

Synthesis and Antimicrobial Activity of Some New 5-(Pyridin-4-yl)-3-thioacetamido-1,2,4-triazole

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Abstract: A series of 5-(pyridin-4-yl)-3-thioacetamide-1,2,4-triazoles (**4a-o**), was synthesized, characterized and evaluated for screening antibacterial and antifungal activities on microorganisms, respectively on five bacteria and and candida tropicalis. The structures of original compounds were confirmed by analytical and spectroscopic (FT-IR, ¹H NMR and ¹³C NMR) methods and elemental analysis. Both the antibacterial and antifungal activities and MIC values of compounds were reported. Among the tested compounds, the most effective compound providing a MIC values of 64 µg mL⁻¹ are **4a,b** aganist *B. subtilis* and *S. typhimurium* and **4o** against *E. coli* only.

Keywords: Triazole, Acetamido, Antimicrobial activity

Introduction

Triazole derivatives are the other major chemical group of antifungal azole derivatives. Nowadays, the most frequently used triazoles are fluconazole and itraconazole. They posses a broad spectrum of antifungal activity and reduced toxicity when compared the imidazole antifungals¹⁻⁶.

The ring-closure reactions of carbohydrazides are well-known and have been thoroughly studied, In these reactions five-membered heterocycles with three heteroatoms such as are formed, 1,3,4-oxadiazoles, 1,3,4-thiadiazole and 1,2,4-triazoles⁷⁻⁹.

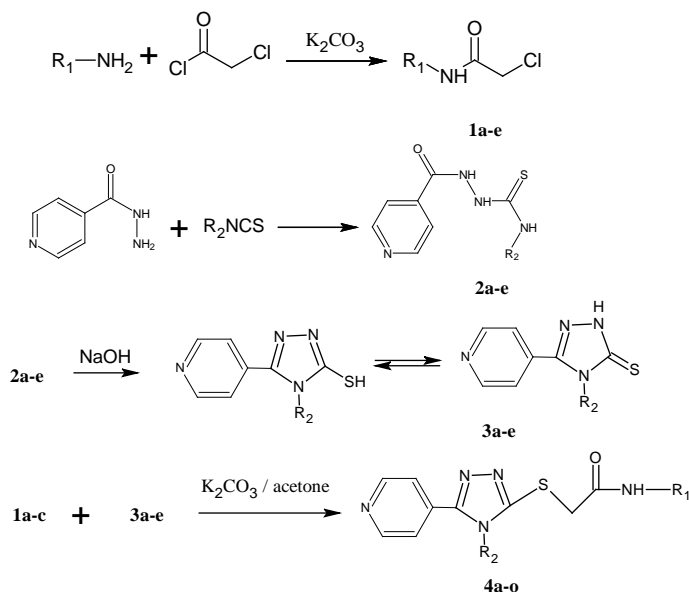
Substituted 1,2,4-triazoles have also been actively studied as bridging ligands coordinating through their vicinal N atoms and some have special structure with interesting magnetic properties¹⁰. In addition there are some studies on electronic structures and thiol-thione tautomeric on equilibrium of heterocyclic thione derivatives¹¹⁻¹³.

Acetanilide derivatives are reported to exhibit a number of biological activities, including anesthetic¹⁴, antipyretic¹⁵, antiinflammatory¹⁶ and antibacterial¹⁷⁻¹⁹ effects. Substitutions including thio^{20,21} alkylthio and alkenylthio^{22,23} derivatees have been carried out primarily at the 3-position of the 1,2,4-triazole ring, as potential antimicrobial agents that will overcome the above mentioned resistance problems. Based on these findings, the present paper deals with the synthesis, characterization and preliminary antimicrobial activity evaluation of some new substances containing triazole rings.

