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The design and synthesis of novel inhibitors of NADH:ubiquinone oxidoreductase

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Abstract—Potent new inhibitors of NADH:ubiquinone oxidoreductase (complex I) have been designed, with the help of molecular modelling, by hybridisation of known complex I inhibitors with inhibitors of cytochrome c oxidoreductase. The most interesting compound was the chromone **7** which was a selective inhibitor of complex I (IC_{50} 15 nM) and showed acaricidal activity against spider mites.

The enzyme complexes NADH:ubiquinone oxidoreductase (E.C. 1.6.99.3; complex I) and cytochrome c oxidoreductase (E.C. 1.10.2.2; complex III or bc_1 complex) are the first and third electron transfer complexes of the mitochondrial respiratory chain. Complex I catalyses the transfer of two electrons from NADH to ubiquinone (coenzyme Q) to give the corresponding ubihydroquinone. The bc_1 complex transfers electrons from the ubihydroquinone to cytochrome c via the Rieske iron–sulfur protein and cytochrome c_1. These redox processes result in translocation of protons across the inner mitochondrial membrane, thereby establishing an electrochemical (proton) gradient which drives the synthesis of ATP.[1]

Several commercial acaricides, including fenazaquine (**1**),[2,3] pyridaben (**2**)[3,4] and tebufenpyrad (**3**)[4] are potent inhibitors of complex I which are believed to bind at, or very close to, a ubiquinone binding site.[1] The naturally occurring chromone stigmatellin (**4**) and its synthetic analogue **5** are potent inhibitors of the bc_1 complex which bind at a ubihydroquinone site.[5,6] These two bc_1 complex inhibitors share the same heterocyclic moiety but have quite different side chains. In contrast, the complex I inhibitors **1**–**3** contain quite different heterocyles but all contain the same 4-*t*-butylphenyl moiety in their side chain. This communication describes the results of our efforts to design new acaricidal complex I inhibitors via hybridisation of the complex I inhibitors **1**–**3** with the bc_1 inhibitors **4** and **5** (Fig. 1).

We postulated that the heterocyclic ring systems in structures **1**–**5** were mimicking a quinone or hydroquinone nucleus and that the lipophilic side chain together with the ring substituents were determining the specificity for either complex I or the bc_1 complex. With the help of molecular modelling, we sought to design new acaricidal complex I inhibitors by changing the ring substituents and lipophilic side chains of the bc_1 inhibiting chromones **4** and **5** to make them more closely resemble those found in the complex I inhibiting structures **1**–**3**. As a result of these efforts, the 2-benzylmercaptochromones **6** and **7** were proposed as potential synthetic targets. They both contained a lipophilic *t*-butylphenyl side chain of the type found in compounds **1**–**3** and an unsubstituted fused phenyl ring as found in fenazaquin (**1**). In addition, the relationship between the lipophilic side chain and the heterocyclic ring carbonyl group closely resembled that seen in pyridaben (**2**). Using the published X-ray crystal structure of tebufenpyrad (**3**)[7] as a starting point, molecular modelling studies suggested that the *t*-butyl carbon atoms and the heterocyclic ring nitrogens (in **1** and **3**)/carbonyl-oxygens (in **2**, **6** and **7**) of structures **1**, **2**, **3**, **6** and **7** could be sensibly overlaid. Initial studies were performed using simple stick models and this work was subsequently semiquantified using computational methods.[8] Thus, the *t*-butyl tertiary-carbons and ring nitrogens/carbonyl oxygens of structures **1**, **2** and **7** were superimposed over the equivalent centres in the X-ray crystal structure of **3**.

