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Synthesis, characterization and biological activity of 2-Aryl -2, 3-dihydro-1*H*-perimidine

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A series of new 2-Aryl-2,3-dihydro-1*H*-perimidine, derivatives (3a - j) were synthesized under reflux and at room temperature by condensation reaction of 1,8-diaminonaphthalene (2) with various substituted benzaldehyde using glacial acetic acid a catalyst. The synthesized compounds were characterized by spectroscopic methods, infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), carbon nuclear magnetic resonance (^{13}C-NMR) and carbon nuclear magnetic resonance-distortionless enhancement by polarization (^{13}C-NMR-DEPT). The synthesized compounds were screened for their biological activity against the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli.* The results showed that 89% of the synthesized compounds were not active against *S. aureus*, while *E. coli* showed 100% sensitivity to the mentioned compound. These results illustrate the marked bactericidal effect of all the synthesized compounds.

Key words: 1, 8-Diaminonaphthalene, 2,3-dihyroperimidin, perimidine.

INTRODUCTION

The derivatives of perimidine described as DNA–intercalating antitumoral agents against carcinogenic lines, a small number of DNA-binding ligands, were assumed to have an important role in cancer chemotherapy such as adriamycin, actinomycine, amsacrine and mitoxantrone. A large number of studies on the structure activity relationships of these compounds have demonstrated the requirement for intercalative binding to DNA for biological activity which has led researcher to design new compounds which have generally been tricyclic or tetracyclic moieties in order to maximize the strength binding to DNA (Ihan et al., 2008; John, 1987). There are several preparative methods for the synthesis of perimidine derivatives. The most commonly method for the preparation of perimidines is the condensation reaction of 1,8-diaminonaphthalene with a carbonyl group which needs special reagent or several reaction conditions (Varsha et al., 2010).

The perimidines have a wide application in industrial field; they are used as dyes (Kazuhoki et al., 2010). Their famous 2,3-Dihydro-1*H*-perimidine (1) (Figure 1) is a saturated form of perimidine at positions 2 and 3 which is a synthetic tricyclic compounds including two nitrogen atoms. Heterocyclic compounds such as perimidine are of a wide interest because they exhibit diverse range of biological activities (Kang and Hsiu, 1984).

Small ring heterocyclic compounds containing nitrogen have been investigated for a long time because of their

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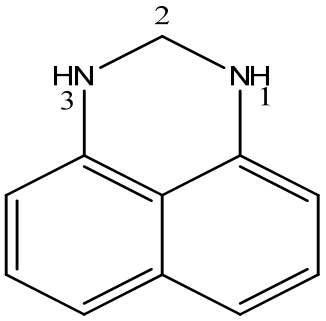

Figure 1. 2,3-Dihydro1*H*-perimidine compound

important medical properties; among these types of molecules is perimidine ring. The biological activities of these types of compounds against some microorganisms were reported in the literature (Kang and Hsiu, 1984).

Their famouse dyes were reported in the literature as solvent black 3. The perimidine derivatives have different uses and importance; they were used as an intermediate inorganic synthesis (Kang and Hsiu, 1984, 1985). Different routs were used in the synthesis of perimidine derivatives, such as microwave irradiation method (Cado and Stephen 1996). 2-Methyl-2-(4-biphenyl) perimidine and 2,2-dimethylperimidine served as an odor sensor which may be useful for discriminating between the odor of human or other mammalian individuals, and they found out that the perimidine monomers and polymers were used in the manufacture of the sensor (Gibson et al., 1999). Corn (1990) investigate novel near infrared absorbing dyes of perimidine and dihydroperimidine. Other spiroperimidine were reported as a photochromic compounds (Davis et al 2005). Herein, the synthesis spectral data biological studies against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* and their comparison with gentamicin of new compounds (3a - j) were achieved. The similar mechanism of action between gentamicin and streptomycin with other aminoglycosides has been approved by many researchers (Jacquelyn, 2008). The bactericidal action of gentamicin was accompanied by inhibition of growth, their results showed that the protein biosynthesis fails within minutes after addition of gentamicin to cultures susceptible bacteria for example Gram-negative bacteria *E. coli*. The mechanism of action of antibiotic on the biosynthesis of DNA and RNA in the Gram-negative bacteria *E. coli* was reported by several investigators, measuring the ^{14}C-labeled thymidine to the DNA of *E. coli* and measuring the incorporation of^{14}C-labeled uracil in the global RNA of the same bacteria. Their result showed the less effect of the antibiotic on the RNA biosynthesis. They found out that nucleic acid biosynthesis is relatively unaffected by gentamicin and they concluded that the gentamicin is a specific inhibiter of protien biosynthesis in susceptible bacteria; gentamicin inhibited the polymerization of phenylalanine in a standard ribosome poly U cell-free system (Fred et al, 1969).

