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SYNTHESIS OF SOME BENZOTHIAZOLE ANILINE DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

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Abstract. Three benzothiazole aniline derivatives **2a**, **2b,** and **2c** were synthesized successfully by the reduction reaction of the respective nitro compound in neutral condition with $Na_2S_2O_4$ in moderate yield. Structures of these derivatives were elucidated by IR, NMR, and MS analysis that referred to a strong agreement between spectral data and structures. Three compounds **2a**, **2b,** and **2c** showed weak antioxidant, antibacterial and antifungal activities.
Keywords: benzothiazole, aniline, antioxidant, antibacterial, antifungal, benzothiazole aniline derivatives.

1. Introduction

Recently, benzothiazole aniline derivatives have been considered with great interest because their derivatives show some outstanding biological activities, especially, anticancer properties [1, 2]. Surprisingly, some simply structural benzothiazole aniline derivatives 2-(4-aminophenyl)benzothiazoles (**I**), and *N*-acetylated derivatives (**II**) have shown surprisingly considerable anticancer against certain cancer cell lines as breast, colon, and ovarian cell lines *in vitro* anticancer screening [3-5]. In addition, benzothiazole aniline derivatives become a core structure for the synthesis of promising anticancer agents [6-8]. In our previous works, we investigated the synthesis of some benzothiazole aniline derivatives has faced the problem which being contaminated with iron (II) when the reduction of nitro compounds to amine compounds was undergone with Fe/HCl reagent [9]. In this work, the benzothiazole aniline derivatives were synthesized by taking advantage of the microwave method in benzothiazole cyclization and sodium dithionite in the conversion of the nitro group to the free amino group in a simple procedure.

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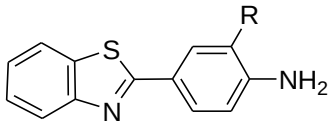

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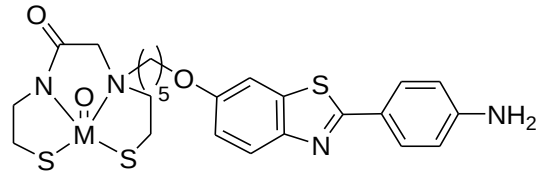

II

2. Content

2.1. Experimental

2.1.1. Chemicals and equipment

Solvents and other chemicals were purchased from Sigma-Aldrich, Merck Corp, Aladdin, Vietnam, or other China's companies and were used as received, unless indicated in detail. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III spectrometer (500 and 125 MHz) using deuterated solvents and tetramethyl silane (TMS) as internal standard. MS spectra were recorded on the SCIEX X500 QTOF system. Melting points were measured using a Gallenkamp melting point apparatus. A domestic Electrolux 800w (2015, Vietnam) microwave oven was used to carry out the reactions.

2.1.2. Synthetic procedure

***** *General method for the synthesis of 2-(4,5-dimethoxy-2-nitrophenyl) benzothiazole* ***(1a);*** *4-(benzothiazol-2-yl)-2-methoxy-3-nitrophenol* ***(1b);*** *2-(3,4-dimethoxy-2-nitrophenyl)benzothiazole* ***(1c)***

A mixture of *o*-aminothiophenol (0.34 mL, 2.1 mmol) and an aldehyde (2.0 mmol) in a 250 mL beaker was irradiated for 3 - 4 min at 400W power level. The progress of the reaction was monitored with TLC every 30 seconds. The mixture was then dissolved in ethyl acetate, *n*-hexane, or ethanol and stood at room temperature to form a solid. The yields of the reaction were up to 98% (**1a**: mp.= 167 °C, **1b**: mp.= 160 °C, **1c**: mp.=165 °C) [9-11].

** General method for the synthesis of benzothiazole aniline derivatives*

Sodium dithionite ($Na_2S_2O_4$, 2.1g, 12 mmol) was added slowly into a refluxed solution of nitro compound **1a**, **1b** or **1c** (2 mmol) in ethanol (50mL) for 30 min. The progress of the reaction was observed with the disappearance of yellow color or TLC in eluent *n*-hexane/ethyl acetate (1:1). The mother liquid was concentrated two third and diluted with cold water (30 mL) to form a precipitate. The crude product was recrystallized in 96% ethanol.

