-benzothiazol ring exhibited a weak activity against *Candida globrata*.



Keywords: Antimicrobial activity; Methyl *N*-arylthiocarbamates; Dimethyl *N*-aryldithiocarbonimidates; 2-Arylamino-2-imidazolines

1. Introduction

N-mono and *N*,*N*-disubstituted dithiocarbamate derivatives have showed antibacterial, antiviral and antifungal activities [1]. Methyl *N*-arylthiocarbamates and dimethyl *N*-aryl dithiocarbonimidates have allowed to synthesis of 2-arylamino-2-imidazolines. 2-Arylamino-2-imidazolines have an interesting chemistry [2–5] and they are effective pharmacophores in medicinal chemistry. 2-Arylamino-2-imidazolines, in particular 2,6-dichlorophenylamino-2-imidazoline (*clonidine*) have a pronounced, hypotensive action, which is coupled with a sedative action. Moreover, some of these compounds also have a more or less pro-

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nounced analgesic action which, however, because of the simultaneous existence of the hypotensive action and the depressant action on the central nervous system, was considered unexploitable. A hypotensive action has also been described for benzoyl derivatives of 2-arylamino-2-imidazoline, especially for the compound 1-benzoyl-2-(2′,6′-dichlorophenyl amino)-2-imidazoline, in the depressant action on the central nervous system and thus the sedative action being substantially less pronounced with this compound [6].

The most recognized of the 2-arylamino-2-imidazolines are *clonidine* and *moxonidine* compounds. These compounds have been shown α_1 and α_2 adrenoceptor activities and more specially, *phentolamine*, which contains an imidazoline ring, is a known α_1-adrenergic antagonist (Scheme 1). Imidazoline I_2 receptors are widely distributed in the body and brain of different species including humans. Functionally these receptors have been implicated in a variety of disease states such as psychiatric disorders, opiate withdrawal Parkinson's and Alzheimer's disease as well as Huntington's chorea [7–10].

[1,3,4]-Thiadiazoles and their derivatives exhibit diverse biological activities possibly due to the presence of =N–C–S moiety [11]. Various phenyl substituted [1,3,4]-thiadiazole-2-amines and their derivatives have recently received significant importance because of their diverse biological properties [12,13]. Prompted by these observations and in continuation of our work on the synthesis of 2-arylamino-2-imidazolines, we thought it worthwhile to synthesis new compounds of 2-arylamino-2-imidazolines having [1,3,4]-thiadizole moiety, with the objective of obtaining new biologically active compounds.

In this study, methyl *N*-arylthiocarbamates (**2a–d**) and dimethyl *N*-aryldithiocarbonimi dates (**2e–i**) were synthesized by reaction of aromatic amines with carbon disulfide (CS_2) and methyl iodide (CH_3I). 2-arylamino-2-imidazolines (**3a–i**) were obtained by treatment 1,2-diaminoethane (DAE) with **2a–i** compounds at 120 °C for 8 h.

This article reports the first synthesis of compounds **2a–e**, **2g–i**, **3e**, **3g–i**. Also reported are synthesis of *o*-chlorobenzyl derivatives of [1,3,4]-thiadiazole-2-yl substituted aminoimidazolines (**3h–i**), which have not been synthesized previously. So far, antimicrobial activity of 2-arylamino-2-imidazolines and their derivatives which having *o*-chloro benzyl group has not been investigated.

2. Chemistry

The reaction of CS_2 and CH_3I with aromatic amine in the presence concentrated aqueous NaOH lead to the formation of methyl *N*-arylthiocarbamates (**2a–d**) and dimethyl *N*-aryldithiocarbonimidates (**2e–i**) which on treatment with bisnucleophile such as DAE give *N*-aryl substituted aminoimidazoline.

In the first part of the study, compounds **2a–i** were synthesized by the treatment of CS_2 and CH_3I with aromatic (or hetero-aromatic) amines in basic medium. In this reaction, different compounds can be synthesized by using various amount of reactive. **2a–d** compounds were synthesized when 1 mol CS_2, 1 mol CH_3I with 1 mol aromatic amine were used. On the other hand, **2e–i** compounds were obtained when 2 mol CS_2, 2 mol CH_3I with 1 mol aromatic amine were used.

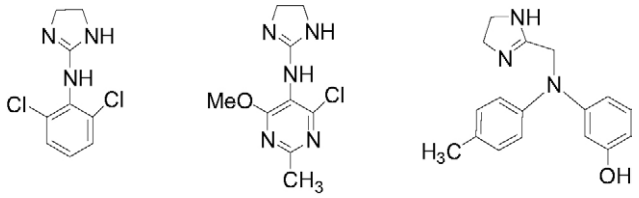
The synthesis of compounds **2a–i**, **3a–i**, **4g**, **h** are presented in Schemes 2,3. Compounds **3a–i** were synthesized by refluxing of **2a–i** with DAE in DMF for 8 h. To strengthen nucleophilic attack of NH_2 on DAE was used excess amount of DAE. Yields of **3h**, **i** compounds were obtained lower than other **3** compounds.

