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ANTHRAQUINONES RELATED TO RHEIN INHIBIT GLUCOSE UPTAKE INTO CHONDROCYTES. A MECHANISM FOR ANTI-OSTEOARTHRITIS DRUGS?

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Abstract: Rhein has been shown to inhibit the uptake of glucose into Ehrlich Ascites tumor cells. In this paper we show that a wide range of anthraquinones related to rhein can also inhibit glucose uptake into chondrocytes, many significantly more than the parent molecule.

Diacetyl rhein (DAR), has been used for the treatment of osteoarthritis (OA)[1,2]. The molecule is completely deacetylated in the body to the active metabolite, rhein[3]. Various studies have shown rhein to have a diverse range of activities[4, 5,6] including inhibition of glucose uptake. One of the more consistent findings from animal models of OA was that there was an initial increase in metabolic rate[7,8] and later, perhaps due to acidosis, cell death. Compounds which could inhibit the uptake of glucose may reduce flux though the glycolytic pathway and hence reduce tissue damage due to the formation of lactate.

Obviously the supply of glucose to the avascular cartilage chondrocytes may greatly influence the behaviour of these cells. Moreover, experiments in fibroblasts have shown a significant factor putatively involved in the osteoarthritic process, interleukin I, stimulates hexose transport by increasing the expression of glucose transporters[9]. Should compounds such as rhein be capable of modulating the uptake of glucose by cartilage cells it may in part explain its beneficial effects in the treatment of osteoarthritis.

Chemistry

Commercially available[10] diacetyl rhein **1** was the starting material for the synthesis of the 4,5-dialkylated anthraquinones in scheme 1. Thus diacetyl rhein **1** was hydrolysed quantitatively to rhein **2** with aqueous sodium carbonate and alkylated to give ethers **3-6** in yields ranging from 46-77%, with either dialkyl sulfates or alkyl halides and potassium carbonate in dioxan/acetone.

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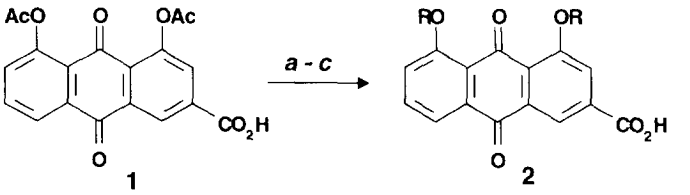
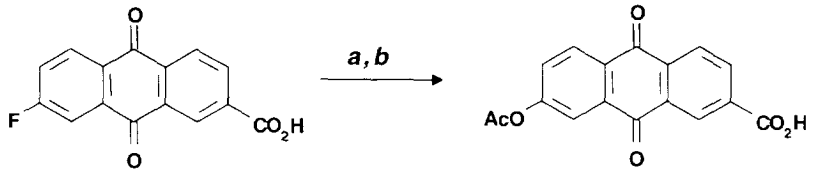


R		% Yield
H	(2)	100
Me	(3)	69
Et	(4)	61
Pr	(5)	77
Bn	(6)	46

a) Na_2CO_3, H_2O, b) RX, K_2CO_3, Dioxan, Acetone, c) NaOH, Dioxan, Water.
(RX = Me_2SO_4, Et_2SO_4, PrI, BnBr)

Scheme1

Mono and trisubstituted anthraquinones were also synthesised, thus the 7-hydroxy and 7-acetoxy analogues were synthesised by nucleophilic substitution of the 7-fluoroanthraquinone-2-carboxylic acid[11] under phase transfer conditions in high yield to give the hydroxyanthraquinone **7** which was subsequently acetylated to give **8**. (scheme 2). Also for this study were prepared 2 tri-substituted anthraquinone carboxylic acids **9** and **10** utilising a directed lithiation strategy developed by Snieckus[12] and published previously.[13]

