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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, ^{1}H 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

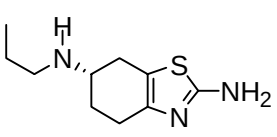
The growing interest in bioactive substituted thiazoles has led to an increasing demand for efficient syntheses of this class of heterocyclic compounds. A large number of research articles have been published during the last few years about the synthesis and pharmacological applications of thiazole derivatives. They have attracted widespread attention due to their diverse biological activities. As medicines, many of them display including anti-HIV[1], anti-inflammatory[2], tubercular activities[3], hypertension[4], anticancer[5], anticonvulsant[6], antibacterial 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 agent[9] and multi-targeted protein tyrosine kinase inhibitor with potential antineoplastic activity (Masitinib)[10] and in agrochemicals fields such as herbicide antidotes[11] (**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-membered[12-14], we designed and synthesized novel substituted 2-aminothiazole as possible antibacterial agents.

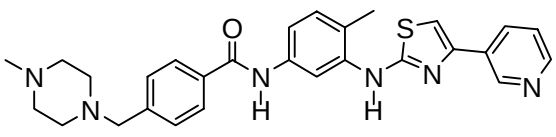
EXPERIMENTAL

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 elementar vario El III Elemental Analyzer. ^{1}H 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).

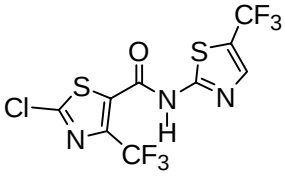
Synthesis of methoxymethylene-3-aryl-thiourea (2): Isothiocyanate was mixed in a 1:1 ratio with the desired imidic acid methyl ester (**1**) in dry ether. The reaction mixture was stirred at room temperature for about 2-3 h. The formed solid was separated by filtration and the precipitates were washed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



Mirapexin
anti-Parkinsonian agent



Masitinib
tyrosine kinase inhibitor targeting KIT



Herbicide antidotes

Scheme-I: Drug candidates with 2-aminothiazole moiety

1-(Methoxy-phenyl-methylene)-3-phenyl-thiourea (2a): Yield 90 %; m.p. 124-126 °C; ^{1}H NMR ($CDCl_3$): δ 8.87 (br. s, 1H), 7.47-7.28 (m, 10 H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$): 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; ^{1}H NMR ($CDCl_3$): δ 9.06 (br. s, 1H), 7.45-7.39 (m, 5 H), 7.27-7.18 (m, 4 H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$): 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; ^{1}H NMR ($CDCl_3$): 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); ^{13}C NMR ($CDCl_3$): 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; ^{1}H NMR ($CDCl_3$): δ 8.76 (br. s, 1H), 7.47-7.36 (m, 10 H), 3.13 (s, 2H), 3.85 (s, 3H); ^{13}C NMR ($CDCl_3$): 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-isothiourea (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-isothiourea (3a): Yield 76 %; m.p. 52-54 °C; ^{1}H NMR ($CDCl_3$): δ 7.43-7.37 (m, 10 H), 3.76 (s, 3H), 2.55 (s, 3H); ^{13}C NMR ($CDCl_3$): 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-isothiourea (3b): Yield 64 %; m.p. 94-96 °C. ^{1}H NMR ($CDCl_3$): δ 7.29-7.42 (m, 10 H), 3.81 (s, 3H), 2.58 (s, 3H); ^{13}C NMR ($CDCl_3$): 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-isothiourea (3c): Yield 67 %; An oil. ^{1}H NMR ($CDCl_3$): 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); ^{13}C NMR ($CDCl_3$): δ 165.8, 163.4, 136.5-125.7 (C_{arom}), 51.3, 47.8, 15.3. FT-IR ($CHCl_3$, ν_{max}, cm^{-1}) 1652, 1643, 1554, 1351, 1310, 816, 768.

1-(1-Methoxy-2-phenyl-ethylidene)-2-methyl-3-phenyl-isothiourea (3d): Yield 73 %; An oil; ^{1}H NMR ($CDCl_3$): δ 7.42-7.47 (m, 10 H), 3.82 (s, 3H), 3.13 (s, 2H), 2.61 (s, 3H); ^{13}C NMR ($CDCl_3$): 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-CH_2-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 zith chloroform, dried over $MgSO_4$ and concentrated under reduced pressure to give the corresponding 2-amino-thiazole **5** witch was purified by recrystallization from ethanol.

4-Phenyl-2-phenylamino-thiazole-5-carboxylic acid ethyl ester (5a): Yield 82 %; m.p. 142-144 °C; ^{1}H NMR ($CDCl_3$): 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); ^{13}C NMR ($CDCl_3$): δ 168.4, 161.5, 158.5, 140.3, 133.7-121.6 (C_{arom}), 60.5, 14.2; FT-IR ($CHCl_3$, ν_{max}, cm^{-1}) 3389, 1709, 1602, 1537, 1442, 1294. Anal. calcd. (%) for $C_{17}H_{14}N_2O_2S$: 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; ^{1}H NMR ($CDCl_3$): δ 9.52 (br. s, 1H), 7.75-7.18 (m, 15 H); ^{13}C NMR ($CDCl_3$): 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 $C_{22}H_{16}N_2OS$: 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; ^{1}H 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); ^{13}C 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 $C_{17}H_{13}ClN_2O_2S$: 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; ^{1}H NMR ($CDCl_3$): δ 9.18 (br. s, 1H), 7.42-7.17 (m, 10 H), 4.51 (s, 2 H), 4.23 (q, 2H, *J* = 7.6 Hz), 1.19 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$): 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 $C_{18}H_{16}N_2O_2S$: 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; ^{1}H NMR ($CDCl_3$): δ 8.13 (br. s, 1H), 7.38-7.21 (m, 10 H), 4.57 (s, 2 H), 4.21 (q, 2H, *J* = 7.6 Hz), 1.23 (t, 3H, *J* = 7.6 Hz); ^{13}C NMR ($CDCl_3$): 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 $C_{18}H_{16}N_2O_2S$: 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; ^{1}H NMR (DMSO-d_6): δ 10.53 (br. s, 1H), 7.53-7.18 (m, 10 H); ^{13}C 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 $C_{16}H_{11}N_3S$: C, 69.29; H, 4.00; N, 15.15. Found (%): C, 69.45; H, 3.88; N, 14.97.

