



One Pot Synthesis and Antimicrobial Activity of Substituted 2-Aminothiazoles

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In this work, we described a preparation of substituted 2-aminothiazoles by two different methods using a one-pot procedure and starting from methoxymethylene-3-aryl-thiourea (**2**). The synthesized 2-aminothiazoles (**5a-h**) were characterized on the basis of elemental analysis and their spectral (IR, ^1H NMR and ^{13}C NMR) data and evaluated their biological activities. The majority of compounds were found to exhibit significant antimicrobial activity.

Keywords: Methoxymethylene-3-aryl-thiourea, 2-Aminothiazoles, Antimicrobial activity.

INTRODUCTION

The growing interest in bioactive substituted thiazoles has led to an increasing demand for efficient syntheses of this class of heterocyclic compounds. A large number of research articles have been published during the last few years about the synthesis and pharmacological applications of thiazole derivatives. They have attracted widespread attention due to their diverse biological activities. As medicines, many of them display including anti-HIV¹, anti-inflammatory², tubercular activities³, hypertension⁴, anticancer⁵, anticonvulsant⁶, anti-bacterial and antifungal^{7,8}. Particularly, the 2-aminothiazole moiety, proven its value in medicinal that has been successfully applied in dopamine agonists, such as mirapexin, the widely used anti-parkinsonian agent⁹ and multi-targeted protein tyrosine kinase inhibitor with potential antineoplastic activity (Masitinib)¹⁰ and in agrochemicals fields such as herbicide antidotes¹¹ (**Scheme-I**).

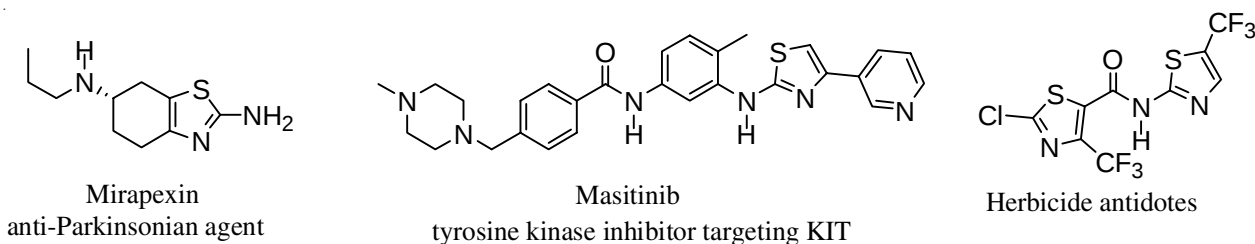
Encouraged by the biological activities of derivatives mentioned above and as a part of the ongoing work of our team on the synthesis and reactivity of biologically active

heterocyclic five-membered¹²⁻¹⁴, we designed and synthesized novel substituted 2-aminothiazole as possible antibacterial agents.

EXPERIMENTAL

Melting points were taken with a Kofler hot staged apparatus and are uncorrected. All reactions were monitored by thin layer chromatography (TLC). IR spectra were determined with a Perkin Elmer 1600 series FTIR spectrometer. Elemental analyses were determined using an elemental vario El III Elemental Analyzer. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Unity spectrometer at 300 MHz using TMS as an internal standard. The bacterial strains and *Aspergillus niger* fungus were obtained from the Centre of the agro food of Tunis (Tunisia).

Synthesis of methoxymethylene-3-aryl-thiourea (2): Isothiocyanate was mixed in a 1:1 ratio with the desired imidic acid methyl ester (**1**) in dry ether. The reaction mixture was stirred at room temperature for about 2-3 h. The formed solid was separated by filtration and the precipitates were washed with dry ether and then recrystallized from absolute ethanol.



Scheme-I: Drug candidates with 2-aminothiazole moiety

1-(Methoxy-phenyl-methylene)-3-phenyl-thiourea (2a): Yield 90 %; m.p. 124-126 °C; ¹H NMR (CDCl₃): δ 8.87 (br. s, 1H), 7.47-7.28 (m, 10 H), 3.85 (s, 3H); ¹³C NMR (CDCl₃): δ 181.4, 167.1, 137.8-122.6 (C_{arom}), 47.2. IR (KBr, ν_{max}, cm⁻¹) 3207, 1658, 1550, 1448, 1340, 1315, 1296, 1054.