Experimental

1a-c, **2a-e**, **3a-e** and **4a-o** compounds were prepared following literature procedures^{24-26,13}, respectively. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected, but checked by differential scanning calorimetry (DSC). The IR spectra were registered with a Mattson 1000 FT-IR spectrophotometer. The NMR spectra were recorded on Varian Gemini 200 MHz and Jeol FX-90Q spectrometers and are reported in ppm (δ) relative to tetramethylsilane as the internal Standard (^1H) or deuteriochloroform (C^{13} NMR 75,5, 49,5 and 22,4 MHz). Elemental analyses were done on ALECOCHNS-938. Starting chemicals were obtained from Merck or Aldrich.

The synthetic route of compounds is outlined in (Scheme 1). In the present work, 2-chloro-*N*-(aryl/alkyl)acetamides (**1a-e**) were prepared for the first time by reacting ($\text{R}_1\text{-NH}_2$) amines with chloroacetyl chloride in accordance with the method described in the literature^{24,25}. The condensation of the acid hydrazide with appropriate aryl/alkyl isothiocyanate resulted in formation of thiosemicarbazides (**2a-e**).



Scheme 1

The thiosemicarbazides (**2a-e**), on refluxing with 2 N sodium hydroxide solution were cyclized into corresponding 5-(pyridin-4-yl)-3*H*-1,2,4-triazole-3-thiole (**3a-e**). Treatment of equimolar quantities of these triazoles (**3a-e**) with 2-chloro-*N*-(aryl/alkyl) acetamides (**1a-c**) in the presence of anhydrous potassium carbonate resulted in the formation of the title compounds (**4a-o**). The characterization data of compounds (**4a-o**) are given in the Table 1 & 2.

General method for the synthesis of compounds (1a-c)

An ice-cooled solution of $\text{R}_1\text{-NH}_2$ (0.05 mol) in acetone was added chloroacetylchloride (0.05 mol in acetone) dropwise and then the solution was kept for 2 h at room temperature. The resulting mixture was poured in ice-cold water and the shiny crystalline product was collected and filtered.

Table 1. Physical data of the prepared compounds **4(a-o)**

Compd.	R ₁	R ₂	M. p. °C	Yield, %	Empirical formula	Calcd. (Found)% CHN
4a	Cyclohexyl	Benzyl	119- 120	75	C ₂₂ H ₂₅ N ₅ OS	64.84(64.88); 6.18(6.21); 17.18(17.19)
4b		Ethyl	91-92	83	C ₁₇ H ₂₃ N ₅ OS	59.10(59.13); 6.71(6.73); 20.27(20.31)
4c		Phenyl	208- 209	78	C ₂₁ H ₂₃ N ₅ OS	64.10(64.14); 5.89(5.92), 17.80(17.82)
4d		Allyl	241- 242	85	C ₁₈ H ₂₃ N ₅ OS	60.48(60.47); 6.49(6.53); 19.59(19.62)
4e		4- methylphenyl	170- 171	69	C ₂₂ H ₂₅ N ₅ OS	64.84(64.86); 6.18(6.22); 17.18(17.23)
4f	4- chlorophenyl	Benzyl	173- 174	68	C ₂₂ H ₁₇ ClN ₅ OS	60.61(60.63); 4.16(4.14); 16.07(16.10)
4g		Ethyl	176- 177	65	C ₁₇ H ₁₆ ClN ₅ OS	54.61(54.64); 4.31(4.28); 18.73(18.74)
4h		Phenyl	211- 212	71	C ₂₁ H ₁₆ ClN ₅ OS	59.78(59.80); 3.82(3.85); 16.60(16.59)
4i		Allyl	261- 262	81	C ₁₈ H ₁₆ ClN ₅ OS	56.63(56.65); 4.18(4.20), 18.15(18.14)
4j		4- methylphenyl	226- 227	78	C ₂₂ H ₁₈ ClN ₅ OS	60.61(60.63); 4.16(4.20); 16.07(16.09)
4k	4- nitrophenyl	Benzyl	191- 192	63	C ₂₂ H ₁₈ N ₆ O ₃ S	59.18(59.22); 4.06(4.05); 18.82(18.85)
4l		Ethyl	187- 188	79	C ₁₇ H ₁₆ N ₆ O ₃ S	53.12(53.14); 4.20(4.21); 21.86(21.87)
4m		Phenyl	151- 153	77	C ₂₁ H ₁₆ N ₆ O ₃ S	58.32(58.34); 3.73(3.74); 19.43(19.42)
4n		Allyl	251- 252	66	C ₁₈ H ₁₆ N ₆ O ₃ S	54.54(54.55); 4.07(4.05); 21.20(21.21)
4o		4- methylphenyl	247- 248	73	C ₂₂ H ₁₈ N ₆ O ₃ S	59.18(59.16); 4.06(4.07); 18.82(18.85)

