

Evaluation of Antimicrobial Activity from a Series of Heterocyclic Compounds Derived from 1,8- Diaminonaphthalene

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ABSTRACT

Four heterocyclic compounds of the 2,3-dihydro-1*H*-perimidine family were synthesized and characterized by conventional spectrometric methods (NMR and MS), then tested on bacterial strains. These compounds coded DAN-B, DAN-DCB, DAN-TCB and DAN-QCB have shown to be active against bacterium strain *Escherichia coli*, *Salmonella typhimurium* S066, *Candida tropicalis* ATCC and *Candida albicans* ATCC 10234. With MIC values of 7.5 mg/mL and 3.75 mg/mL, DAN-B and DAN-DCB are respectively active against *Escherichia coli*, while DAN-TCB and DAN-QCB are active with MIC value of 15 mg/mL. *Salmonella typhimurium* S066 is sensitive to DAN-B, DAN-TCB and DAN-QCB compounds with MIC value of 30 mg/mL for each of them. All compounds except DAN-DCB are active on *Candida tropicalis* ATCC 13803. As for *Candida albicans* ATCC 10234, it is sensitive to all our

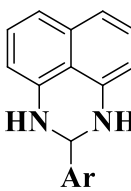
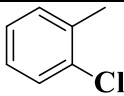
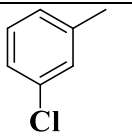
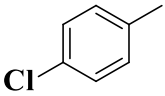
compounds with MIC values of 0.45 mg/mL for DAN-B, 15 mg/mL for DAN- DCB and DAN TCB then 7, 5 mg/mL for DAN-QCB.

Keywords: 2,3-dihydro-1*H*-perimidine, heterocyclic, perimidine, bacterium and MIC

INTRODUCTION

Drug resistance due to mutating pathogens is an increasingly recurrent phenomenon. Faced with this problem, researchers in synthetic chemistry use heterocyclic compounds of the 2,3-dihydro-1*H*-perimidine family to find alternative solutions. Indeed, 1*H*-perimidins are six-membered heterocyclic [1-7]. They were first discovered by Aguiar in 1874 [8]. They are characterized by either a bond deficiency or an excess of Π bond [9]. 1*H*-perimidines are used as intermediates in dyes, dyeing in polymerization [10] as well as for polyester fibers [11]. They are of great interest [12 - 14] thanks to their biological activities, and constitute an important source of antioxidant power [15]. Tuo et al. [16], showed that compound 2,3 dihydro-1*H*-perimidin-2-yl)-phenyl (DAN-B) showed antibacterial activities on *Escherichia coli* ATCC 25922, *Salmonella typhimurium* SO66, *Staphylococcus aureus* CIP 4.83, *Staphylococcus epidermidise* CIP 54124, with respective inhibition concentration values of 7.5 mg/mL; 30mg/mL; 7.5 mg/mL and 1.87 mg/mL. For antifungal activity, inhibition values of 3.75 mg/mL are recorded for *Candida Albicans* ATCC 10234 and 0.45 for *Candida tropicalis* ATCC 13803. Substitution of chlorine in the *ortho*, *meta* and *para* position on the phenyl ring, makes it possible to synthesize the compounds studied. In addition to compound DAN-B, the other three compounds were characterized by classical spectroscopic methods (NMR and MS). The objective of this work is to synthesize, characterize and experimentally evaluate the antimicrobial activity of our compounds. These antimicrobial activities were carried out using the Agar disk diffusion method on the following strains of bacteria: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* SO66, *Staphylococcus aureus* CIP 4.83, *Staphylococcus epidermidise* CIP 54124, on *Candida Albicans* ATCC 10234 and *Candida tropicalis* ATCC 13803. The Table 1 below gives the codes of the compounds synthesized and the structures of the aryl groups (Ar). These compounds are obtained by the condensation of 1,8 diaminonaphthalene and benzaldehyde derivatives.