2-(benzothiazol-2-yl)-4,5-dimethoxyaniline (**2a**) as a yellow solid after recrystallization in ethanol (62%, mp. 167 ºC); IR (ν (cm^{-1}): 3474, 3308, 3200 – 2500 (br.), 3006, 2937, 2836, 1625, 1582, 1559, 1526, 1289, 1250, 1174, 1083, 1067, 1015. ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm), *J* (Hz): 8.00 (d, *J* = 7.5, 1H), 7.91 (d, *J* = 8.0, 1H), 7.46 (td, *J* = 7.0, 1.0, 1H), 7.34 (td, *J* = 7.0, 1.0, 1H), 7.13 (s, 2H), 7.02 (s, 1H), 6.51 (s, 1H), 3.79 (s, 3H), 3.74 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm):

168.2, 153.5, 153.2, 144.2, 140.2, 132.1, 126.2, 124.3, 121.4, 121.4, 112.8, 104.7, 99.6, 56.5, 55.3; ESI-MS m/z: $[M+H]^+$, 287.1 au; $[M-H]^-$, 284.9 au.

3-amino-4-(benzothiazol-2-yl)-2-methoxyphenol **(2b)** as a yellow solid after recrystallization in ethanol (70%, mp. 170 ^oC); ^1H NMR (500 MHz, DMSO-d_6), δ (ppm), *J* (Hz): 9.78 (s, 1H), 8.0 (d, *J* = 6.5, 1H), 7.92 (d, *J* = 6.5, 1H), 7.46 (td, *J* = 6.0, 1.0, 1H), 7.35 (td, *J* = 6.0, 0.5, 1H), 7.27 (d, *J* = 7.0, 1H), 7.03 (s, 1H, NH_2), 6.28 (d, *J* = 7.5, 1H), 3.74 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 168.9, 153.3, 152.4, 142.6, 133.4, 132.0, 126.3, 126.0, 124.5, 121.5, 121.4, 107.2, 105.8, 59.0; ESI-MS m/z: $[M+H]^+$, 272.8 au; $[M-H]^-$, 270.8 au.

6-(benzothiazol-2-yl)-2,3-dimethoxyaniline **(2c)** as a white solid after recrystallization in ethanol (72%, mp. 164 ^oC); ^1H NMR (500 MHz, DMSO-d_6), δ (ppm), *J* (Hz): 8.04 (dd, *J* = 7.5, 0.5 Hz, 1H), 7.95 (dd, *J* = 7.5, 0.5 Hz, 1H), 7.48 (td, *J* = 7.5, 1.5 Hz, 1H), 7.42 (d, *J* = 9.0 Hz, 1H), 7.39 (td, *J* = 7.5, 1.5 Hz, 1H), 7.02 (s, 2H, NH_2), 6.48 (d, *J* = 9.0 Hz, 1H), 3.85 (s, 3H), 3.73 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 168.7, 154.2, 153.2, 141.9, 134.3, 132.2, 126.3, 125.9, 124.8, 121.7, 121.6, 108.9, 101.3, 59.2, 55.7, ESI-MS m/z: $[M+H]^+$, 286.8 au.