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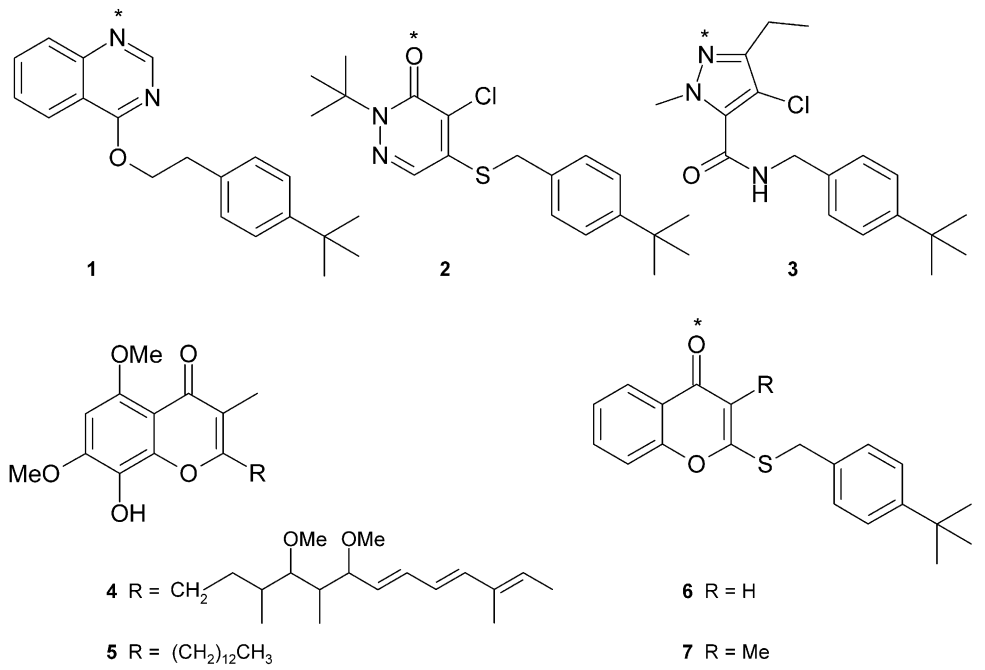


Figure 1. Inhibitors of the electron transfer complex I and the bc_1 complex. The heterocyclic ring nitrogens (in **1** and **3**) and carbonyl-oxygens (in **2**, **6** and **7**) used in the molecular modelling overlay are marked with an asterisk (*).

All structures were then conformationally minimised within the imposed constraints that the *t*-butyl tertiary-carbons and the ring nitrogens/carbonyl-oxygens, respectively, were confined to stay within 0.15 Å of each other. The resulting overlaid structures are shown in Figure 2 and considering the relatively few constraints that were imposed, the degree of overlap was considered very encouraging. In particular, all four heterocyclic rings ended up in a very similar plane and the side chains connecting the heterocyclic and *t*-butylphenyl moieties were all well aligned. The minimised structure for tebufenpyrad (**3**) remained very close to the published X-ray crystal structure.[7]