5-Aryl substituted-[1,3,4]-thiadizole amines (**1h**, **i**) were synthesized by cyclization of aryl carboxylic acids and thiosemicarbazide in phosphorous oxychloride [11–14]. The reaction of compounds **1h**, **i** with CS_2 and CH_3I and NaOH led to the formation dimethyl *N*-[1,3,4]-thiadiazole-2-yl substituted dithiocarbonimidates (**2h**, **i**). The treatment of compounds **2h**, **i** with DAE resulted in the formation of [1,3,4]-thiadiazole-2-yl substituted aminoimidazolines **3h**, **i** (Scheme 3)**.** Compounds **4h**, **i** were synthesized via nücleophilic attack of N on imidazoline ring (endocyclic nitrogen atom) to chloride-bearing C atom of 2-benzyl chloride compound. Synthesis of *o*-chlorobenzyl derivatives of compounds **3h**, **i** were carried out by treatment [1,3,4]-thiadiazole-2-yl aminoimidazolines (**3h**, **i**) with 2-benzyl chloride in DMSO and basic medium (Scheme 3).

2a–i, **3e**, **3g–i** and **4h**, **i** were synthesized and characterized for the first time in this present study. The synthesized some compounds were tested in vitro for their antimicrobial activity.

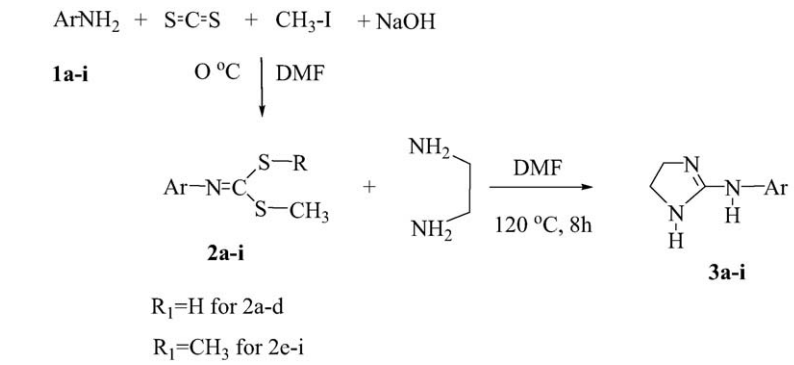
3. Result and discussion

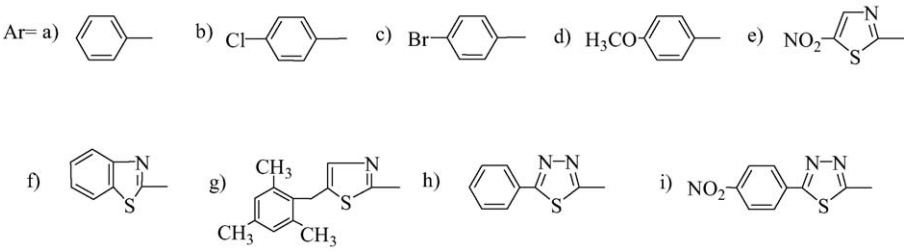
Compounds **2a–d** have showed thioimidate–thioamide tautomerization. Thioamide form is more dominant according



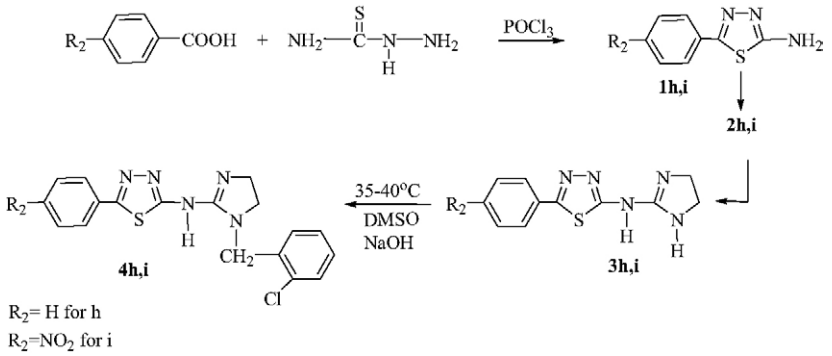
Clonidine *Moxonidine* *Phentolamine*

Scheme 1. Imidazoline derivatives containing a 2-aminoimidazoline or an imidazoline ring which are known to interact with α-adrenergic receptors.