Table 2. Spectral data of the prepared compounds **4a-o**

Compd	IR (KBr): ν (cm^{-1})	$^1\text{H-NMR}$ δ (ppm)
4a	1668 (C=O), 1614 (C=N)	7.00-8.69 (m, 10H, Ar-H and -NH), 5.22(s, 2H, -N-CH ₂), 3.84 (s, 2H, S-CH ₂), 3.76-3.78 (m, 1H, cyclohexyl -N-CH), 1.23-1.84 (m, 10H, cyclohexyl-CH ₂).
4b	1670 (C=O), 1610 (C=N)	7.25-8.81 (m, 5H, Ar-H and -NH), 4.03-4.09 (q, 13H, -CH ₂ -CH ₃), 3.85 (s, 2H, -S-CH ₂), 3.82-3.83 (m, 1H, cyclohexyl -N-CH), 1.25-1.86 (m, 13H, -CH ₂ -CH ₃ and cyclohexyl-CH ₂).
4c	1658 (C=O), 1614 (C=N)	7.24-8.56 (m, 10H, Ar-H, and -NH), 3.85 (s, 2H, S-CH ₂), 3.79-3.82 (m, 1H, , cyclohexyl -N-CH), 1.25-1.188 (m, 10H, cyclohexyl-CH ₂).
4d	1675 (C=O), 1609 (C=N)	7.43-8.85 (m, 5H, Ar-H and -NH), 5.82 (ddt, 1H, N-CH ₂ -CH=CH ₂), 5.38 (dd, 1H cis N- CH ₂ -CH=CH ₂), 5.12 (dd, 1H, trans N- CH ₂ -CH=CH ₂), 4.61 (d, 2H, -N-CH ₂), 3.92 (s, 2H, S-CH ₂), 3.87-3.88 (m, 1H, cyclohexyl -N-CH), 1.25-1.89 (m, 10H, cyclohexyl-CH ₂).
4e	1678 (C=O), 1618 (C=N)	7.11-8.57 (m, 9H, Ar-H, and -NH), 3.78 (s, 2H, -S-CH ₂), 3.71-3.73 (m, 1H, cyclohexyl -N-CH), 2.47 (s, 3H, CH ₃), 1.25-1.89 (m, 10H, cyclohexyl-CH ₂).
4f	1660 (C=O), 1614 (C=N)	10.43 (br, 1H, -NH), 7.06-8.72 (m, 13H, Ar-H), 5.31 (s, 2H, -N-CH ₂), 3.99 (s, 2H, S-CH ₂).
4g	1680 (C=O), 1613 (C=N)	10.51 (s, 1H, -NH), 7.20-8.83 (m, 8H, Ar-H), 3.97-4.09 (m, 4H, S-CH ₂ and -CH ₂ -CH ₃), 1.44 (t, 3H, CH ₂ -CH ₃).
4h	1660 (C=O), 1614 (C=N)	10.66 (br, 1H, -NH), 7.05-8.54 (m, 13H, Ar-H), 4.21 (s, 2H, S-CH ₂).
4i	1674 (C=O), 1617 (C=N)	10.23 (br, 1H, -NH), 7.27-8.66 (m, 8H, Ar-H), 6.11 (ddt, 1H, N-CH ₂ -CH=CH ₂), 5.27 (dd, 1H, cis N- CH ₂ -CH=CH ₂), 5.09 (dd, 1H, trans N- CH ₂ -CH=CH ₂), 4.69 (d, 2H, -N-CH ₂), 3.91 (s, 2H, S-CH ₂).
4j	1659 (C=O), 1615 (C=N)	10.40 (s, 1H, -NH), 7.13-8.58 (m, 12H, Ar-H), 3.94 (s, 2H, S-CH ₂)
4k	1674 (C=O), 1614 (C=N)	10.19 (br, 1H, -NH), 7.11-8.87 (m, 13H, Ar-H), 5.29 (s, 2H, -N-CH ₂), 3.95 (s, 2H, S-CH ₂).
4l	1678 (C=O), 1617 (C=N)	10.23 (br, 1H, -NH), 7.47-8.87 (m, 8H, Ar-H), 4.15 (q, 2H, -CH ₂ -CH ₃), 3.87 (s, 2H, S-CH ₂), 1.41 (t, 3H, CH ₂ -CH ₃).
4m	1665 (C=O), 1619 (C=N)	10.33 (br, 1H, -NH), 7.25-8.88 (m, 13H, Ar-H), 3.95 (s, 2H, , S-CH ₂).
4n	1648 (C=O), 1616 (C=N)	10.31 (br, 1H, -NH), 7.56 8.64 (m, 8H, Ar-H), 6.21 (ddt, 1H, N-CH ₂ -CH=CH ₂), 5.28 (dd, 1H, cis N- CH ₂ -CH=CH ₂), 5.05 (dd, 1H, trans N- CH ₂ -CH=CH ₂), 4.74 (d, 2H, -N-CH ₂), 3.89 (s, 2H, S-CH ₂).
4o	1665 (C=O), 1620 (C=N)	10.11 (br, 1H, -NH), 7.19-8.72 (m, 12H, Ar-H), 3.96 (s, 2H, S-CH ₂), 2.39 (s, 3H, CH ₃).

