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Synthesis and antigiardial activity of formononetin analogues

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The synthesis and antigiardial activity of formononetin analogues is hereby reported. Formononetin, an isoflavone commonly obtained from the Leguminoseae family has previously been reported to possess antigiardial activity. In this paper, 10 analogues of formononetin were synthesized and tested for antigiardial activity. One compound was found to be active, 3 were moderately active and 6 were found to be inactive. All the 10 analogues were found to be inactive against other protozoa such as *Trichomonas vaginalis* and *Tritrichomonas foetus*.

Keywords Antigiardial, Formononetin, Activity

INTRODUCTION

Protozoal infections are a major global problem causing long term disability and death, with severe medical and psychological consequences around the world. Giardiasis, caused by *Giardia lamblia* (syn. *Giardia intestinalis*, *Giardia duodenalis*), is estimated to affect up to 33% of the population in developing countries [1]. Other reports indicate that the prevalence of human giardiasis in developing countries is about 20% and in some cases up to 43% while the prevalence in developed countries is about 5% [2]. Giardiasis is characterized by diarrheal disease and malabsorption syndrome in the severe cases. The infection is the most common cause of waterborne outbreaks of diarrhea in the United States and is occasionally seen as a cause of food-borne diarrhea [3]. The treatment of giardiasis has involved the use of nitroimidazoles such as metronidazole, benzimidazoles such as albendazole, nitazoxanide, paromomycin, furazolidine and quinacrine (Figure 1) [4-7]. Resistance against the drug of choice, metronidazole, has been reported [1,7]. There is therefore a need to search for new drug molecules for the management of giardiasis.

Formononetin is an isoflavone that is widespread in the Leguminoseae family. It has previously been reported to possess various pharmacological activities such as the inhibition of proliferation of prostate, breast and colon cancer cells, wound and bone fracture healing, antihypertensive activity as well as anti-infective activities especially against giardiasis where it is reported to be more potent than metronidazole, the drug of choice in the treatment of giardiasis [8-11]. The mechanism of action for antigiardial activity has been proposed to be rapid detachment of trophozoites [12]. The antigiardial activity of the naturally occurring analogues of formononetin such as daidzein has been reported, but there is a need to report on the activity of synthetic analogues. This paper reports the synthesis and antigiardial activity of formononetin analogues.

EXPERIMENTAL

Synthesis of formononetin analogues was carried out by modification of rings A and B as well as the benzopyranone linker. Modifications on the benzopyrone linker were achieved through various synthetic schemes (Scheme 1) to yield 2 compounds **1** (**PCK03**) and **2 (PCK05)**). Modifications on ring A and ring B were achieved through similar synthetic schemes. For ring A modifications, the starting materials had varied substituents at either position 5 or 6 while for ring B modifications, the scheme started with protection of the para-hydroxyl group on ring A (Scheme 2) to yield compounds 3 to 8 (**PCK33C**, **PCK36B**, **PCK39B**, **PCK43B**, **PCK44E** and **PCK46C**). Compounds 9 (**PMK01**) and 10 (**PMK02**) were obtained commercially.