Scheme 2. Synthesis of methyl *N*-arylthiocarbamates, dimethyl *N*-heteroaryldithioimidocarbonates and 2-arylamino-2-imidazolines.



Scheme 3. Synthesis of 2-chloro substituted benzyl derivatives of compounds **3h**, **i**.

to IR, ^{1}H-NMR and ^{13}C-NMR data at room temperature. In the ^{1}H-NMR spectrum of compounds **2a–d** were observed at 2.46–2.62 (SCH_3) and 8.91–9.95 ppm (SH, controlled by changing with D_2O) integrating for three proton and one proton, respectively. The IR spectra of compounds **2a–i** showed SCH_3 absorption between 830 and 726 cm^{-1}. In addition, the IR spectra of compounds **2a–d** displayed SH absorption between 3214 and 3110 cm^{-1}. Besides the IR spectra of compounds **2e** and **2h** showed to two different NO_2 absorption peak both 1530 and 1350 cm^{-1}. The ^{1}H-NMR spectra of compounds **2e–i** displayed no signals belonging to SH group; instead, SCH_3 signal appeared between 2.48 and 2.65 integrating for six protons. In the ^{13}C-NMR spectra of compounds **2a–i**, S–CH_3 and C=N signals appeared δ 16.2 and 142.5 ppm, respectively.

Compounds **3a–i** have showed imine–enamine tautomerization. The imine–enamine tautomerization of 2-arylamino-2-imidazolines was investigated by means of ^{1}H-NMR spectroscopy. In the ^{1}H-NMR (200 and 90 MHz) spectra of **3a–i** singlets integrating for four hydrogens were observed at δ 3.78–3.76 ppm, respectively. These compounds are effectively symmetrical because of imine–enamine dynamic tautomerization, which makes the CH_2 protons equivalent. At the same time this effect causes lowering of the order of the spectra ($\Delta\upsilon/J = 0$). In the IR spectra of compounds **3a–i** displayed two different NH absorption (stretching) 3450–3250 cm^{-1}.

In the ^{1}H-NMR spectra of compounds **4h**, **i** the signals belonging to 2-benzyl chloride (aliphatic CH_2) were observed at 4.70 ppm while the signals belonging to endocyclic imida-

zoline NH disappeared. The signals belonging to exocyclic NH were appeared at 5.49 ppm for **4h** and 8.19 ppm for **4i** compounds. The IR spectra at **4h**, **i** compounds showed additional peaks at 750 and 1140 cm^{-1} due to *o*-substituted phenyl group.

3.1. Biological evaluation

We have designed and synthesized novel compounds of 2-arylamino-2-imidazolines class, in order to investigate the relationships between antimicrobial activity and structure. The minimum inhibitory concentration (MIC) of the synthesized compounds was determined against the Gram-positive *Bacillus subtilis*, *Staphylococcus aureus*, the Gram-negative *Escherichia coli*, *Salmonella typhimurium* and the yeast *Candida globrata* and *Candida tropicalis* using a standard broth dilution technique. Their antimicrobial activity was compared to ampicillin and fluconazol as standard drugs. All the MIC results are presented in Table 1.

The obtained data reported that compounds were able to inhibit the growth of the selected microorganisms in vitro showing MIC values between 512 and 8 μg ml^{-1}.

All the synthesized compounds showed some degree of antimicrobial activities against test microorganisms. Among the synthesized compounds, methyl *N*-4-chlorophenyl-thiocarbamate **2b** was found the most active derivative at an MIC value of 8 μg ml^{-1} against *S. aureus*. Compounds **2a–c**, **2e–g**, **3e** and **3f** might be slightly more active than others against some of the test microorganisms.

Compounds **2b** and **c** which having electron withdrawing substituents such as, chloride or bromide, respectively, on phenyl group were found to be the most active compounds against the Gram-positive bacteria *S. aureus* ATCC 6538, while the compound **2a** which bearing a phenyl group showed poor activity towards *S. aureus* ATCC 6538 and *B. subtilis* ATCC 6633. Dimethyl *N*-heteroaryldithioimidocarbonates bearing5-substitutedthiazolering(**2e**and**2g**),dimethyl*N*-heteroaryldithioimidocarbonates having benzothiazole ring (**2f**) and 2-arylamino-2-imidazoline bearing thiazole ring (**3e**) exhibited promising activity against *S. aureus*. In addition, compounds **2f** and **3e** showed poor antifungal activity towards *C. globrata*. Also, all of the other compounds exhibited poor activity against *S. aureus*, *E. coli*, *B. subtilis* and *C. globrata*.

