ANTICOCCIDIAL ACTIVITY OF NOVEL SEMI-SYNTHETIC ANALOGUES OF FRENOLICIN B (PART I)

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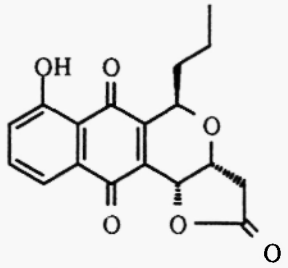
Abstract: Semi-synthetic aromatically substituted analogues of the naphthopyranquinone, frenolicin B **1**, have been produced and their biological activity as anticoccidial agents investigated *in vivo*.

INTRODUCTION

Coccidiosis is a devastating disease which causes severe economic losses in the poultry industry. Existing anticoccidials are becoming less effective as resistance has built up to many of the agents in commercial use and so the need to discover and develop new classes of coccidiostats which are not cross-resistant with existing agents is a significant challenge to the Animal Health industry.

The naphthopyranquinone, frenolicin B **1**, has previously been reported to possess *in vivo* anticoccidial activity in broilers against the protozoan parasite *Eimeria tenella*.[1] Our *in vivo* studies confirmed that Frenolicin B **1** was efficacious in a standard 7 day chick model[2] with activity at 25-50ppm when dosed in feed against a polyether ionophore antibiotic resistant field isolate of *Eimeria tenella*. This encouraging efficacy led us to investigate the potential for semi-synthetic analogues of **1** to deliver improved anticoccidial activity.



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RESULTS AND DISCUSSION

Initial efforts focused on modifications of the phenolic hydroxyl group present in **1**. O-Methylation of **1** was readily achieved using with methyl iodide and silver (I) oxide to give the methyl ether **2** (97%).[3] In an analogous fashion the allyl ether **3** was prepared (89%) and then subjected to a reductive Claisen rearrangement[4] (zinc/glacial acetic acid in toluene at 100°C.) to yield **4** (30%) (Figure 1).

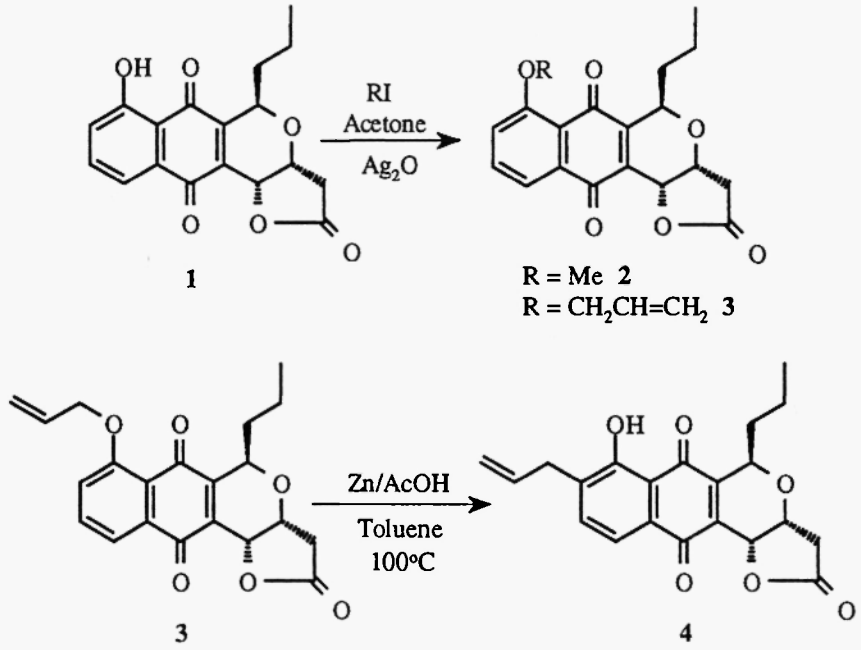
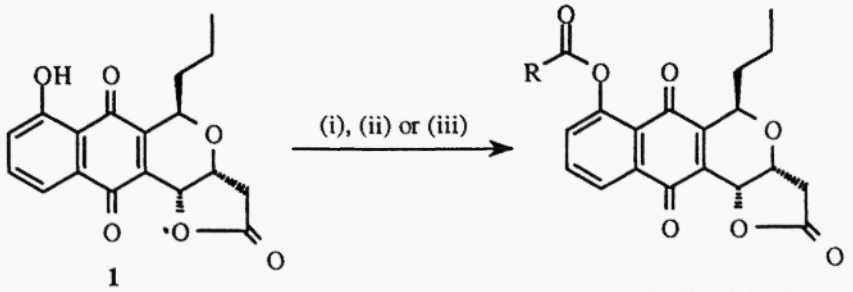