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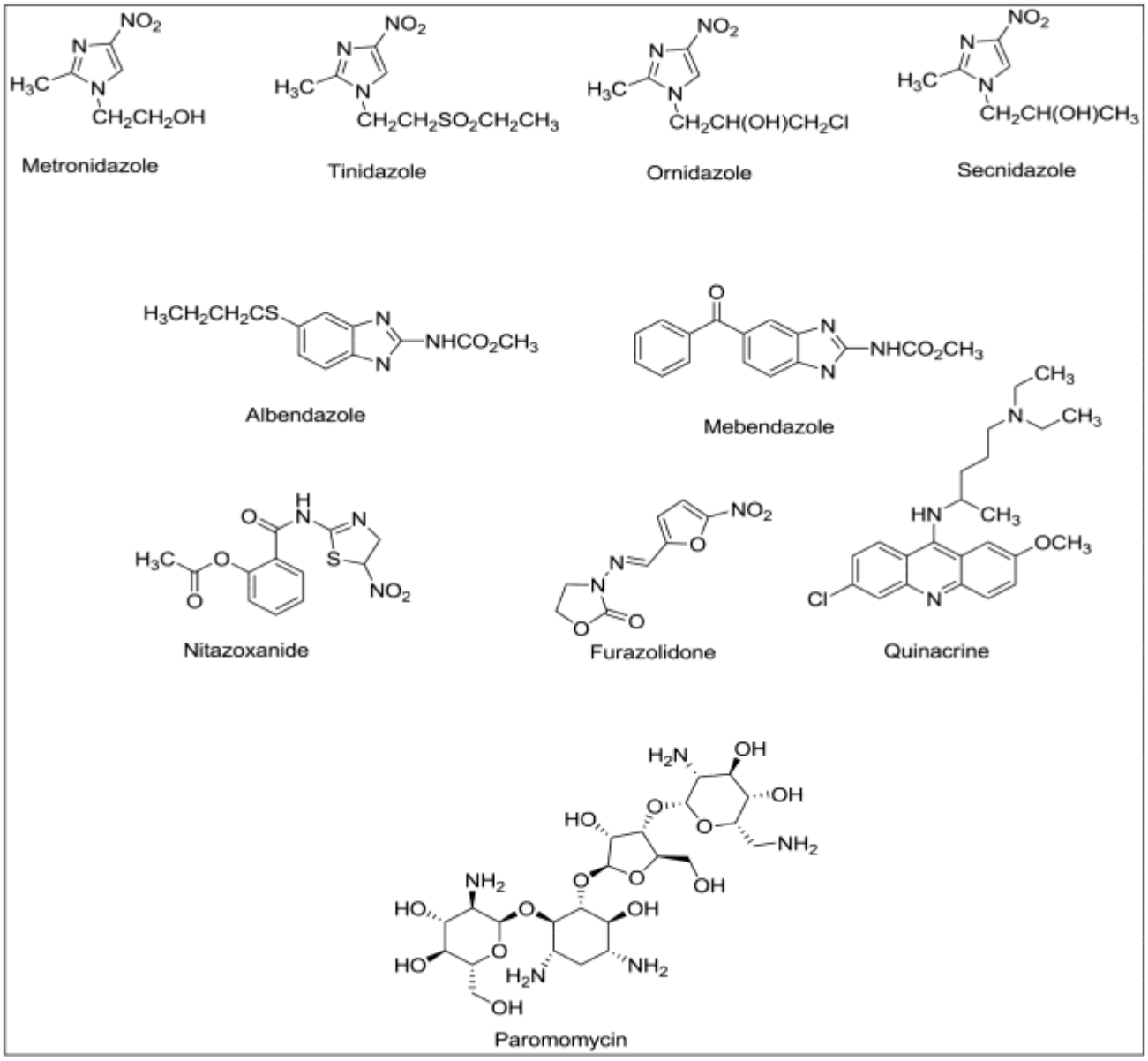
