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SYNTHESIS AND ANTI-TUMOUR ACTIVITY OF 6-METHYL DERIVATIVES OF FLAVONE-8-ACETIC ACID (FAA)

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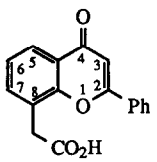
Abstract. A range of 16 derivatives of flavone-8-acetic acid (FAA) with a 6-methyl substituent have been prepared and their anti-tumour activity evaluated *in vitro* against a panel of human and murine tumour cell lines and *in vivo* against MAC 15A. While many of the compounds show activity comparable to FAA *in vitro*, this essentially disappears *in vivo*, possibly due to degradation before the compounds can reach the tumour site.

Although flavone-8-acetic acid **1** (FAA, NSC347512, LM975) initially seemed a promising new agent for the treatment of solid tumours,[1,2] its good activity against murine tumour models was not reflected in humans and clinical trials gave disappointing results.[3] As some of us have discussed in a recent review,[4] the mechanism of action of this compound is not well understood despite extensive studies, and synthesis and evaluation of structural analogues might provide some new insight. The range of analogues for which activity data is available is in fact somewhat limited. Since the original report[1] where a good number analogues were examined, only one paper has reported activity data for simple analogues.[5] A further large number of compounds have been described in patents,[6] but only for a few of these has the activity been reported. Useful *in vivo* activity has been reported for analogues of **1** with a 1-cyclopentenyl or 1-cyclohexenyl substituent in place of phenyl at the 2-position,[7] for a wide range of substituted xanthenone-4-acetic acids **2**,[8] and most recently for the flavone-8-carboxylic acid derivatives **3**.[9] We report here the preparation and *in vitro* and *in vivo* activity of some new simple derivatives of **1** with a methyl group at the 6-position.



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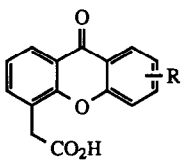

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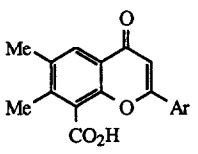

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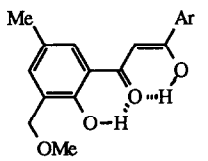

5'

Synthesis.

The compounds were prepared following a patent procedure[10] starting from 2-hydroxy-3-methoxymethyl-5-methylacetophenone **4**. This was in turn obtained in good yield from *p*-cresol by acetylation and Fries rearrangement,[11] chloromethylation using HCl/formaldehyde, and reaction with MeOH/Fe powder.[10] Generation of the dianion of **4** using NaH and condensation with the required aromatic ester then gave the 1,3-diones **5**. Interestingly these were shown by their ^{1}H and ^{13}C NMR data to exist almost entirely in one of the two possible

Biotransformation represents an increasingly common methodology for the synthesis of enantiopure organic molecules, and adaptation of this approach for organometallic π-complexes provides a logical extension, despite the difference in type of chirality (planar *vs* carbon-centered), sensitivity to photo-oxidation and general lack of compatibility with aqueous media. Recently, kinetic resolution of organometallic π-complexes, (arene)$Cr(CO)_3$ and (diene)$Fe(CO)_3$, have been achieved by using biocatalysts such as pig liver esterase or baker's yeast in aqueous system.[8] Since the (arene)chromium and (diene)iron complexes are difficult to dissolve in water, a method to deal with these complexes is restricted to those that can cover up the these disadvantages. It is also desirable that a reagent or catalyst can be separated easily from the products after reaction. The use of a lipase as the catalyst for the resolution of enantiomers provides a good method because the lipase works in an organic solvent under an anaerobic atmosphere, and it is easily separated from the reaction products by filtration. As part of our program dealing with the use of planar chirality in organic synthesis, we wish to report here a kinetic resolution of (arene)chromium and (diene)iron complexes by lipase in an organic solvent.[9]

Results and Discussion

Kinetic Resolution of (Arene)chromium Complexes by Lipases.