Table 1: Structures of compounds synthesized.

	compound	Ar
	DAN-B	
	DAN-DCB	
	DAN-TCB	
	DAN-QCB	

MATERIAL AND METHODS

Material

Benzene-1,8-diaminonaphtalène, Benzaldehyde, 2-Chlorobenzaldehyde, 3-Chlorobenzaldehyde, and 4-Chlorobenzaldehyde were procured from Aldrich and used without further purification. All organic solvents were purchased from Merck and dried before use. Melting points were determined in capillary tube using an MPD Mitamura Riken Kogyo (Japan) electrothermal melting point apparatus and are uncorrected. The ^1H NMR spectra were recorded on a Bruker-Avance-300 spectrometer, operating at 300 MHz. The mass spectra were recorded on a TOF LCT Premier (WATERS) Spectrometer coupled to an HPLC Alliance 2695 chain.

Methods

Synthesis of compounds:

Synthesis of 2-phenyl-2,3-dihydro-1H-perimidine (DAN-B):

Benzaldehyde (19.60 mmol) and 1,8-diaminonaphtalène (19.60 mmol) were dissolved in ethanol (50 ml). The mixture was heated at reflux for 07 hours to give a green precipitate. The precipitate obtained was filtered and rinsed in ethanol (Rf: 0.87 in hexane/acetate d'ethyle (2;1)).

Synthesis of 2-(2-chlorophenyl)-2,3-dihydro-1H-perimidine (DAN-DCB):

2-chlorobenzaldehyde (17.77 mmol) and 1,8-diaminonaphtalène (17.77 mmol) were dissolved in ethanol (60 ml). The mixture was heated at reflux for 05 hours 20 to give a brown precipitate. The precipitate obtained was filtered and rinsed in ethanol (Rf: 0.76 in hexane/acetate d'ethyle (2;1)).

Synthesis of 2-(3-chlorophenyl)-2,3-dihydro-1H-perimidine (DAN-TCB):

3-Chlorobenzaldehyde (17.63 mmol) and 1,8-diaminonaphtalène (17.63 mmol) were dissolved in ethanol (60ml). The mixture was heated at reflux for 05 hours 20 to give a brown precipitate. The precipitate obtained was filtered and rinsed in ether (Rf: 0.90 in hexane /acetate d'ethyle (2;1)).

Synthesis of 2-(4-chlorophenyl)-2,3-dihydro-1H-perimidine (DAN-QCB):

4-Chlorobenzaldehyde (10 mmol) and 1,8-diaminonaphtalène (10 mmol) were dissolved in ethanol (60 ml). The mixture was heated at reflux for 04 hours 10 to give a brown precipitate. The precipitate obtained was filtered and rinsed in ethanol (Rf: 0.81 in hexane /acetate d'ethyle (2;1)). The general synthesis of the four compounds is shown in Figure 1 below.

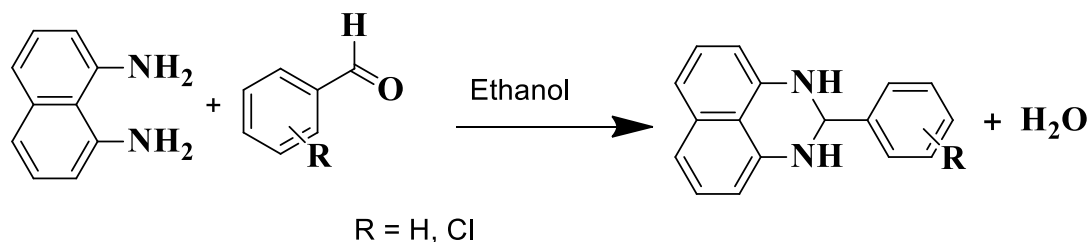


Figure 1: The general synthesis route of compounds

Antimicrobial Activity

In order to determine the pure compounds active against the strains tested, the method of diffusion by well on agar, as described by Bakkiyaraj S. [17] and Irshad S. [18], was used. Thus, a screening of the antibacterial and antifungal activities of our pure products was carried out.

