



Research Paper

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ANTITRICHOMONAL ACTIVITY OF 1,3-DIARYL-2-PROPEN-1-ONES ON *TRICHOMONAS GALLINAE*

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Abstract

1,3-diaryl-2-propen-1-ones were synthesized by the Claisen - Schmidt condensation method. *T. gallinae* parasites isolated from domestic pigeon were cultured *in vitro*. The *in vitro* susceptibility of *T. gallinae* was evaluated in multi-well plates at 37°C.

Four of the synthetic compounds produced significant antitrichomonal activity against *T. gallinae*. The minimal lethal concentrations (MLCs) (produced by) 2'-hydroxy-4-methoxychalcone, 2'-hydroxy-2,4'-dimethoxychalcone, 2'-hydroxy-4-chlorochalcone, 3,4,4'-Trimethoxychalcone and 4-hydroxycychalcone were 100.0, 0.78, 50.0, 50.0 and 3.13 µg/ml respectively. The results indicate that 2'-hydroxy-2,4'-dimethoxychalcone and 3 other synthetic 1,3-diaryl-2-propen-1-ones possess potent antitrichomonal activity against *T. gallinae*. However, the studies on cytotoxicity effect showed that all the active chalcones demonstrated a very low haemagglutination titre values ranging between 0.57 – 4.06 suggesting their low toxicity profile.

Keywords: *Trichomonas gallinae*, 1,3-diaryl-2-propen-1-ones, *in vitro* activity

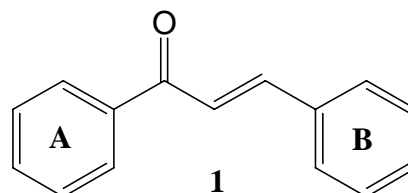
Introduction

Trichomonas gallinae is a flagellated protozoan living in the upper digestive tract and in various organs of different avian groups, especially columbiformes (doves and pigeons). The domestic pigeon, *Columba livia*, (Anth) is the primary host of this parasite as well as certain dove species such as white-wing doves. *T. gallinae* is the causative agent of canker in pigeons and causes a variety of pathologic manifestations depending on the strain of the parasite and species of bird it infects (Baker, 1986; Cooper and Petty, 1988; Henderson et al., 1988; Jessup, 1980). The pathologic changes associated with *T. gallinae* infection of the upper digestive tract of birds range from mild inflammation of the mucosa to large

caseous lesions that block the lumen of the esophagus. Pigeons however are more susceptible to secondary organ invasion (liver, air sacs, lung, and brain) by virulent strains of the parasite. Necrotic lesions develop in these organs leading to the death of the host (Stabler, 1954).

The effectiveness of a drug (2-amino-5-nitrothiazole), against pigeon trichomonosis caused by the protozoan *Trichomonas gallinae* has been reported (Stabler and Mellentin, 1951), and this led to the discovery of the activity of a nitroimidazole derivative (metronidazole) against *T. vaginalis* (Cosar and Julou, 1959). The latter resulted in the opportunity for a significant improvement in the treatment of trichomonad infections in humans (Forsgren and Forssman, 1979). Metronidazole was also shown to be effective against *T. gallinae* (Bussieras et al., 1961). Metronidazole has been the drug of choice for treatment of human urogenital trichomonosis since its discovery (Krieger et al., 1985). In avian veterinary medicine, several nitroimidazoles including metronidazole, dimetridazole, ronidazole and carnidazole, have been developed as effective drugs against *T. gallinae* (Franssen and Lumeij, 1992). In man, cases of clinical resistance to nitroimidazoles are rare despite their extensive use worldwide (Edwards, 1993). However, resistance to drugs in avian trichomonosis management has been reported using three isolates from wild birds (Munoz et al., 1998).

In our continued efforts at examining synthesized chalcone and related compounds for possible biological activities, a number of chalcones [2-9] and 1,3-diaryl-2-propen-1-ones [10-11] were examined for antitrichomonal activities.



