Synthesis and Antibacterial Activities of *2-Amino-N-(P-Chlorophenyl) Acetamide Derivatives*

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Abstract- A series of 2-amino-N-(p-Chlorophenyl) acetamide derivatives (5a ~ 5d) were synthesized by the reaction of 2-bromo-N-(p-Chlorophenyl) acetamide (3) with various amine (4a ~ 4d) at room temperature. The antibacterial activities of all the compounds were evaluated against four bacterial strains (Acinetobacter baumannii, Pseudomonas aeruginosa and Staphylococcus aureus) and showed moderate to high activities.

Indexed Terms- N-phenylacetamide, Antibacterial Activity, Synthesis.

I. INTRODUCTION

Over the past decade, antibiotic resistance is one of the most important global public health problems due to antibiotics abuse[1]. Hence, it is an urgent need for overcoming multidrug resistance *(*MDR*)* to develop new antibacterial drugs. The *N*-phenylacetamide derivatives are highly identified for their pharmacological and biological activities including anti-*Helicobacter pylori*[2], anticonvulsant[3], antimicrobial[4-5], and HIV-1 inhibitor[6]. Some approved drugs have the N-phenylacetamine component in their structure including Practolol (a beta-adrenergic antagonist), Inosine pranobex (an antiviral drug), and Etidocaine (a local anesthetic drug). In this study, a series of 2-amino-N-(p-Chlorophenyl) acetamide derivatives were designed. Their antibacterial activities were examined against *Acinetobacter baumannii* ATCC19606, *Pseudomonas aeruginosa* ATCC27853, *Pseudomonas aeruginosa* ATCC29260 and *Staphylococcus aureus* ATCC6538p strains.

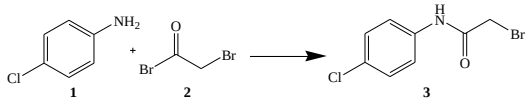
II. MATERIALS

Ethyl acetate and Dichloromethane were purchased from Duksan Pure Chemicals. *n*-Hexane and Tetrahydrofuran were purchased from Macron Fine Chemicals. Pyridine, 3-Fluoroaniline, Bromoacetyl bromide, Octylamine, Butylamine and Piperidine were purchased from Alfa Aesar Chemicals. 4-Chloroaniline was purchased from Acros Organics Chemicals. Potassium carbonate was purchased from VETEC (sigma-aldrich) Chemicals. All reagents were used as received without any further purification.

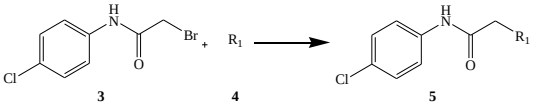
III. EXPERIMENTAL

The series of 2-amino-N-(p-Chlorophenyl) acetamide derivatives was synthesized. The 2-bromo-N-(*p*-Chlorophenyl) acetamide were condensed with various amine at room temperature in presence of CH_2Cl_2 and saturated potassium carbonate solution. The structures of resulted derivatives were confirmed by ^{1}HNMR, ^{13}CNMR and Mass spectral analysis.

Scheme 1. Synthesis of 2-bromo-N-(*p*-Chlorophenyl) acetamide (3)



Scheme 2. Synthesis of 2-amino-N-(p-Chlorophenyl) acetamide derivatives (5a ~ 5d)



	R_1		R_1
4a	$CH_3(CH_2)_3NH_2$	**5a**	$CH_3(CH_2)_3NH$
4b	$CH_3(CH_2)_7NH_2$	**5b**	$CH_3(CH_2)_7NH$
4c	$C_5H_{11}N$	**5c**	$C_5H_{10}N$
4d	3-$FC_6H_4NH_2$	**5d**	3-FC_6H_4NH

The starting material, 2-bromo-N-(p-chlorophenyl) acetamide (3), were prepared via amination reaction of 4-Chloroaniline and Bromoacetyl bromide (Scheme 1). The structure analysis of compounds 3 was confirmed by ^{1}H and ^{13}CNMR spectral analysis; the CH_2 signal of acetamide moiety appeared at □ 4.014 and 29.320 ppm, respectively. Nucleophilic substitution of compounds 3 with different substituted

amines such as butylamine, octylamine, piperidine and 3-fluoroaniline afforded in the formation of 2-amino-N-(p-Chlorophenyl) acetamide derivatives (5a ~ 5d)(Scheme 2).

