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STRUCTURAL REQUIREMENTS FOR RESPIRATORY INHIBITION BY ROTENOIDS; IS AN INTACT B/C RING SYSTEM ESSENTIAL?

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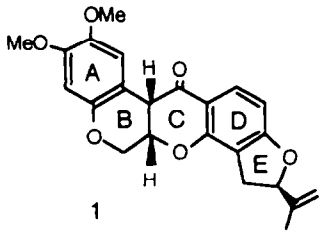
Abstract: Guided by structural comparisons between the classical electron transport inhibitor rotenone and other compounds with a similar mode of action, a new set of inhibitors has been designed and synthesized which relate to rotenone but lack the core B/C ring structure previously considered of major significance. Although less active than rotenone, significant levels of inhibition have been found for several analogues.

The natural *O*-heterocycle rotenone **1** is the major biologically active component of Derris resin, the crude root extract of *Derris elliptica*.[1] The use of ground Derris root as an insecticide was recorded in the scientific literature in 1848,[2] and the preparation still has applications as a botanical insecticide. Rotenone also shows antifeedant[3] and piscicidal properties[4] (useful in fish farming), and blocks microtubule formation.[5] It is a classical blocker of mitochondrial electron transport, acting as an inhibitor of NADH - ubiquinone dehydrogenase in Complex 1,[6] and it has long been used as a tool in the study of bioenergetics.



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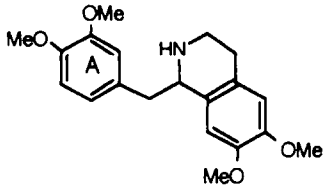
Several investigations of structure - activity relationships have been reported,[7] using mainly natural rotenoids and their transformation products. The information available to us suggests that few rotenoid relatives in which the B/C ring system is altered are active,and that the cleavage of these rings results in major loss of activity. In order to make progress in this area, it appeared to us to be very desirable to design and synthesize active structural variants in which the core rotenoid tetracycle is not obligatory. We decided to look for inspiration in structural comparisons between rotenone and other inhibitors believed to act at the same site. These include the natural products

piericidin A,[8] myxalamide D,[9] and papaverine[10] (tetrahydropapaverine **2** is shown, *v.i.*), and the synthetic benzimidazole **3**[11a] related to piericidin and ubicidin inhibitors.[11b]



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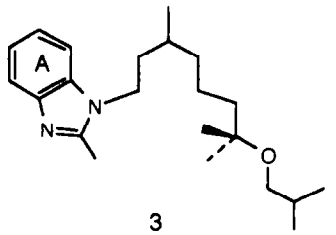

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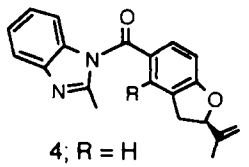
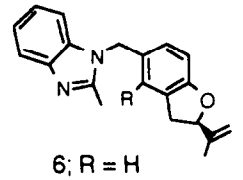
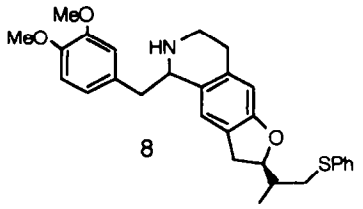
We have examined structural relationships within this group through modelling studies[12] which suggest that it would be possible to devise active rotenone analogues which do not contain the B/C system. The essential elements appear to be rings A,D, and E, or connected substructures, while B,C appear to determine the spatial disposition of these units. Thus tetrahydropapaverine **2** (a better fit to rotenone than the parent alkaloid) and benzimidazole **3** contain aromatic rings overlaying rotenone ring A, with the rest of the molecules connecting only to rotenone rings D and E. Similar observations can be made for piericidin, myxalamide and other compounds outside the scope of this communication. We decided to test the validity of these necessarily speculative notions through synthesis of 'hybrid' compounds **4** - **7** in which the 2-methylbenzimidazole unit of **3** is linked to isopropenylbenzofuran moieties of the rotenone D/E type, and of the tetrahydroisoquinolines **8** and **9**, related to both **1** and **2**. Compound **8** is the immediate synthetic precursor of **9**; discussion of the synthetic work will appear elsewhere. Thus structures **4**, **5**, **6**, **7**, **8**, and **9** represent the rotenoid system in which the rings B/C have been drastically remodelled.



