Synthesis and Estimation of the Insecticide and Antibacterial Activities for Some New Amide Derivatives

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Abstract: *In this work, new compounds of amide derivatives (C1-C3) were synthesized through the conversion reaction of* p*-chloroaniline to diazonium salt (B1), which reacts with aniline to form a new azo-compound (B3). Synthesized of* p*-alkoxybenzoic acid (A1-A3) and reacts with* $SOCl_2$ *to form A4-A6 compounds that react with B3 compound to form amide compounds (C1-C3). The synthesized derivatives were tested by docking analysis and characterized via FTIR,* ^{1}*H-NMR spectra. In the docking study, the interaction diagram also displays many van der Waals interactions, which are used to estimate the synthetic compounds' activity as insecticides like anti-termites. Heptyl came in first on the binding score, followed by octyl and then nonyl. Due to the compounds' modified conformation in interacting with the enzyme's binding pocket, the length of the alkyl residue of the derivative adversely impacted their binding inhibition. The synthesized compounds (C1 and C3) give a good result as anti-*E. coli *and anti-*Staphylococcus *strains.*

Keywords: *amide; azo; insecticide; bacterial*

■ INTRODUCTION

The amide's function is unarguable and of primary importance, being the constituent of natural and synthetic polymers and found in a wide variety of bioactive small molecules prepared both by nature and in the laboratory. In addition, amides have been employed as reaction partners in diverse transformations, representing the source for both the carbonyl and amine groups [1].

The amide functional group is one of the most important in organic synthesis, with a wide range of applications in different fields. For example, therapeutic peptides have demonstrated their effectiveness in pharmaceutical chemistry [2], and approximately 25% of marketed drugs and two-thirds of drug candidates contain at least one amide function. Furthermore, the use of amides in material and polymer chemistry is of great importance, such as for OLED applications, dentistry, or nanocomposites. In organic synthesis, amides are also significant, owing to their outstanding reactivity as nucleophiles [3]. A peptide bond exists between the nitrogen and carbon of the carbonyl group [4-5].

Amides are a kind of substance that has been used in a variety of industries, including pharmaceuticals, agrochemistry, and materials research. Asides have had a significant impact on the pharmaceutical industry, as evidenced by the significant number of medications having an amide component and the high fraction of amide-linking processes performed by medicinal chemists [6]. Various chemical processes use amides as catalysts, ligands, reagents, solvents, and substrates. Because of their usefulness, new amide synthesis techniques are continually being developed [7-8].

The reaction of acid chlorides with amines is the most effective and widely used procedure for the manufacture of amides. Azo dyes are chemical

compounds that include the N=N functional group. They are a commercially important class of azo compounds [9]. Azo dyes now provide a more significant part of the dye chemical industry, and their relevance is likely to grow in the coming years. They are essential in the management of the dye and printing markets. These dyes are made using a simple diazotization and coupling method. A variety of techniques and adjustments are used to achieve the appropriate color characteristics, yield, and particle size of the dye for better dispersibility. The most widely used dyes, accounting for around 60% of all dye manufacturing, are azo dyes [10]. Azo dyes account for more than 70% of all dyes used in the industry [11]. The most widely used synthetic colorants are azo dyes, which are used in textiles, printing, and paper.

Escherichia coli (*E. coli*) is one of the most common Gram-negative foodborne pathogens, which usually is used as an indicator bacterium in tests for fecal contamination of food [12]. Staphylococcal infections, commonly called staph infections, are caused by a genus of bacteria called *Staphylococcus*. The most common human pathogen is *S. aureus*. A pathogen is an organism that causes disease [13]. Insecticides are chemicals that are used to keep insects under control by killing them or stopping them from acting in undesired or damaging ways. Their structure and mode of action are used to classify them. Many pesticides work by blocking the enzyme cholinesterase in the insect's brain. Other pesticides work as growth regulators or endotoxins [14].

