Research Article

Confirmed Mechanism for 1,8-Diaminonaphthalene and Ethyl Aroylpyrovate Derivatives Reaction, DFT/B3LYP, and Antimicrobial Activity of the Products

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Received 2 May 2018; Accepted 10 September 2018; Published 10 December 2018

Academic Editor: Xinyong Liu

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Two new series of perimidine derivatives were prepared from the reaction of 1,8-diaminonaphthalene with ethyl aroylpyrovate followed by coupling for the products. The structures, the mechanism, and the tautomeric forms of the products have been discussed on the basis of spectral data aided with the computational study. Applying Gaussian09 software, the reaction mechanism was assured. The energy contents were computed after optimization for all proposed structures. The likely extracted compound has a reduced heat during formation and internal energy which coincides with experimental outcomes. The antimicrobial activity of all products was screened, and their results indicated the presence of five derivatives more potent than the reference drugs used. The simulation procedure was executed by Autodock 4.2 tools over the expected compounds yielded (**12** and **21**). This computational technique asserts on the drug behavior inside the causative organism proteins. The organisms here match with that used in the antimicrobial and antifungal study.

1. Introduction

It was reported that the reaction of 1,2-diaminoaromatic compound **1** with 1,2-dicarbonyl compounds **2** or **3** afforded the quinoxaline or pyrazin-2(1*H*)-one derivatives **4** and **5** [1, 2]. However, condensation of 1,8-diaminonaphthalene with benzil **2** or alkyl carboxylate derivative of 3-furanone **7** furnished the perimidine derivatives **8** and **9**, respectively [3–5] (Schemes 1 and 2). Perimidine ring (peri-naphthofused pyrimidine systems) is a heterocycle containing two nitrogen atoms which is the unusual system with an excess and deficiency of *p*-electrons simultaneously [6, 7]. This feature makes them susceptible to electrophilic [8, 9] and nucleophilic attack [9]. The biological activities of perimidines have attracted our attention due to their wide range of application, for example, antitumor, antimicrobial, antifungal, and antiulcer [10–12]. Also, some of them were applied as coupler substances for preparation of oxidation dyestuffs, hair colouring compositions [13, 14], and fluorescent molecular sensors [15].

On the other side, ethyl aroylpyrovates **10** contain three different carbonyl groups with a different polarity and reactivity which condensed with various reagents to afford new heterocyclic compounds [16, 17]. It was proven that the most reactive carbonyl is that adjacent to the ester group [1, 18] (Scheme 3).

From all the previous screening, the author was interested herein to investigate the reactivity of ethyl aroylpyrovate **10** in the reaction with 1,8-diaminonaphthalene and elucidate the product or products of such reaction. This due to the previous reaction can afford one or more of the seven products which will be illustrated (Scheme 4). Moreover, Gaussian09 computation software was considered to establish a well view about the reaction mechanism [19]. DFT/B3LYP is a suitable method for executing the optimization process [20] in the gas phase and ethanol solvent for comparative assertion. Essential parameters have been considered as heat during formation, frontier energy gap, dipole moment, and oscillator strength. Such parameters were intended due to their convinced impact in

SCHEME 1: Reaction of 1,2-diaminoaromatic compound **1** with dicarbonyl compounds **2** and **3** [1, 2].

SCHEME 2: Condensation of 1,8-diaminonaphthalene with benzyl **2** or alkyl carboxylate derivative of 3-furanone **7** [3–5].

stability differentiation between all possible outputs [21]. As well as investigating the antimicrobial activity for novel synthesized derivatives was also intended. The simulation procedure for antimicrobial behavior was also considered to strengthen the results.

2. Experimental

2.1. Physical Techniques. All melting points were uncorrected and measured on the Gallenkamp electric melting point apparatus (capillary method, Gallenkamp Co., London, UK). Infrared spectra were determined on a Mattson 5000 FT-IR spectrometer (Shimadzu Co., Kyoto, Japan, not all frequencies are reported) using KBr discs. The nuclear magnetic resonance NMR spectra were determined using a WP 300 spectrometer (Bruker Co., Billerica, MA, USA) at 300 MHz for ^1H or 75.5 MHz for ^{13}C using TMS as the internal standard. The mass spectra were recorded on a Qp-2010 mass spectrometer (Shimadzu, Tokyo, Japan) at 70 eV (EI mode). Elemental analyses (C, H, and N) were

determined on the PerkinElmer 2400 analyzer. The reactions were pursued by TLC (aluminum sheets, Merck), and the spots were disclosed by exposure to the UV lamp.

2.2. Synthesis of Ethyl 2-(2-Oxo-2-arylethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (12a–e). In ethanolic solution, a mixture of 1,8-diaminonaphthalene **6** (0.40 g, 2.5 mmol) and ethyl aroylpyrovate **10** (2.5 mmol) was refluxed for 5 hrs. The reaction mixture was allowed to cool at room temperature, and then, the solid formed was collected by filtration, dried, and recrystallized from ethanol.