1-(4-Chloro-phenyl)-3-(methoxy-phenyl-methylene)-thiourea (2b): Yield 73 %; m.p. 136-138 °C; ¹H NMR (CDCl₃): δ 9.06 (br. s, 1H), 7.45-7.39 (m, 5 H), 7.27-7.18 (m, 4 H), 3.85 (s, 3H); ¹³C NMR (CDCl₃): δ 180.7, 166.9, 134.7-126.8 (C_{arom}), 49.1. FT-IR (KBr, ν_{max}, cm⁻¹) 3202, 1654, 1552, 1448, 1351, 1319, 1283, 1089.

1-Benzyl-3-(methoxy-phenyl-methylene)thiourea (2c): Yield 81 %; m.p. 106-108 °C; ¹H NMR (CDCl₃): δ 7.85 (br. s, 1H), 7.52-7.37 (m, 5 H), 7.21-7.13 (m, 5 H), 3.92 (s, 2H), 3.81 (s, 3H); ¹³C NMR (CDCl₃): δ 181.2, 167.4, 141.3-127.1 (C_{arom}), 53.8, 47.2. FT-IR (KBr, ν_{max}, cm⁻¹) 3197, 1656, 1554, 1447, 1351, 1310, 1282, 1056.

1-(1-Methoxy-2-phenyl-ethylidene)-3-phenyl-thiourea (2d): Yield 62 %; m.p. 114-116 °C; ¹H NMR (CDCl₃): δ 8.76 (br. s, 1H), 7.47-7.36 (m, 10 H), 3.13 (s, 2H), 3.85 (s, 3H); ¹³C NMR (CDCl₃): δ 179.5, 167.1, 137.2-125.1 (C_{arom}), 48.4, 32.8. FT-IR (KBr, ν_{max}, cm⁻¹) 3217, 1657, 1548, 1439, 1349, 1308, 1282, 1062.

Synthesis of methoxymethylene-isothiurea (3): To a suspension of 20 mmol of sodium hydride in 50 mL of dry THF, 10 mmol of methoxymethylene-3-aryl-thiourea (**2**) was added at room temperature under nitrogen atmosphere. After stirring for 1 h, 10 mmol of methyl iodide in 20 mL of THF was added. The mixture was stirred for an additional 18 h. Then, the solution was quenched by distilled water. The extraction was carried out with ether. After the usual work up, the solvent was evaporated under reduced pressure was either dried under vacuum (**3c-d**) or treated with pentane and recrystallized from methanol (**3a-b**).

1-(Methoxy-phenyl-methylene)-2-methyl-3-phenyl-isothiurea (3a): Yield 76 %; m.p. 52-54 °C; ¹H NMR (CDCl₃): δ 7.43-7.37 (m, 10 H), 3.76 (s, 3H), 2.55 (s, 3H); ¹³C NMR (CDCl₃): δ 166.4, 165.7, 137.2-123.5 (C_{arom}), 46.8, 15.27. FT-IR (KBr, ν_{max}, cm⁻¹) 1653, 1645, 1550, 1340, 1327.

1-(4-Chloro-phenyl)-3-(methoxy-phenyl-methylene)-2-methyl-isothiurea (3b): Yield 64 %; m.p. 94-96 °C. ¹H NMR (CDCl₃): δ 7.29-7.42 (m, 10 H), 3.81 (s, 3H), 2.58 (s, 3H); ¹³C NMR (CDCl₃): δ 166.7, 165.2, 142.7-124.2 (C_{arom}), 47.3, 14.9. FT-IR (KBr, ν_{max}, cm⁻¹) 1650, 1648, 1552, 1357, 1331.

1-Benzyl-3-(methoxy-phenyl-methylene)-2-methyl-isothiurea (3c): Yield 67 %; An oil. ¹H NMR (CDCl₃): 7.09-7.22 (m, 5 H), 7.35-7.48 (m, 5 H), 3.87 (s, 2H), 3.73 (s, 3H); 2.49 (s, 3H); ¹³C NMR (CDCl₃): δ 165.8, 163.4, 136.5-125.7 (C_{arom}), 51.3, 47.8, 15.3. FT-IR (CHCl₃, ν_{max}, cm⁻¹) 1652, 1643, 1554, 1351, 1310, 816, 768.