The compounds **2h**, **i** and **3h** which have [1,3,4]-thiadiazole ring showed a poor antimicrobial effect against test organisms (256 μg ml^{-1}). Similarly, compounds **4h** and **i** which contain *o*-chlorobenzyl group on endocyclic nitrogen atom of [1,3,4]-thiadiazol-2-yl amino-2-imidazolins showed the same weak effect against test organisms (256 μg ml^{-1}).

Compounds **2g**, **3g** which have 2,4,6-trimethyl benzyl group and **3i** exhibited poor antifungal activity towards *C. tropicalis*. Likewise, compounds **2f** and **2g** indicated poor antibacterial effect against *E. coli*.

These results indicated that the standard antibiotic was only active by an extra fourfold and eightfold in comparison to **2b** and **c**, respectively, which demonstrates the potential efficacy.

Table 1
Antimicrobial activity results (MIC values in μg ml^{-1}) of synthesized compounds with the standard drugs

Sample	S.t	E.c	B.s	S.a	C.g	C.t
2a	256	256	128	128	256	256
2b	256	256	256	8	256	256
2c	256	256	256	16	256	256
2d	256	256	256	256	256	256
2e	256	256	256	64	256	256
2f	256	512	256	64	128	256
2g	256	512	256	64	256	512
2h	256	256	256	256	256	256
2i	256	256	256	256	256	256
3b	256	256	256	256	256	256
3e	256	256	256	64	256	256
3f	256	256	256	256	128	256
3g	256	256	512	256	256	512
3h	256	256	256	256	256	256
3i	256	256	256	256	256	512
4h	256	256	256	256	256	256
4i	256	256	256	256	256	256
Ampicilin	2	2	2	2	–	–
Fluconazol					8	8

In vitro activity of the selected compounds and the reference drugs (MIC values in μg ml^{-1}). B.s: *B. subtilis* ATCC 6633, S.a: *S. aureus* ATCC 6538 P, E.c: *E. coli* ATCC 25922, S.t: *S. typhimurium* NRRL B 4420, C.g: *C. globrata* ATTC 66032, C.t: *C. tropicalis* ATCC 13803.

4. Experimental

4.1. Chemistry

Aromatic and hetero-aromatic amines were obtained from commercial sources, except for hetero-aromatic amines (**1g–i**) which were used to synthesis of **2g–i** and **3g–i** compounds. **1h**, **i** compounds were prepared following literature procedures [12–14]. Melting points (uncorrected) were determined with a Gallenkamp apparatus. IR spectra were measured on a Mattson model FT-IR spectrometer (potassium bromide disks), and ^1H spectrum were recorded on FX 90Q JEOL and 200 MHz Bruker AC instruments. Chemical shift values are reported in parts per million (δ) relative to tetramethyl silane as an internal standard. Elemental analyses were performed on a LECO model 932 instrument.

4.1.1. General method for the synthesis of compounds (2a–d)

To a well-stirred cold solution of aromatic amines (0.04 mol) in DMF (20 ml) were added aqueous NaOH (20 M, 4 ml), carbon disulfide (3.0 ml, *d*: 1.26 g cm^{-3}, 0.05 mol), and methyl iodide (3.1 ml, d: 2.28 g cm^{-3}, 0.05 mol) in sequence at intervals of 30 min and stirring was continued for 2–4 h. The mixture was then poured into cold water and the resulting solid was washed with water and recrystallized from ethanol solvent to afford the desired compound (Scheme 2). The following products were obtained.

4.1.1.1. Methyl N-*phenylthiocarbamate (**2a**).* (Yield 57%), yellow powder, m.p. 92–93 °C. Analysis (Calc./found %): for $C_8H_9NS_2$ C: 2.42/51.94, H: 4.95/4.79, N: 7.64/7.35, S: 34.99/34.53; IR (KBr) (υ, cm^{-1}), 3110 (S–H stretching), 2920 (aliphatic C–H stretching), 1600 (C=N stretching), 775 (SCH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ (ppm) 2.46 (s, 3H, SCH_3), 7.4 (s, 5H, Ar–H), 9.40 (1H, SH).

4.1.1.2. Methyl N-*4-chlorophenylthiocarbamate (**2b**).* (Yield 64%), orange powder, m.p. 112–114 °C. Analysis (Calc./found %): for $C_8H_8ClNS_2$ C: 44.13/44.24, H: 3.70/3.50, N: 6.43/6.27, S: 29.45/28.06; IR (KBr) (υ, cm^{-1}), 2932 (aliphatic C–H stretching), 1617 (C=N stretching), 507 (C–Cl); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ: 2.66 (s, 3H, SCH_3), 7.40–7.60 (m, 4H, Ar–H), 9.85 (1H, SH).