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were *p*-chloroaniline (C_6H_6ClN), ethanol (C_2H_6O), aniline (C_6H_7N), thionyl chloride ($SOCl_2$), 4-hydroxybenzoic acid ($C_7H_6O_3$, 99% purity, Sigma Aldrich, USA), potassium carbonate (K_2CO_3), 1-heptylbromide ($CH_3(CH_2)_6Br$), 1-octylbromide ($CH_3(CH_2)_7Br$), nonylbromide ($CH_3(CH_2)_8Br$, 97% purity Merck, Germany), high-quality absolute ethanol and dimethylformamide (DMF, 99% purity Merck, Germany).

Instrumentation

The instrumentations used in this study were Fourier-transform infrared (FTIR) spectroscopy (ALPHA II, Bruker, Germany), and ^{1}H-NMR hydrogen nuclear magnetic resonance (NMR 400 MHz, ACF 400, Bruker, Germany).

Procedure

General procedure for the synthesis of azo compound (B3) [15]

In an ice water bath, add 6 mL of 10% HCl solution to *p*-chloroaniline (0.01 mol) with 3 drops of HCl in a test tube. The test tube contained 0.02 mol (1.38 g) of $NaNO_2$ in 7 mL of distilled water. In the ice bath (5 °C), the first solution was added to another solution in the test tube. A 0.01 mol of aniline solution in 10 mL of 10% NaOH was added in test tube No.3. Then, in test tube No.3, add the solution mixture. Finally, the final product was filtered, and the precipitate was collected, which led to the necessary products being made. The melting point of B3 was 204–209 °C and has yellow color.

General procedure for the synthesis of p*-alkoxybenzoic acid compounds (A1-A3) [16]*

Dissolved 15 mL of ethanol with 0.01 mol (1.38 g) 4-hydroxybenzoic acid and alkyl bromide (R: heptyl, octyl, and nonyl) and refluxed for 24 h. The solution was added to 0.02 mol (1.2 g) of K_2CO_3, diluted in a small quantity of water (around 5 mL), and heated for 1–3 h. The solvent was evaporated, and an equivalent volume of water was added, followed by heating the solution until it became pure. Acidification with HCl resulted in the formation of a solid precipitate.

Synthesis of 4-alkoxybenzoyl chloride (A4-A6) [17]

For 3–4 h, a mixture of each 0.01 mol of 4-alkoxybenzoic acid (A1–A3) and 15 mL of $SOCl_2$ was refluxed with a few drops of DMF as solvent. After evaporating the excess $SOCl_2$, the result is a dark brown precipitate of 4-alkoxybenzoyl chloride. Finally, the acid chloride residue was utilized straight for the further reaction without purification.

Synthesis of N*-(4-(4-Chloro-phenylazo)-phenyl)-4-alkoxy-benzamide compounds (C1-C3) [18]*

The compound (A4-A6) 0.001 mol was dissolved in 15 mL of pyridine and added by 0.001 mol of B3. The solution was stirred at 25 °C for 24 h. Following that, the