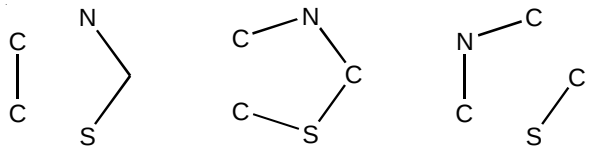
(4-Chloro-pheny)-1-(4-phenyl-2-phenylamino-thiazol-5-yl)-methanone (5g): Yield 72 %; m.p. 142-144 °C; ^{1}H NMR

(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 $C_{22}H_{15}ClN_2OS$: 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; ^{1}H 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 $C_{17}H_{13}N_3S$: C, 70.08; H, 4.50; N, 14.42. Found (%): C, 70.23; H, 4.38; N, 14.57.

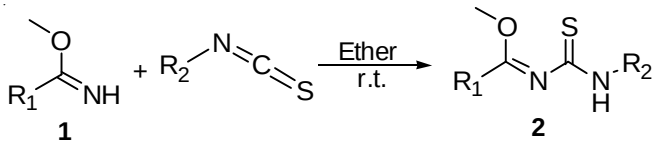
RESULTS AND DISCUSSION

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



A **B** **C**

The literature shows that the scheme A and C are the most commonly used and known the Hantzsch reaction[15]. We show in the present research article that the pattern B is very convenient way allowing access to this type of compound. The synthetic pathways depicted in **Schemes II** and **III** outlines, the chemistry of the present study. Our synthesis began with the starting material methoxymethylene-3-aryl-thiourea (**2**), which are easily prepared in good yield following the reaction of imidic acid methyl ester (**1**) with isothiocyanate (**Scheme-II**).

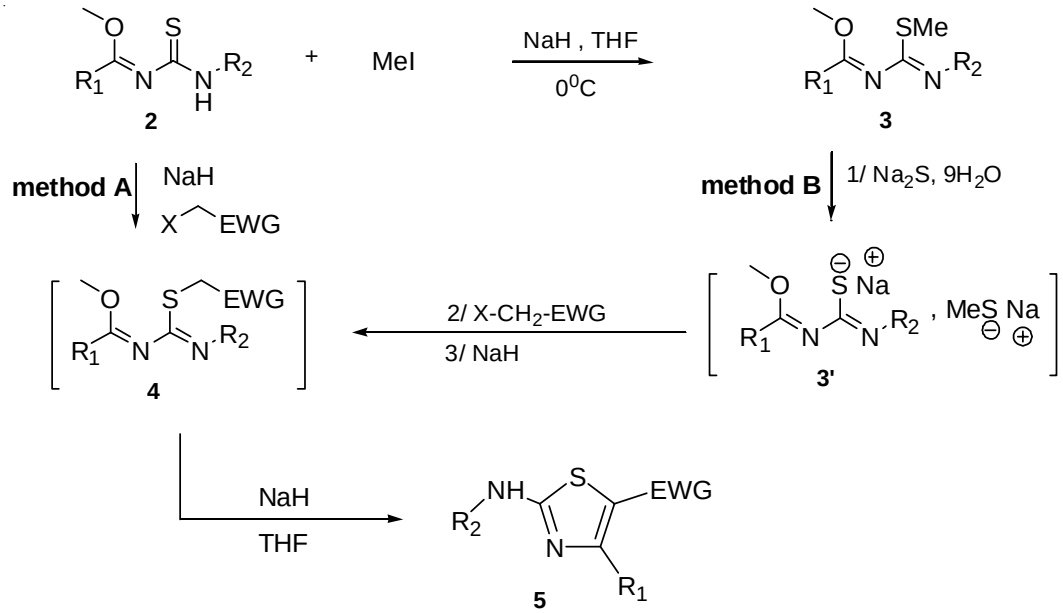


1 **2**

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.

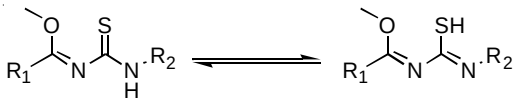


X = Cl, Br R_1 = Ph, Ph-CH_2- R_2 = Ph, 4-Cl-Ph , Ph-CH_2-
EWG = CN, CO_2Et , Ph-CO- , 4-Cl-Ph-CO-

Compounds **5**	R_1	R_2	EWG	Yield* (%)
5a	Ph	Ph	CO_2Et	82
5b	Ph	Ph	Ph-CO	78
5c	Ph	4-Cl-Ph	CO_2Et	73
5d	Ph	Ph-CH_2-	CO_2Et	76
5e	Ph-CH_2-	Ph	CO_2Et	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



Thioamide form **Thioamidic acid form**

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 heterocycles[16-18]. 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 thioimidic thioamide-acid equilibrium in such compounds.

This success has lead 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 µg/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 = CO_2Et) 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 attached to the thiazole nucleus has no significant influence on the antimicrobial activity.

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

Compd.	Diameter of growth inhibition zone (mm)			
	Antibacterial activity		Antifungal activity	
	P. aeruginosa	*E. coli*	*S. aureus*	*A. niger*
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	-

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 = CO_2Et) showed potent antifungal activity.

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