A. Synthesis of 2-Bromo-N-(4-chlorophenyl) acetamide (3)

The 4-Chloroaniline (1, 5.08g, 40 mmol) and saturated K_2CO_3 (35 mL) in CH_2Cl_2 (60 mL) were stirred at ice-bath until all the compounds dissolved[7]. Then, bromoacetyl bromide (2, 4.43 mL, 50 mmol) was added dropwise over 5 min and stirred at ice-bath for 1 hour. After completion of the reaction, the CH_2Cl_2 was removed by rotary evaporator, residual liquid was extracted by ethyl acetate (60 mL × 1). The organic phase was washed subsequently with deionized-water (100 mL × 2). After being dried over anhydrous $MgSO_4$, filtered, and ethyl acetate was removed under reduced pressure by rotary evaporator to get the crude product (3). The crude product was purified by flash chromatography (n-hexane : ethyl acetate =4 : 1). The yield is 80%. GCMS (EI) m/z (relative intensity) : $[M+2]^+$ 249(25.7), M^+ 247(19.6), 168(0.6), 127(100), 113(2), 77(2.8); ^{1}HNMR (400 MHz, $CDCl_3$) : δ 4.014 (s, 2H, CH_2), 7.308 (t, J=2.0Hz, 1H, Ar-H), 7.330 (t, J=3.2Hz, 1H, Ar-H), 7.478 (t, J=2.4Hz, 1H, Ar-H), 7.501 (t, J=2.8Hz, 1H, Ar-H), 8.192(s, 1H, NH); ^{13}CNMR (100 MHz, $CDCl_3$): δ 29.320 (CH_2), 121.229 (Ar), 129.145 (Ar), 130.292 (Ar), 135.427 (Ar), 163.488(C=O).

B. General Procedure for the Synthesis of 2-amino-N-(p-Chlorophenyl) acetamide derivatives (5a ~ 5d)

The 2-Bromo-N-(4-chlorophenyl) acetamide (2.49 g, 10 mmol) and saturated K_2CO_3 (10 mL) in dichloromethane (20 mL) were stirred at room temperature until all the compounds dissolved[7]. After stirred 5 min, substituted amine (10 mmol) were added by a disposable syringe and the mixture was reacted for 3 hours to complete the reaction. Then, the dichloromethane was removed under vacuum. The mixture was added deionized-water (10 mL) and extracted with ethyl acetate (20 mL x 1), the organic layer was then washed twice with 50 mL deionized-water. After the solution was dried over anhydrous $MgSO_4$, filtered and ethyl acetate was removed by rotary evaporator to afford the crude product (5a ~5d). The crude product was purified by flash chromatography (n-hexane: ethyl acetate = 4: 1)

C. The spectra of 2-amino-N-(p-Chlorophenyl) acetamide derivatives (5a ~ 5d)

1) 2-(butylamino)-N-(4-chlorophenyl) acetamide (5a) : GCMS (EI) m/z (relative intensity): $[M+2]^+$ 242(6.6), M^+ 240(36.4), 1978(12.7), 185(3.5), 153(13.9), 140(37.8), 127(100), 111(12.4); ^{1}HNMR (400 MHz, $CDCl_3$) : δ 0.933(t, J=7.2Hz, 3H, CH_3), 1.248(m, 4H, CH_2), 2.034(s, 1H, NH), 2.657(t, J=7.2Hz, 2H, CH_2), 3.351(s, 2H, CH_2), 7.264 (t, J=2Hz, 1H, Ar-H), 7.286 (t, J=3.2Hz, 1H, Ar-H), 7.525 (t, J=2Hz, 1H, Ar-H), 7.547(t, J=3.2Hz, 1H, Ar-H), .419 (s, 1H, NH); ^{13}CNMR (100 MHz, $CDCl_3$) : δ 13.844(CH_3), 20.242(CH_2), 32.169(CH_2), 49.993(CH_2), 52.910(CH_2), 120.474 (Ar), 128.878(Ar), 128.946 (Ar), 136.266 (Ar), 170.117(C=O). The yield is 60%.

2) 2-(octylamino)-N-(4-chlorophenyl) acetamide (5b) :. GCMS (EI) m/z (relative intensity) : $[M+2]^+$ 298(18), M^+ 296(38), 197(33), 184(6), 169(9), 153(13), 128(100), 111(11); ^{1}HNMR (400 MHz, $CDCl_3$) : δ 0.868(t, J=6.4Hz, 3H, CH_3), 1.262(m, 12H, CH_2), 2.022(s, 1H, NH), 2.629(t, J=6.8Hz, 2H, CH_2), 3.331(s, 2H, CH_2), 7.247(t, J=2.0Hz, 1H, Ar-H), 7.269(t, J=2.8Hz, 1H, Ar-H), 7.514(t, J=2.0Hz, 1H, Ar-H), 7.536(t, J=2.8Hz, 1H, Ar-H), 9.426(s, 1H, NH); ^{13}CNMR (100 MHz, $CDCl_3$) : δ 13.998(CH_3), 22.553(CH_2), 27.092(CH_2), 29.176(CH_2), 29.328(CH_2), 30.002(CH_2), 31.707(CH_2), 50.235(CH_2), 52.834(CH_2), 120.429 (Ar), 128.802 (Ar), 128.863(Ar), 136.229 (Ar), 170.117(C=O). The yield is 61%.