1-(1-Methoxy-2-phenyl-ethylidene)-2-methyl-3-phenyl-isothiurea (3d): Yield 73 %; An oil; ¹H NMR (CDCl₃): δ 7.42-7.47 (m, 10 H), 3.82 (s, 3H), 3.13 (s, 2H), 2.61 (s, 3H); ¹³C NMR (CDCl₃): δ 165.8, 162.7, 138.4-123.6 (C_{arom}), 51.4, 33.7, 13.9. FT-IR (KBr, ν_{max}, cm⁻¹) 1653, 1647, 1531, 1349, 1308.

Synthesis of 2-aminothiazole 5 (method A): To a suspension of 20 mmol of sodium hydride in 50 mL of dry THF was

added dropwise under nitrogen atmosphere with stirring at room temperature a solution of methoxymethylene-3-aryl-thiourea (**2**) (10 mmol) in THF (20 mL). After stirring for 1 h, Br-CH₂-EWG (10 mmol) dissolved in THF (30 mL) was added. The reaction mixture was stirred for a period of 12 h and hydrolyzed with water (10 mL). The organic layer was extracted with chloroform, dried over MgSO₄ and concentrated under reduced pressure to give the corresponding 2-aminothiazole **5** which was purified by recrystallization from ethanol.

4-Phenyl-2-phenylamino-thiazole-5-carboxylic acid ethyl ester (5a): Yield 82 %; m.p. 142-144 °C; ¹H NMR (CDCl₃): δ 9.67 (br. s, 1H), 7.54-7.22 (m, 10 H), 4.23 (q, 2H, *J* = 7.6 Hz), 1.18 (t, 3H, *J* = 7.6 Hz); ¹³C NMR (CDCl₃): δ 168.4, 161.5, 158.5, 140.3, 133.7-121.6 (C_{arom}), 60.5, 14.2; FT-IR (CHCl₃, ν_{max}, cm⁻¹) 3389, 1709, 1602, 1537, 1442, 1294. Anal. calcd. (%) for C₁₇H₁₄N₂O₂S: C, 65.79; H, 4.55; N, 9.03. Found (%): C, 65.58; H, 4.76; N, 9.19.

Phenyl-(4-phenyl-2-phenylamino-thiazol-5-yl)-methanone (5b): Yield 78 %; m.p. 136-138 °C; ¹H NMR (CDCl₃): δ 9.52 (br. s, 1H), 7.75-7.18 (m, 15 H); ¹³C NMR (CDCl₃): δ 186.3, 163.4, 145.5, 141.1, 137.3-120.5 (C_{arom}); FT-IR (KBr, ν_{max}, cm⁻¹) 3393, 1693, 1598, 1538, 1444, 1302. Anal. calcd. (%) for C₂₂H₁₆N₂O₂S: C, 74.13; H, 4.52; N, 7.86. Found (%): C, 73.91; H, 4.74; N, 8.03.

2-(4-Chloro-phenylamino)-4-phenyl-thiazole-5-carboxylic acid ethyl ester (5c): Yield 73 %; m.p. 160-162 °C; ¹H NMR (DMSO-*d*₆): δ 8.47 (br. s, 1H), 7.45-7.22 (m, 9 H), 4.19 (q, 2H, *J* = 7.6 Hz), 1.22 (t, 3H, *J* = 7.6 Hz); ¹³C NMR (DMSO-*d*₆): δ 168.4, 160.4, 157.0, 141.2, 135.3-122.1 (C_{arom}), 59.9, 14.8; FT-IR (KBr, ν_{max}, cm⁻¹) 3398, 1697, 1692, 1590, 1532, 1466, 832, 764. Anal. calcd. (%) for C₁₇H₁₃ClN₂O₂S: C, 59.21; H, 3.80; N, 8.12. Found (%): C, 59.47; H, 4.07; N, 4.32.