Tricarbonyl(η^6-arene)chromium complexes exist as two enantiomeric forms when the phenyl ring is substituted with different groups at the *ortho*- or *meta*-positions. The preparation of enantiomerically pure (arene)chromium complexes has great potential for the stereoselective transformations and asymmetric synthesis.

In the first attempt, racemic tricarbonyl(*o*-substituted benzylalcohol)chromium (*rac*-**1**) was resolved with isopropenyl acetate in the presence of different lipase. We subjected the $Cr(CO)_3$ complexes of *o*-methyl, *o*-methoxy and *o*-trimethylsilyl (TMS) derivatives of benzylalcohol as the substrates to be resolved and the results are summarized in Table 1. The reaction was terminated after ca. 50% conversion by filtration of the enzyme to give optically active hydroxyl complexes and the corresponding acetate complexes. Transacetylation of *rac* **1** (R = Me) in the presence of a lipase from *Pseudomonas cepacia* (Amano PS) at 25 °C gave the (1*R*,2*S*)-alcohol **2a** in 47% yield with >99% ee along with the corresponding (1*S*,2*R*)-acetate complex **3a** in 48% yield with 98% ee. The absolute configuration of the resolved complexes **2a** and **3a** was determined by comparison of specific rotation values of authentic compounds[1a,3b] derived from resolved tricarbonyl(*o*-methyl benzaldehyde)chromium by Davies procedure.[3i,j,k] For the *o*-methoxy substituted chromium complex **1b**, reaction in the presence of Amano AK from *Pseudomonas sp.* produced the (1*R*,2*S*)-alcohol complex **2b** in 47% yield with 95% ee together with the (1*S*,2*R*)-acetate complex **3b** in 46% yield with 96% ee, respectively. These enzymes recognize and attack selectively the alcohol with the (1*S*,2*R*)-configuration. On the other hand, a lipase Amano AY from *Candida rugosa* reacted with the (1*R*,2*S*)-alcohol, the antipode to that for the reaction with Amano PS and Amano AK, although the enantioselectivity was unsatisfactory (entries 5,8,10). In the case of *o*-trimethylsilyl substituent **1c**, no transesterification with isopropenyl acetate occurred by use of lipase, Amano PS, Amano AK and PPL due to steric hindrance of the Me_3Si group. However, Toyobo A from *Pseudomonas aeruginosa* resolved racemic *o*-TMS complex **1c** to afford the (1*R*,2*S*)-alcohol **2c** in 48% yield with 85% ee and the (1*S*,2*R*)-acetate **3c** in 47% yield with 84% ee though the resolution was slightly decreased (E[10] = 33, entry 9).

Chemosensitivity was assessed using an MTT assay[19] following the continuous (96 hours) exposure of cell lines to each compound. All results were expressed in terms of % survival, taking the control absorbance values to represent 100% survival. From the dose response curves constructed, IC_{50} values were estimated. FAA was reconstituted in saline. Solubility problems were encountered with compounds **9e,f**, **11a,b,d** and **12a,d** in that all proved insoluble in physiologically acceptable solvents and so chemosensitivity data could not be obtained. The final concentration of solutions used was less than 0.01% and solvent controls were used throughout.

The responses of human and murine tumour cell lines to FAA **1** and its analogues are presented in the Table. The results presented clearly demonstrate that FAA and its analogues are cytotoxic *in vitro*. Cytotoxic potency however is low with the majority of compounds inducing cell kills at IC_{50} values of greater that 100 $\mu g\ mL^{-1}$. In the case of MAC 15A cells the most active compounds were **10b** and **10f** with **10a** and **10e** being the least active. The remaining compounds **9a-d** and **10d** were of comparable cytotoxicity to **1**.