2.2. Results and discussion

2.2.1. Synthesis

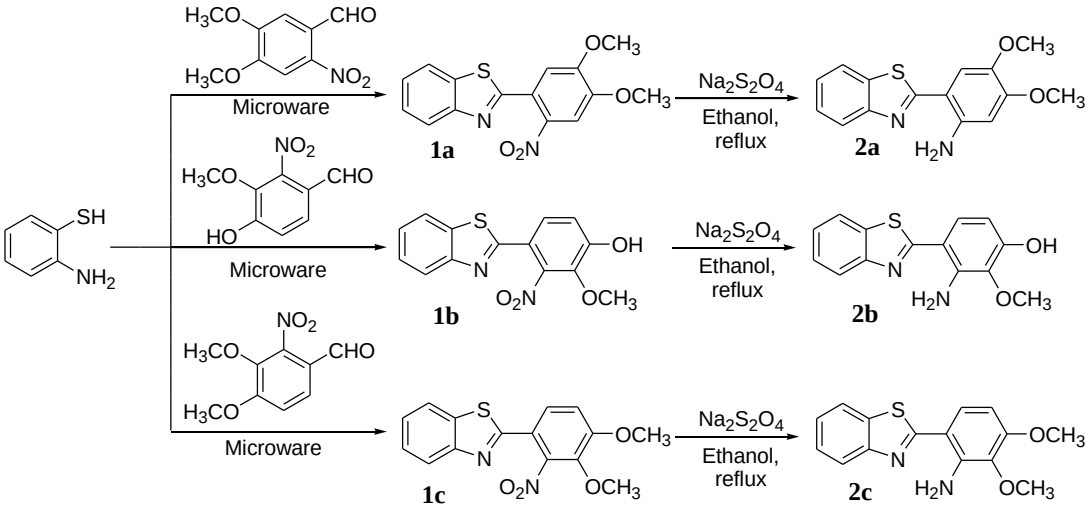

Figure 1. Synthesis of benzothiazole aniline derivatives 2a, 2b and 2c

The synthesis of benzothiazole aniline derivatives was shown in Scheme 1. The reduction of the nitro group to the amino group was optimized by using some classic methods, Table 1 for the conversion of **1b** to **2b**. All entries were carried out up to 20 hours and monitored with thin layer chromatography (TLC). All entries 1, 3, and 5 took place in water slowly or had no reaction due to the small solubility of substrates in water. Surprisingly, Fe/NH_4Cl did not work in this case because the acidity of ammonium chloride might be weaker than the salt of the respective amine compound and the free amine was formed and then oxidized immediately resulting in a black solution as observed. When Fe/HCl was used, the products obtained were salts and

contain iron. Therefore, $Na_2S_2O_4$ was chosen as the reaction agent. Unfortunately, as soon as the reagents $Na_2S_2O_4$/NaOH/H_2O were added (entry 5), the reaction solution turned black due to the reason that in the basic condition, free aniline was formed and oxidized by oxygen in the air. The reason for this observation was the aniline black because the benzene ring contains 3 donating electron groups –OCH_3, -NH_2, and O^- that raises the electron density on the benzene ring, consequently, it is oxidized quickly.

Table 1. Reduction optimization results for compound 1b

Entry	Reagent	Solvent	Time (h)	Observation	Yield (%)
1	Fe/NH_4Cl	H_2O	18h, reflux	Black solution	0
2	Fe/NH_4Cl	C_2H_5OH	20h, reflux	Black solution	0
3	Fe/con. HCl	H_2O	12h, reflux	Yellow solution	45
4	Fe/con. HCl	C_2H_5OH	7h, reflux	Yellow solution	60 (salt)
5	$Na_2S_2O_4$/ NaOH	H_2O	0.5h, reflux	Black solution	0
6	$Na_2S_2O_4$	C_2H_5OH	7h, reflux	Pale yellow solid	62 (free amine)

2.2.2. Structural determination

The structures of the benzothiazole aniline **2a, 2b,** and **2c** were determined by modern spectroscopic methods [12].

To understand the structures of these benzothiazoles, compound **2b**, one of the most complicated compounds, was chosen to study IR, ^{1}H NMR, ^{13}C NMR, and MS spectra. First of all, the negative mass spectrum showed a base pick at *m/z* 271 au and the positive mass spectrum showed a peak at *m/z* 273 au. The molecular weight of compound **2b** must be 272 g.mol^{-1} matching with the designed product, expectedly, Figure 2.