2.2.1. Ethyl 2-(2-Oxo-2-phenylethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (12a). Dark red solid (yield, 70%), m.p. = 190–192°C. IR (KBr) ν_{max} 3435, 3317 (NH), 1725, 1628 (2C=O). ^1H NMR (DMSO- d_6) δ 1.33 (t, J = 7.2 Hz, 3H, CH_3), 3.89 (s, 2H, CH_2), 4.32 (q, J = 7.2 Hz, 2H, CH_2), 6.60–8.08 (m, 11H, Ar-H), 10.80 (brs, 2H, 2NH); MS (EI): m/z (%): 360 (M^+ , 20), 347 (25), 256 (40), 170 (20), and 92 (100). Analysis for $\text{C}_{22}\text{H}_{20}\text{N}_2\text{O}_3$ (360.41): calcd: C, 73.32; H, 5.59; and N, 7.77%. Found: C, 73.15; H, 5.39; and N, 7.60%.

2.2.2. Ethyl 2-(2-Oxo-2-(p-tolyl)ethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (12b). Dark red solid (yield, 74%), m.p. = 172–174°C. IR (KBr) ν_{max} 3443, 3316 (NH), 1717, 1610 (2C=O). ^1H NMR (DMSO- d_6) δ 1.33 (t, J = 7.0 Hz, 3H, CH_3), 2.37 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 4.32 (q, J = 7.0 Hz, 2H, CH_2), 6.60–7.92 (m, 10H, Ar-H), 10.89 (brs, 2H, 2NH); MS (EI): m/z (%): 374 (M^+ , 10), 350 (60), 344 (100), 336 (63), 328 (38), 313 (52), 285 (28), 275 (25), 241 (18), and 134 (12). Analysis for $\text{C}_{23}\text{H}_{22}\text{N}_2\text{O}_3$ (374.44): calcd: C, 73.78; H, 5.92; and N, 7.48%. Found: C, 73.59; H, 5.81; and N, 7.26%.

2.2.3. Ethyl 2-(2-(4-Chlorophenyl)-2-oxoethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (12c). Dark red solid (yield, 68%), m.p. = 180–182°C. IR (KBr) ν_{max} 3434, 3316 (NH), 1716, 1627 (2C=O). ^1H NMR (DMSO- d_6) δ 1.32 (t, J = 7.0 Hz, 3H, CH_3), 3.86 (s, 2H, CH_2), 4.33 (q, J = 7.0 Hz, 2H, CH_2), 6.49–7.98 (m, 10H, Ar-H), 10.82 (brs, 2H, 2NH); MS (EI): m/z (%): 396 (M^+ +2, 10), 395 (M^+ +1, 11), 394 (M^+ , 25), 361 (26), 347 (28), 319 (52), 277 (75), 257 (100), 111 (14), 105 (54), and 91 (32). Analysis for $\text{C}_{22}\text{H}_{19}\text{ClN}_2\text{O}_3$ (394.85): calcd: C, 66.92; H, 4.85; and N, 7.09%. Found: C, 66.78; H, 4.74; and N, 6.89%.

2.2.4. Ethyl 2-(2-(4-Fluorophenyl)-2-oxoethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (12d). Dark red solid (yield, 73%), m.p. = 168–170°C. IR (KBr) ν_{max} 3429 (br. NH), 1671, 1610 (2C=O). ^1H NMR (DMSO- d_6) δ 1.22 (t, J = 7.0 Hz, 3H, CH_3), 3.13 (s, 2H, CH_2), 3.67 (q, J = 7.0 Hz, 2H, CH_2), 6.29–8.04 (m, 10H, Ar-H), 10.50 (brs, 2H, 2NH); ^{13}C NMR (DMSO- d_6) δ 11.91 (CH_3), 35.4 (CH_2), 42.69 (CH_2), 100.42 (C2), [113.85, 115.26, 115.55, 118.25, 119.33, 120.57, 122.49, 127.63, 128.41, 130.42, 133.46, 135.16, 148.63, 149.05 (Ar-C)], 163.20 (C=O), 196.23 (C=O). MS (EI): m/z (%): 378 (M^+ , 12), 377 (M^+ -1, 3.4), 367 (28), 360 (22), 348 (6), 336

SCHEME 3: The X-ray analyses which prove the reactivity of carbonyl group-2 of ethyl aroylpyrovate **10** [1, 18].

SCHEME 4: Reaction of 1,8-diaminonaphthalene **6** with ethyl aroylpyrovate derivatives **10a-e**.

(27), 332 (12), 304 (14), 168 (2.4), 156 (1.1), 122 (2.3), 93 (27), 92 (23), and 77 (45). Analysis for $C_{22}H_{19}FN_2O_3$ (378.40): calcd: C, 69.83; H, 5.06; and N, 7.40%. Found: C, 69.61; H, 4.94; and N, 7.52%.