General method for the synthesis of compounds (2a-e)

0.01 Mole of isonicotinohydrazide in (50 mL) of absolute ethanol was heated until it dissolved. 0.01 mole appropriate substituted isothiocyanate derivatives (R_2NCS) was added and the mixture was refluxed for 5 h. After the completion of the reaction, the crude product which precipitated on cooling was filtered, washed with diethyl ether, dried and crystallized with suitable solvents.

General method for the synthesis of compounds (3a-e)

A solution of (0.01 mole **(2a-e)**) in 50 mL 1 N NaOH solution was heated under reflux for 5 h. The mixture was cooled and acidified to pH 3 with concentrated hydrochloric acid. The precipitate was filtered and washed several times with distilled water. The pure compounds were obtained following crystallization with suitable solvents.

General method for the synthesis of compounds (4a-o)

A suspension of acetamide (**(1a-c)**) (0.005 mol) in acetone (10 mL), was added an equimolar amount of triazole (**(3a-e)**). An equimolar K_2CO_3 was added as a catalyst and the solution was stirred for 10 h. The solid was evaporated. The residue was dispersed with water to obtain **(4a-o)**.

In the present work, 15 new compounds (**(4a-o)**) were synthesized. The structures of them were elucidated by spectral data. When chloroacetyl chloride was added to amines in the presence of potassium carbonate, the compounds (**(1a-c)**) were obtained in 85-90% yields. Synthetic methods for preparation of the title heterocycles from the isothiocyanate adducts of hydrazides are known. The treatment of isonicotinohydrazide with isothiocyanates gave thiosemicarbazides (**(2a-e)**), respectively, in nearly quantitative yields. On the treatment of thiosemicarbazides (**(2a-e)**), with aqueous sodium hydroxide, 1,2,4-triazole derivatives were obtained in 75-85% yields (**(3a-e)**). The reaction of 2-chloro-*N*-(aryl/alkyl)acetamides (**(1a-c)**), 1,2,4-triazoles (**(3a-e)**) and anhydrous potassium carbonate in acetone gave the 5-(pyridin-4-yl)-3-thioacetamido-1,2,4-triazoles in 63-83% yields (**(4a-o)**).