Chalcones and other biogenetically related compounds are collectively called flavonoids and many of their derivatives have been tested for biological activities which includes molluscicidal (Adewunmi *et al*, 1987) and antimicrobial (Gabor *et al*, 1967) activities. They have also been found to exhibit anti-inflammatory (Viana *et al*, 2003; Pushkar and Balawant, 2001; Tewtrakul *et al*, 2003), anti-oxidant (Miranda *et al*, 2000; Repetlo and Lesuy, 2002) and analgesic (Viana *et al*, 2003; Azarifar and Ghasemnejad, 2003) activities. An interesting chalcone from natural product 2',6'-dihydroxy-4'-methoxychalcone isolated from *Piper aduncum* (Piperaceae) produced significant antiprotozoal activity against *Leishmania amazonensis* (Torres-Santos et al., 1999). There have been no reports of chalcones or any of their derivatives for anti-trichomonal activity. In view of the problems associated with drug resistance in the treatment of avian *T. gallinae*, some synthesized chalcones and related compounds were evaluated as potential anti-trichomonal agents.

Material and Methods

Synthesis of 1,3-diaryl-2-propen-1-ones

Using Claisen - Schmidt condensation method (Adewunmi et al., 1987), appropriate acetophenones were condensed with benzaldehydes to give 3, 4,- methylenedioxy-2¹-hydroxychalcone [2], 2¹-hydroxy-4-methoxychalcone [3], 2¹-hydroxy-3,4,4¹-trimethoxychalcone [4], 2¹-hydroxy-2,4¹-dimethoxychalcone [5], 2¹-hydroxy-4-chlorochalcone [6], 2-methoxy-4¹-hydroxychalcone [7], 3, 4, 4¹-trimethoxychalcone [8], 4-hydroxychalcone [9], 1-Phenyl-3-(2-thienyl)-2-propen-1-one [10] and 1-Phenyl-3-(2-furyl)-2-propen-1-one [11] in 65-81% yield.

Structural elucidations of the compounds were carried out using Gallenkamp apparatus for melting point determination. The IR spectra were run on a Pye Unicam SP 3-300 IR spectrophotometer as potassium bromide pellets. ¹Hmr spectra were taken on a Bruker WM 300 nuclear magnetic resonance using tetramethylsilane (TMS) as internal standard and deuteriodimethyl sulphoxide as solvent. Mass spectra were determined using MAT 44S spectrometer at 70ev while the elemental analyses were obtained with Carlo Erba 1106 elemental analyzer.

Biological assays

Samples of *Trichomonas gallinae* from the buccal cavity and oesophagus of the local pigeon (*C. livia*) were taken with a cotton swab to detect the presence of the protozoa in the birds' upper digestive tract. The parasites were cultured xenically *in vitro*, in Locke-egg (LE) medium (NIH modification of Boeck and Drbohlav's medium) (Von Brand et al., 1943) without antibiotics, pH 7.2, supplemented with 10% heat inactivated bovine serum, at 37 °C. Previous studies have shown that the presence of antibiotics in the culture medium affected the pathogenicity level and decreased the haemolytic activity of *T. gallinae* isolates (Stabler, 1954). Isolates were subcultured every 48 h. The trichomonads in the logarithmic phase of growth and subcultured every 48 h exhibited more than 95% mobility and normal morphology.

The method used in the *in vitro* assay was essentially as previously described (Meingassner and Thurner, 1979). Sterile, multi-well plates were used to incubate the isolates with the corresponding drug dilutions. Different assays, with each compound were performed in triplicate. The plates were incubated for 48 h at 37°C under aerobic conditions. The wells were examined after 24 and 48 h of incubation with an inverted microscope. The MLCs for each drug were thus obtained by means of the observation with the microscope. These MLCs were defined by the lowest drug concentrations in which no motile organisms were seen or no growth was detected after 24 and 48 h of cultivation, respectively.

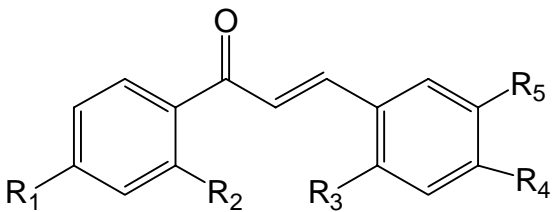
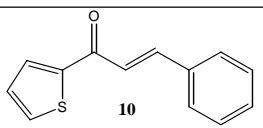
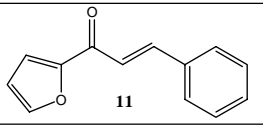
Cytotoxicity assay

The cytotoxicity of the 10 chalcones were monitored by haemagglutination activity using formaldehyde fixed bovine erythrocytes as described by Peumans et al., (1982), Sadique et al., (1989) and Wang et al., (1995).

