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3,5-Bis(3-dimethylaminomethyl-4-hydroxybenzylidene)-4-piperidone and related compounds induce glutathione oxidation and mitochondria-mediated cell death in HCT-116 colon cancer cells

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ABSTRACT

This study aims at investigating the cytotoxicity and some of the modes of action of 3,5-bis(3-dimethylamino-4-hydroxybenzylidene)-4-piperidone trihydrochloride **3** and two related compounds **2** (which lacks the dimethylaminomethyl groups) and **4** (which has an additional dimethylaminoethyl substituent in both aryl rings) in order to ascertain the contribution of dimethylaminoethyl substituent to bioactivity. The bioactivities of **2**–**4** were compared with curcumin **5**. Both **2** and **3** displayed submicromolar GI_{50} values towards HCT-116 cells and were significantly more potent than **4**, **5** and 5-fluorouracil (5-FU). All of the compounds displayed greater toxicity towards HCT-116 cells than human CRL-1790 non-malignant colon cells. In HCT-116 cells, the compounds **2**, **3** and **5** increased the ratio of oxidised to reduced glutathione and destabilized the mitochondrial membrane potential. Both **2** and **5** produced an increase in mitochondrial superoxide and a burst in intracellular reactive oxygen species in HCT 116 cells. In addition, **2** and **4** stimulated respiration in rat liver mitochondria while **2** and **5** induced mitochondrial swelling. The results suggest that **2** and **5** cause oxidation or cross-linking of the thiols which control the mitochondrial permeability transition.

A number of conjugated unsaturated ketones have antineoplastic properties.[1] An important feature of these molecules is that they interact with thiols but have little or no affinity for reacting with amino and hydroxyl groups[2,3] which are found in nucleic acids. Hence these compounds may be devoid of the genotoxic properties of a number of anticancer drugs.[4] Of particular interest are compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl group which have the potential to allow successive interactions with cellular thiols. Various studies revealed that after an initial thiol alkylation, a second chemical insult is more damaging to tumours than non-malignant cells[5] and hence tumour-selective toxicity may result.

The dienone **1** was reported from our laboratory as a thiol alkylating anticancer agent which possesses IC_{50} values in the low micromolar range towards human Molt4/C8 and CEM T-lymphocytes as well as murine L1210 cells.[6] The compounds **2**–**4** were designed with the aspiration of increasing cytotoxic potency based on the following considerations (Fig. 1). In the case of **2**, the placement of a 4-hydroxy group onto the aryl rings may enable hydrogen bonding with cellular constituents to occur. Furthermore **2** has the potential to be converted into the corresponding tautomers **A** and **B**. In addition, one-electron oxidation of **2** caused by molecular oxygen and/or reactive oxygen species (ROS) could give rise to the free radical **C**. The addition of a 3-dimethylaminomethyl group to the aryl rings of **2** led to **3**. In addition to the possibilities of tautomer and free radical formation, deamination of **3** could lead to the generation of the orthoquinone methide **D**, thereby creating a further site for thiol alkylation. Furthermore in the acidic milieu of certain tumours,[7] protonation of **3** leading to **E** can occur. The result is an electron-attracting substituent in the aryl rings which will diminish the electron density on the olefinic carbon atoms and hence increase the rate of thiol alkylation. Also, the positive charge may facilitate uptake into tumour cell mitochondria, due to their often increased electrochemical membrane potential.[8] Finally the dienone **4** was suggested which, in addition to producing the chemically reactive species that might originate from **2** and **3**, may also lead to **F** in which the exocyclic methylene group in particular would be predicted to have high electrophilic properties.