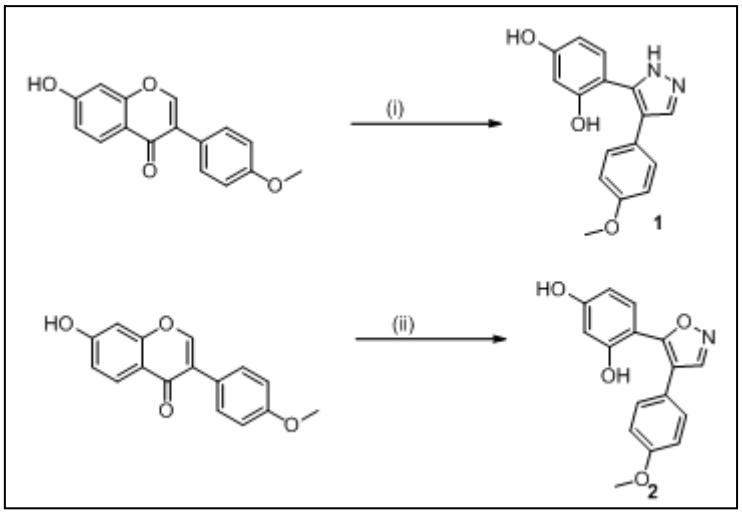
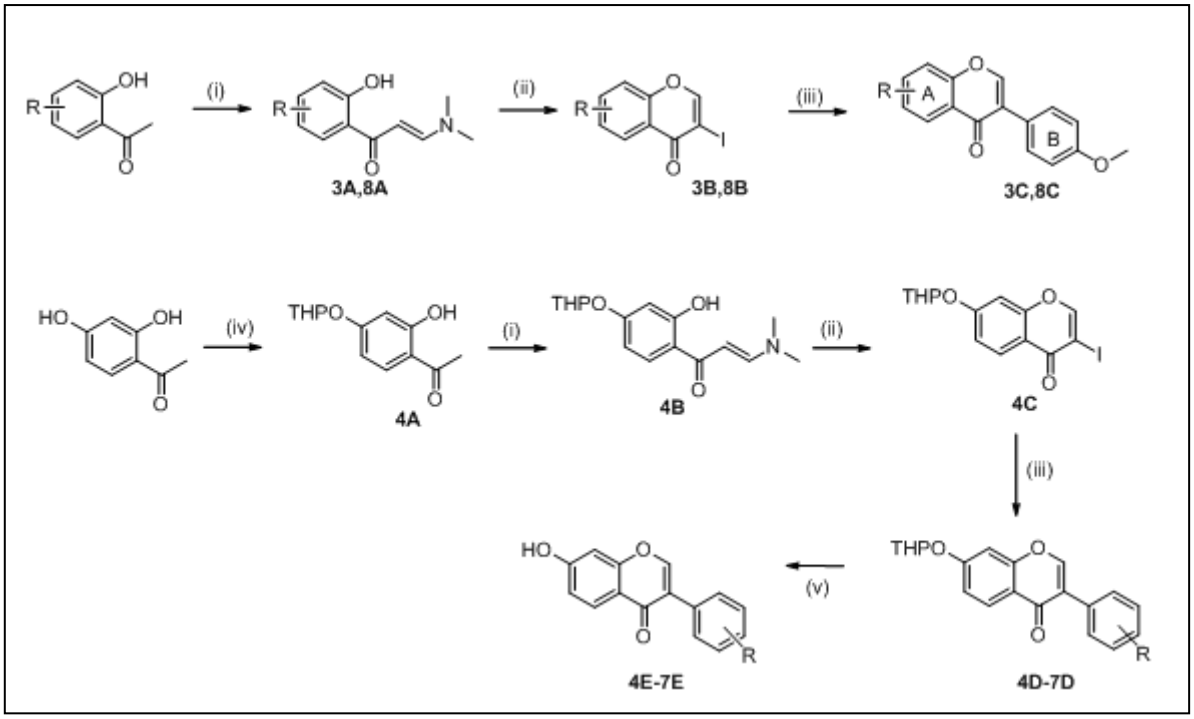