2.2.5. Ethyl 2-(2-(4-Bromophenyl)-2-oxoethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (12e). Dark red solid (yield, 75%), m.p. = 175–176°C. IR (KBr) $\nu_{\max}$ 3442, 3318 (NH), 1717, 1627 (2C=O). 1H NMR (DMSO- d_6) δ 1.33 (t, J = 7.0 Hz, 3H, CH_3), 3.87 (s, 2H, CH_2), 4.34 (q, J = 7.0 Hz, 2H, CH_2), 6.47–7.99 (m, 10H, Ar-H), 10.91 (brs, 2H, 2NH); MS (EI): m/z (%): 441 (M^+ +2, 1.8), 440 (M^+ +1, 12), 439 (M^+ , 3), 424 (34), 412 (23), 392 (14), 381 (100), 380 (23), and 154 (1.1).

Analysis for $C_{22}H_{19}BrN_2O_3$ (439.31): calcd: C, 60.15; H, 4.36; and N, 6.38%. Found: C, 60.03; H, 4.27; and N, 6.19%.

2.3. Coupling Reaction of Compounds 12a-e to Synthesize Perimidine Derivatives 21a-e. To a stirred solution of ethyl 2-(2-aryl-2-oxoethyl)-2,3-dihydro-1H-perimidine-2-carboxylate derivatives **12a-e** (1 mmole) in ethanol, sodium hydroxide solution (0.04 g of NaOH in 15 mL ethanol with two drops of water), was added and the reaction mixture was cooled in an ice bath to 0–5°C. To the resulting solution, while being stirred, was added dropwise over a period of 10 min a solution of aniline diazonium chloride, prepared as usual by diazotizing aniline (1 mmole) in

hydrochloric acid (6 M, 1 mL) with sodium nitrite (1 M, 1 mL). The whole mixture was then left in a refrigerator overnight. The precipitated solid was filtered, washed with water, and finally crystallized from ethanol/dioxane to give the respective hydrazone derivatives **21a–e**.

2.3.1. 2-[N-Phenyl-2-oxo-2-phenyl-ethanehydrazonoyl]-1H-perimidine (21a). Dark brown solid (yield 76%), m.p. >300°C [22], IR ν : br 3427 (2NH), 1623 (CO) cm^{-1} , ^1H NMR (DMSO- d_6) δ : 6.72–7.80 (*m*, 16H, Ar-H), 13.62 (*s*, 2H, 2NH); MS *m/z* (%) 390 (M^+ , 5), 314 (M^+ -Ph, 35), 298 (M^+ -PhNH, 23), 285 (M^+ -PhCO, 6), 167 ($\text{C}_{11}\text{H}_7\text{N}_2$, 5), 139 (12), 127 (5), 105 (5), 93 (5), and 84 (12). Analysis for $\text{C}_{25}\text{H}_{18}\text{N}_4\text{O}$ (390.45): calcd: C, 76.91; H, 4.65; and N, 14.35%. Found: C, 76.74; H, 4.46; and N, 14.09%.

2.3.2. 2-[N-Phenyl-2-oxo-2-(4-methyl)phenyl-ethanehydrazonoyl]-1H-perimidine (21b). Dark brown solid (yield 69%), m.p. = 162–164°C, ^1H NMR (DMSO- d_6) δ : 2.28 (*s*, 3H, CH_3), 7.40–8.35 (*m*, 15H, Ar-H), 10.90 (*s*, 2H, 2NH); MS *m/z* (%) 404 (M^+ , 15), 403 (M^+ -1, 21), 388 (M^+ - CH_3 , 39), 330 (M^+ - $\text{C}_3\text{H}_5\text{O}_2$, 74), 310 (43), 298 (100), 266 (24), 165 (6), 148 (5), 121 (13), 118 (12), and 91 (4). Analysis for $\text{C}_{26}\text{H}_{20}\text{N}_4\text{O}$ (404.47): calcd: C, 77.21; H, 4.98; and N, 13.85%. Found: C, 76.94; H, 4.76; and N, 13.66%.

2.3.3. 3-[N-Phenyl-2-oxo-2-(4-chloro)phenyl-ethanehydrazonoyl]-1H-perimidine (21c). Dark brown solid (yield 68%), m.p. = 150–152°C, IR ν : 3386, 3184 (2NH), 1644 (CO) cm^{-1} , ^1H NMR (DMSO- d_6) δ : 7.05–8.39 (*m*, 15H, Ar-H), 13.45 (*s*, 2H, 2NH); MS *m/z* (%) 424 (M^+ , 10), 387 (21), 369 (100), 336 (65), 313 (32), 308 (45), 299 (63), 284 (64), 111 (7), 87 (22), and 71 (33). Analysis for $\text{C}_{25}\text{H}_{17}\text{ClN}_4\text{O}$ (424.89): calcd: C, 70.67; H, 4.03; and N, 13.19%. Found: C, 70.54; H, 3.89; and N, 13.36%.