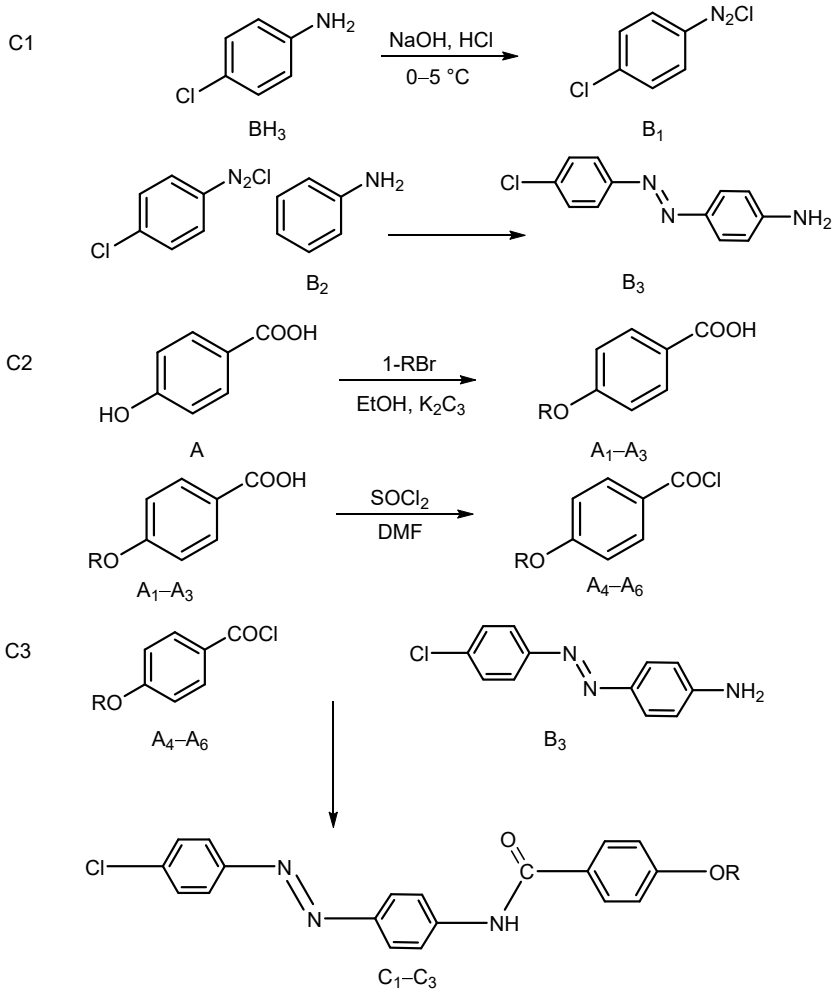
mixtures were put into ice cubes, filtered, and rinsed with distilled water.

Docking analysis

Acetylcholinesterase (AChEcrystallographic)'s 3D structure was obtained from the protein data bank (PDB code: 6xyu). Using Swiss Protein Viewer, the enzyme structure was subjected to energy reduction (version 4.1). Following a series of preparation procedures, the co-crystallized ligands and water molecules were taken out, the structure was adjusted, and polar hydrogens were added. ChemDraw extreme (version 18.0) was used to draw out the synthesized compounds, and the sdf files were then saved and transformed using Open Babel software to PDB files. Using Autodock Tools (version 1.5.6) [19] and a pdbqt file format, the docking operation was carried out after energy reduction for both the enzyme and the receptor was complete. Autodock Vina was used to perform molecular docking along with Auto Grid software with a grid box of size (30 × 30 × 30) and a grid center (39.787 * 53.325 * 5.243), which represent the x, y, and z dimensions, respectively. The docking parameters were set to default values, and ten conformations were generated. Discovery Studio [20] is used for generating visualization images.

■ RESULTS AND DISCUSSION

4-Alkoxybenzoic acid (A1-A3) was synthesized via the reaction of 4-hydroxybenzoic acid with alkyl bromide (1-bromoheptane, 1-bromooctane, and 1-bromononane). From Scheme 1, an azo dye can only be



Scheme 1. Routs for the synthesized compounds C1–C3

manufactured using two organic molecules, a coupling component and a diazonium salt. The diazonium salt interacts as an electrophile with an electron-rich coupling element, such as aniline, through an electrophilic aromatic substitution process. The amine group is positioned at para to the aryl diazonium ion. The described compounds were synthesized through the condensation reaction between previously produced compounds (B3) and 4-alkoxybenzoyl chloride (A4-A6). The physicochemical properties of the synthesized compounds are listed in Table 1.

Insecticide activity and the efficacy of compounds C1, C2, and C3 as pesticides against *Aphidoidea* insects were studied in the Department of Plant Protection (Al-Muthanna Agriculture Directorate) in Al Muthanna Governorate. The concentrations of 3000, 4000, and 5000 ppm of the prepared compounds C1, C2, and C3, which have been dissolved with paraffin. The synthesized compounds were used as pesticides by spraying the solution on the insect using the spray method. The synthesized compounds that were used as pesticides in the application gave positive results by killing the insects at the different concentrations used. The efficacy of compounds C1, C2, and C3 as pesticides against *Aphidoidea* insects. The synthesized compounds used as pesticides in the application gave positive results by killing the insects at different concentrations at different times, as shown in Table 2.

