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Research Article

Synthesis of New p-Alkylaminophenol Compounds and Investigation of Their Antimicrobial and Antioxidant Activity

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Abstract

Alkylaminophenols are synthetic derivatives well known for their anticancer activity. In this work, we report the antimicrobial and antioxidant activity of such compounds. A series (4,5,6) of alkylaminophenol compounds were prepared with fairly good yields by Petasis reaction. Synthesized compounds were characterized by ^{1}H-NMR and ^{13}C-NMR. The obtained compounds were tested against Gram-positive and Gram-negative bacteria. The compound 4 showed antimicrobial activity at a concentration of 10 mg/mL, while the 6 compound showed activity on S. aureus. Phenolic compounds have attracted attention due to their proximal and antioxidant activities. Since alkylaminophenols have phenolic structure, their antioxidant activity has been investigated. Thus, drug-active substances with high antioxidant capacity against diabetes, heart disease, and cancer were synthesized.

Keywords: Alkylaminophenol, antimicrobial, anticancer, antioxidant

Yeni p-alkilaminofenol Bileşiklerinin Sentezi ve Antimikrobiyal ve Antioksidan Aktivitelerinin Araştırılması

Öz

Alkilaminofenoller antikanser aktivitelerine sahip iyi bilinen organik yapılardır. Bu çalışmada, yüksek verimlerle petasis reaksiyonuyla bir seri (4,5,6) alkilaminofenol bileşiği sentezlendi. Sentezlenen bileşikler 'H-NMR ve ^{13}C-NMR ile karakterize edildi. Ardından,bu bileşiklerin antimikrobiyal ve antioksidan aktivitelerini incelendi. Elde edilen bileşikler, Gram pozitif ve Gram negatif bakterilere karşı test edildi. 4 nolu bileşiğin , 10 mg / mL konsantrasyonda antimikrobiyal aktivite gösterirken, 6 nolu bileşiğin, S. aureus'ta aktivite gösterdiği görüldü. Fenolik bileşikler proksimal ve antioksidan aktivitelerinden dolayı dikkat çekici yapılardır. Alkilaminofenoller fenolik yapıya sahip olduklarından antioksidan aktiviteleri de araştırılmıştır. Diyabete, kalp hastalığına ve kansere karşı yüksek antioksidan kapasiteye sahip ilaç aktif maddeleri; ilk kez sentezlenmiştir.

Anahtar kelimeler: Alkilaminofenol, antimikrobiyal, antikanser, antioksidan

1. Introduction

Cancer is a leading cause of death worldwide. The emergence of different types of the same disease ensures that treatments and drugs are used differently. In recent years, alkylaminophenol based compounds have been frequently used in chemotherapy because of their anticancer and antioxidant activity. It is known to be effective, especially in bone cancer. The ability to act as free radical scavengers

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and the presence of a phenol group in their structure required research into their biological properties (Neto 2016; Doan 2016; Doan and Nguyen 2017; Larson 1988; Cotelle 1996; Velioğlu 1998; Zheng 2001)

Alkylaminophenols are heterocyclic structures synthetically obtained by the reaction of an amine, aldehyde, and boronic acid (Ulaş 2019; Petasis 1993; Mandai 2012; Mandai et. 2012; Candeias 2009; Candeias 2010; Rosholm 2015) We began the study by synthesizing a series of alkylaminophenol and then we performed structural characterization of these compounds. At the last stage, we investigated antimicrobial and antioxidant activities.

2. Materials and methods

^{1}H and ^{13}C spectra were recorded on Agilent 600 spectrometer (600 and 150 MHz, respectively) in $CDCl_3$ with TMS as the internal standard. Melting points were measured on a Buchi B-540 digital melting point apparatus. All chemicals and solvents were purchased from commercial sources and used without further purification.