2.3.4. 2-[N-Phenyl-2-oxo-2-(4-fluoro)phenyl-ethanehydrazonoyl]-1H-perimidine (21d). Dark brown solid (yield 70%), m.p. = 157–159°C, IR ν : br 3436 (2NH), 1630 (CO) cm^{-1} , ^1H NMR (DMSO- d_6) δ : 6.80–8.37 (*m*, 15H, Ar-H), 12.60 (*s*, 2H, 2NH); MS *m/z* (%) 408 (M^+ , 22), 371 (62), 305 (24), 269 (17), 252 (12), 169 (8), 165 (6), 134 (12), 106 (11), 95 (2), 84 (33), and 65 (100). Analysis for $\text{C}_{25}\text{H}_{17}\text{FN}_4\text{O}$ (408.44): calcd: C, 73.52; H, 4.20; and N, 13.72%. Found: C, 73.33; H, 4.05; and N, 13.65%.

2.3.5. 2-[N-Phenyl-2-oxo-2-(4-bromo)phenyl-ethanehydrazonoyl]-1H-perimidine (21e). Dark brown solid (yield 63%), m.p. >300°C, IR ν : 3375, 3225 (2NH), 1620 (CO) cm^{-1} , ^1H NMR (DMSO- d_6) δ : 7.27–8.10 (*m*, 15H, Ar-H), 10.91 (*s*, 1H, 1NH), 12.95 (*s*, 1H, NH); MS *m/z* (%) 470 (M^+ +1, 12), 468 (M^+ -1, 7), 450 (16), 426 (22), 384 (59), 365 (59), 155 (2.3), 76 (6), and 65 (13). Analysis for $\text{C}_{25}\text{H}_{17}\text{BrN}_4\text{O}$ (469.34): calcd: C, 63.98; H, 3.65; and N, 11.94%. Found: C, 63.78; H, 3.56; and N, 11.69%.

2.4. Theoretical Implementation

2.4.1. Geometrical Optimization. Gaussian09 program [19] is software used to investigate all the proposed structures **12–18** and **21** (Ar = Ph). DFT/B3LYP method is a suitable method used to investigate the physicochemical properties of proposed tautomeric forms. 3-21G is the base set used to accomplish the comparative investigation. Essential files yielded from this implementation (log and chk files). These files were visualized over the program screen to extract important parameters. HOMO and LUMO images were also obtained. Other additive energies according to optimized structures applying HyperChem 8.1 program were computed. Molecular mechanics (MM⁺) method was followed by semiempirical AM1 one to extract log files. All parameters were obtained after reaching to the equilibrium state in the gas phase.

2.4.2. Molecular Docking. Utilization for AutoDock tools 4.2 was to execute the docking process between the proposed drug and protein receptors of variable bacteria and fungi. *Aspergillus niger* (1ukc), *Bacillus subtilis* (1ydo), *Escherichia coli* (5o22), *Geotrichum candidum* (1thg), *Klebsiella pneumoniae* (3gdz), *Pseudomonas aeruginosa* (2w7q), *Salmonella typhimurium* (1af7), *Staphylococcus aureus* (1bqb), *Staphylococcus epidermidis* (3kp3), and *Streptococcus pyogenes* (2esr) were the microorganism protein receptors used in this investigation. Such process was accomplished after the addition of Gasteiger charges over all compound atoms. The nonpolar atoms of hydrogen were incorporated to effect on the multiplication of rotatable bonds. The presence of hydrogen atoms with Kollman unite charge type is an essential preliminary feature [23]. The affinity maps by $\times\times$ Å grid points with 0.375 Å spacing were adapted using the Autogrid program [24]. Electrostatic terms and Vander Waals force were evaluated after adapting the Autodock parameter sets and the dielectric function of distance dependent, respectively. The docking simulation process to be the completed Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method must be used [25]. The torsion of the investigated compound and occasionally the initial position as well as the orientation must be set. The rotatable torsions must be released completely through the docking process. Each docking process was accomplished through tens following runs and adjusted to be block after reaching 250000 energy considerations. Throughout the process, the size was settled to be 150, a translational step of 0.2 Å, and quaternion and torsion steps were of 5.

3. Results and Discussion

3.1. Chemistry. Scheme 4 illustrates all possible products **12–18** from the reaction of 1,8-diaminonaphthalene **6** and ethyl aroylpyrovate **10** in refluxing ethanol. Such possibilities were tested using Gaussian 09 software to optimize their structural forms (Figure 1) and computed essential parameters. These parameters are significant in asserting the reaction mechanism built on experimental aspects. The TLC experiment and the spectral data (^1H , ^{13}C NMR, mass, and IR) of the products indicate the isolation of only one product

FIGURE 1: Continued.