Figure 1. Examples of antigiardial compounds of clinical significance.



Scheme 1: *Reagents and conditions*: (i) $N_2H_4.H_2O$; EtOH, reflux, 60-80°C, 2h (ii) Hydroxylamine hydrochloride, N-methylmorpholine, EtOH, Reflux, 80°C, 8h



Scheme 2: *Reagents and conditions*: (i) DMF-DMA, 95°C, 3h (ii) I_2, pyridine, $CHCl_3$, rt, 12h, (iii) $ArB(OH)_2$, Pd/C, Na_2CO_3, DME/H_2O, 45°C, 4h (iv) DHP, PPTS, DCM, rt, 4h (v) p-TsOH, MeOH, THF, 60°C, 2h

For antiprotozoal activity, the isolate 30236 of *T. vaginalis* from American Type Culture Collection (ATCC) and the isolate TFK of *T. foetus* isolated by Dr. H. Guida (Embrapa, Rio de Janeiro, Brazil) from the urogenital tract of a bull were used. The isolate WB of *G. lamblia* kindly donated by Dr. Adriana Lanfredi Rangel (FIOCRUZ, Bahia, Brazil) was used. The results were qualitatively expressed considering viable trophozoites (normal morphology and motility) compared to untreated parasites [13, 14]. Compounds that revealed potential activity against *G. lamblia* in the screening assay were tested in the quantitative assay.

RESULTS

All the compounds tested were inactive against *T. vaginalis* and *T. foetus*. This corroborates well with previously reported data where formononetin was found to be inactive against other protozoa tested [11,12]. Four compounds exhibited some antigiardial activity (Table 1).

The compound **PMK02** presented the highest activity against *G. lamblia* trophozoites at 1.0 µM (Figure 2). As expected, the positive control metronidazole (100 µM) reduced 100% of parasite viability (data not shown).

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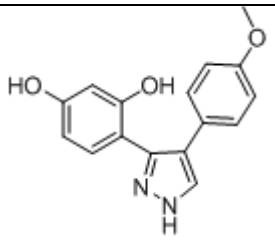
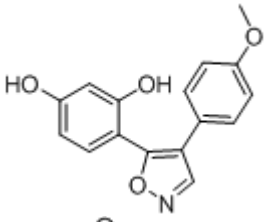
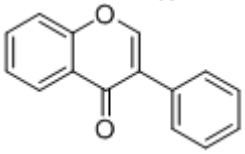
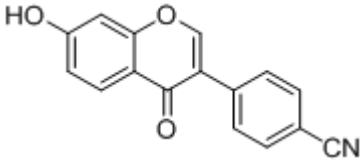
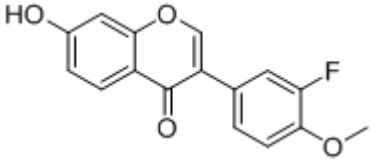
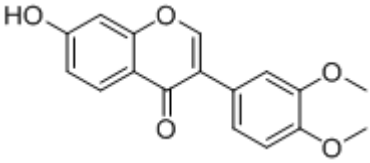
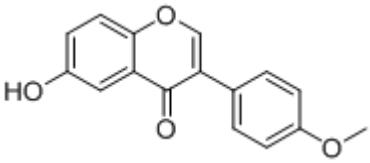
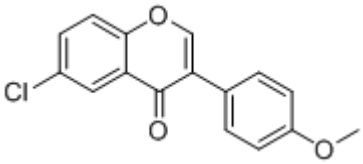
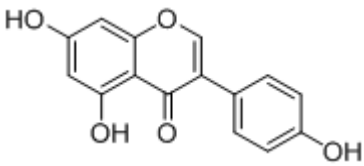
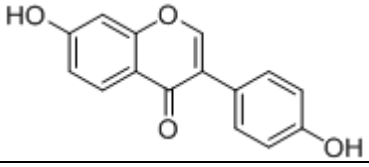
Table 1: Antiprotozoal activity of formononetin analogues

	MW	STRUCTURE	Solubility	*T. vaginalis*	*T. foetus*	*G. lamblia*
PCK03	282.29		>200	Inactive	Inactive	Inactive
PCK05	283.28		>200	Inactive	Inactive	Moderately active
PCK33C	222.24		80	Inactive	Inactive	Active
PCK36B	263.25		80	Inactive	Inactive	Moderately active
PCK39B	286.25		40	Inactive	Inactive	Inactive
PCK43B	298.29		80	Inactive	Inactive	Inactive
PCK44E	268.26		20-40	Inactive	Inactive	Moderately active
PCK46C	286.71		10-20	Inactive	Inactive	Inactive
PMK01	270.24		40	Inactive	Inactive	Inactive
PMK02	254.24		20-40	Inactive	Inactive	Active