MATERIALS AND METHODS

Measurement

Melting points were determined by open capillary method and are uncorrected. The infra red (IR) spectra in (KBr pellets) were recorded on a thermo mattson IR 300 Spectrophotometer and Bio-rad Merlin FTIR spectroscopy, Mod FTS 3000. The nuclear magnetic resonance (^{1}H-NMR and ^{13}C-NMR) spectra were recorded on Brucker (300 MHz) at Al-Albayt University-Jordon. Mass spectra (MS) were recorded on High resolution mass Bruker Daltonics Data Analysis 3.4, and the gas chromatography-mass spectrometry (GC-MS) (EI), Shimatzu, Japan, at Al-Albayt University-Jordon. Thin layer chromatography (TLC) was carried out using silica gel coated aluminium sheets DC-Aloufoline 20 × 20 cm Kieseigel 60 F_{254} pre-coated Germany Merck.

Synthesis of 2-Aryl-2, 3-dihydro-1*H*-Perimidine (3 a-e)

1,8-Diamino naphthalene (1.5820 g, 0.01 mole) was dissolved in 10 ml absolute ethanol. The soluble appropriate aldehydes (0.02 mole) in 10 ml absolute ethanol when added is followed by the addition of few drops of glacial acetic acid. The reaction mixture was stirred for 24 h at room temperature. The products were filtered, washed with cold absolute ethanol, recrystallized from absolute ethanol. The physical properties and yield percent are recorded in Table 3.

Synthesis of 2-Aryl-2, 3-di hydro-1*H*-Perimidine (3f-j)

The same procedure as described earlier was carried out under reflux for (1 to 3 h). Progress of the reaction was monitored by TLC (chloroform). At completion, the mixture was cooled to room temperature and filtered. The solid products were washed with ethanol and dried in an oven at 60°C. The products were filtered and recrystallized from appropriate solvent. The physical properties are listed in Table 3.

Biological study

The sensitivity of 2-Aryl-2,3-dihydro-1H-perimidine (3 a to j) were carried out against two kinds of bacteria, Gram-positive *S. aureus* and Gram-negative bacteria *E. coli* using disc agar diffusion method (Shakhawan 2001). The tests were performed using Muller Hinton agar, the medium was prepared using nutrient agar for preservation of pure culture, then sterilized by autoclave, and poured in Petri dish to a depth of 4 mm. Activation of each type of bacteria Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) was done before culturing on the nutrient agar in a nutrient broth which was used for dilution of bacterial and cultivation of culture isolates for 24 h in 37°C, then inoculation of the plates. The discs of the synthesized compounds were prepared by mixing a compound with KBr powder (1:3). The mixture was pressed under pressure KBr which has been used as a blank disc. The dried surface of the Muller Hinton agar plate was streaked; five dried discs were placed on the surface of the cultured media per petri dish. The plates were then incubated at 37°C for 18 to 24 h. Microbial growth was indicated by measuring the diameter of the zone of inhibition.