3) 2-(piperidin-1-yl)-N-(4-chlorophenyl) acetamide (5c) : GCMS (EI) m/z (relative intensity) : $[M+2]^+$ 254(2.9), M^+ 252(8.1), 168(4.3), 154(9.2), 126(100), 111(32); ^{1}HNMR (400 MHz, $CDCl_3$) : δ 1.578(m, 6H, CH_2), 2.463(t, J=4.8Hz, 4H, CH_2), 2.996(s, 2H, CH_2), 7.194 (d, J=2.0Hz, 1H, Ar-H), 7.221 (d, J=2.0Hz, 1H, Ar-H), 7.467 (d, J=2.0Hz, 1H, Ar-H), 7.484 (d, J=2.0Hz, 1H, Ar-H), 9.69(s, 1H, NH); ^{13}CNMR (100 MHz, $CDCl_3$) : δ 23.311(CH_2), 25.986(CH_2), 54.607(CH_2), 62.435(CH_2), 120.338 (Ar), 128.643 (Ar), 136.130 (Ar), 168.760(C=O). The yield is 60%.

4) 2-(3-fluorophenylamino)-N-(4-chlorophenyl) Acetamide (5d) : GCMS (EI) m/z (relative intensity) : $[M+2]^+$ 280(28.3), M^+ 278(8.7),

166(0.13), 153(0.42), 140(0.5), 124(100), 75(5.1); ^{1}HNMR (400 MHz, $CDCl_3$) : δ 3.914(s, 2H, CH_2), 4.453(s, H, NH), 6.379 (dt, J=2.4Hz, J=10.8Hz, 1H, Ar-H), 6.449 (dd, J=2.0Hz, J=8.0Hz, 1H, Ar-H), 6.559 (td, J=2.4Hz, J=8.4Hz, 1H, Ar-H), 7.183 (q, $J_{1,2}$=8.4Hz, $J_{1,3}$=14.8Hz, 1H, Ar-H), 7.269 (m, 2H, Ar-H), 7.474 (m, 2H, Ar-H), 8.401(s, 1H, NH); ^{13}CNMR (100 MHz, $CDCl_3$) : δ 49.575(CH_2), 100.710(Ar), 106.453(Ar), 109.157(Ar), 121.100(Ar), 129.061(Ar), 129.525(C-Cl), 130.846(Ar), 135.655(Ar), 148.517(Ar), 165.169(C-F), 168.223(C=O). The yield is 58%.

^{1}HNMR and ^{13}CNMR spectra were run at 400 MHz, on a Varian Unity plus & Mercury Plus, using TMS as an internal standard in deuterated chloroform. The mass spectra were recorded on Agilent Technologies 6890N Network GC System and Agilent Technologies 5975 inert Mass selective Detector (MSD, electron energy, 70 eV).

D. Antibacterial Activity

The antibacterial activities were conducted by the disc diffusion method. All the 2-amino-N-(p-Chlorophenyl) acetamide derivatives were measured in vitro for their antibacterial activity against various microorganisms included Acinetobacter baumannii ATCC19606, Pseudomonas aeruginosa ATCC27853, Pseudomonas aeruginosa ATCC29260, and Staphylococcus aureus ATCC6538p. Each test compounds were dissolved in Ethyl acetate to get a concentration of 0.1g/mL. The disc (6 mm in diameter) was impregnated with 30 μL of each test solution and placed on cation-adjusted Mueller Hinton agar medium[8]. The plates were incubated at 37 ℃ for 12~16 hours and the inhibition zones measured in mm. Discs impregnated with Ethyl acetate were used as the negative *control* and tetracycline discs as antibacterial activity reference standard. The results were shown in Table 1.

Table 1. Antibacterial activity of the 2-amino-N-(p-Chlorophenyl) acetamide derivatives against the test microorganisms.

Compounds	Disk Inhibition Zone (DIZ, mm)			
	Ab 19606	Pa 27853	Pa 29260	Sa 6538p
5a	24.0	12.5	13.5	14.0
5b	32.0	23.5	24.5	15.0
5c	16.3	8.0	23.0	8.0
5d	20.5	8.0	8.0	23.5
Ea	8.0	8.0	8.0	8.0
Tc	33.0	24.0	22.5	35.0

Ea : Ethyl acetate, Tc : Tetracycline, Ab: *Acinetobacter baumannii*, Pa: *Pseudomonas aeruginosa*, Sa: *Staphylococcus aureus*

IV. RESULTS AND DISCUSSION

The series of 2-amino-N-(p-Chlorophenyl) acetamide derivatives (5a ~ 5d) were synthesized with moderate to good yields and the structures were confirmed by ^{1}HNMR, ^{13}CNMR and Mass spectral analysis.

To evaluate the antibacterial activities, all the compounds were assayed by the disc diffusion method against four different strains included *Acinetobacter baumannii* ATCC19606, *Pseudomonas aeruginosa* ATCC27853, *Pseudomonas aeruginosa* ATCC29260 and *Staphylococcus aureus* ATCC6538p. The results of antibacterial properties are shown in Table 1. Based on the disc inhibition assay, compound 5b showed significant antibacterial activity against the strains of *A. baumannii* ATCC19606 (DIZ=32.0 mm), *P. aeruginosa* ATCC27853 (DIZ=23.5 mm) and *P. aeruginosa* ATCC29260 (DIZ=24.5 mm), respectively. Compound 5d possessed the highest antibacterial effect, with Disk Inhibition Zone (DIZ) value 23.5 mm, against *S. aureus* ATCC6538p strain.

All the compounds exhibited moderate to high antibacterial ability against all the tested microorganism strains.

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