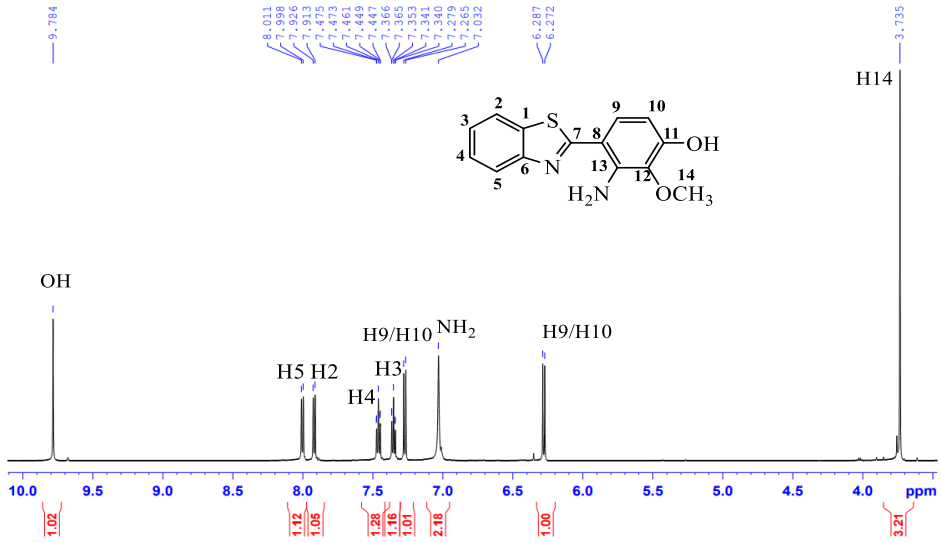

Figure 2. ^{1}H NMR spectrum of compound 2b

Secondly, ^{1}H NMR spectrum of compound **2b** indicated 12 protons including a proton at δ = 9.78 ppm for H of OH phenolic, 2 protons at δ = 7.03 ppm for H (NH_2), 3 protons at δ = 3.73 ppm for H14. In addition, there were two doublet peaks at δ = 7.27 and 6.28 ppm with a splitting constant of about 7.0 ÷ 7.5 Hz which were for either H9 or H10. There were two doublet peaks at δ = 8.01 ppm (J = 6.5 Hz) and δ = 7.92 ppm (J = 6.5 Hz) which were assigned for H5 and H2. Moreover, there were two triplet-doublet peaks at δ = 7.46 with a splitting constant of about J = 6.0; 1.0 Hz and at δ = 7.35 ppm with a splitting constant of about J = 6.5; 0.5 Hz which should be for H3 or H4. Because proton H3 has two *ortho*- interactions with H2 and H4, one *meta*-interaction with H5; proton H4 has two *ortho*- interactions with H3 and H5, one *meta*-interaction with H2. ^{13}C NMR spectrum of compound **2b** indicated 14 peaks associated with 14 carbon atoms. Certainly, a peak at 58.99 ppm was assigned for C14. The remaining 13 signals are for aromatic carbons. NMR spectral analysis of other compounds **2a** and **2c** was shown in the experimental section.

2.2.3. Biological activities of benzothiazole aniline derivatives

Three benzothiazole aniline derivatives **2a**, **2b,** and **2c** were tested for antioxidant, antibacterial and antifungal activities. The test results are presented in Table 2 and Table 3.

Table 2. **Antibacterial and antifungal activities**

Nº	Sample	Minimum inhibitory concentration (MIC, μg.mL^{-1})							
		Bacterium Gr(-)		***Bacterium Gr(+)***		***Mold***		***Yeast***	
		E. coli	*P. aeruginosa*	*B. subtilis*	*S. arueus*	*A. niger*	*F. oxysporum*	*S. cevevisiae*	*C. albicans*
1	**2a**	200	-	-	-	-	200	-	-
2	**2b**	-	-	200	-	-	150	150	-
3	**2c**	-	-	-	-	-	150	200	-

Test results have shown that all samples exhibited tested antimicrobial activity against at least 1 bacterial strain or 1÷2 fungal strain with MIC values between 150 - 200 μg.mL^{-1}.

Table 3. Antioxidant activities

Nº	Sample	SC (%)	SC_{50} (μg.mL^{-1})
	Ascorbic acid	86.53 ± 0.3	12.6
	DPPH*	0 ± 0	-
1	**2a**	34.72 ± 0.4	> 1000
2	**2b**	72.09 ± 0.8	306.31
3	**2c**	19.32 ± 0.5	> 1000

SC (%): Percent of inhibition and scavenging at 1000 μg.mL^{-1}; SC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity;
**DPPH: 2,2-diphenyl*-1-picrylhydrazyl.

Test results have shown that only compound **2b** showed weak antioxidant activity in 306.31 μg.mL^{-1} comparing to 12.6 μg.mL^{-1} of ascorbic acid. These results also indicated that benzothiazole aniline derivatives **2a** and **2c** without OH phenolic group didn't show any significant antioxidant activity [13]. However, the combination of NH_2

and OH phenolic groups seemed to act more on fungi. For example, **2a** showed weak antibacterial activity against 2 strains: *E. Coli* and *F. Oxysporum*; **2c** also inhibited against mold and yeast strains only. Whereas, **2b** exhibited against three strains: *B. subtilis*, *F. oxysporum* and *S. cevevisiae* in 150μg.mL^{-1}.

3. Conclusions

Three new benzothiazole anilines were synthesized by the reduction reaction of the respective nitro compound with sodium dithionite in ethanol in moderate yield. Structures of benzothiazole aniline were determined with spectroscopy methods such as NMR and MS. Compound **2b** exhibited weak antioxidant activity with SC_{50} = 306.31μg.mL^{-1}, compounds **2a**, **2b,** and **2c** exhibited weak antibacterial and antifungal activity with MIC = 150÷200 μg.mL^{-1}.

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