2-Benzylamino-4-phenyl-thiazole-5-carboxylic acid ethyl ester (5d): Yield 76 %; m.p. 160-162 °C; ¹H NMR (CDCl₃): δ 9.18 (br. s, 1H), 7.42-7.17 (m, 10 H), 4.51 (s, 2H), 4.23 (q, 2H, *J* = 7.6 Hz), 1.19 (t, 3H, *J* = 7.6 Hz); ¹³C NMR (CDCl₃): δ 168.5, 162.0, 158.1, 139.8, 134.5-121.4 (C_{arom}), 60.2, 49.3, 14.6; FT-IR (KBr, ν_{max}, cm⁻¹) 3396, 1696, 1594, 1528, 1485. Anal. calcd. (%) for C₁₈H₁₆N₂O₂S: C, 66.64; H, 4.97; N, 8.64. Found (%): C, 66.42; H, 4.15; N, 8.83.

4-Benzyl-2-phenylamino-thiazole-5-carboxylic acid ethyl ester (5e): Yield 81 %; m.p. 160-162 °C; ¹H NMR (CDCl₃): δ 8.13 (br. s, 1H), 7.38-7.21 (m, 10 H), 4.57 (s, 2H), 4.21 (q, 2H, *J* = 7.6 Hz), 1.23 (t, 3H, *J* = 7.6 Hz); ¹³C NMR (CDCl₃): δ 168.3, 161.8, 156.3, 139.4, 136.2-121.4 (C_{arom}), 60.6, 36.0, 14.5; FT-IR (KBr, ν_{max}, cm⁻¹) 3406, 1703, 1602, 1531, 1476. Anal. calcd. (%) for C₁₈H₁₆N₂O₂S: C, 66.64; H, 4.97; N, 8.64. Found (%): C, 66.47; H, 4.12; N, 8.56.

4-Phenyl-2-phenylamino-thiazole-5-carbonitrile (5f): Yield 88 %; m.p. 150-152 °C; ¹H NMR (DMSO-*d*₆): δ 10.53 (br. s, 1H), 7.53-7.18 (m, 10 H); ¹³C NMR (DMSO-*d*₆): δ 160.1, 151.5, 139.2, 133.8-120.5 (C_{arom}), 114.2; FT-IR (KBr, ν_{max}, cm⁻¹) 3395, 2224, 1605, 1538, 1481. Anal. calcd. (%) for C₁₆H₁₁N₃S: C, 69.29; H, 4.00; N, 15.15. Found (%): C, 69.45; H, 3.88; N, 14.97.

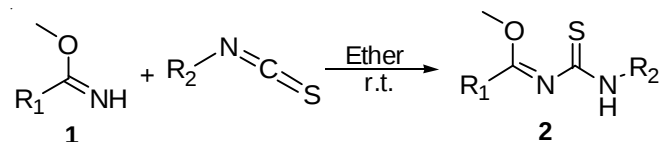
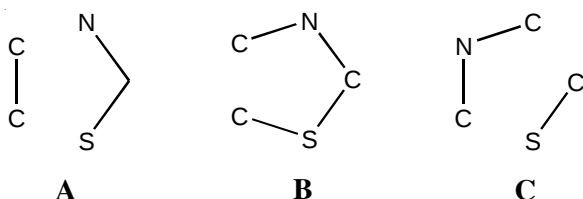
(4-Chloro-phenyl)-1-(4-phenyl-2-phenylamino-thiazol-5-yl)-methanone (5g): Yield 72 %; m.p. 142-144 °C; ¹H NMR

(DMSO- d_6): δ 9.47 (br. s, 1H), 7.63-7.21 (m, 14 H); ^{13}C NMR (DMSO- d_6): δ 189.7, 162.1, 147.2, 140.5, 138.1-121.3 (C_{arom}); FT-IR (KBr, ν_{max} , cm^{-1}) 3393, 1696, 1602, 1537, 1463, 835, 762. Anal. calcd. (%) for $\text{C}_{22}\text{H}_{15}\text{ClN}_2\text{OS}$: C, 67.60; H, 3.87; N, 7.17. Found (%): C, 67.73; H, 4.03; N, 7.04.