In the IR spectrum of **(4a-o)**, the most characteristic absorptions are at $1648-1680\text{ cm}^{-1}$ ν (C=O), $1609-1620\text{ cm}^{-1}$ ν (C=N). In the $^1\text{H-NMR}$ spectra of compounds (**(4a-o)**), NH peaks were observed as broad at about 10.11-10.66 ppm region. In addition, compounds which are including S- CH_2 peaks were observed as singlets at about 3.84-4.21 ppm region. All the other aromatic and aliphatic protons were observed at expected regions.

Antimicrobial activity

For determining both antibacterial and antifungal activity, the synthesized compounds and the control drugs were dissolved in absolute dimethylsulfoxide (DMSO). Further dilutions were prepared at the required quantities of 1024, 512, 256, 128, 64, 32, 16, 8 and $4\text{ }\mu\text{g mL}^{-1}$ 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 (CLSI). 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 CLSI^{27,28}.

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* ATCC 25922, *S. typhimurium* NRRL B 4420 and the yeast-like fungi; *C. globrata* ATCC 66032 and *C. tropicalis* ATCC 13803. Ampicillin and flucanazole were used as antibiotic reference for bacteria and yeast, respectively (obtained from Firat University at the Department of Biology, Turkey).

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 °C²⁶. 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.

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

Results and Discussion

We have designed and synthesized novel compounds of 5-(pyridin-4-yl)-3-thioacetamido-1,2,4-triazoles 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 glabrata* 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 3.

Table 3. Antimicrobial activity results (MIC values in µg mL⁻¹) of synthesized compounds with the standard drugs

Sample	<i>E.c</i>	<i>B.s</i>	<i>S.t</i>	<i>S.a</i>	<i>C.t</i>	<i>C.g</i>
4a	128	64	64	1024	1024	1024
4b	128	64	64	512	512	-
4c	128	128	128	256	1024	-
4d	256	256	256	512	1024	-
4f	128	128	128	512	-	-
4g	128	256	256	1024	-	-
4i	256	256	256	512	512	512
4j	256	256	256	512	512	1024
4k	128	128	256	512	256	256
4l	256	256	256	256	512	-
4m	128	128	128	256	1024	-
4n	128	256	256	512	1024	1024
4o	64	256	256	256	512	512
Ampicilin	2	2	2	2	-	-
Fuluconazol					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. aureus* ATCC 6538 P, *E.c*: *E. Coli* ATCC 25922, *S.t*: *S. typhimurium* NRRL B 4420, *C.g*: *C. glabrata* ATCC 66032, *C.t*: *C. tropicalis* ATCC 13803

The obtained data reported that compounds were able to inhibit the growth of the selected microorganisms *in vitro* showing MIC values between 64 and 1024 µg mL⁻¹. The synthesis of some compounds (**4**), showed some degree of antimicrobial activity against test microorganisms. Among the synthesized compounds, the most active derivative at an MIC

values of $64 \mu\text{g mL}^{-1}$ are (**4a,b**) against *B. subtilis* and *S. typhimurium* and (**4o**) against *E. coli* only. Compounds (**4a,b**), (**4o**) might be slightly more active than others against some of the test microorganisms.

Compounds (**4a,b**) and (**4o**) which having electron withdrawing substituents such as, benzyl / ethyl and 4-methylphenyl, respectively, on cyclohexyl and 4-nitro-phenyl group were found to be the most active compounds against *B. Subtilis*, *S. typhimurium* and *E. Coli*.

The lowest effective substances are (**4d**, **4i**, **4j** and **4l**) against all the microorganisms studied. Interestingly, despite all the substances have very similar structures, their antimicrobial activities are very different.

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