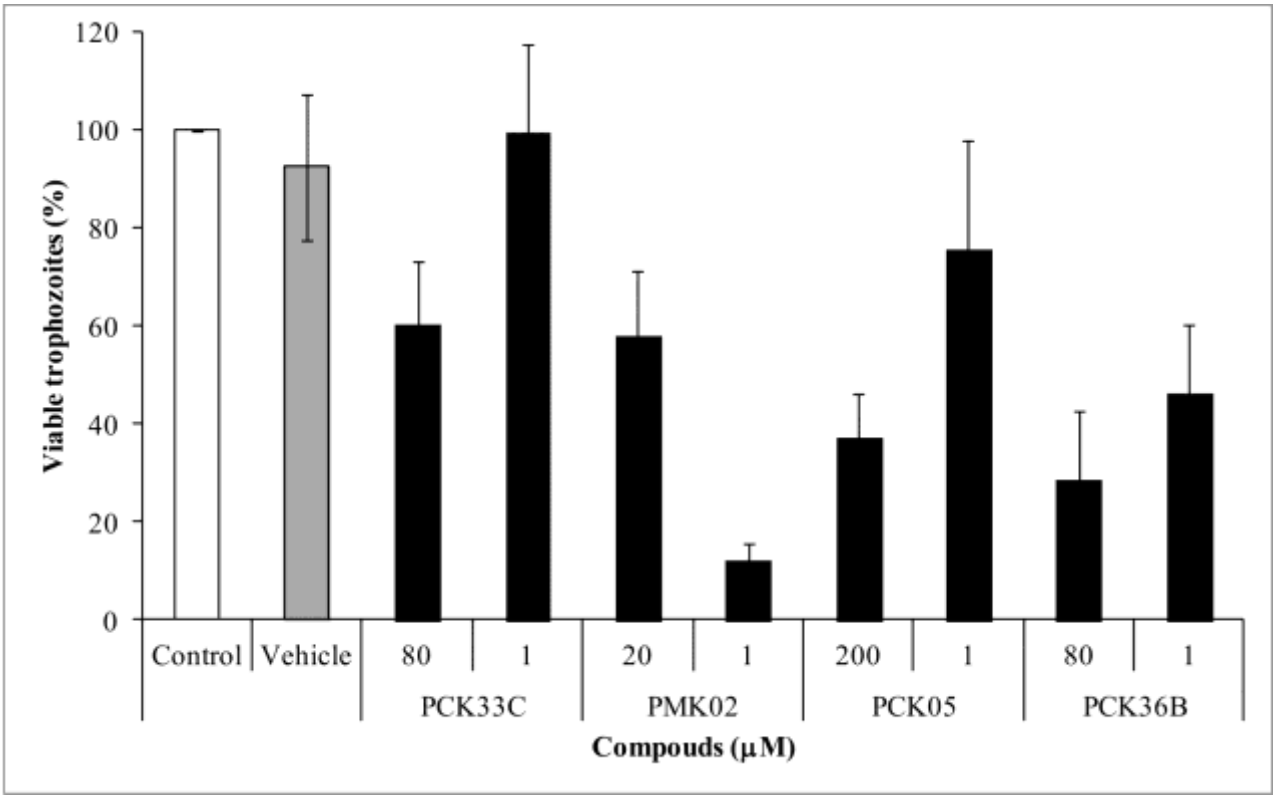


Figure 2. Antigiardial activity of four analogues of formononetin.
Control means only trophozoites in TYM medium (without compounds), and vehicle means control for solubilization with 0.6% DMSO. Bars represent the mean ± SD of four different experiments (parasite suspensions), all in triplicate.

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