4-Benzyl-2-phenylamino-thiazole-5-carbonitrile (5h): Yield 62 %; m.p. 114-116 °C; ^1H NMR (DMSO- d_6): δ 9.03 (br. s, 1H), 7.37-7.23 (m, 10 H), 4.53 (s, 2 H); ^{13}C NMR (DMSO- d_6): δ 159.8, 149.3, 139.7, 134.0-121.2 (C_{arom}), 114.1, 35.2; FT-IR (KBr, ν_{max} , cm^{-1}) 3397, 2225, 1601, 1539, 1485. Anal. calcd. (%) for $\text{C}_{17}\text{H}_{13}\text{N}_3\text{S}$: C, 70.08; H, 4.50; N, 14.42. Found (%): C, 70.23; H, 4.38; N, 14.57.

RESULTS AND DISCUSSION

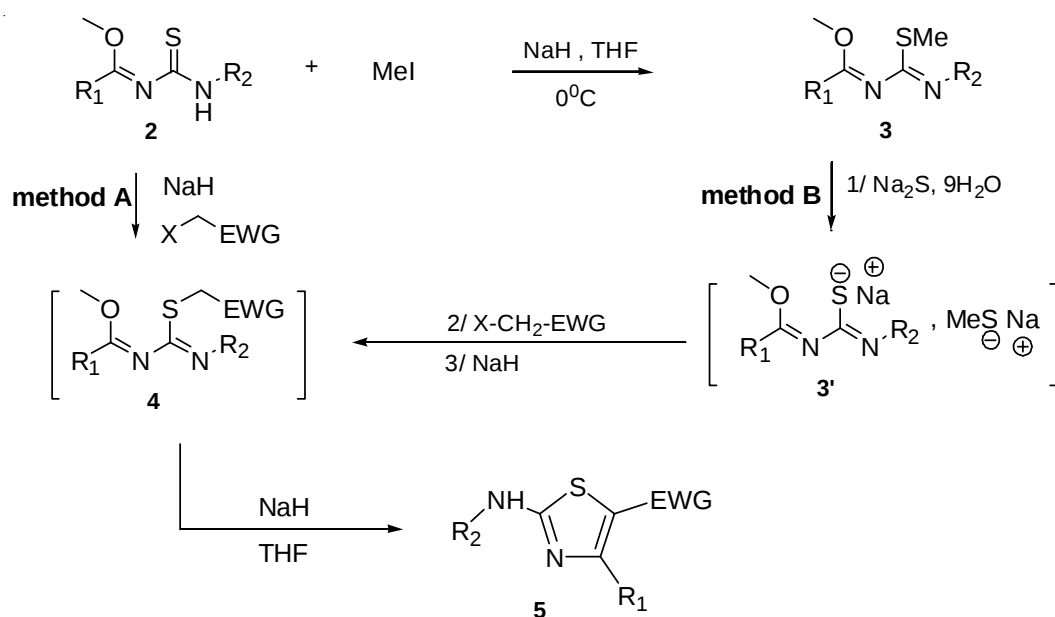
To access the thiazole ring, three reaction schemes (A, B and C) are possible.



Compounds 2, 3	R ₁	R ₂	Yield (%)
2a, 3a	Ph	Ph	90, 76
2b, 3b	Ph	4-Cl-Ph	73, 64
2c, 3c	Ph	Ph-CH ₂ -	81, 67
2d, 3d	Ph-CH ₂ -	Ph	62, 73

Scheme-II

Compounds **2** behave as 1,3-bielectrophilic reagents. However, in certain conditions, the thioamide function they can be in equilibrium with its tautomeric form thioimidic acid.

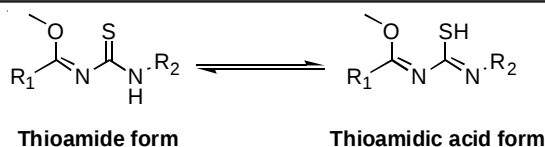


X = Cl, Br R₁ = Ph, Ph-CH₂- R₂ = Ph, 4-Cl-Ph, Ph-CH₂-
EWG = CN, CO₂Et, Ph-CO-, 4-Cl-Ph-CO-

Compounds 5	R ₁	R ₂	EWG	Yield* (%)
5a	Ph	Ph	CO ₂ Et	82
5b	Ph	Ph	Ph-CO	78
5c	Ph	4-Cl-Ph	CO ₂ Et	73
5d	Ph	Ph-CH ₂ -	CO ₂ Et	76
5e	Ph-CH ₂ -	Ph	CO ₂ Et	81
5f	Ph	Ph	CN	88
5g	Ph	Ph	4-Cl-Ph-CO	72
5h	Ph-CH ₂ -	Ph	CN	62

*Yield calculated using method A.