Preparation and Fixation of bovine erythrocyte:

Bovine (*Bos taurus*) erythrocytes fixed with formalin were prepared according to the modified procedures of Sadique et al., (1989). Fresh blood sample was collected from N'Dama, (a representative of *B. taurus* breed) into a sterile conical flask containing 3.8% (w/v) trisodium citrate and mixed thoroughly. Then, 20 ml of blood was centrifuged at

Table 1: Antitrichomonal activities of synthetic chalcone compounds on *T. gallinae* at 24 and 48 hrs.

Compounds						MLC ($\mu\text{g/ml}$) at 24 h	MLC ($\mu\text{g/ml}\pm\text{SD}$) at 48 h
	R1	R2	R3	R4	R5		
[2] 3,4methylenedioxy-2'-hydroxychalcone	OH	H	H	Methylenedioxy		>100	>100
[3] 2'-Hydroxy-4-methoxychalcone	H	OH	H	OCH ₃	H	>100	100 \pm 0
[4] 2'-Hydroxy-3,4,4'-trimethoxychalcone	OCH ₃	OH	H	OCH ₃	OCH ₃	>100	> 100
[5] 2'-Hydroxy-2,4'-dimethoxychalcone	OCH ₃	OH	OCH ₃	H	H	100	0.78 \pm 0
[6] 2'-Hydroxy-4-chlorochalcone	H	OH	H	Cl	H	>100	50 \pm 0
[7] 2-Methoxy-4'-hydroxychalcone	OH	H	OCH ₃	H	H	>100	> 100
[8] 3,4,4'-Trimethoxychalcone	OCH ₃	H	H	OCH ₃	OCH ₃	>100	50 \pm 0
[9] 4Hydroxycychalcone	H	H	H	OH	OH	>100	3.13 \pm 0
 10						>100	>100
 11						>100	> 100
Metronidazole						100	0.78 \pm 0

4000 rpm for 10 min. on a Gallenkamp centrifuge. The packed red blood cells were washed with 10 mM phosphate buffer saline (PBS) pH 7.2 until a clear supernatant was

obtained. The washed packed RBC were suspended in (5% v/v) formaldehyde - phosphate buffer saline (1:12.3 v/v) solution. The mixture was left at room temperature for 24 h. The final fixed RBC were washed and centrifuged with PBS 3 times, and preserved with 0.1% methyl parabene to prevent microbial growth and stored at 4° C.

Heamagglutination assay:

100 µl of PBS was pipetted into 96 well microtitre plates. The first row was used as control without the compounds. The compounds (100 µl) were added into the first well of the second row, and a 2-fold serial dilution was made until the last well in row three. Then 50 µl of fixed bovine erythrocytes was added to all the wells. They were incubated at room temperature for 1 h. The presence of buttons in the centre of the well indicates no agglutination and the heamagglutination titre value of the drugs were estimated as the reciprocal of the last dilution showing agglutination

Chemicals

All the chemicals were obtained from various sources, all are of Analar grade. Formaldehyde (Sigma), Ethanol (BDH), Methanol (BDH), Sodium tri citrate (BDH), DMSO -Dimethyl sulfoxide (Sigma), DPPH (Sigma), Ascorbic acid (BDH Laboratories), Methyl paraben (Sigma), Sodium chloride (BDH), Na₂HPO₄ and NaH₂PO₄.

Results

Eight of the ten 1,3-diaryl-2-propen-1-ones examined were chalcones [2-9] while two were 1-Phenyl-3-(2-thienyl)-2-propen-1-one [10] and 1-Phenyl-3-(2-furyl)-2-propen-1-one [11]. The results of the effects of the synthetic compounds on *T. gallinae* are shown in Table 1. The cytotoxicity/haemagglutination assay results are presented in Table 2. All the compounds exhibited very low haemagglutination (HA) titre values with a wide range of concentrations at which agglutination occur on fixed bovine erythrocytes.