4; R = H
5; R = OMe



6; R = H
7; R = OMe



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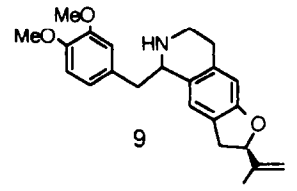

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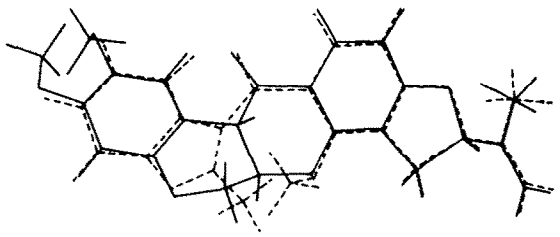
These products were then tested[13] for their ability to block NADH dehydrogenase in a preparation of submitochondrial particles obtained from blow fly flight muscle,[14] the conversion of NADH to NAD being monitored spectrophotometrically. The results, shown in the Table, are expressed as pI_{50} *i.e.* -log IC_{50}, and assume that all the activity is confined to a single stereoisomer.

Compound	pI50
(1)	8.70
(4)	7.82
(5)	6.60
(6)	7.30
(7)	6.82
(8)	7.90
(9)	6.76

Table; Inhibitory activities *vs.* NADH dehydrogenase



Overlay for Compounds **1** (—) and **5** (- - -)

The data show that all the compounds are good inhibitors, the best two **4** and **8** with relative activity 13.4 and 16% of rotenone, respectively. This indicates that it is is possible to replace rotenone rings B and C with retention of modest activity, and it is encouraging that these two compounds contain quite different replacements for the B/C system. Benzimidazole **4** has no ring C, and dihydropyran ring B is mimicked by the imidazole, and in the tetrahydroisoquinoline **8** neither rings B nor C are present.

Some reduction in activity is displayed by the remaining compounds, and this may perhaps be understood by their relative abilities to take on conformations close to that of rotenone. X-Ray studies[15] have revealed that rotenone can adopt two conformations in the crystalline state, although only one is present in solution.[16] The preferred shape has rings A/B and rings C/D/E in separate (approximate) planes forming a bend across the B/C junction. In compound **4**, the benzimidazole has a plane equivalent to the rotenone A/B plane; the dihydrobenzofuran and conjugated carbonyl group form a second plane, probably slightly twisted from the first to avoid steric clashes with the 2-methyl substituent of the imidazole. Removal of the carbonyl with loss of conjugation allows greater conformational freedom to **6**, with some reduction of activity. Introduction of a methoxyl group in **5** and **7**, to parallel the rotenone ring C oxygenation, brings major steric problems with the imidazole methyl giving rise to major conformation change, and substantially altering the relative orientations of ring A and the dihydrobenzofuran away from the arrangement in rotenone. As an illustration, the best fit for rotenone and compound **5** is shown in the figure; the conformation required for **5** is *ca.* 19 kcal mol^{-1} above its minimum, showing the extent of steric strain in adopting this conformation. In compound **8**, the isoquinoline nitrogen overlays the site of the rotenone carbonyl oxygen, and is maintained in the correct situation with respect to ring D by the fused ring system. It is interesting that compound **8**, with a bulkier but more lipophilic ring D attachment, is the more active of the pair. In summary, we have shown that it is possible to radically redesign the B/C ring system of the rotenoids, by calling on information obtained from structural comparisons with other inhibitors, with retention of moderate inhibitory activity. Two new types of inhibitor have emerged without an extensive exercise in analogue synthesis, and we believe that further work in this area will be fruitful.

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12 Molecular modelling was carried out within the SYBYL software package, using a multifit routine to predict energetically viable molecular overlays

13 * The particle suspension (2mm^3) was added to buffer (100mm^3, 0.3M sucrose, 20mM potassium phosphate, 2mM EDTA, pH 7.6) in a semimicro cuvette (1ml). The sample (1mm^3 solution in acetone) was added and incubated at 30° for 15min The sample was diluted with reaction buffer (880mm^3, 0 16M aspartate, 5mM EGTA, 0 02mM magnesium chloride, pH 7 4) The assay was initiated by the addition of NADH (20mm^3, 10mM solution) Conversion of NADH to NAD was monitored at 340nm spectrophotometrically with time at 30°C

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