2.1. General procedure for the synthesis of p- alkylaminophenols

Salicylaldehyde (5 mmol) was added to a stirred mixture of amine (5 mmol) and arylboronic acid (5 mmol) in 1,4-dioxane at reflux and stirred for 24-36h.The resultant solution was diluted with H_2O and extracted with EtOAc. The organic layer washed with brine and dried over Na_2SO_4. The product was obtained by column chromatography on silica gel with n-hexane-EtOAc (9:1) as eluent. Evoparation of the solvent afforded the aminoalkylphenol as colorless to yellow oil or white to yellow solid (Kaboudin 2018).

2-(benzo[d][1,3]dioxol-5-yl(pyrrolidin-1-yl)methyl)phenol (4)

Yield 1.20 g (81%), yellow solid, mp 95-96 ºC .^{1}H NMR spectrum (600 MHz, $CDCl_3$), δ, ppm (J, Hz):1.84 (m, 4H); 2.50-2.64 (m, 4H); 4.30 (s, 1H); 5.91(d, J=16.8, 2H); 6.71(t, J=7.8, 2H); 6.86 (d, J=7.8, 1H); 6.89 (d, J=7.8, 1H); 6.94 (d, J=7.2, 1H); 7.05 (s, 1H); 7.11 (t, J= 7.2, 1H); 12.23 (s, 1H). ^{13}C NMR (CDCl3, 150 MHz): δ, ppm: 23.4; 53.1; 75.4; 101.0; 107.9; 108.0; 108.1; 109.7; 116.9; 119.1; 121.2; 122.2; 126.7; 128.2; 128.3; 136.2; 147.0; 147.9; 156.5.

2-(benzo[d][1,3]dioxol-5-yl(piperidin-1-yl)methyl)phenol (5)

Yield 1.34 g (86%,) Yellow solid, mp 108-109 ºC.^{1}H NMR (600 MHz, $CDCl_3$): δ(ppm) = 1.47-1.65 (m, 7H); 2.42 (broad s, 3H); 4.38 (s, 1H); 5.92 (d, J=15, 2H); 6.69-6.73 (m, 2H); 6.82-6.85 (m, 2H); 6.89 (d, J=7.8, 1H); 6.96 (s, 1H); 7.11 (t, J=7.8, 1H); 12.51 (s, 1H).^{13}C NMR (CDCl3, 150 MHz): δ, ppm: 24.1; 26.1; 76.2; 101.1; 108.0; 108.1;108.2; 109.8; 116.9; 119.0; 122.2; 125.6; 128.3; 129.1; 133.4; 136.6; 146.2; 146.3; 147.2; 147.9; 157.0.

2-(azepan-1-yl(benzo[d][1,3]dioxol-5-yl)methyl)phenol (6)

Yield 1.52 g (94%), Yellow solid, mp 79-80 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ(ppm) = 1.64-1.71 (m, 8H); 2.71 (s, 4H); 4.66 (s, 1H); 5.93 (d, J=10.8, 2H); 6.69 (t, J=6.6, 1H); 6.75 (d, J=7.8, 1H); 6.86 (d, J=6.6, 3H); 7.00 (s,1H); 7.12 (s, 1H); 12.59 (s, 1H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ, ppm: 26.4; 28.0; 53.5; 75.4; 101.1; 108.1; 108.7; 109.8; 110.0; 116.9; 118.8; 122.2; 122.3; 122.4; 126.2; 128.4; 128.5; 128.6; 128.8; 133.5; 147.2; 148.0; 157.2

2.2. Antimicrobiyal Activity of 4, 5, 6

Disc diffusion assay was performed to determine the antimicrobial activity of the newly synthesis compounds with disc diffusion method according to the National Committee for Clinical Laboratory Standards Guidelines (NCLLS 1997). A suspension of the tested microorganism (0.1 ml of 108 cells per ml) covered the surface of agar plates. Filter papers of 6 mm diameter are wetted at various concentrations and placed on inoculated agar plates. Ten microliters of the test compounds were filled into sterile filter paper discs (6 mm) and put in inoculated plates. The seeded plates were incubated at 37°C for 24 h and 30°C for 48 h for bacteria and fungi, respectively. Imipenem (IPM) and Erythromycin were used as positive controls for bacteria and fungi, respectively. All tests were made in triplicate and the antimicrobial activity was indicated as diameter of inhibition zones (mm). Values are presented as means ±SD of three parallel measurements.