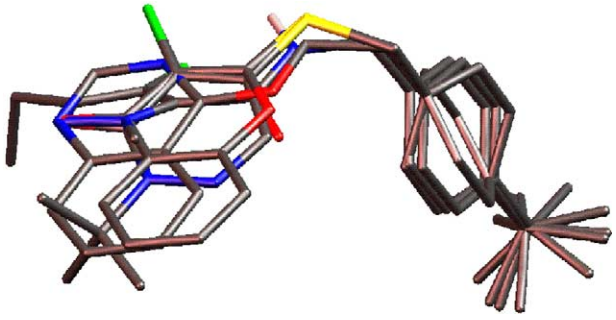
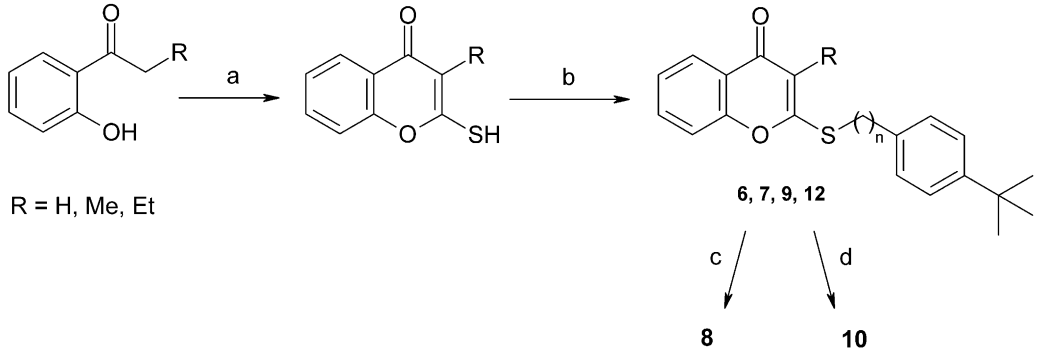
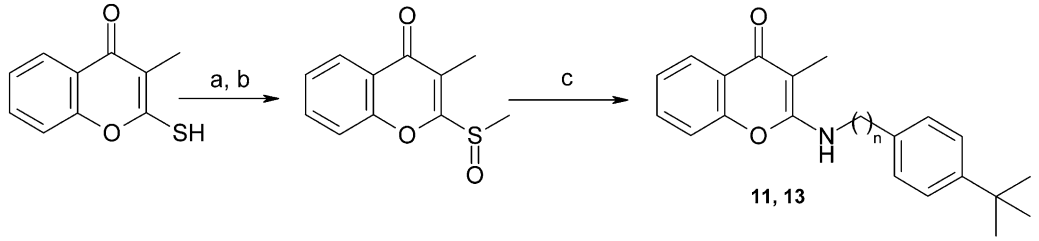


Figure 2. Diagram showing the superimposed structures **1**, **2**, **3** and **7**.

Based upon the results of the molecular modelling studies described above, the chromones **6** and **7** were synthesised by adapting the methodology described by Bantick and Suschitzky[9] as summarised in Scheme 1. Biochemical testing on submitochondrial membranes prepared from house fly flight muscles[10] showed that compounds **6** and **7** inhibited the NADH-dependent reduction of cytochrome c (IC_{50} 500 and 8 nM, respectively) but had no effect on succinate-dependent cytochrome c reduction. These results indicated that the new chromones were binding to complex I and not to the bc_1 complex. Under the same assay conditions, the Stigmatellin analogue **5**[11] was (as expected[5]) a potent inhibitor of the bc_1 complex (IC_{50} 2 nM) but had no effect on



Scheme 1. Reagents and conditions: (a) 3 equiv *t*-BuOK, 1.1 equiv CS_2, toluene, rt, 4 days then AcOH to pH 5; 30–67%; (b) 1.5 equiv 4-*t*-$BuC_6H_4(CH_2)_nOH$, 1.5 equiv $EtO_2CN{=}NCO_2Et$, 1.5 equiv PPh_3, THF, rt, 24 h; 30–64%; (c) from compound **6** (R = H): 1 equiv NCS, AIBN, CCl_4, Δ, 5 h; 44%; (d) from compound **7** (R = Me): 3 equiv MCPBA, CH_2Cl_2, rt, 16 h; 92%.



Scheme 2. Reagents and conditions: (a) 1 equiv K_2CO_3, 1.1 equiv MeI, acetone, rt, 20 h; 75%; (b) 1 equiv MCPBA, CH_2Cl_2, 0 °C, 16 h; 67%; (c) 1.1 equiv 4-*t*-$BuC_6H_4(CH_2)_nNH_2$, MeCN, 50 °C, 24 h; 57–72%.