According to Table 2, the chalcones and the reference drugs produced various degrees of protection on the bovine RBC membrane and exhibited very low haemagglutination (HA) titre values ranging between 0.57 – 4.06. The order of protection of these agents was: 2'-Hydroxy-4-chlorochalcone [6] > 3,4,4'-Trimethoxychalcone [8] > 2'-Hydroxy-4-methoxychalcone [3] = Acetylsalicylic acid [ASA] > 2'-Hydroxy,2,4-dimethoxychalcone[5] = Metronidazole [MTZ] > 4-Hydroxychalcone [9]. (This was shown in the following concentrations through which agglutination occur in decreasing order respectively: 1.75 ± 0.56, 1.50 ± 0.00, 1.25 ± 0.34, 1.25 ± 0.34, 0.375 ± 0.00, 0.375 ± 0.00 and 0.25 ± 0.06).

Discussion

The chalcones examined were those with hydroxyl substituent at position 2' [2-6] and those without substituent at position 2' [7-9]. Five of the chalcones (3, 5, 6, 8 and 9) showed antitrichomonal activity at concentrations equal to and below 100 µg/ml. It is interesting to observe that 1-phenyl-3-(2-thienyl)-2-propen-1-one [10] and 1-phenyl-3-(2-

furyl)-2-propen-1-one [11] were not active. This suggests that 1-thienyl, and 1-furyl substituents do not enhance anti-trichomonal activities of 1,3-diaryl-2-propene-1-ones. The observed activity of chalcones (3, 5 and 6) is not surprising because of the 2'-hydroxyl substituent which could form flavones or isoflavones through intra-molecular cyclisation leading to enhanced activity. Compound [4] showed little anti-trichomonal activity compared with compound [5] which is the most active product. The difference in potency may be due to the intramolecular formation of methylenedioxy substituent by the 3,4-dimethoxy substituent on [4] as witnessed in the inactivity of 2'-hydroxy-3,4-methylenedioxychalcone [2].

The five synthesized chalcones, metronidazole and acetylsalicylic acid were subjected to the cytotoxicity abilities on bovine fixed RBC to investigate any correlation between their cytotoxic abilities through haemagglutination titre assay and anti-trichomonal activities. We therefore use this method to explain the safety and efficacy of synthesized chalcones against trichomonal infection. It appears that the low cytotoxicity ability of [3], [6], [8] and acetylsalicylic acid has contributed to their ineffectiveness against *T. gallinae*. In this way, they have very low titre values, an indication of low cell destructive property thereby leaving these organisms without lethal effects. However, metronidazole (MTZ), [5] and [9] produced high HA values indicating higher cytotoxicity than that of [3], [6], [8] and acetylsalicylic acid. In other words, there appears to be some correlation between the anti-trichomonal activity of the compounds and the haemagglutination titres.

The most active compound, 5, was almost as potent as metronidazole against *T. gallinae*. Although the active compounds were not tested against metronidazole-resistant strains of *T. gallinae*, the targets of these compounds may be different from that of metronidazole. Whether or not this renders cross-resistance unlikely must be tested in future studies. More work needs to be done on other derivatives of 1,3-diaryl-2-propene-1-ones to decide whether they can be successfully developed for use in chemotherapy of trichomonosis. In particular, the nature of the substitutions at R₁, R₂, R₃ and R₄, should be studied further.

Table 2: Cytotoxicity activities of Five Chalcones, Metrinidazole (MTZ) and Acetylsalicylic acid (ASA) on formaldehyde fixed bovine erythrocytes. Values are Means \pm SEM of triplicate tests.

Treatments	Concentrations where agglutination occurs (mg/ml)	Heamagglutination Titre values
2'-Hydroxy,2,4-dimethoxychalcone[5]	0.375 \pm 0.00	2.67
4-Hydroxychalcone [9]	0.25 \pm 0.06	4.06
2'-Hydroxy-4-methoxychalcone [3]	1.25 \pm 0.34	0.80
2'-Hydroxy-4-chlorochalcone [6]	1.75 \pm 0.56	0.57
3,4,4'-Trimethoxychalcone [8]	1.50 \pm 0.00	0.67
Metrinidazole [MTZ]	0.375 \pm 0.00	2.67
Acetylsalicylic acid [ASA]	1.25 \pm 0.34	0.80

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