FIGURE 1: The optimized geometries of all suggested derivatives (a–g) **12–18** and (h–j) **21A–C**.

in each case for such reaction. Their mass spectra show the molecular ion peak corresponding to elimination of only one water molecule which matches strongly with both structures **12** or **13**. Structure **13** was ruled out based on the data of IR and ^{13}C NMR spectra. This is due to the presence of two absorption bands for the two carbonyl groups characteristic for ester and aryl groups at $1725\text{--}1716$ and $1628\text{--}1610\text{ cm}^{-1}$. The low value of the second carbonyl group can be attributed to the conjugation with aryl moiety and the hydrogen bond with the NH group. Moreover, all ^1H NMR spectra of the products **12a–e** revealed the presence of triplet and quartet signals for CH_3CH_2 integral ester groups near 1.33 and 4.32 ppm. As well as, the singlet signal for the CH_2 group was found near 3.87 ppm (see Experimental). Another evidence for the structures **12a–e** is the value of C2 concerning to the perimidine ring in the ^{13}C NMR spectrum of compound **12d** (as an example for series prepared). Such was appeared at down field ($\delta = 100.42\text{ ppm}$) due to the presence of electron withdrawing ester group at the same carbon. However, the C2 in structure **13** appeared at the up-field value near $\delta = 81.8\text{ ppm}$ [5]. All previous concerns were assured from optimizing characteristic parameters executed from the DFT/B3LYP method (Table 1). 3-21G base set leads to produce best distribution for atomic contents of each compound in the gas phase and ethanol. The reduced formation energy values for compounds **12** and **13** point to the truth of the oriented mechanism proposed. The excellence for the formation energy value of compound **12** propose its priority as the only one product yielded from the condensation reaction. The high value of dipole moment of such compound (**12**) is suitable for the high difference in electronegativity inside the compound. Moreover, the reduced oscillator strength of such compound is suitable for facilitated electronic transition inside the compound (**12**) which coincides with its reactivity. This proposal was also confirmed by applying HyperChem 8.1 program which provides data (Table 2) and confirms the previous aspects. The reduced content energies (total energy, binding energy, and heat during formation) of compound **12** in comparing with others (**13–18**) confirm its high stability. This leads to its prior in production without conflict.

In continuation, the reactivity of the active methylene group in ethyl 2-(2-aryl-2-oxoethyl)-2,3-dihydro-1H-perimidine-2-carboxylate (**12a–e**) toward the coupling reaction with diazotized aniline in ethanol in the presence of

NaOH has been investigated as shown in Scheme 5. Mass, IR, ^1H NMR, and elemental analyses confirmed the chemical structure of coupling products as the structure **21a–e**. The disappearance of the carbonyl absorption band of the ester group in all IR spectra of all derivatives (**21a–e**) proved that the coupling reaction proceeds via the *Japp-Klingemann* reaction [26] with elimination of the ethyl format molecule (Scheme 5). The actual tautomeric structure of the coupling products **21A–C** was studied and confirmed based on computational data extracted. The minimized internal energies (total energy and binding energy) beside the formation energy (Tables 1 and 2) of **21** tautomers suggest their high stability which outweigh their production from the coupling reaction and indicate that the most stable tautomer is **21A**. The HOMO and LUMO levels (Figures 2 and 3) were extracted over the optimized structures for comparison. The HOMO level appeared is very concentrated in the compounds over 1,8-diaminonaphthalene moiety of the perimidine ring in **21**. However, the LUMO level appeared is concentrated in the Ar-CO moiety which considered a good receptor for electrons as appeared in the coupling reaction. The two levels have the same positions in the coupling products (**21a–e**) which verify the same electron density attitude after reaction.

3.2. Biological Activity. The agar diffusion technique was used in screening the antimicrobial activity of all derivatives **12a–e** and **21a–e** [27] against two fungi, namely, *A. Niger* and *G. candidum* in comparison with *Amphotericin B* as a reference drug. Four species of each of Gram-positive and Gram-negative bacteria were tested against standard antibacterial agents as *ampicillin* and *gentamicin*, respectively (Tables 3 and 4). A suspension solution of the organisms was added to the sterile nutrient agar media at 45°C , and the mixture was transferred to sterile Petri dishes and allowed to solidify. Holes of 6 mm in diameter were made using a cork borer. A solution of each perimidines **12a–e** or **21a–e** or the reference drugs (5 mg/mL) in dimethylsulfoxide (DMSO) was prepared. The amount tested of either the synthesized compounds or reference drugs was $100\text{ }\mu\text{L}$. Dimethylsulfoxide (DMSO) was used as a negative control. The plates were left for 1 h at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time between the applications of different solutions.

TABLE 1: DFT/B3LYP (3-21G) parameters over all proposed tautomers (12-18 and 21A-C).