Figure 1

Acetylation of **1** with acetic anhydride gave the acetate **5** (62%), whilst reaction with succinic acid mono-*t*-butyl ester and dicyclohexylcarbodiimide (DCC) followed by deprotection with trifluoroacetic acid, yielded the succinate ester **6** (26% over 2 steps). The glycine ester **7** was synthesised *via* a DCC mediated coupling of N-Boc-glycine with **1** followed by deprotection to the primary amine with trifluoroacetic acid (21% over 2 steps) (Figure 2).



(i) Acetic anhydride
(ii) (a) succinic acid mono-*t*-butyl ester, DCC
(b) Trifluoroacetic acid
(iii) (a) N-Boc-glycine, DCC
(b) Trifluoroacetic acid

(i) R = Me **5**
(ii) R = $CH_2CH_2CO_2H$ **6**
(iii) R = CH_2NH_2 **7**

Figure 2

Conversion of **1** to the triflate derivative **8** was readily achieved upon reaction of **1** with triflic anhydride (77%). Subsequent reaction of **8** with phenyl boronic acid and Pd(0) catalysis under standard Suzuki coupling conditions yielded the biaryl analogue **9** (13%)(Figure 3).

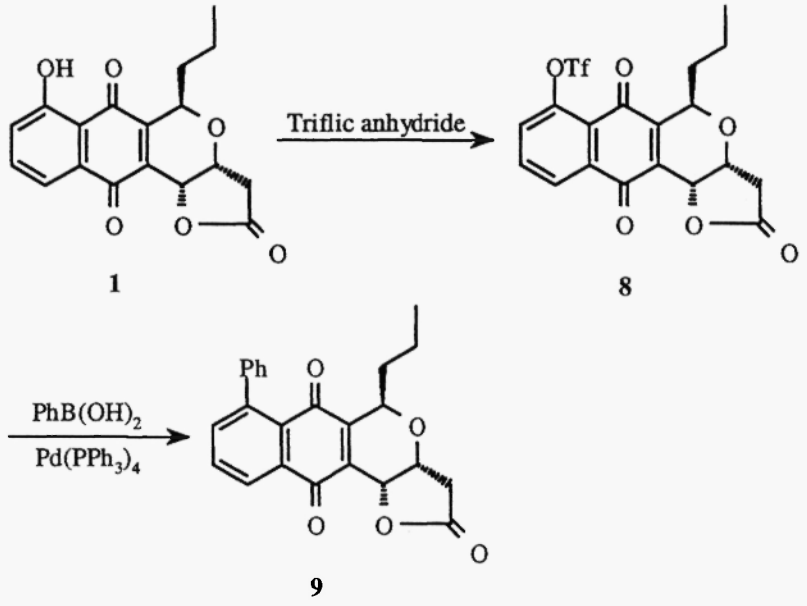


Figure 3

Aromatic electrophilic substitution of **1** yielded a range of analogues. Chlorination with a saturated solution of chlorine in glacial acetic acid gave solely the *para* chloro derivative **10** (15%) whilst bromination with a saturated solution of bromine in glacial acetic[5] acid gave a 2:1 mixture of both

the *ortho* and *para* substituted compounds **11** and **12** (60%) which were easily separated by column chromatography on silica (Figure 4).