2.3. Antioxidant Activity of all alkylaminophenol compounds

Antioxidant activity was tested via 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity with small modifications in 96-well microplate (Dimitrova 2010 and Sharma 2009). DPPH was dissolved in methanol at 0.004 % concentration. Test compounds were dissolved in Dimethyl Sulfoxide (DMSO). Concentrations were 1.25, 2.5, 5, 10 mg/ml, respectively. Each well contained a solution of 200 µl DPPH-methanol. 10 µl of serial concentration of each test compound and controls were added separately into each well, Ascorbic acid (11.8 mM), Butylated hydroxytoluene (60 mM) and DMSO were also used as controls. Then microplates were incubated at room temperature for 30 minutes, in the dark. After incubation, the absorbance of test compounds the antioxidant activity was measured at 517 nm by the microplate reader. All tests were made in triplicate. DPPH free radical scavenging activity was determined with the following equation:

Inhibition of DPPH % = [(AbsControl - AbsSample) / AbsControl] x 100

AbsControl = The absorbance of DPPH-methanol solution

AbsSample = The absorbance of test compounds/controls mixed with DPPH-methanol solution, separately

3.Results and Discussion

3.1.Chemistry

This study aims to synthesize biologically active alkylaminophenol compounds. The Petasis reaction was chosen as a synthesis reaction. As well konown, this multicomponent reaction generally occur between an aldehyde, secondary amine, and boronic acid. The reaction was affected by the solvent, temperature, and atmosphere conditions. Therefore, the targeted compounds were synthesized by selecting an optimized procedure. A catalyst is usually needed (Frauenlob 2012; Shi 2012; Reddy 2015) to perform the reaction; however, no catalyst (Ying 2010) was used in this study.