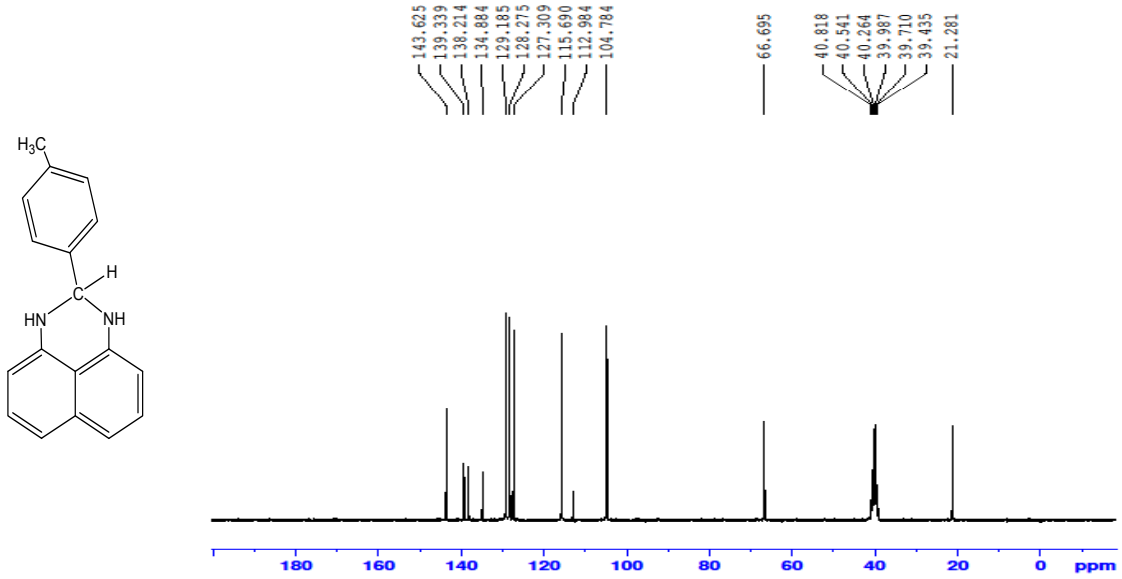

Figure 2. ^{13}C-NMR spectrum of compound (3e).

RESULTS AND DISCUSSION

The reaction of substituted benzaldehydes with primary diamines (2) afforded 2-Aryl-2,3-dihydro-1*H*-Perimidine (3a - j). The desired compounds prepared in different conditions while no analogues reactions using room temperature have yet been described. Thus 1,8-diaminonaphthalene was allowed to react with various substituted benzalaldehydes in absolute ethanol using glacial acetic acid as a catalyst for 24 h at room temperature. The rapid condensation was monitored by TLC using (chloroform as eluent), while the substituted benzaldehydes (f - j) did not react at room temperature. Therefore the desired compounds were prepared by condensation reaction under reflux for 1 to 3 h.

The possible mechanism of this reaction first involves the formation of azomethine bond followed by nucleophilic attack by the basic nitrogen of the second free amino group. Cyclization takes place with the formation of the final product. The structure of the title compounds were characterized based on their physical, analytical and spectral data. From the IR spectra of compounds 3a to j, a new sharp N - H stretching bands were observed at 3401 to 3364 cm^{-1}. The existence of the N - H stretching bands confirmed the cyclization at the positions 1, 2, 3 and the disappearance of four bands belong to two NH_2 stretching for symmetric and asymmetric vibration of the NH_2 groups at 3412, 3386, 3332, 3304 cm^{-1} in IR spectrum of 1, 8 to diaminonaphthalene which supported the formation of the products (Table 4). The position of the bond was also confirmed by the ^{1}H - NMR spectra of the compounds (Table 5). The singlet at δ 5.35 to 6 ppm in the spectrum of 3a - j showed the C-H proton supported by observation of a signal in the ^{13}C - NMR at δ 61.378 - 66.628 ppm (Akbar et al., 2009). In addition, the appearance of multiplets signals at δ 6.6 - 8.1 ppm for ten protons belongs to phenyl and naphthyl rings, which were recorded as 10 signals in^{13}C - NMR spectrum (Figure 2). The singlet signal belongs to two N - H of perimidine moiety seen at δ 7.4 ppm in ^{1}-HNMR spectrum (Table 5). The ^{13}C-NMR-DEPT-135 spectrum showed 8 signals assigned to protonated carbon for compound (3h) and both compounds (3e and i) showed 5 signals for 5 protonated carbon and the CH_3 carbon was observed at δ 21.263 ppm (Table 5).

The electron ionization mass spectrometry (EIMS) of 2 compounds, 3e and 3h, which were chosen as prototypes, were obtained. The MS of the compound 3e showed a molecular ion peak(M^+) with high intensity at m/z 259%, other peaks were observed due to fragments as a result of loss of protons, CH_3, C_6H_3, H_2 molecules, followed by loss of CH and N atom. C_2, which is another fragmentation, was recorded from the origin compound while the MS of compound 3h was calculated from molecular ion peak m/z 290.1 and showed peaks due to fragments, supporting the expected structures.