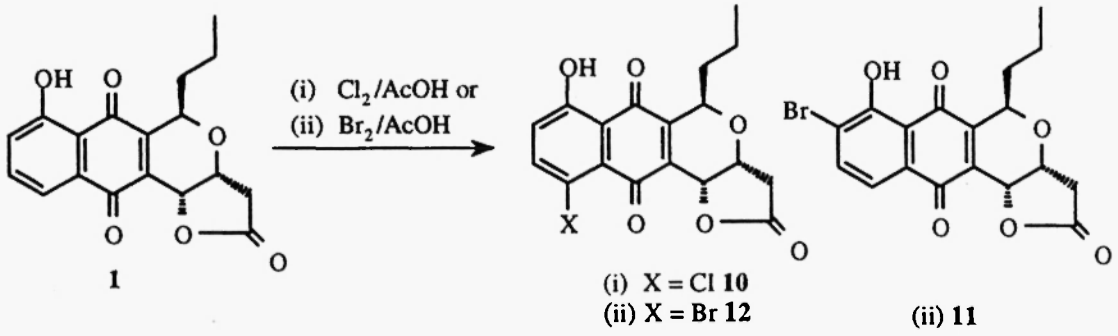


Figure 4

Nitration of **1** using nitronium tetrafluoroborate in acetonitrile yielded two products; namely the *ortho* and *para* analogues **13** and **14** in 1:1 ratio (64%). These regioisomers were readily separated by chromatography (Figure 5).

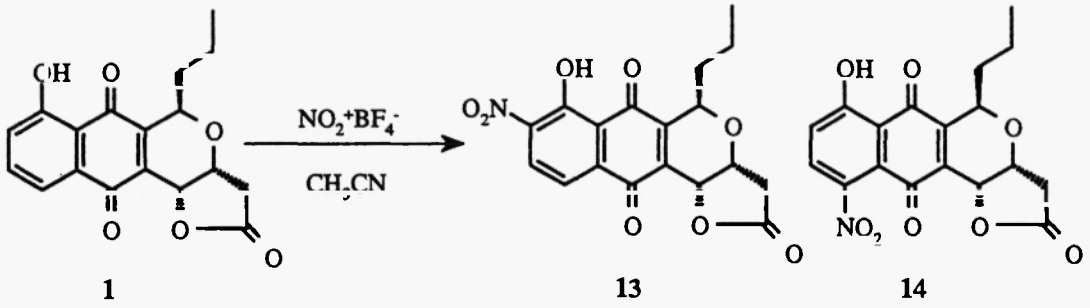


Figure 5

Attempted reduction of the nitro groups in both **13** and **14** using palladium on charcoal yielded a ring opened intermediate **15** which was then oxidatively cyclised with pyridine and air to give the anilines **16** (25% from **13**) and **17** (25% from **14**) (Figure 6).

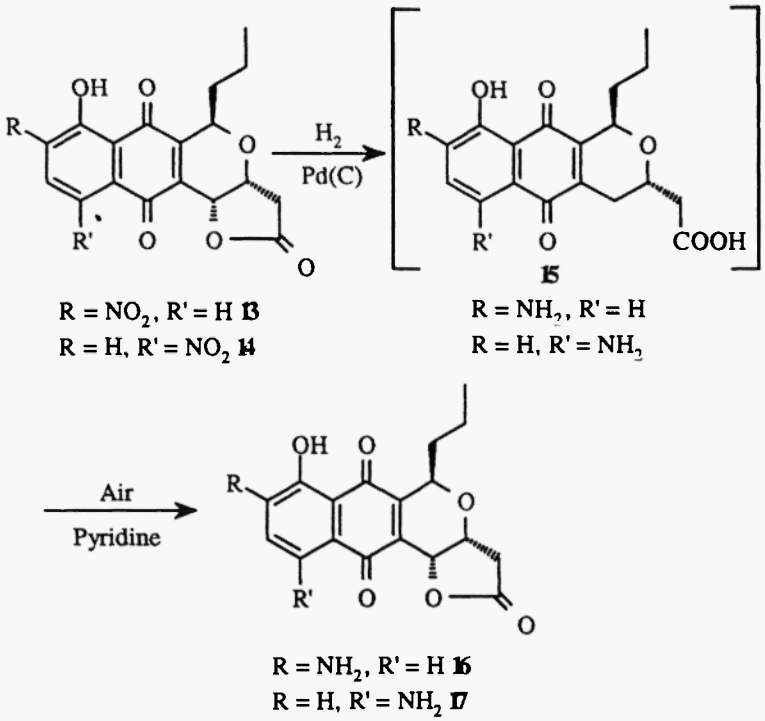


Figure 6

The anilines **16** and **17** were readily diacetylated in an excess of acetyl chloride and diisopropylethylamine to give **18** (21%) and **19** (60%). Prolonged heating of **16** with triethyl orthoacetate under acid catalysis yielded the benzoxazole analogue **20** (36%) (Figure 7).