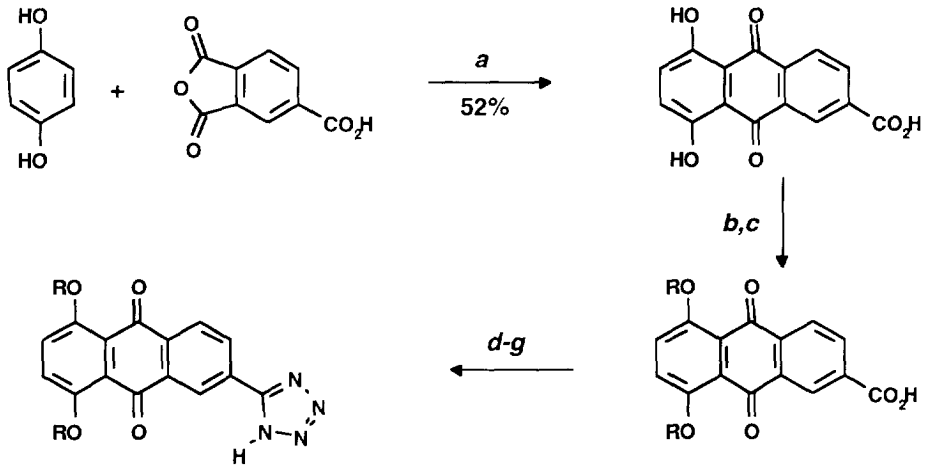


Step	% Yield
a (OH, 7)	98
b (OAc, 8)	61

a) *n*-Bu_4NCl, NaOH, PhCl, H_2O, 110°C, 6h, b) Ac_2O, H_2SO_4.

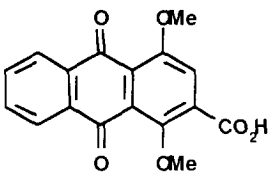
Scheme 2

To further understand the structure activity requirements of substitution around the anthraquinone nucleus 5,8-disubstituted anthraquinones were synthesised (scheme 3).



R	% Yield
Me (16)	61

R	% Yield
Ac (11)	92
Me (12)	69
Et (13)	61



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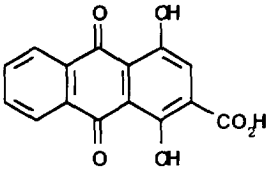

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a) $AlCl_3$, NaCl, 170°C, 2h, b) RX, K_2CO_3, Dioxan, Acetone, c) NaOH, Dioxan, Water

d) $SOCl_2$, Pyridine, DCE, 80°C, e) *t*-$BuNH_2$, 0.5h, 20°C, f) PCl_5, Toluene, 95°C,

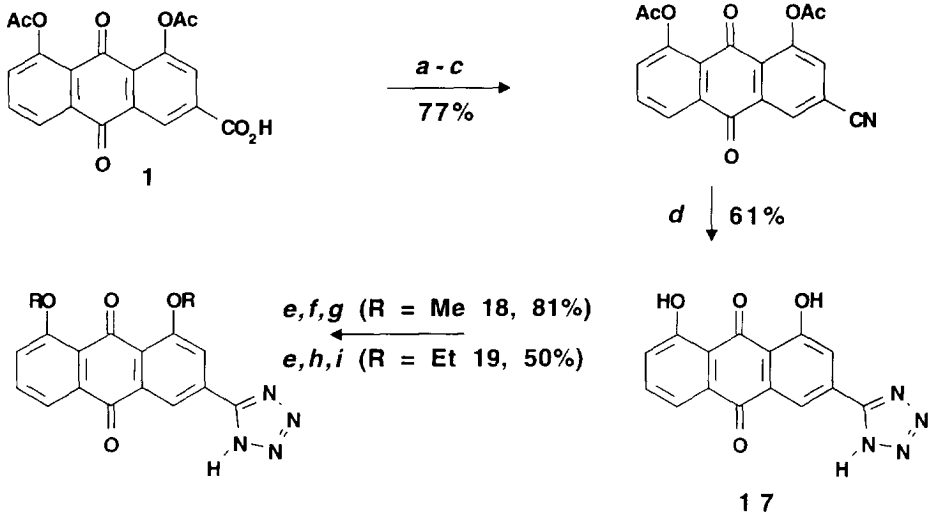
g) NaN_3, Et_3NHCl, DMF, H_2O. (RX = Me_2SO_4, Et_2SO_4)

Scheme 3

Thus Friedel-Crafts reaction between hydroquinone and benzene tricarboxylic anhydride provided 5,8-dihydroxyanthraquinone-2-carboxylic acid which was alkylated and esterified then hydrolysed to provide the acetate **11** and ethers **12** and **13** in 61-92% yield. 1,4-disubstituted analogues **14** and **15** were synthesied by analogous electrophilic substitution and this has been published.[14] Acid **12** was subsequently converted to the dimethoxytetrazole **16** by the formation of the *t*- butylamide followed by von Braun reaction to synthesise the carbonitrile with phosphorius pentachloride in toluene. The nitrile was converted to the tetrazole with sodium azide and triethylamine hydrochloride in DMF.