The indexes	Compounds proposed									
	12	13	14	15	16	17	18	21A	21B	21C
DFT (in the gas phase)										
E (A.U.)	-1179.0538	-1179.0176	-1024.8445	-1024.8229	-1024.7594	-1102.9729	-1102.8973	-1250.7728	-1250.7425	-1250.7516
HOMO (ev)	-0.16943	-0.17047	-0.18788	-0.20387	-0.20432	-0.20184	-0.19332	-0.16835	-0.18688	-0.17179
LUMO (ev)	-0.06024	-0.04643	-0.07797	-0.07446	-0.07475	-0.07489	-0.07923	-0.08974	-0.05425	-0.08767
ΔE_{L-H} (ev)	0.10919	0.12404	0.10991	0.12941	0.12957	0.12695	0.11409	0.07861	0.13263	0.08412
Dipole moment (debye)	5.0333	2.9408	2.4273	2.3373	6.1572	3.3955	4.8856	6.4436	3.3095	3.0171
Oscillator strength	0.0008	0.0001	0.0312	0.0250	0.1241	0.0032	0.0250	0.0009	0.0027	0.0190
DFT (in ethanol)										
E (A.U.)	-1179.0712	-1179.0348	-1024.8601	-1024.8385	-1024.7798	-1102.9871	-1102.9147	-1250.7924	-1250.7603	-1250.7632
HOMO (ev)	-0.18124	-0.18105	-0.19124	-0.20708	-0.20530	-0.20738	-0.20142	-0.18347	-0.19622	-0.18512
LUMO (ev)	-0.06190	-0.05161	-0.08301	-0.08130	-0.08213	-0.08088	-0.08783	-0.08816	-0.06388	-0.08547
ΔE_{L-H} (ev)	0.11934	0.12944	0.108139	0.12578	0.12317	0.1265	0.11359	0.09531	0.13234	0.09965
Dipole moment (debye)	6.2687	3.9833	2.9013	3.0273	8.6666	4.7047	6.8986	8.3543	4.2647	4.5073
Oscillator strength	0.0012	0.0002	0.0369	0.0211	0.5074	0.004	0.0377	0.0038	0.0032	0.0098

TABLE 2: MM⁺ internal energies computed over all proposed tautomers (12-18 and 21A-C).

Function	Compounds proposed									
	12	13	14	15	16	17	18	21A	21B	21C
Total energy	-102992.175	-102982.874	-87772.794	-87765.524	-87763.048	-94928.863	-94899.331	-105923.799	-105925.533	-105917.534
Total energy (kcal/mol)	-164.1285	-164.1136	-139.8748	-139.8632	-139.8593	-151.2787	-151.2317	-168.8003	-168.8031	-168.7903
Binding energy (a.u.)	-5236.8731	-5227.5718	-4456.1687	-4448.8985	-4446.4225	-4988.7493	-4959.2172	-5579.3923	-5581.125	-5573.1271
Heat during formation (ΔH_f) (kcal/mol)	-30.5761	-21.2748	36.1772	43.4475	45.9235	53.7847	83.3168	142.2527	142.5192	148.5179
$\Delta\Delta H_f$ (kcal/mol)	0.0000	9.3013	66.753	74.0236	76.4996	84.3608	113.8929	0.0000	0.2665	6.2652

SCHEME 5: Coupling reaction of compounds 12a–e.

FIGURE 2: Continued.

FIGURE 2: (a–j) Frontier orbitals regarding the HOMO level for optimized structures.

FIGURE 3: Continued.

FIGURE 3: (a–j) Frontier orbitals regarding the LUMO level for optimized structures.

TABLE 3: The activity of the tested compounds **12a–e** and **21a–e** against two fungi species.

Compound number	<i>Aspergillus niger</i>	<i>Geotrichum candidum</i>	Compound number	<i>Aspergillus niger</i>	<i>Geotrichum candidum</i>
12a	19.4 ± 0.63 (83.3%)	18.1 ± 0.58 (71.8%)	21a	17.3 ± 1.2 (74.2%)	19.3 ± 1.2 (76.6%)
12b	23.7 ± 0.72 (101.7%)	24.4 ± 1.2 (96.8%)	21b	15.5 ± 2.1 (66.5%)	14.2 ± 2.1 (56.3%)
12c	19.6 ± 0.63 (84.4%)	21.6 ± 0.63 (85.71%)	21c	24.1 ± 1.2 (103.4%)	23.6 ± 1.2 (93.7%)
12d	22.8 ± 1.2 (97.9%)	20.7 ± 0.72 (82.1%)	21d	19.2 ± 0.72 (82.4%)	17.3 ± 0.72 (68.7%)
12e	19.4 ± 0.63 (83.3%)	25.6 ± 0.63 (101.6%)	21e	12.9 ± 0.72 (55.4%)	18.5 ± 0.72 (73.4%)
Amphotericin B	23.3 ± 0.58	25.2 ± 0.72	Amphotericin B	23.3 ± 0.58	25.2 ± 0.72

Data are expressed in the form of mean ± SD.

The plates were then incubated at 37°C for 24 h and observed for antimicrobial activity. The diameters of the inhibition zone were measured and compared with the respective reference drug. The observed inhibition zones were

measured in millimeter beyond well diameter. Each treatment was replicated three times, and the average value is recorded. Also, the percentage value of inhibition zones compared to reference drugs was recorded (Tables 3 and 4).

TABLE 4: The activity of the tested compounds **12a–e** and **21a–e** against four G⁺ bacteria and four G[−] bacteria fungi species.