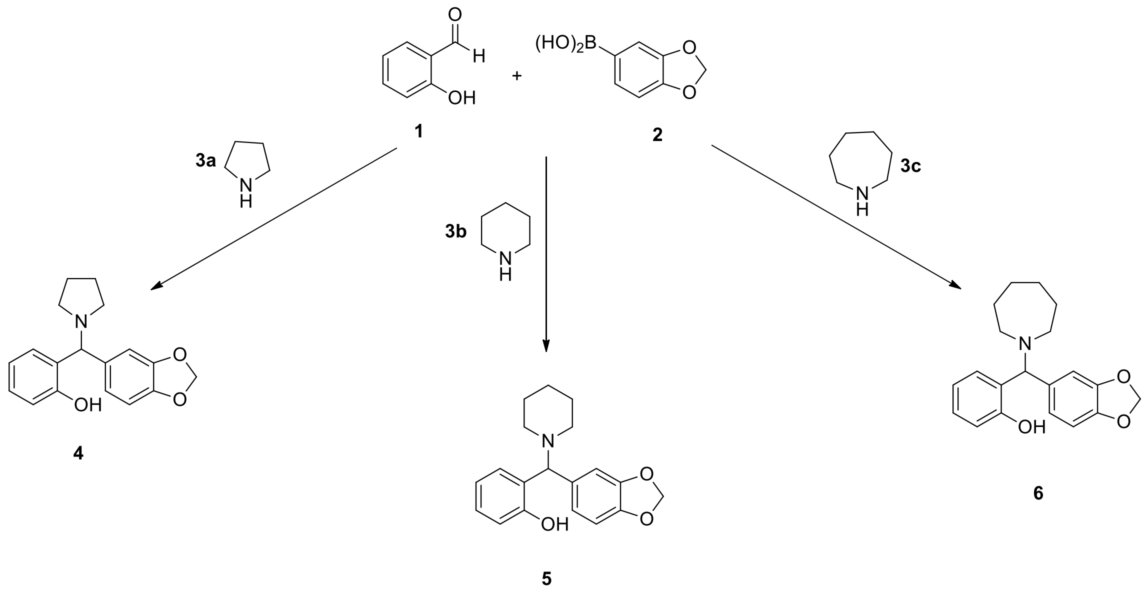

Figure 1 Synthesis of Alkylaminophenols

In the reaction (Figure 1) Therefore, salicylaldehyde **1**; 3,4- (methylenedioxy) phenylboronic acid **2** were reacted with three different cyclic secondary amines. Three new compounds with high yields 2- (benzo [d] [1,3] dioxol-5-yl (pyrrolidin-1-yl) methyl) phenol; **4**, 2- (benzo [d] [1,3] dioxol-5 yl (piperidin-1-yl) methyl) phenol; **5**, 2- (azepan-1-yl (benzo [d] [1,3] dioxol-5-yl) methyl) phenol **6** were synthesized.

The reaction occurred with the formation of iminium ion between the secondary amine and the aldehyde, then by alkylaminophenol via the nucleophilic addition of boronic acid (Figure 2).

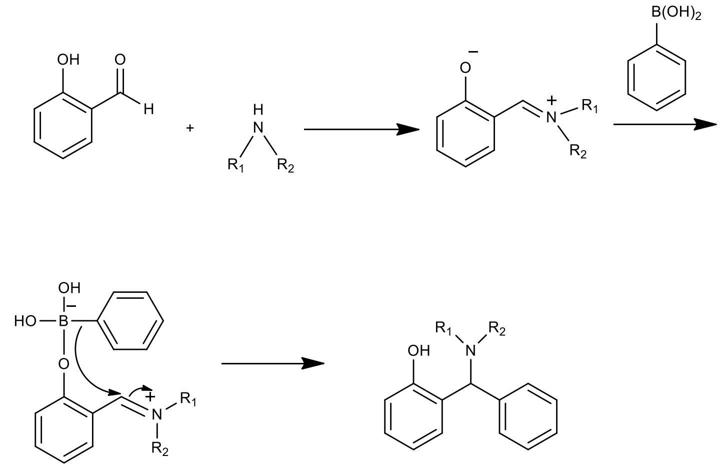

Figure 2 Petasis reaction mechanism between salicylaldehyde, boronic acid and secondary amines

In the structure analysis of the compounds, it was found that the specific chiral C-H proton of the alkylaminophenols was about 4.00-5.00 ppm, and the O-H peak bound to the aromatic ring was between 12.00 and 13.00 ppm. After the structure analysis was completed, antimicrobial and antioxidant capacity of these compounds were investigated.

3.2.Biological assays

3.2.1 Antimicrobial activity

The Antimicrobial activity of test compounds was evaluated against two Gram (-) negative Escherichia coli and Pseudomonas aeruginosa, two Gram (+) positive Staphylococcus aureus and Streptococcus pyogenes bacteria, and a yeast Candida albicans. Salmonella typhimurium (TA98 and TA100) strains with Rfa mutation were tested for checking whether test compounds (concentration of 10 mg/ml) passed through bacterial membrane.

4 test compound showed antimicrobial activity against S. pyogenes at 10 mg/ml and showed weak effects against other microorganisms at the same dose. **6** test compound showed activity against S. aureus. Since the test compounds have to enter the cell to show antimicrobial activity, the compounds should also be checked whether they enter the cell. For this reason, it was investigated whether the absence or weak of antimicrobial activity was related to the test compounds that were passed through the cell membrane. For this purpose, the test compounds were tested by using Ames Test strains TA98 and TA100. According to these results, it can be suggested that the tested compounds may have passed through the cell membranes.

