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Antimicrobial Evaluation of Some Pyridone and Hydantoin N-glycosides Carrying Pyrene Moiety

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In our previous work, some of imidazole and pyridone carrying pyrene derivatives were synthesized before. The potential antimicrobial effects of the synthesized compounds were screened for qualitative (zone of inhibition) and quantitative antibacterial activity (MIC) by agar diffusion test and microtitration methods, respectively, using a variety of microorganisms (*S. Typhimurium* and *P. aeruginosa*) as Gram –ve bacteria, (*S. aureus*) as Gram +ve bacteria and (*C. albicans* and *A. flavus*) as yeast. Some of them exhibited promising activities and the other compounds exhibited moderate activity when compared to standard reference drug.

Key words: Imidazole derivatives; Pyridones; glycosides; Antimicrobial activities.

In view of the emergence of drug-resistant microbial strains, the search for new antibacterial and antifungal chemotherapeutics has become an increasingly important part of medicinal chemistry. Infections caused by bacteria and fungi remain a major health concern due to the development of resistance to existing antimicrobial agents. The increasing incidences of Gram-positive and Gram-negative bacterial resistance to antibiotics such as chloramphenicol, streptomycin, tetracycline, sulfadiazine, bacitracin and azithromycin have caused life-threatening infectious diseases[1,2]. On the other hand, the systemic and dermal fungal infections such as Candidiasis, Cryptococcosis and Aspergillosis have significantly increased due to the resistance to the currently available antifungal azoles[3,4]. The emergence of multiple drug resistant microorganisms has caused major health concern worldwide. Therefor, there is an increasing need to design and synthesize new antimicrobial agents with broad spectrum of activities. A large number of nitrogen-containing heterocycles have been claimed by researchers all around the world because of its versatile and eminent biological profile [5–7]. The pyridine nucleus is of considerable interest as this ring is the key constituent in a range of bioactive compounds, both naturally occurring and synthetic, and often of considerable

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complexity[8]. Many publications report the biological properties of pyridinecarbonitriles as being anticancer[9], anti-inflammatory, analgesic[10], antibacterial[11], anti-HIV agents[12] and anti-Alzheimer[13]. Also, Hydantoins are important heterocyclic scaffolds that induce prominent biological effects[14]. Numerous applications have been found for hydantoin derivatives due to their anticonvulsant[15], antimicrobial[16] and anticancer agents[17]. In hope to discover a novel antimicrobial agents, a series of pyridone and hydantoin N-glycosides derivatives carrying pyrene ring are screened for their antibacterial and antifungal activities.

EXPERIMENTAL

All melting points were uncorrected and determined by the Electro-thermal IA 9100 melting point apparatus. The infrared (IR) spectra were recorded using potassium bromide disc technique on Schimadzu 435 IR Spectrophotometer at Microanalytical unit, National Research Centre. ^{1}H NMR spectra were performed on a Bruker AC 250 FT NMR spectrometer at 250 MHz or a Varian Gemini 2000 NMR spectrometer at 300 MHz and on Jeol-ex 270 Hz NMR Spectrophotomete at University of Southern Denmark, Denmark. ^{13}CNMR spectra were recorded on a Bruker AC 250 FT NMR spectrometer at 63 MHz or a Varian Gemini 2000 NMR spectrometer at 75 MHz at University of Southern Denmark, Denmark. Mass Spectra were recorded on Finnigan MAT SSQ 710 and Fast atom bombardment mass spectra (FAB ms) were recorded on a Kratos 50TC spectrometer at University of Southern Denmark, Denmark. Elemental analysis (C, H and N) were carried out at Microanalytical unit, Cairo University.

MATERIALS AND METHOD

Antimicrobial assay

Bacterial strains

The antibacterial activity of the eleven chemical compounds was tested against 3 different bacterial strains isolated from food of animal origin; two Gram –ve bacteria (*S. Typhimurium* and *P. aeruginosa*) and one Gram +ve bacteria (*S. aureus*) which were isolated from meat by products of animal origin and are highly hazardous to human as they cause food intoxication. The strains were streaked onto Muller Hinton agar plates (oxoid). Also, chemicals were tested for their antimycotic activities against two yeast (*C. albicans and A. flavus*) using Sabaroud Dextrose agar plates (Difco). Six wells were formed in each plate using sterile Pasteur pipette. The strains were prepared in dilution equivalent to 0.5 Mc-Farland.

Agar well diffusion test[18]

The sterilized media (autoclaved at 120^{o}C for 30 mins) were allowed to cool, then agar was poured in plates, after solidification wells were done using sterile Pasteur pipette then 50 mL of the prepared chemicals (100 mg/mL DMSO) were added in wells after streaking the agar surface with the bacterial strains or mycotic strains then plates were allowed to sterile and incubated at 37^{o}C for 24 hrs to allow bacterial growth and at 28^{o}C for 24-72 hrs for mycotic growth. After incubation the zone of inhibition was measured and tabulated in mm [table 1].