The compounds synthesized were evaluation evaluated against various types of Gram-positive and Gram-negative bacteria. All compounds give good results by inhibiting the zone growth of each bacteria used, as shown in Table 3.

Fig. S1 explained FTIR for compound A2, the broadband at 3423 cm^{-1} for OH group, 3116 and 3084 cm^{-1} for C–H aromatic, 2977 and 2831 cm^{-1} for C–H aliphatic, 1729 cm^{-1} detected for C=O and 1614 cm^{-1}

Table 1. Physicochemical properties of synthesized compounds B3, C1–C3

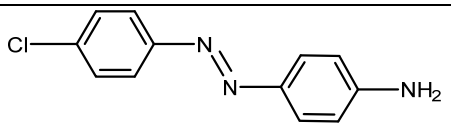
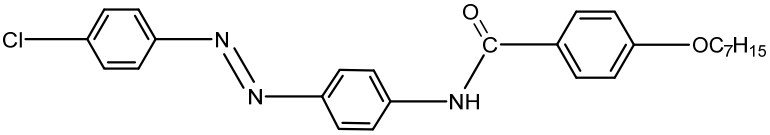
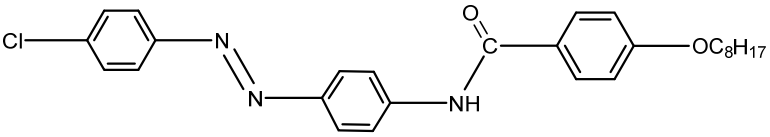
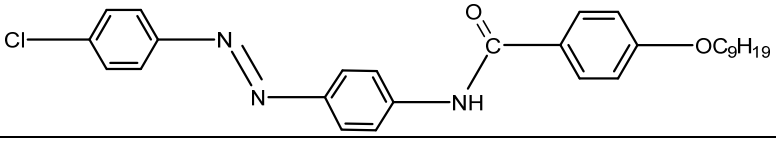
Compound No.	Structure	Yield %	M.p °C	Color
B3		76	204–209	Yellow
C1		72	183–188	Brown
C2		74	171–175	Dark Brown
C3		78	158–162	Orange

Table 2. Anti-insect activities of the compounds C1–C3

Compound No.	Time (min) to kill Aphidoidea insects		
	3000	4000	5000
C1	3.00	2.00	1.75
C2	4.00	3.00	2.75
C3	4.00	3.50	2.25

Table 3. Antibacterial activities of the compounds C1–C3

Bacteria name	Zone of inhibition (mm)	
	C1	C3
Staphylococcus	+	++
Escherichia coli	++	+

Note: (+) less than 13 mm, and (++) more or equal to 13

for C=C aromatic [21]. Fig. S2 explained FTIR for compound A3, the broadband at 3443 cm^{-1} for the OH group, 3033 cm^{-1} for C–H aromatic, 2968 and 2866 cm^{-1} for C–H aliphatic, 1716 cm^{-1} detected for the C=O and 1601 cm^{-1} for C=C aromatic [21]. The FTIR spectrum of compound B3 in Fig. S5 showed the two bands 3543 and 3406 cm^{-1} (NH_2), 3077 cm^{-1} (C–H aromatic stretching), 1614 cm^{-1} (C=C), 1578 cm^{-1} (N=N), 1112 cm^{-1} (C–N bending), 716 cm^{-1} (C–Cl). The FTIR spectrum of compound C2 in Fig. S7 showed 1251 cm^{-1} (C–O), 3084 and 3116 cm^{-1} detected for C–H of stretching aromatic rings, the peaks at 2977 cm^{-1} returned to C–H aliphatic stretching, 1711 cm^{-1} detected for carbonyl of amide, the wide band 3421 cm^{-1} for N–H group, 1413–1530 cm^{-1} (C=C aromatic stretching) [21]. The FTIR spectrum of compound C3 in Fig. S8 showed the bands 3569 cm^{-1} (N–H), 2983 cm^{-1} (C–H aliphatic stretching), 1666 cm^{-1} (C=O), 1582 cm^{-1} (N=N), 1270 cm^{-1} (C–O), 1121 cm^{-1} (C–N), 630 cm^{-1} (C–Cl).