Compound number	Gram-positive bacteria					Gram-negative bacteria		
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Bacillus subtilis</i>	<i>Streptococcus pyogenes</i>	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhimurium</i>
12a	19.3 ± 0.58 (81.4%)	17.4 ± 0.58 (77.7%)	26.7 ± 1.2 (82.4%)	NA	NA	24.3 ± 0.63 (95.7%)	21.9 ± 0.58 (96.9%)	20.4 ± 0.72 (87.6%)
12b	22.8 ± 0.58 (96.2%)	21.6 ± 0.72 (96.4)	29.3 ± 0.58 (90.4%)	NA	NA	24.2 ± 0.72 (95.3%)	20.3 ± 0.63 (89.8%)	21.6 ± 0.63 (92.7%)
12c	22.6 ± 0.85 (95.4%)	19.6 ± 1.2 (87.5%)	28.3 ± 0.63 (87.3%)	NA	NA	25.2 ± 0.58 (99.2%)	18.6 ± 1.2 (82.3%)	19.9 ± 0.63 (85.4%)
12d	21.2 ± 0.63 (89.5%)	19.6 ± 0.58 (87.5%)	27.3 ± 0.53 (84.3%)	NA	NA	24.2 ± 1.2 (95.3%)	22.1 ± 0.44 (97.8%)	19.9 ± 0.72 (85.4%)
12e	23.1 ± 0.63 (97.5%)	22.7 ± 0.72 (101.3%)	28.1 ± 0.53 (86.7%)	NA	NA	18.5 ± 1.2 (72.8%)	19.2 ± 1.2 (85.0%)	20.4 ± 0.58 (87.6%)
21a	16.7 ± 0.63 (70.5%)	14.4 ± 0.72 (64.3%)	19.2 ± 0.63 (59.3%)	NA	NA	17.8 ± 1.2 (70.1%)	17.3 ± 1.2 (76.5%)	19.6 ± 0.58 (84.1%)
21b	15.2 ± 1.2 (64.1%)	19.8 ± 0.67 (88.4%)	24.3 ± 0.58 (75.0%)	NA	NA	17.9 ± 0.58 (70.5%)	13.3 ± 0.58 (58.8%)	18.4 ± 1.2 (79.0%)
21c	22.4 ± 0.63 (94.5%)	20.9 ± 0.72 (93.3%)	23.8 ± 0.63 (73.5%)	NA	NA	24.3 ± 1.2 (95.7%)	22.5 ± 1.2 (99.6%)	26.3 ± 0.58 (112.9%)
21d	13.5 ± 1.2 (57.0%)	15.3 ± 0.44 (68.3%)	18.2 ± 0.58 (56.2%)	NA	NA	19.3 ± 0.63 (76.0%)	16.3 ± 0.63 (72.1%)	19.3 ± 0.58 (82.8)
21e	15.3 ± 0.43 (64.6%)	18.6 ± 0.58 (83.0%)	22.4 ± 0.53 (69.1%)	NA	NA	16.4 ± 0.53 (64.6%)	20.4 ± 0.53 (90.3%)	18.7 ± 0.63 (80.3%)
Ampicillin	23.7 ± 0.63	22.4 ± 1.2	32.4 ± 0.72	24.5 ± 0.63	—	—	—	—
Gentamicin	—	—	—	—	22.3 ± 0.58	25.4 ± 1.2	22.6 ± 0.63	23.3 ± 0.58

NA: no activity; data are expressed in the form of mean ± SD.

TABLE 5: Docking energy values (k cal/mol) for two best tautomers complexed with variable protein receptors.

Ligands	pK ₁ –pK ₂	Receptor	Estimated free energy of binding	Estimated inhibition constant (K _i) (μM)	vdW + bond + desolve energy	Electrostatic energy	Total intercooled energy	Frequency	Interaction surface
Compound 12a	10.33–0.97	1ukc	−6.55	15.80	−6.22	−0.07	−6.29	20%	585.749
		1ydo	−5.81	55.13	−5.52	+0.01	−5.50	10%	569.54
		5o22	−4.67	375.04	−4.54	−0.17	−4.71	10%	487.392
		1thg	−4.72	346.39	−4.15	−0.09	−4.24	40%	473.189
		3gdz	−5.35	119.29	−5.07	−0.15	−5.22	10%	581.063
		2w7q	−7.92	1.56	−7.58	−0.03	−7.61	30%	723.71
		1af7	−6.89	8.84	−7.97	−0.26	−8.24	30%	796.368
		1bqb	−5.06	196.91	−5.72	+0.08	−5.64	50%	681.073
		3kp3	−5.32	126.03	−5.25	+0.10	−5.15	30%	585.688
		2esr	−6.76	11.05	−6.71	−0.05	−6.76	40%	585.867
Compound 21a		1ukc	−4.70	359.18	−6.01	−0.07	−6.08	10%	639.464
		1ydo	−5.54	87.07	−6.65	−0.14	−6.79	20%	558.58
		5o22	−4.41	585.48	−5.73	−0.03	−5.76	10%	520.673
		1thg	−4.73	342.60	−6.00	−0.04	−6.04	20%	542.067

$$\text{The \% activity} = \frac{\text{Mean zone of inhibition by test compound (mm)}}{\text{Mean zone of inhibition by standard (mm)}} \times 100. \quad (1)$$

The obtained results of antifungal activity reflected variable activities of the tested compounds. Among the tested perimidine derivatives **12b** and **21c** was the most potent derivatives against *Aspergillus niger* with inhibition zones exceed the *Amphotericin B* (101.7% and 103.4%, respectively). In addition, compound **12e** showed

activity with 101.6% inhibition percent in comparison with the reference standard used (Table 3). The other derivatives revealed the activity ranging from excellent to good.