4.1.1.3. Methyl N-*4-bromophenylthiocarbamate (**2c**).* (Yield 65%), brown powder, m.p. 133–135 °C. Analysis (Calc./found %): for $C_8H_8BrNS_2$ C: 36.65/36.12, H: 3.08/3.45, N: 5.34/4.98, S: 24.46/24.58; IR (KBr) (υ, cm^{-1}), 3214 (S–H stretching), 2971 (aliphatic C–H stretching), 1643 (C=C stretching), 1585 (C=N stretching) 726 (SCH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ: 2.62 (s, 3H, SCH_3), 7.44–7.60 (m, 4H, Ar–H), 8.91 (1H, SH).

4.1.1.4. Methyl N-*4-methoxyphenylthiocarbamate (**2d**).* (Yield 63%), light brown crystal, m.p. 109–111 °C. Analysis (Calc./found %): for $C_9H_{11}NOS_2$ C: 50.67/50.08, H: 5.20/5.37, N: 6.57/6.54, S: 30.06/30.56; IR (KBr) (υ, cm^{-1}), 3158 (S–H stretching), 3078 (aromatic C–H stretching), 2962–2921 (aliphatic C–H stretching), 1607 (C=C stretching), 1589 (C=N stretching), 1253 (OCH_3 stretching), 830 (S–CH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ: 2.62 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 6.95–7.26 (m, 4H, aromatic protons) 9.17 (1H, SH).

*4.1.2. General method for the synthesis of compounds (**2e–i**)*

To a solution of aromatic (or heteroaromatic) amine (0.1 mol) in DMF (75 ml), aqueous 20 M sodium hydroxide (5.5 ml, 0.11 mol) was added with stirring at room temperature. After 10 min carbon disulfide (3.3 ml, *d*: 1.26 g cm^{-3}, 0.055 mol) was added and stirring was continued for 30 min. Then aqueous 20 M sodium hydroxide (3 ml, 0.06 mol) and carbon disulfide (1.8 ml, 0.0275 mol) were added. This operation was finally repeated 10 min later. After 30 min the reaction was placed in an ice bath, methyl iodide (12.5 ml, *d*: 2.28 g cm^{-3}, 0.2 mol) was added drop wise and stirring was continued for 2 h. The mixture was then poured into ice cooled water and the precipitate thus obtained was filtered, washed with water, dried and recrystallized from ethanol. The following products were obtained.

4.1.2.1. Dimethyl N-*(5-nitro-1,3-thiazol-2-yl) dithioimidocarbonate (**2e**).* (Yield 59%), yellow powder, m.p. 174–175 °C (ethanol). Analysis (Calc./found %): for $C_6H_7N_3O_2S_3$ C: 28.90/28.39, H: 2.83/3.06, N: 16.85/15.34, S: 38.58/38.79; IR (KBr) (υ, cm^{-1}), 3095 (aromatic C–H stretching), 2979–2930 (aliphatic C–H stretching), 1678 (C=C stretching), 1621 (C=N stretching), 1530 (Ar–NO_2 asymmetric stretching) and 1350 (symmetric stretching), 841 (S–CH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ (ppm) 2.65 (s, 6H, SCH_3), 8.36 (s, 1H, thiazole ring proton).

4.1.2.2. Dimethyl N-*(1,3-benzothiazol-2-yl) dithioimidocarbonate (**2f**).* (Yield 69%), yellowish powder, m.p. 101–102 °C (ethanol). Analysis (Calc./found %): for $C_{10}H_{10}N_2S_3$ C: 47.21/48.39, H: 3.96/4.06, N: 11.01/11.34, S: 37.81/38.77; IR (KBr) (υ, cm^{-1}), 2995 (aliphatic C–H stretching), 1592 (C=C stretching), 1510 (C=N stretching), 833 (S–CH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ (ppm) 2.59 (s, 6H, SCH_3), 6.90–7.30 (m, 2H, Ar–H), 7.50–7.86 (2H, m, Ar–H).

4.1.2.3. Dimethyl N-*[5-(2,4,6-trimethylbenzyl)thiazole-2-yl] dithioimidocarbonate (**2g**).* (Yield 74%), orange power, m.p. 91–92 °C (ethanol). Analysis (Calc./found %): for $C_{16}H_{20}N_2S_3$ C: 57.10/58.12, H: 5.99/5.19, N: 8.32/8.45, S: 28.58/28.97; IR (KBr) (υ, cm^{-1}), 2920 and 2855 (aliphatic C–H stretching), 1612 (C=C stretching), 1523 (C=N stretching), 756 (S–CH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ (ppm) 2.26 (s, 9H, mesitylene CH_3), 2,58 (s, 6H, SCH_3), 3,92 (s, 2H, aliphatic CH_2), 6,21 (s, 1H, thiazole ring proton), 6.87–7.26 (m, 2H, Ar–H).