	in vitro IC_{50} value ($\mu g\ mL^{-1}$)											*in vivo* % TGI at dose (mg/kg)		
Comp-ound	MAC 15A	MAC 16	MAC 26	WEHI	K562	HCLO	HT-29	DLD1	HCT 18	HRT 18	BM	100	200	300
1	150	180	208	—	310	>500	390	>500	245	95	—	—	83	—
9a	165	300	95	210	140	270	180	250	190	80	180	0	0	0
9b	100	42	170	85	—	110	220	170	380	170	280	3	0	20
9c	120	—	130	33	48	190	—	—	—	110	62	0	18	8
9d	160	210	95	98	110	260	500	245	170	95	235	11	0	0
9e	—	—	—	—	—	—	—	—	—	—	—	39	59	45
9f	—	—	—	—	—	—	—	—	—	—	—	46	43	8
10a	210	250	400	—	300	>500	370	>500	410	450	—	32	4	16
10b	68	170	>500	—	130	>500	390	>500	>500	>500	—	39	27	25
10d	100	220	290	—	185	>500	300	400	350	490	—	14	36	0
10e	350	335	>500	—	300	>500	500	>500	>500	>500	—	36	56	94†
10f	60	270	500	—	330	>500	430	>500	>500	>500	—	21	21	36
12d	—	—	—	—	—	—	—	—	—	—	—	6	15	0

† 4/5 deaths Vehicle for *in vivo* tests: **1,9a,9d-f** 20% Cremophor/saline, **9b,c** 10 % NaOH/saline, **10a-f** saline, **12d** arachis oil

***In vivo* Biological Results.** Pure strain NMRI mice were used from the Bradford Clinical Oncology Unit inbred colony. The development of several adenocarcinoma of the colon in NMRI mice from primary tumours induced by the prolonged administration of 1,2-dimethylhydrazine has been described elsewhere.[20] The tumour line used in this study was MAC 15A grown subcutaneously as a poorly differentiated, solid tumour in NMRI mice which has previously been shown to respond to FAA.[21] Chemotherapy began when tumours had reached a size that could be accurately measured and had an established vasculature. Anti-tumour activity was assessed by tumour weights. All drugs were administered intraperitoneally at comparable doses to **1** which was used as a positive control.

The results of the *in vivo* studies are also presented in the Table. In the case of **1** good anti-tumour activity was observed (83% tumour inhibition) with significant differences ($T_{0.01}$) between treated and control tumour weights. No significant differences between treated and control tumour weights were observed in mice treated with **9a-c,f**, **10a,b,d,f**, **11a,b,d** and **12a,d**. In the case of **9d** treated tumour weights at 300 mg kg^{-1} were significantly greater ($T_{0.05}$) than control tumour weights. Significant ($T_{0.05}$) activity was observed with **9e** at 200 and 300 mg kg^{-1} (59 and 45% tumour inhibition respectively) and **10e** at 200 mg kg^{-1} (56% tumour inhibition).

At 300 mg kg^{-1} **10e** was toxic (4/5 deaths). In a repeat experiment **10e** at 250 mg kg^{-1} was not active and no hemorrhagic necrosis was observed with any of the compounds tested.

The results of this study clearly demonstrate that a very narrow structure activity relationship exists. Whilst the cytotoxic potency of all these compounds are comparable *in vitro* , the presence of a single methyl group on the molecule **9a** effectively abolishes the activity against MAC 15A tumours *in vivo*. Substitution by an OH group at the 6-position of **1** was previously reported to completely remove its activity against C38 *in vivo*.[1] It seems possible that the main effect of the 6-methyl group here is to provide a handle for degradation of the compounds *in vivo* before they can reach the tumour site. If this is the case, then the good *in vitro* activity observed for electron rich aryl groups such as methoxyphenyl, furyl and thienyl may translate to improved *in vivo* activity for the corresponding 6-unsubstituted FAA analogues. We are currently determining the activity of a wide range of these prepared by a new improved route and the results will be reported shortly.

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