Similarly, diacetyl rhein was converted to 4,5-bisacetoxy anthraquinone-2-carbonitrile (scheme 4). Deacetylation accompanied tetrazole formation onconversion of the nitrile to the

4,5-dihydroxtetrazole **17**. For synthesis of the alkylated tetrazoles protection of the tetrazole with trityl chloride was needed before alkylation with either dimethyl or diethyl sulfate gave alkylated tetrazoles **18** and **19.**



a) $SOCl_2$, Pyridine, DME, 80°C, b) *t*-$BuNH_2$, 0.5h, 20°C, c) PCl_5, Toluene, 95°C, d) NaN_3, Et_3NHCl, DMF, H_2O , e) Ph_3CCl, Et_3N, CH_2Cl_2, f) Me_2SO_4, Cs_2CO_3, Dioxan, MEK, g) HCl, EtOAc, MeOH, h) Et_2SO_4, Cs_2CO_3, Dioxan, MEK, i) HCl, CH_2Cl_2, EtOH.

Scheme 4

Biological evaluation and discussion

Chondrocytes were prepared from the metacarpophalangeal joints of mature cows obtained soon after slaughter. The cartilage was digested with trypsin followed by collagenase. Cells were recovered by filtration and plated at a density of 1-2x 10^7 cells / ml into 24 well type I collagen-coated plates (5x10^6/well). The cells were cultured in DMEM low glucose (1g/l) medium supplemented at pH 7.4 with HEPES, BES, TES , ascorbate and 0.5%FCS. For short term incubations the absence of glutamine was not critical.

Following attachment, anthraquinones dissolved in DMSO were added to a final concentration in the test mixture of 200, 100, 50 and 10 μM and a final DMSO concentration of 0.1%. Aliquots of the medium were removed as soon as the compounds were added (t= 0h) and then again after 3 hours (t= 3h). Glucose was determined by liquid chromatography using a Dionex A1-450 workstation with a Carbopac PA-100 eluted isocratically with 100mM NaOH. Detection was by pulsed amperometry[15].

Table 1. Inhibition of glucose uptake by anthraquinones. All compounds were tested at 50μM. Results show the inhibition obtained at 50μM from a 4 point inhibition curve ranging from 10 to 200μM.

COMPOUND NO.	LILLY NO.	INHIBITION @ 50μM (%)
1	245108	45.6
2	170988	3.0
3	290273	-1.9
4	310114	18.5
5	310194	56.7
6	310040	2102.9
7	270660	16.9
8	270661	1.6
9	310065	4.8
10	310415	22.1
11	310124	14.8
12	310126	25.3
13	310323	37.7
14	290530	14.0
15	290570	24.9
16	310298	81.8
17	290333	102.8
18	310036	36.2
19	310137	92.0
PHLORIZIN		11.9
CATECHIN		12.1
PHLORETIN		180.0

The compounds examined all showed dose response kinetics for the inhibition of glucose uptake across the range examined. Compounds were tested in batches of 10 and each included **2** (rhein) and N-(4,5-dimethoxy-9,10-anthraquinon-2-yl) methane sulphonamide (which gave a robust reproducible response(~69.5%)) for normalisation purposes. The percentage uptake of glucose by chondrocytes in the 3 hour incubation period was 71.1±4.1 (n=4) at 50μM rhein, 67.7±7.5 (n=4) at 100μM and 55.8±8.7(n=5) at 200μM rhein, compared with 73.3±2.6 (n=5) for controls treated with an equivalent amount of DMSO alone.

Table 1 shows the effects of the anthraquinones examined at 50μM on the inhibition of glucose utilisation by chondrocytes. In addition to the anthraquinones, 3 other compounds were tested as comparitors : phlorizin, phloretin and catechin. The reason for including phloretin was that it is effective in inhibiting glucose uptake in sebaceous glands . Phlorizin is the glycosylated

form of phloretin and is considerably less active. Catechin has been included since it too has been suggested to be effective in osteoarthritis and is structurally similar to the rhein family of anthraquinones.

At 50μM, rhein (**2**, the active metabolite of diacetyl rhein) had virtually no activity in inhibiting glucose uptake. Whereas Floridi[4], showed that at 50μM, rhein was capable of inhibiting glucose uptake in Ehrlich ascites cells by about 50%. This may indicate that the mechanisms for glucose uptake in the two cell types are different. Discordance between the results of other studies[3,4,5] and those obtained in this study may therefore relate to the different cell types used. Phlorizin blocks reabsorption of glucose from the kidney tubule and will allow quantitative production and secretion of glucose from succinate (or other TCA intermediates) fed to these animals[16]. This compound was however, inactive in this study. Two compounds in this study showed inhibition of glucose uptake significantly greater than 100% ; phloretin (180.0%) and **6** (2102.9%). These data suggest that there is a net efflux of glucose from the cells possibly by enhanced gluconeogenesis and/or a reversal of glucose transporters. The relative lack of effect of rhein and phlorizin in this study may suggest that a different glucose transporter is utilised by chondrocytes, which could account for the differences in response of rhein in differing cell types.

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