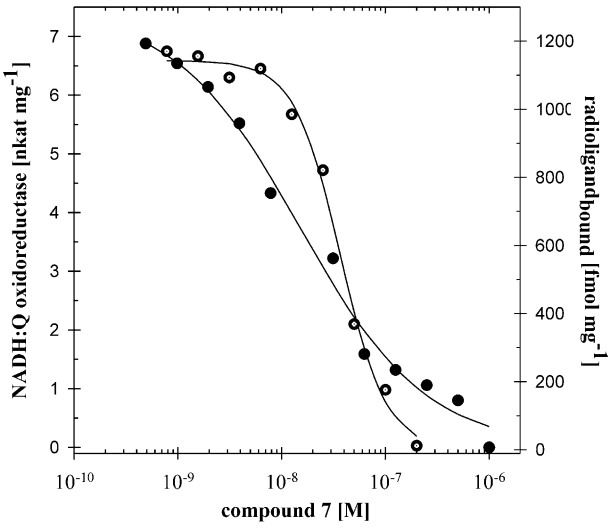
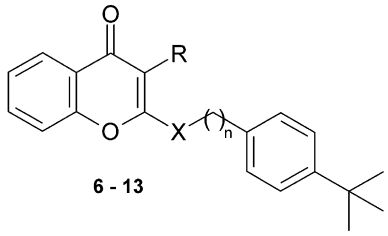


Figure 3. Inhibition of purified NADH:Q oxidoreductase and inhibition of specific radioligand binding to purified complex I from *Musca domestica* as a function of chromone **7** concentration. Flight muscle complex I (25 μg protein per mL) was purified as described; inhibition of enzyme activity (●); inhibition of specific radioligand binding (○).[10]

complex I activity. Further testing showed that compound **7** inhibited purified soluble house fly complex I[10] with an apparent IC_{50} of 15 nM (Fig. 3), whereas compound **6** was much less active (IC_{50} = 118 nM). The enzymatic results were further substantiated by equilibrium binding data:[10] Compound **7** inhibited the specific binding of the radiolabelled complex I inhibitor ^{3}H-AE F119209[12] in a concentration-dependent manner (Fig. 3) with an IC_{50} of 38 nM and an apparent K_i of 25 nM. Taken together, the biochemical results indicate that the chromone **7** occupies the same, or a closely overlapping, binding site to the known complex I inhibitors **1**–**3** and ^{3}H-AE F119209. Biological testing of compound **7** showed that it possessed acaricidal activity against spider mites (LD_{50} ca. 50 ppm) whereas chromones **5** and **6** were inactive.[13]

In order to probe the structure–activity requirements of this new lead, a number of other chromone analogues of compound **7** were prepared according to Schemes 1 and 2[9] and tested for biochemical[10] and biological[13] activity. The results (Table 1) showed that the 3-chloro compound **8** maintained biochemical and some biological activity, whereas the 3-ethyl substituted compound **9** was inactive. Oxidation of the sulfur (compound **10**) led to a 20-fold drop in inhibitory potency and loss of biological activity. Replacement of the sulfur by NH

Table 1. Inhibition of complex 1 (membrane bound) and acaricidal activity of the chromones **6**–**13**



Compd	R	X	*n*	Complex I IC_{50} (nM)[10]	TETRUR activity @ 300 ppm (%)[13]
6	H	S	1	500	0
7	Me	S	1	8	100
8	Cl	S	1	6	50
9	Et	S	1	>200	0
10	Me	SO_2	1	160	0
11	Me	NH	1	20	0
12	Me	S	2	10	80
13	Me	NH	2	200	0

(compound **11**) gave a reasonable inhibitor (IC_{50} 20 nM) which was, however, biologically inactive. The effect of elongating the connecting chain between the chromone and *t*-butylphenyl moieties was also investigated. Inhibitory potency and significant biological activity were maintained in the sulfur analogue **12** but the NH analogue **13** was inactive. Overall, there is a reasonably good correlation between in vitro and in vivo activity, whereby compounds with IC_{50} values of ≤10 nM were biologically active.

In conclusion, we have succeeded in designing and synthesising potent new inhibitors of complex 1, starting out from known bc_1 inhibitors.[14] The most interesting compound, chromone **7**, is a selective inhibitor of complex I (IC_{50} 15 nM) and exhibits acaricidal activity against spider mites.

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