Antimicrobial activities

Experiments were performed to evaluate the activities of the synthesized compounds against two species of bacteria *S. aureus and E. coli*. Anti-microbial study was assessed by measuring the minimum inhibitory zone (using disk agar diffusion method) and the results were

Table 1. Perimidine's antibacterial activity measured by the disc diffusion method.

Compound	R	*S. aureus* (mm)	*E. coli* (mm)
3a	-H	20	25
3c	4-OCH_3	NIZ	40
3d	2-Br	NIZ	42
3e	4-CH_3	NIZ	26
3f	4-Br	NIZ	42
3g	2-OH	NIZ	44
3h	2-NO_2	NIZ	40
3i	4-NO_2	NIZ	40
3j	4-Cl	NIZ	40
Gentamicin			26

Values are represented as mean inhibition zone (mm), highly reactive (Inhibition zone > 24 mm), Active (inhibition zone 20 to 24 mm), NIZ = no inhibition zone.

represented in Table 1. The biological interest of perimidine derivatives were recorded in the literatures (Kang and Hsiu, 1984). Therefore the antibacterial study was done and the activity was determined by the disc diffusion method at the concentration of 50 µg per disk. All the synthesized used compounds were tested for their antibacterial activity against both bacteria *S*. *aureus* and *E. coli*. The gentamicin was chosen as a standard antibacterial agent. The synthesized compounds were more active against *E. coli* and showed negative effect against *S. aureus*. The compound 3a was moderately active against both the gram-positive and the gram-negative tested bacteria, whereas the most effective compound of perimidine derivatives was (3g) against *E. coli* indicating that the presence of (OH) group in 3g caused potential antibacterial activity, while both compounds (3a and 3e) were compared to (3g), they showed the lower activity against *E. coli*. Compounds 3a - j were found to exhibit more activity than the standard drug gentamicin that has a wide effect on the *E. coli.*

The results showed the effect of substituents on the activity of perimidine derivatives against both bacteria. Gram-positive bacterial cell walls contain peptidoglycan and teichoic or teichuronic acid, and the bacterium may or may not be surrounded by a protein or polysaccharide envelope. Gram-negative bacterial cell walls contain peptidoglycan, lipopolysaccharide, lipoprotein, phospholipid and protein. The critical attack site of anti-cell-wall agents is the peptidoglycan layer. This layer is essential for the survival of bacteria in hypotonic environments; loss or damage of this layer destroys the rigidity of the bacterial cell wall, resulting in death (Harold et al 1996). Gentamicine is a type of antibacterial agent that inhibits protein synthesis, the aminoglycosides irreversibly bind to the 30S ribosome and freeze the 30S initiation complex (30S-mRNA-TRNA) so that no further initiation can occur, the aminoglycosides also slows down protein synthesis that has already initiated and induced misreading of the mRNA (David et al., 2007; Marie-Paule et al., 1999; Davis, 1987). It may also destabilize bacterial membranes, inhibit the polymerization of phenyl alanine in a standard ribosome polymerization. Fragoso and Ciferri have been concerned with gentamicin-induced misreading of RNA code words as indicated by stimulations of ambiguous incorporation of certain amino acids in cell-free system, employing synthetic polyribonucleotides as model messenger RNAs (Fred et al, 1969). The increased activity may be attributed to enhancement of lipophilicity due to incorporation of aromatic benzene ring and substituent NO_2, CH_3 groups at meta and para positions with the presence of two N-H groups; these compounds tend to be highly bound to plasma protein, the more lipophilic compound the greater compound the greater binding. Table 2 investigates the percentage of sensitivity of the bacteria species under the study which was 11% for *S. aureus* while sensitivity of *E. coli* was 100%.

Table 2. The percentage of the active compounds against *S. aureus* and *E. coli* susceptibility.

Types of bacteria	Sensitive (%)	Resistance (%)
S. aureus	11	89
E. coli	100	0

Conclusion

In the present work, a series of new 2-Aryl-2, 3-dihy dro-1*H*-perimidines (3a - j) were synthesized and characterized by spectral studies. All the synthesized compounds were evaluated for their antibacterial activities against *S. aureus* and *E. coli* microorganisms by agar diffusion method.

Table 3. Some physical properties of 2-Aryl-2, 3-dihydro-1*H*-perimidines (3a to j).