4 test compound showed antimicrobial activity against all microorganisms at 10 mg/ml. 6 test compound showed activity against S. aureus. Neither **4** nor **6** showed antimicrobial activity against microorganisms in increasing (20, 40 and 80 mg/ml) concentrations (Table 2).

Table 2. Antibacterial activity of the test compounds.

Test compound	Dose mg/ml	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenes*	*C. albicans*
4	1.25	7.0 ± 1.0	-	7.3 ± 0.5	-	-
	2.5	7.0 ± 1.0	7.0 ± 0	7.3 ± 0.5	-	-
	5	7.0 ± 0	7.0 ± 0	7.4 ± 0.6	7.0 ± 1.0	7.0 ± 1.0
	10	10.0 ± 1.0	9.0 ± 0	7.3 ± 0.5	14.0 ± 1.0	7.0 ± 1.0
5		-	-	-	-	-
6	1.25	-	-	-	-	-
	2.5	-	-	8.3 ± 0.5	-	-
	5	-	-	9.0 ± 1.0	-	-
	10	-	-	9.0 ± 1.0	-	-
DMSO	15 µl	-	-	7.0 ± 0	-	-
Imp.	Disk	26.3 ± 1.5	27.4 ± 1.4	26.4 ± 1.2	26.4 ± 1.2	26.4 ± 1.2
Erytr.	Disk	22.6 ± 1.5	23.0 ± 1.2	23.0 ± 1.2	23.0 ± 1.2	23 ± 1.2

3.2.2 Antioxidant activity

DPPH free radical scavenging effect was found out at 1.25, 2, 5, and 10 mg/ml, and the results supply over 50% inhibition in these antioxidant assays were seen in Table 3, respectively. As depicted in Table 3, the antioxidant activity of the test compounds showed increasing activity dependent on the increasing concentration. The highest antioxidant activity was observed at 10 mg/ml concentration of all test compounds. It was calculated as DMSO inhibited DPPH-methanol solution at 5%, AA inhibited at 70.68% and BHT inhibited at 68.64%, respectively. All the results are indicated in Table 3.

Table 3 DPPH free radical scavenging activity

Samples	**DPPH%**	**Samples**	**DPPH%**	**Samples**	**DPPH%**
Control	0	Control	0	Control	0
Ascorbic acid	70.68 ± 0.03	Ascorbic acid	70.68 ± 0.03	Ascorbic acid	70.68 ± 0.03
BHT	68.64 ± 0.01	BHT	68.64 ± 0.01	BHT	68.64 ± 0.01
DMSO	5.56 ± 0.03	DMSO	5.56 ± 0.03	DMSO	5.56 ± 0.03
4 -1,25	17.67 ± 0.05	**5** -1,25	12.34 ± 0.06	**6** -1,25	16.02 ± 0.03
4 -2,5	26.04 ± 0.07	**5** -2,5	16.99 ± 0.04	**6** -2,5	21.24 ± 0.10
4 -5	35.34 ± 0.07	**5** -5	25.72 ± 0.02	**6** -5	23.64 ± 0.03
4 -10	47.29 ± 0.05	**5** -10	37.51 ± 0.01	**6** -10	38.20 ± 0.01

Values are means ± S.D. n = 3, P < 0.05, importantly dissimilar with Student's t-test.

4. Conclusion

In summary, novel alkylaminophenol compounds with high antioxidant and biological activity were synthesized. Compound 4 exhibited antimicrobial activity against all microorganisms at 10 mg/ml, while compound 6 showed activity against S. aureus. Compound 5 showed no antimicrobial activity. The absence of antimicrobial activity may be important to deliver the drug to the cell without killing the cell. As previously reported, the antioxidant activity of the newly synthesized compounds may be due to phenolic groups in chemical structures, and our test results have confirmed this.

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