4.1.2.4. Dimethyl N-*{5-phenyl- [1,3,4]-thiadiazol-2-yl} dithioimidocarbonate (**2h**).* (Yield 62%), orange powder, m.p. 111–113 °C (ethanol). Analysis (Calc./found %): for $C_{11}H_{11}N_3S_3$ C: 46.95/46.65, H: 3.94/3.38, N: 14.93/14.23, S:34.18/ 34.37; IR (KBr) (υ, cm^{-1}), 2968 (aliphatic C–H stretching), 1619 (C=C stretching), 1578 and 1537 (C=N stretching), 751 (S–CH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ (ppm) 2.64 (s, 6H, SCH_3), 7.47–7.99 (m, 5H, Ar–H).

4.1.2.5. Dimethyl N-*{5-(4-nitrophenyl) [1,3,4]-thiadiazol-2-yl} dithioimidocarbonate (**2i**).* (Yield 55%); orange powder, m.p. 250–251 °C (ethanol). Analysis (Calc./found %): for $C_{11}H_{10}N_4O_2S_3$ C: 40.48/39.56, H: 3.09/3.32, N: 17.16/17.19, S: 27.47/28.09; IR (KBr) (υ, cm^{-1}), 2930–2900 (aliphatic C–H stretching), 1633 (C=C stretching), 1592 (C=N stretching), 1510 (Ar–NO_2 asymmetric stretching) and 1349 (symmetric stretching), 846 (S–CH_3 stretching); ^{1}H-NMR ($CHCl_3$-*d*, 90 MHz) δ (ppm) 2.48 (s, 6H, SCH_3), 8.31–8.52 (m, 4H, Ar–H).

*4.1.3. Synthesis of 2-arylamino-2-imidazolines (**3a–i**)*

A solution of **2a–i** (0.04 mol) in DMF (15 ml) was added to a solution of 1,2-diaminoethane (0.08 mol) in DMF (15 ml) with stirring at room temperature. The reaction mixture was maintained at 120 °C for 8 h. Then the mixture was cooled and added to ice-cold water. The resulting solid was washed with water, dried and recrystallized from appropriate solvent.

4.1.3.1. N-*(Phenyl)*-N-*(4,5-dihydro-1H-imidazol-2-yl) amine (**3a**).* (Yield 63%), white powder, m.p. 115–116 °C (ethanol). Analysis (Calc./found %): for $C_9H_{11}N_3$ C: 67.06/68.11, H: 6.88/7.24, N: 26.07/26.83; IR (KBr) (υ, cm^{-1}), 3277 (imidazoline N–H stretching), 3257 (N–H stretching), 1660 (C=N stretching), 1537 (N–H bending); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 3.48 (s, 1H, NH), 3.73 (s, 4H, imidazoline CH_2), 6.30 (broad peak, 1H, imidazoline NH), 7.12–8.20 (m, 5H, Ar–H).

4.1.3.2. N-*(4-Chlorophenyl)*-N-*(4,5-dihydro-1H-imidazol-2-yl) amine (**3b**).* (Yield 71%); white powder, m.p. 147–148 °C (ethanol). Analysis (Calc./found %): for $C_9H_{10}Cl\ N_3$ C: 55.25/56.13, H: 5.15/5.74, N: 21.48/22.05; IR (KBr) (υ, cm^{-1}), 3284 (imidazoline N–H stretching), 3245 (N–H stretching), 1681 (C=N stretching), 1594 (N–H bending); ^{1}H-NMR (DMSO-d_6, 200 MHz): δ (ppm) 3.67 (s, 1H, NH), 3.76 (s, 4H, imidazoline CH_2), 6.24 (broad peak, 1H, imidazoline NH), 7.05–7.40 (m, 4H, Ar–H).

4.1.3.3. N-*(5-Nitro-1,3-thiazol-2-yl)*-N-*(4,5-dihydro-1H-imidazol-2-yl) amine (**3e**).* (Yield 46%), white powder, m.p. 133–135 °C (chloroform). Analysis (Calc./found %): for $C_6H_7N_3O_2S$ C: 33.80/34.19, H: 3.31/3.59, N: 32.85/33.21, S: 15.04/15.19; IR (KBr) (υ, cm^{-1}), 3358 and 3317 (N–H stretching), 1681 (C=C stretching), 1619 (C=N stretching), 1544 (Ar–NO_2 asymmetric stretching), 1465 (N–H bending) 1359 (Ar–NO_2 symmetric stretching); ^{1}H-NMR (DMSO-d_6, 90 MHz) δ (ppm) 3.66 (s, 4H, imidazoline CH_2), 8.19 (s, 2H, NH), 8.35 (s, 1H, thiazole ring proton).