Minimum inhibitory conc. (MIC)[19]

It was carried out on ELISA plates were 2 fold several dilution of each compound was carried out then 100 ¼L of the tested strains were added in each well then plates were incubated at 37^{o}C/12 hrs or at 28^{o}C/24-27 hrs for bacterial and mycotic plates respectively, then MIC readings were tabulated which indicate the highest dilution of the compounds which cause complete hindrance for the tested strains and gives clear supernatant and absence of precipitation (Table 2).

RESULTS AND DISCUSSION

In continuation of our previous work, a series of pyridone and hydantoin N-glycosides derivatives incorporating pyrene ring 1-11 (Fig. l) were synthesized in advance[20]. Herein, we used these compounds for evaluation as antimicrobial activities agents.

Antimicrobial Screening

Compounds 1-8 showed no. activity on G +ve bacteria (*S. aureus*) as well as compounds no . 1, 6, 7, 8 showed no activity against G-ve or G +ve bacteria. On the contrary compounds no. 2, 3 and 4 were effective against G-ve bacteria. Only, *S. typhimurium* and *Ps. aeruginosa* showing zone of inhibition equals 15 and 12, 11 and 11, 10 and 12 respectively followed by compound no. 9, 10, 11

were effective against G –ve and G +ve bacteria with zone of inhibition with *S. Typhimurium*, *Ps. aeruginosa* and *S. aureus* equals 11, 14 and 12, 10, 14 and 14, 9, 12 and 11 respectively (Table 1).

For mycotic activity compounds no. 6, 7, 10, 11 showed no activity against tested strains *C. albicans* and *A.flavus*. On the other hand compounds from 1 to 5 as well as 8 and 9 were effective against tested strains, the highest effect was for compounds 1, 3 and 9 showing zone of inhibition equals 13, 13 and 11, 14 respectively.

As for MIC, it was carried out for the compounds using 2 fold serial dilutions for each compound. Results revealed low activity of tested compounds comparing with the reference drugs (Table 2).

Against *S. Typhimurium*, compound no. 5 showed the best results compared with the reference drug TOB where both gave MIC equal 3.125 against *Ps. aeruginosa the* best results were shown with compounds no. 9 and 10 which are the same as the reference drug TOB showing MIC equals 6.25. Against *S. aureus* compounds no. 3, 4 and 5 showed no hindrance while on the other hand, compound no. 10 showed best MIC equal 6.25 among the tested compounds compared with reference drugs TOB (0.19) and Ciprofloxacin (0.097). The antimycotic activities revealed that

Table 1. The antimicrobial activity of tested compounds against bacterial and fungal strains isolated from animal byproduct origin using agar disc diffusion test

Compounds	G -ve bacteria		G +ve bacteria	Yeast	
	S. Typhimurium	*Ps. aeruginosa*	*S. aureus*	*C. albicans*	*A.flavus*
1	-ve	-ve	-ve	13	13
2	10	12	-ve	-ve	11
3	15	12	-ve	13	13
4	11	11	-ve	10	13
5	10	11	-ve	10	13
6	-ve	-ve	-ve	12	-ve
7	-ve	-ve	-ve	-ve	-ve
8	-ve	-ve	-ve	11	11
9	11	14	12	11	14
10	10	14	14	-ve	-ve
11	9	12	11	11	-ve
Tobromycin	15	14	27	-	-
Fluconazole100 µg/l	-	-	-	15	14
Ciprofloxacin100 µg/l	28	25	28		

Table 2. The antimicrobial activity of tested compounds against bacterial and fungal strains isolated from animal byproduct origin using minimum inhibitory concentration test

Compounds	G -ve bacteria		G +ve bacteria	Yeast	
	S. Typhimurium	*Ps. aeruginosa*	*S. aureus*	*C. albicans*	*A.flavus*
3	3.125	25	-	12.5	12.5
4	50	50	-	100	12.5
5	100	50	-	100	25
9	50	6.25	50	50	12.5
10	100	6.25	6.25	-ve	-ve
11	100	50	50	-ve	-ve
TOB	3.125	6.25	0.19	-	-
Fluconazole	-	-	-	0.97	0.97
CIB	0.19	0.39	0.097	-	-

compound no. 3 showed the hindrance effect against *C.albicans* and *A.flavus* with MIC = 12.5 followed by compounds no. 9 and 4 showing antimycotic activity 50, 12.5 and 100, 12.5 respectively, and it discussed as follow:

Reaction of pyene-1-aldehyde (1) with 2-thio(oxo)hydantoin in glacial acetic acid in the presence of sodium acetate afforded the corresponding 5-((pyren-1-yl)methylene)-2-thio(oxo)hydantoin derivatives 2a,b, respectively. Also, compound 1 was condensed with acetyl derivatives and malononitrile or ethyl cyanoacetate in the presence of ammonium acetate to afford the corresponding pyridone derivatives 3a-d and 4a-e, respectively. The latter compound 4a was reacted with 2-deoxy-3,5-di-*O*-(p-toluyl)-±-*D*-erythropentafuranosyl chloride in DMF in the presence of triethyl amine in room temperature to afford the corresponding N-ribosyl pyridine derivative 5. Additionally, compound 2b was treated with 2-deoxy-3,5-di-*O*-(p-toluyl)-±-*D*-erythropentafuranosyl chloride in DMF in the presence of TEA to afford 1-ribosyl hydantoin derivative 6. But, when compound 2a reacted with alkyl halides, namely, methyl iodide, chlorom ethylmethylsulfide, chloromethylethyl ether or methylbromoacetate in dry DMF in the presence of anhydrous potassium carbonate at room temperature afforded the corresponding S-alkylated derivatives 7a-d, respectively. Treatment of 7 with 2-(*E*)-monosaccharide hydrazones in heating methanol gave the corresponding 5-(pyren-1-yl) methylene)-2-((2-(*E*)-polyhydroxy-alkylidene)hydrazono) hydantoin 8a-c, respectively.

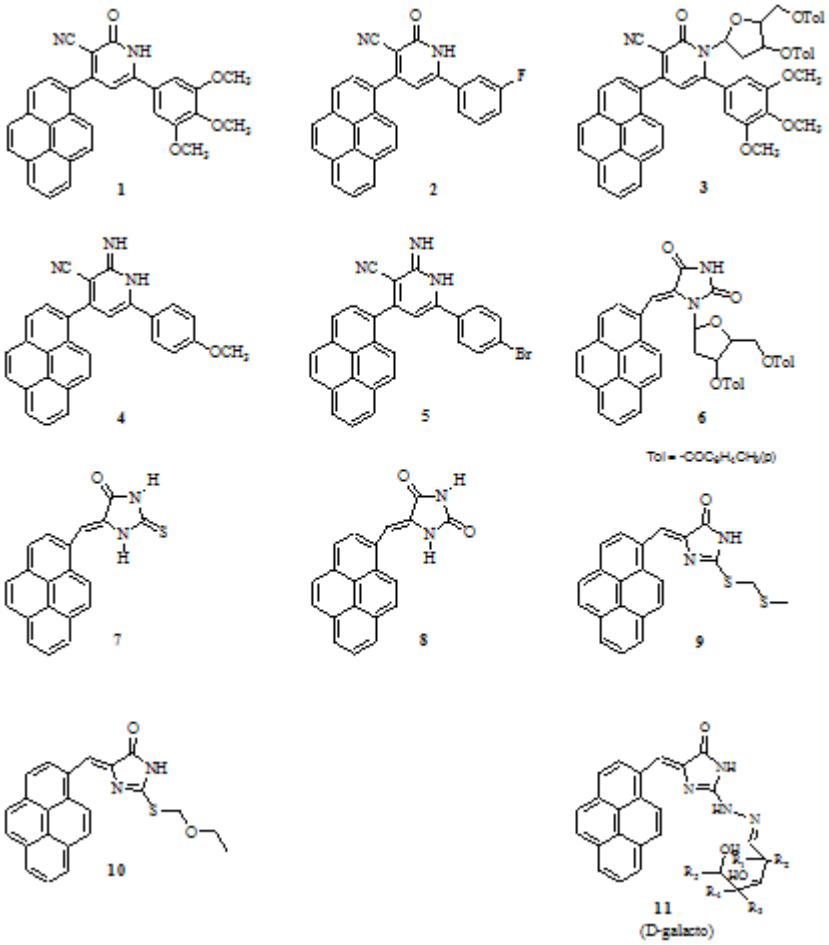

Fig. 1. Chemical strutcture for tested cornpounds 1-11

CONCLUSION

In summary, a new series of pyrenyl nucleoside derivatives incorporating pyridine and hydantoin moieties have been screened for their *in vitro* antibacterial activity using topramycin as the standard drug (100 µg/mL). They were also evaluated for their *in vitro* antifungal activity using fluconazole as a standard antifungal drug (100 µg/mL). The results of preliminary bioassays indicate that a number of these compounds exhibit antibacterial activities as well as antifungal stains, which are comparable to commercially available drugs.Compound **3** showed the best results against (*S. Typhimurium*) the same effect as compared with the reference drug TOB. Also, compounds **9** and **10** showing MIC equals 6.25 against *Ps. aeruginosa* the same effect as the reference drug TOB. The modification of the Pyridine ring of the parent compound offers a promising prospect and more active analogues are expected to be found.

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