^{1}H-NMR (DMSO-d_6 500 MHz) spectra of the compounds A1 and A3 are shown in Fig. S3 and S4, δ 10.9 (s, 1H) and 11.5 (s, 1H) ppm detected for the proton of acid group (OH), δ 7.8–7.2 (m, 2H) ppm for aromatic protons, δ 4.1 ppm for OCH_2 protons, δ 1.7–1.2 (m, 6H) ppm for CH_2 protons, and δ 0.90 and 0.92 (m, 2H) ppm for methyl protons [22]. The ^{1}H-NMR spectrum of compound B3 is shown in Fig. S6 (DMSO-d_6 500 MHz): δ 7.8–7.0 (m, 4H) ppm for aromatic protons and δ 11.8 (s, 1H) ppm for amino protons. The ^{1}H-NMR spectrum of compound C1 is shown in Fig. S9, δ 7.8–6.9 (m, 7H) ppm detected for aromatic protons, δ 3.9 (m, 1H) ppm for OCH_2 protons, δ 3.2–1.2 (m, 7H) ppm for $(CH_2)_5$ protons, δ 0.8 (m, 2H) ppm for methyl protons, and the peak at δ 11.1 (s, 1H) detected for NH. The ^{1}H-NMR spectrum of compound C2 is shown in Fig. S10, δ 7.9–6.9 (m, 7H) ppm detected for aromatic protons, δ 4.0 (m, 1H) ppm for OCH_2 protons, δ 1.20–1.27 (m, 9H) for $(CH_2)_6$ protons, δ 0.8 (m, 2H) ppm for methyl protons, and the peak at δ 11.1 (s, 1H) ppm detected for NH [22].

In docking analysis, the docking simulations were done to explore the possible mechanistic inhibition of AChE. The binding scores of the synthesized compounds are ranked according to their binding affinities. The results show that heptyl derivatives have the best binding scores compared to others. Fig. S11 demonstrates the interactions between the target enzyme and an octyl derivative. The most noticeable interaction was a formed halogen bonding between ASP437 and the chloro of the compound. At the same time, the straight alkyl residue of a heptyl derivative forms an alkyl interaction. The interaction diagram also shows multiple van der Waals interactions. The ranking order of the binding score was heptyl > octyl > nonyl. The length of the alkyl residue of the derivative negatively affected their binding inhibition, which could be attributed to changing the conformation of the compounds to interact with the binding pocket of the enzyme. Fig. S12 and S13 show the binding modes for octyl and nonyl, respectively [23].

■ CONCLUSION

In conclusion, three new amide derivatives, C1-C3, have been synthesized. Furthermore, the newly synthesized amide compounds, C1 and C3, act as bacterial inhibitors, including *S. aureus*, and *E. coli bacteria*. Finally, the first quarter was critical as an antidote to earthworms. The heptyl group was ranked first, followed by octyl, and the nonyl group in the structures of final synthesized compounds because the length of the derivative's alkyl residue had a detrimental impact on their binding inhibition by an effect on the AChE enzyme. The synthesized compounds give an excellent result as anti-bacteria.

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■ SUPPORTING INFORMATION

Amide compounds as organic molecules that are synthesized and characterized by using FTIR and ^{1}H-NMR in addition to using docking analysis.

■ AUTHOR CONTRIBUTIONS

Dina Saleem and Zinah Hussein Ali conducted the experiment, Mustafa Sabri Cheyad and Baneen Salam

Rasool conducted the FTIR and ^{1}H-NMR spectroscopy and molecular docking analysis, and Abbas Khudhair Abbas wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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