4.1.3.4. N-*(1,3-Benzothiazol-2-yl)*-N-*(4,5-dihydro-1H-imidazol-2-yl) amine (**3f**).* (Yield 66%), white powder, m.p. 131–132 °C (ethanol). Analysis (Calc./found %): for $C_{10}H_{10}N_4S$ C: 55.02/55.63, H: 4.62/4.96, N: 25.37/26.41, S: 14.69/15.19. IR (KBr) (υ, cm^{-1}), 3281 (imidazoline N–H stretching), 3220 (N–H stretching), 1611 (C=N stretching), 1528 (N–H bending); ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 1.74 (s, 1H, NH), 3.78 (s, 4H, imidazoline CH_2), 7.09–7.64 (m, 4H, Ar–H), 8.01 (broad peak, 1H, imidazoline NH).

4.1.3.5. N-*[5-(2,4,6-Trimethylbenzyl) thiazole-2-yl]*-N-*(4,5-dihydro-1H-imidazole-2-yl) amine (**3g**).* (Yield 66%), light gray powder, m.p. 172–174 °C (toluene). Analysis (Calc./found %): for $C_{16}H_{20}N_4S$ C: 63.97/63.85, H: 6.71/6.56, N: 18.65/18.34, S: 10.67/10.07. IR (KBr) (υ, cm^{-1}), 3382 and 3372 (N–H stretching), 2923 (C–H stretching), 1626 (C=C stretching), 1532 (C=N stretching), 1471 (N–H bending); ^{1}H-NMR (DMSO-d_6, 90 MHz) δ (ppm) 2.15 (s, 2H, NH), 2.26 (s, 9H, 3 × CH_3), 3.66 (s, 4H, imidazoline CH_2), 3.91 (s, 2H, aliphatic CH_2), 5.76 (s, 1H, thiazole ring proton), 6.87 (m, 2H, Ar–H).

4.1.3.6. N-*(5-Phenyl-[1,3,4]-thiadiazol-2-yl)*-N-*(4,5-dihydro-1H-imidazol-2-yl) amine (**3h**).* (Yield 57%), yellowish crystal, m.p. 192–194 °C (toluene). Analysis (Calc./found %): for $C_{11}H_{11}N_5S$ C: 53.86/54.03, H: 4.52/4.66, N: 28.56/28.41, S: 13.07/13.65. IR (KBr) (υ, cm^{-1}), 3317 and 3297 (N–H stretching), 1614 (C=C stretching), 1528 (C=N stretching), 1443 (N–H bending); ^{1}H-NMR (DMSO-d_6, 90 MHz) δ (ppm) 3.76 (s, 4H, imidazoline CH_2), 7.45 (s, 5H, Ar–H); 7.55 (s, 2H, NH).

4.1.3.7. N-*{5-(4-Nitrophenyl)-[1.3.4]-thiadiazole-2-yl}*-N-*(4,5-dihydro-1H-imidazol-2-yl) amine (**3i**).* (Yield 44%), yellow powder, m.p. 239–240 °C (ethanol). Analysis (Calc./found %): for $C_{11}H_{10}N_6O_2S$ C: 45.51/45.11, H: 3.47/3.70, N: 28.95/28.56, S: IR (KBr) (υ, cm^{-1}), 3396 and 3218 (N–H stretching), 1626 (C=C stretching), 1538 (C=N stretching), 1508 (Ar–NO_2 asymmetric stretching), 1465 (N–H bending) 1350 (Ar–NO_2 symmetric stretching); ^{1}H-NMR (DMSO-d_6, 90 MHz) δ (ppm) 3.58 (s, 4H, imidazoline CH_2), 7.53–8.29 (m, 4H, Ar–H), 8.09 (s, 2H, NH).

*4.1.4. Synthesis of 2-benzyl chloride derivatives of compounds **3h**, **i***

To a mixture of *N*-[5-(4-nitrophenyl)-1.3.4-thiadiazole-2-yl)-*N*-(4,5-dihydro-1*H*-imidazol-2-yl) amine (0.91 g, 3.14 mmol) and finely powdered NaOH (0.5 g, 12.5 mmol) in DMSO (7 ml) was added drop wise 2-benzyl chloride (0.35 ml, 3.45 mmol). The resulting solution was stirred at 35–40 °C for 1 h. Then water was added to the reaction mixture, and the solid that precipitated was collected crystallization from ethanol.