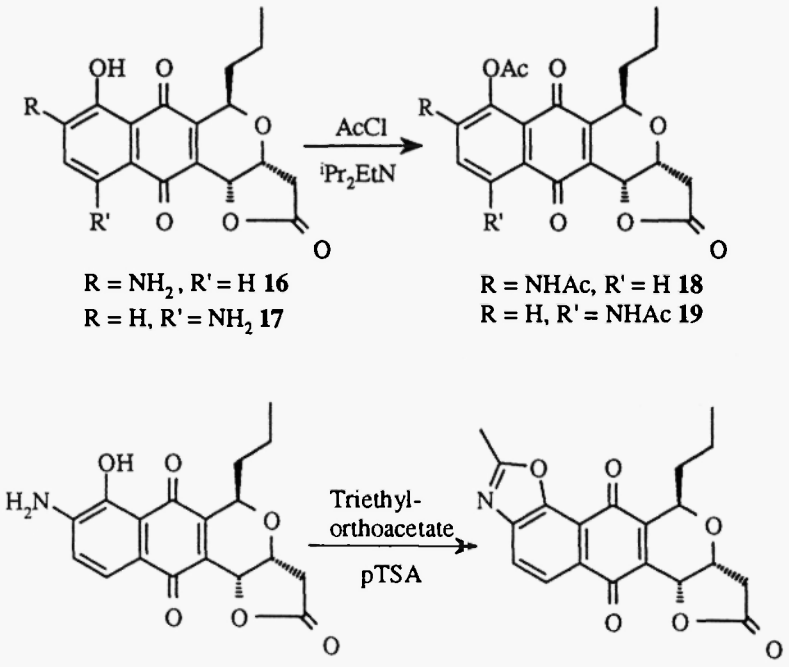


Figure 7

The anticoccidial activities of some of the analogues described herein were measured using a standard 7 day *in vivo* chick model utilising polyether ionophore resistant field isolates of *Eimeria tenella*.[2] The results of these tests are shown below (Table 1) where the effective dose is defined as the in feed dose (ppm) required to give a mean (n=5) reduction in cecal lesion score of >80% with respect to non-medicated,controls.

Table 1

Compound	Effective Dose (ppm)	Compound	Effective Dose (ppm)
1	25-50	2	>50
3	>100	4	>50
5	50	6	12.5
7	12.5	8	>100
9	>100	10	>100
11	100	12	>50
14	>50	17	>50
18	>50	19	100

In general these results indicate that there are limited opportunities to improve *in vivo* activity *via* modification of the aromatic ring system of frenolicin B. *Ortho* substituted compounds **4, 11** and **18** are less potent than **1** which indicates that substitution at this position is detrimental to anticoccidial activity. Similarly, *para* substituted analogues **10, 14, 17** and **19** show reduced anticoccidial activity *in vivo*.

Replacement of the phenolic hydroxyl group as in **9** or substitution at that position to give a hydrolytically stable product as in **2, 3** and **8** also leads to a reduction in *in vivo* anticoccidial efficacy.

The most promising analogues synthesised were the acetate based analogues **5-7**. These analogues were designed as pro-drugs and possess various chemical stabilities at differing aqueous pH. The simple acetate **5** retained moderate *in vivo* anticoccidial activity, whilst the more aqueous soluble succinate ester **6** and glycine ester **7** show improved potency *in vivo* with good anticoccidial efficacy demonstrated at 12.5 ppm when dosed in feed. Further work is underway to find the optimal pro-drug group that gives improved *in vivo* efficacy coupled with good chemical stability.

CONCLUSION

Several aromatically substituted analogues of frenolicin B have been synthesised and their anticoccidial efficacy measured *in vivo*. In general, these analogues were less potent than frenolicin

B, however, the more aqueous soluble ester pro-drugs **6** and **7** demonstrated improved *in vivo* activity when compared to frenolicin B.

ACKNOWLEDGEMENTS

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