Antibacterial Testing

The bacterial cultures: *Escherichia coli* ATCC 25922, *Salmonella typhimurium* SO66, *Staphylococcus aureus* CIP 4,83, and *Staphylococcus epidermidice* CIP 54124 were obtained from Microbiology Laboratory of Swiss Scientific Research Center of Abidjan (Cote d'Ivoire). The bacterial cultures were incubated at 37°C for 18 hours by inoculation into nutrient agar. Schiff bases were stored dry at room temperature and dissolved 60 mg/mL then 3,75 mg/ml in dimethylsulfoxide (DMSO). Antibacterial activities of each compound were evaluated by the agar disc-diffusion method. Mueller Hinton Agar Media (15 cm³) kept at 45°C was poured in the Petridishes and allowed to solidify. Poured Petri plates (9 cm) were incubated with 50µL of normal saline solution of above culture media (10⁵-10⁶ bacteria per mL). Discs injected with prepared perimidine (50µL) were applied on the solid agar medium by pressing tightly. The Petri plates were placed at 37°C for 18 hours. At the end of period the inhibition zones formed on media were measured with a zone reader in millimeters.

Antifungal Testing

Pathogenic strains of *Candida albicans* ATCC 10234 and *Candida tropicalis* ATCC 13803 were obtained from the Microbiology Laboratory of Swiss Scientific Research Center in Cote d'Ivoire. Perimidines were stored dry at room temperature and dissolved 60mg/mL in dimethylsulfoxide (DMSO). Antifungal activities of each compound were evaluated by the agar disc-diffusion method. Sabarod's agar media (15 cm³) kept at 45°C was poured in the Petridishes and allowed to solidify. Sterile, filter paper discs of 10mm diameter were impregnated with prepared perimidine (50µl) and were placed on to the media, seeded with fungus. The plates were then incubated at 37°C for 1-3 days. At the end of period the inhibition zones formed on media were measured with a zone reader in millimeters.

RESULTS AND DISCUSSION

MS Study

The mass spectra (HR-ESI-MS) of the title compounds showed peaks corresponding to the molecular ions at m/z 281.1 [M + H]⁺, that allowed to propose C₁₇H₁₃N₂Cl empirical formula for the compounds.

¹H NMR and ¹³C NMR Spectroscopy

Synthesis of 2-phenyl-2,3-dihydro-1H-perimidine (DAN-B):

yield: 82%, mp: 206°C. ¹H-NMR (CDCl₃): δ (ppm) = 5.31 (s, 1H, CH), 3.51 (m, 2H, NH), 6.47-8.08 (m, 10H, CH_{arom}). ¹³C-NMR (CDCl₃): δ (ppm) = 60.97, 112.49, 117.98, 119.21, 127.08, 129.08, 133.33, 134.39, 143.20, 155.42. Anal calcd for C₁₇H₁₄N₂

2-(2-Chlorophenyl)-2,3-dihydro-1H-perimidine (DAN-DCB):

Yield 47%, mp 196°. C. 1H-NMR (CDCl₃): δ (ppm) = 5.95 (s, 1H, CH), 3.50 (m, 2H, NH), 6.56-7.97 (m, 10H, CH_{arom}). 13C-NMR (CDCl₃): δ (ppm) = 63.81, 106.13, 117.73, 126.96, 127.66, 129.71, 130.19, 133.19, 141.59 Anal calcd for C₁₇H₁₃N₂Cl

2-(3-chlorophenyl)-2,3-dihydro-1H-perimidine (DAN-TCB):

Yield 46.71%, mp 140°. C. 1H-NMR (CDCl₃): δ (ppm) = 5.35 (s, 1H, CH), 3.61 (m, 2H, NH), 6.46-7.58 (m, 10H, CH_{arom}). 13C-NMR (CDCl₃): δ (ppm) = 76.48, 106.16, 118.26, 124.33, 129.84, 130.23, 131.21, 134.96, 141.78, 142.26. Anal calcd for C₁₇H₁₃N₂Cl

2-(4-Chlorophenyl)-2,3-dihydro-1H-perimidine (DAN-QCB):

Yield 46.71%, mp 134°. C. 1H-NMR (CDCl₃): δ (ppm) = 5.40 (s, 1H, CH), 3.66 (m, 2H, NH), 6.49-8.35 (m, 10H, CH_{arom}). 13C-NMR (CDCl₃): δ (ppm) = 67.80, 77.48, 106.11, 113.55, 114.03, 117.02, 118.23, 127.02, 129.16, 129.43, 135.00, 146.76. Anal calcd for C₁₇H₁₃N₂Cl

The resonance of protons had been assigned on the basis of their integration and multiplicity pattern.