Scheme-III



Thus, these compounds are transformed into 1,4-electrophilic-nucleophilic reagents. This equilibrium has been exploited by many researchers to synthesize a wide range of sulfur heterocycles¹⁶⁻¹⁸. The first test performed on the methyl iodide in the presence of sodium hydride as a base was successful. We isolated after hydrolysis, the corresponding product and thus verified the existence of the thioimide-thioamide-acid equilibrium in such compounds.

This success has led us to believe that the introduction of a relatively active methylene promote cyclization to thiazole. By increasing the mobility of hydrogen in the α -position with respect to sulfur using alkyl halides activated by electron-withdrawing groups, we finally got the expected 2-aminothiazoles (**5**) (method A) via the non-isolated intermediates **4** with yields ranging from 62 to 88 %. We operated in the presence of a double amount of sodium hydride (**Scheme-II**).

The methoxymethylene-isothiourea (**3**) can be engaged in a one-pot three-step procedure (method B) and sodium sulfide was added to form the thiolate (**3'**) that reacted with activated halides and finally, cyclization was completed by reacting intermediate **4** with sodium hydride (**Scheme-II**). It is noted that the best yields were obtained in this procedure by using 2 equivalents of activated halides. This can be explained by considering that the activated halide reacts first with sodium methylthiolate, which is more nucleophilic than the intermediate formed thiolate (**3'**). It is noted that the yields obtained by this method are low and vary from 20 to 30 %.

Antimicrobial activity: The synthesized 2-aminothiazoles (**5a-h**) were evaluated for their *in vitro* antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli* and the fungus *Aspergillus niger*. Standard drugs like ampicillin and griseofulvin were used for the comparison purpose. The antimicrobial was assayed by using the agar disc-diffusion method^{19,20} at 50 $\mu\text{g/mL}$ (in DMSO). The nutrient agar plates were incubated at 37 °C for 24 h. The plates were examined and the inhibition of zone was measured in millimetres. The results are summarized in Table-1.

The results in the Table-1, generally show that the majority of the tested 2-aminothiazoles were considerably active and showed varying degrees of inhibition against all the tested bacteria. On the other hand, it is noted that the antimicrobial activity is closely related to electron-attached group in position 5 of the thiazole ring. However, thiazoles **5f** and **5g** (EWG = CN) showed moderate to good activity against bacteria but were insignificantly active against *A. niger*. The best antibacterial activity was displayed by the thiazole **5f** and the Gram-negative bacteria *E. coli* is considered the most sensitive among the tested organisms. Thiazoles **5a**, **5c-e** (EWG = CO₂Et) showed good activity against fungi (*A. niger*) and are moderately effective against all strains of bacteria. The introduction of a chloro group at position 4 of the aromatic ring

TABLE-1
ANTIMICROBIAL SCREENING RESULTS
OF 2-AMINOTHIAZOLES (**5a-h**)

Compd.	Diameter of growth inhibition zone (mm)			
	Antibacterial activity		Antifungal activity	
	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>A. niger</i>
5a	12	9	11	13
5b	11	17	13	9
5c	9	12	11	17
5d	5	-	9	15
5e	8	5	-	13
5f	12	19	8	-
5g	13	16	11	8
5h	11	18	12	5
Griseofulvin	-	-	-	24
Ampicillin	15	22	19	-

attached to the thiazole nucleus has no significant influence on the antimicrobial activity.

Conclusion

A one-pot procedure was developed to prepare substituted 2-aminothiazoles using two different methods starting from methoxymethylene-3-aryl-thiourea. All the synthesized thiazoles have been tested for *in vitro* antimicrobial activities. The antimicrobial activity is closely related to electron-attached group in position 5 of the thiazole ring. The thiazole **5f** (EWG = CN) showed potent antibacterial activity and **5c** (EWG = CO₂Et) showed potent antifungal activity.

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