No.	R	MF	Yield%	MP ˚C	Color	R_f Chloroform
3a	-H	$C_{17}H_{14}N_2$	52	198-200	Green	0.73
3b	4-F	$C_{17}H_{13}FN_2$	75	180-182	Pale violet	0.89
3c	4-OCH_3	$C_{18}H_{16}N_2O$	60	160-162	Pale violet	0.94
3d	2-Br	$C_{17}H_{13}BrN_2$	55	182-184	Pale pink	0.75
3e	4-CH_3	$C_{18}H_{16}N_2$	65	161-164	Pale pink	o.64
3f	4-Br	$C_{17}H_{13}BrN_2$	70	108-110	Greenish yellow	o.779
3g	2-OH	$C_{17}H_{14}N_2O$	68	188-191	Yellow	o.5
3h	2-NO_2	$C_{17}H_{13}N_3O_2$	60	170-174	Red	o.74
3i	4-NO_2	$C_{17}H_{13}N_3O_2$	90	202-204	Orange	o.6
3j	4-Cl	$C_{17}H_{13}ClN_2$	98	167-169	Yellow	0.56

Table 4. Assignments of characteristic frequencies (cm^{-1}) of IR spectra for the prepared 2-Aryl-2, 3-dihydro-1*H*-perimidine (3a to j).

Compounds	N-H str.	C-H str. (aromatic)	C-H str.	N-H Def.	*C=Cstr.*	*o-p-m* Subs.
3a	3368	3024	2987	1602	1490	699, 749
3b	3401	3040, 306	2800	1599	1506	815
3c	3365	3037	2795	1598	1512	813
3d	3380	3054	2800	1602	1589	747
3e	3365	3037, 2915	2795	1598	1483	814
3f	3347	3042	2849	1602	1588	812
3g	3368	3065	-	1602	1489	753
3h	3357	3357	2852	1601	1518	758
3i	3364	3069,3046	2799	1600	1512	815
3j	3379	3049	2800	1589	1488	817

Table 5. Chemical shift of ^{1}H-NMR and ^{13}C-NMR spectra data for some prepared 2-Aryl-2, 3-dihydro-1H-Perimidine (3a-j).Solvent: DMSO d_6

Compound	(δ) ppm
3b	^{1}H-NMR s, 1H6.7 (N-H); m, 10H6.5(C-H) Ar.; s, 1H5.4(C-H) Perimidine moiety
3e	^{1}H-NMR s, 1H 6.7(N-H); m,10H 6.4-7.4(C-H)Ar.; s,1H5.3(C-H) Perimidine moiety; s,3H 2.3(CH_3).^{13}C-NMR C_1:138.186 $C_{2,2^-}$:129.66 $C_{3,3}$:127.289C_4:143.59C_5:66.628 $C_{6,^-6}$:139.32$C_{7,7^-}$;104.734$C_{8,8^-}$:128.252$C_{9,9^-}$:115.637C_{10}:134.846
3h	^{1}H-NMR s,1H 6.8(N-H); m,10H6.5-8(C-H)Ar.; s,1H5.8(C-H) Perimidine moiety. ^{13}C-NMR $C_{1:}$147.871$C_{2,^-2}$,127.14 $C_{3,3^-}$:129.493 C_4:150.294 C_5:63.359 $C_{6,6}$:142.516 $C_{7,7}$:105.041 $C_{8,8^-}$:123.855 $C_{9,9^-}$:116.045 C_{10}: 134.751. $C_{11:}$112: 832
3i	^{1}H-NMR s,1H 6.(N-H); m,10H(C-H)Ar.; s,1H5.8(C-H) Perimidine moiety. ^{13}C-NMR $C_1C_{1:}$147.871 $C_{2,^-2}$,127.14 $C_{3,3^-}$:129.493 C_4:150.294 C_5:63.359 $C_{6,6}$:142.516 $C_{7,7}$:105.041 $C_{8,8^-}$:123.855 $C_{9,9^-}$:116.045 C_{10}: 134.751 $C_{11:}$112: 832

From the results we concluded that most of the synthesized compounds were not effective against *S. aureus*, except the compound 3a which has clear activity against *S. aureus*, while all of the synthesized compounds (100%) showed a wide effect against *E. coli*, as well as the entire synthesized compounds compared to standard drug gentamicin and they showed higher effect than gentamicin.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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