Antimicrobial Activity of Compounds

The results of the antibacterial screening of the compounds DAN-B, DAN-DCB, DAN-TCB and DAN-QCB are presented in table 2. It emerges from this analysis that the values of the diameters of the zones of inhibition are between 9 and 16 mm.

Table 2: Mean diameters (mm) of the zones of inhibition and values of the minimum inhibitory concentrations (MIC) of the compounds DAN-DCB, DAN-TCB and DAN-QCB.

Average diameters of the Inhibition zones (mm)									Valeur des CMI (mg / ml)			
Bacteria	<i>E. coli</i>		<i>S. typhi</i>		<i>S. aureus</i>		<i>S. epid.</i>		<i>E. coli</i>	<i>S. typhi</i>	<i>S. aureus</i>	<i>S. epid.</i>
Concentrations (mg / mL) C1= 60, C2= 15												
Compounds	C1	C2	C1	C2	C1	C2	C1	C2				
DAN-B	16	14	10	0	15	12	14	10	7,5	30	7,5	1,87
DAN-DCB	12	15	0	0	16	10	12	09	3,75	ND	15	7,5
DAN-TCB	13	11	12	0	12	10	15	11	15	30	15	15
DAN-QCB	14	10	12	0	12	0	16	0	15	30	30	30
Witness												
Gen.	30	29	30	29	32	29	30	29	0,007	0,007	0,007	0,007

Values are the means of three replicates; **Gen**: Gentamicin, **S. Typhi** : Salmonella typhimurium, **S.Epid.** : Staphylococcus epidermidise, ND : Not Determined.

In this series of molecules where the phenyl ring of the DAN-B compound is substituted by chlorine, all the compounds studied show antibacterial activity except the DAN-DCB compound which is also ineffective against the *Salmonella typhimurium* SO66 strain. With an MIC value of 3.75 mg/mL, the *ortho*-substituted compound DAN-DCB shows the strongest antibacterial activity of the whole series against *Escherichia coli* ATCC 25922. It appears from this analysis that the compound DAN-DCB indicates the lowest CMI values, so it is the most active of the series. It therefore seems obvious to us that the halogenation of the phenyl ring of the

compound 2,3- dihydro-1*H*-perimidin-2-yl)-phenyl by chlorine is capable of modifying the biological behavior of the latter.

Antifungal activity of test compounds

The results of the antifungal activities of the compounds studied are recorded in Table 3 below.

Table 3: Mean diameters (mm) of the zones of inhibition and values of the minimum inhibitory concentrations (MIC) of the compounds.

Average diameters of the Inhibition zones (mm)					Values des CMI (mg / mL)	
Yeasts	<i>Candida albicans</i>		<i>Candida tropicalis</i>		<i>Candida albicans</i>	<i>Candida tropicalis</i>
Concentrations (mg / mL) C1= 60, C2=15						
Compounds	C1	C2	C1	C2		
DAN-B	12	10	10	09	3,75	0,45
DAN-DCB	11	09	0	0	15	ND
DAN-TCB	12	11	14	13	15	7,5
DAN-QCB	13	12	15	13	7,5	7,5
Witness						
Amph. B	30	29	30	29	0,007	0,007

Values are the means of three replicates, **Amph. B:** Amphotericine B, ND: Not Determine

This analysis highlights the antifungal activity of the compounds studied. Except DAN-DCB compound which is inactive against the *Candida tropicalis* fungus, all the other compounds are active against the two fungi used. The unsubstituted compound (DAN-B) displays the lowest MIC values of 3.75 mg/mL and 0.45 mg/mL, therefore the most active.

CONCLUSION

In this work, the biological study shows that our compounds DAN-DCD show the best antibacterial activity against *Escherichia coli* and DAN-B compound exhibits the best activities on the other strains. We can therefore say that the substitution of the phenyl ring of the unsubstituted compound (DAN- B) decreases its antimicrobial activity. For the rest of this study, we plan to evaluate the antioxidant activity of these compounds and use other substituents in order to investigate the activity of new molecules of this family.

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