Investigating the Gram-positive bacterial activity results in Table 4, a highly potent activity was recorded for compounds **12e**, **12b**, and **21c** against *S. epidermidis* with

FIGURE 4: Docking complexes for compound **12** with variable protein receptors. (a) 1ukc; (b) 1ydo; (c) 5o22; (d) 1thg; (e) 3gdz; (f) 2w7q; (g) 1af7; (h) 1bqb; (i) 3kp3; (j) 2esr.

FIGURE 5: Hb plots for the compound **12**-protein complexes. (a) 1ukc; (b) 1ydo; (c) 5o22; (d) 1thg; (e) 3gdz; (f) 2w7q; (g) 1af7; (h) 1bqb; (i) 3kp3; (j) 2esr.

inhibition percentages 101.3%, 96.4%, and 93.3%, respectively. The same derivatives in addition to **12c** showed excellent activity when compared with *ampicillin* (the inhibition percentages ranging from 97.5 to 94.5%).

Focusing on Gram-negative bacteria, all tested perimidine derivatives have no activity against *P. aeruginosa*, and in contrast, compound **21c** is more reactive than *gentamicin* against *S. typhimurium* with inhibition percentage

112.9%. The same derivative **21c** also has activity similar to *gentamicin* against the Gram-negative bacteria *Klebsiella pneumonia* and *Escherichia coli* as shown in Table 4. In addition, perimidine derivatives **12a–d** and **21e** was equipotent to *gentamicin* against some species (Table 4).

3.3. Molecular Docking Study. In the recent few decades, advanced development happened in the drug designing industry. Among this study was the computational method used to predicate the inhibition feature of the proposed drug against causative organism proteins. This study was achieved using AutoDock 4.2 tools to establish a comparative insight on the degree of inhibition toward pathogens. The receptors had chosen attributing to the tested bacteria and fungi in the experimental work. 1ukc and 1thg were the protein receptors used which attribute to *Aspergillus niger* and *Geotrichum candidum* fungi. Whenever, 1ydo, 5o22, 3gdz, 2w7q, 1af7, 1bqb, 3kp3, and 2esr were the protein receptors used, they attribute to *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes* bacteria. The data extracted upon docking complexes with proven compounds (**12** and **21A**) were displayed in Table 5. The values of free energy of binding and the inhibition constant display a moderate interaction between the two compounds and 1ukc and 1thg receptors. The values of total intercooled energy reflect the stability of docking complexes for such broad interaction surface. The docking complexes (Figure 4; Supplementary Figure 1S) display H-bonding in the corner only with protein helix [28]. This conclusion coincided with a little extent of H-bonding that appeared in the Hb plots (Figure 5; Supplementary Figure 2S) which exhibit interaction over the siding part in broad helix. However, variable features appeared against bacterial pathogen proteins. The best interaction was displayed with 1af7 and 1bqb receptors attributing to *Salmonella typhimurium* and *Staphylococcus aureus* organisms. This is concluded from the reduced energies (Table 5) over broad interacting surfaces. However, moderate inhibition was expected with the two tested compounds (**12** and **21A**) versus rest proteins. The docking complexes (Figure 4; Supplementary Figure 1S) display high occlusion for the two compounds with 1af7 or 1bqb protein helix. This was confirmed upon the Hb plots (Figure 5; Supplementary Figure 2S) which display the best distribution of intra-H-bonding with two reported receptors. But, on the other side, a little extent for H-bonding was recorded upon rest protein helix though broad interaction surfaces. Finally, this simulation process coincided by an excellent way with the experimental screening work which displays a moderate inhibition against most organisms considered.

4. Conclusion

The mechanism of the reaction of 1,8-diaminonaphthalene with ethyl aroylpyrovate was discussed using all possible spectroscopic tools as well as the computational study by applying Guassian09 software. In addition, the antimicrobial

activity of all products was screened. The results of such study indicated that three derivatives **12b**, **12e**, and **21c** have impacts on the fungi and bacteria more than the reference drugs used. However, the theoretical study of the antimicrobial activity of two derivatives **12a** and **21a** as examples of the synthesized series using AutoDock 4.2 tools indicated that such derivatives have moderate activity for all fungi and bacteria species.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The author declares that there are no conflicts of interest.

Supplementary Materials

Figures are given for docking complexes for compound **21A** with variable protein receptors (Figure 1S), Hb plots for the compound **21A**-protein complexes (Figure 2S), and spectral data for all compounds such as FTIR spectra (Figures S3–S9), mass spectra (Figures 10S–19S), and NMR spectra (Figures S20–S28) are available on the journal's website. (Supplementary Materials)

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