*4.1.4.1. [1-(2-Chlorobenzyl)-4,5-dihydro-1H-imidazole-2-yl]-[5-phenyl-[1,3,5]-thiadiazol-2-yl] amine (**4h**).* (Yield 64%), orange powder, m.p. 153 °C (ethanol). Analysis (Calc./found %): for $C_{18}H_{16}ClN_5S$ C: 58.45/58.65, H: 4.36/3.28, N: 18.93/19.08, S: 8.67/7.93. IR (KBr) (υ, cm^{-1}), 3314 (N–H stretching), 3075 (aromatic C–H stretching), 2954–2858 (C–H stretching), 1676 (C=C stretching), 1592 (C=N stretching), 507 (C–Cl); ^{1}H-NMR (DMSO-d_6, 90 MHz,) δ (ppm) 3.27 (s, 4H, imidazoline CH_2), 4.67 (s, 2H, aliphatic CH_2), 5.49 (s, 1H, NH), 7.50–7.82 (m, 9H, Ar–H).

*4.1.4.2. [1-(2-Chlorobenzyl)-4,5-dihydro-1H-imidazole-2-yl]-[5-(4-nitrophenyl)[1,3,5]thiadiazol-2yl] amine (**4i**).* (Yield 58%), orange powder, m.p. > 300 °C (THF, toluene). Analysis (Calc./found %): for $C_{18}H_{15}ClN_6O_2S$ C: 52.11/52.78, H: 3.64/3.62, N: 20.26/20.19, S: 7.73/7.89. IR (KBr) (υ, cm^{-1}), 3331 (N–H stretching), 3067 (aromatic C–H stretching), 2930–2872 (C–H stretching), 1674 (C=C stretching), 1592 (C=N stretching), 1516 (Ar–NO_2 asymmetric stretching), 1455 (N–H bending), 1349 (Ar–NO_2 symmetric stretching); ^{1}H-NMR (DMSO-d_6, 90 MHz,) δ (ppm) 3.30 (s, 4H, imidazoline CH_2), 4.73 (s, 2H, aliphatic CH_2), 7.45 (m, 8H, Ar–H), 8.39 (s, 1H, NH).

4.2. Microbiology

For determining both antibacterial and antifungal activity, the synthesized compounds and the control drugs were dis-

solved in absolute dimethylsulfoxide (DMSO). Further dilutions were prepared at the required quantities of 1024, 512, 256, 128, 64, 32, 16, 8 and 4 μg ml^{-1} on the microorganisms at the concentrations studied. The stock solutions were prepared in DMSO and DMSO had no effect on the microorganisms in the concentrations studied. Antimicrobial activities of compounds were determined by using broth dilution method by the National Committee for Clinical Laboratory Standards (NCCLS). MIC which is the lowest concentration of a compound that completely inhibits microbial growth, was determined by a standard broth dilution technique adapted from the NCCLS [15,16].

Two Gram-positive, two Gram-negative bacteria and two yeast-like fungi were used as quality control strains. Tested microorganisms were the Gram-positive *B. subtilis* ATCC 6633, *S. aureus* ATCC 6538 P, the Gram-negative *E. coli* ATTC 25922, *S. typhimurium* NRRL B 4420 and the yeast-like fungi; *C. globrata* ATCC 66032 and *C. tropicalis* ATCC 13803. Ampicillin and fluconazole were used as antibiotic reference for bacteria and yeast, respectively (obtained from Firat University at the Department of Biology, Turkey).

4.2.1. Antibacterial and antifungal assays

Bacterial cultures were obtained in Mueller–Hinton broth (Difco) for all the bacterial strains after 24 h of incubation at 37 ± 0.1 °C. The yeasts were propagated in Sabouraud dextrose broth (Difco) after incubation for 24 h at 25 ± 0.1 °C [15]. Testing was carried out in Mueller–Hinton broth and Sabouraud dextrose broth at pH 7.4 for bacteria and yeast, respectively. The final inoculum size was 10^5 CFU ml^{-1} for bacteria and fungi, respectively.

Test compounds were dissolved in DMSO at an initial concentration of 1024 μg ml^{-1} and then were serially diluted in culture medium to 4 μg ml^{-1}.

A set of tubes containing only inoculated broth was kept as control. Antibacterial activity was determined after incubation for 24 h at 37 °C for bacteria and after incubation for 48 h at 25 °C for the yeasts. MIC was defined as the lowest concentrations of the compounds that inhibited visible growth of the microorganism. Every experiment in the antibacterial and antifungal assays was replicated twice to define the MIC values.

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