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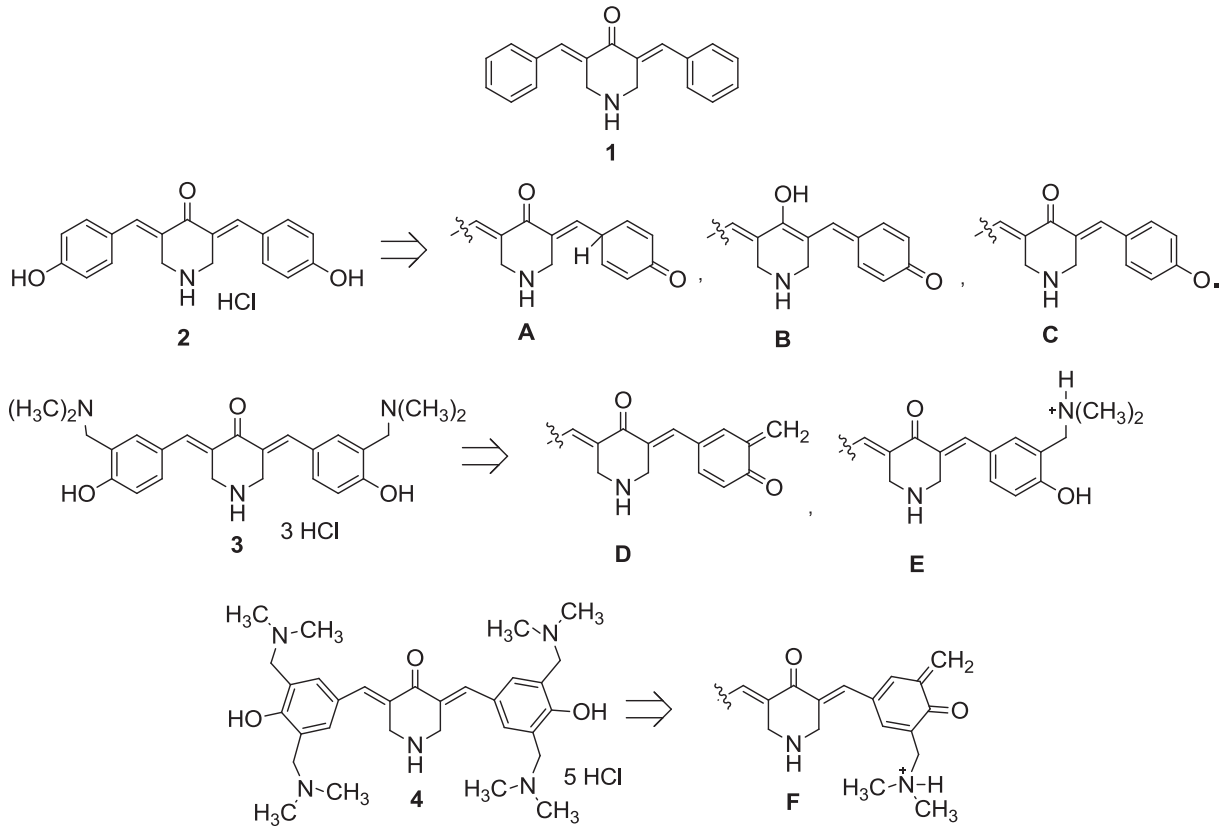

Fig. 1. The structures of **1**–**4** and the partial structures of the possible cytotoxic species derived from **2**–**4** which are designated **A**–**F**.

A very recent report outlined the syntheses of **2**–**4** and their evaluation against Molt4/C8, CEM and L1210 cells.[9] The average IC_{50} values of **2**–**4** against these three cell lines were 38.3, 0.78 and >500 μM, respectively. The same investigation revealed that **3** caused apoptosis as revealed by internucleosomal DNA fragmentation in human HSC-2 squamous cell carcinomas and human HL-60 promyelocytic leukemia cells as well as activation of caspases-3 and -7 in HSC-2 cells. In addition, **3** cleaved PARP1 in both HSC-2 and HL-60 cells.

The objective of the present study was to investigate the biochemical pathways by which **3** and the related compounds **2** and **4** exert their cytotoxic properties towards neoplastic cells. In particular, such studies may reveal some of the reasons for the huge disparity between the cytotoxic potencies of these three compounds towards HCT-116 cells. In addition, there is currently a considerable interest in curcumin **5** (Fig. 2) as an anticancer agent.[10] This compound contains 3-aryl-2-propenoyl and 2-arylvinyl groups which are present in **2**–**4**. Thus a comparison of some of the biological properties of **5** with **2**–**4** was pursued. Furthermore since a major interest in these laboratories is in colon cancer, the investigations were designed using human HCT-116 colon cancer cells. Comparison of the effects of **2**–**5** with 5-fluorouracil (5-FU) was conducted since 5-FU is used clinically in treating colon cancers.

One of the ways in which these compounds may exert their anticancer activities is through effects on mitochondria. Mitochondria are an anticancer target due to their key involvement in apoptosis and energy metabolism, and some differences between mitochondria of normal and cancer cells.[11–13] Moreover, mitochondrial thiol status and ROS generation are key factors controlling the mitochondrial permeability transition in the trigger for apoptosis.[14,15] Therefore we undertook these studies to investigate the ability of these compounds to induce cell death involving intracellular thiol oxidation, ROS generation and the mitochondrial permeability transition.

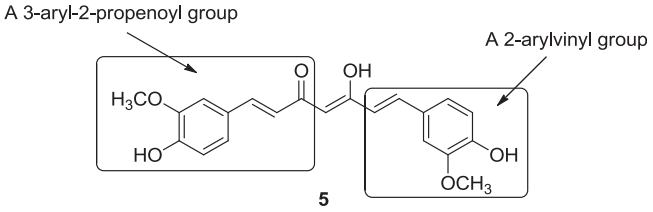

Fig. 2. The structure of curcumin **5**.

Each of the unsaturated ketones **2**–**5** and 5-FU were evaluated against human HCT-116 colon cancer cells which is a neoplasm having metastatic properties. The results are portrayed in Table 1. The GI_{50} values of **2** and **3** against HCT-116 cells were both submicromolar. However **4** had approximately one tenth of the potencies of **2** and **3** towards these cells. Both **2** and **3** were approximately 4 times more potent than **5** and had six times lower GI_{50} values than 5-FU. The compounds were also evaluated against human non-malignant CRL-1790 colon cells and these results are presented in Table 1. The GI_{50} values of each compound were greater towards CRL-1790 than HCT-116 cells and hence all of the compounds demonstrate tumour-selective toxicity. Of particular interest are the observations that **2**–**5** had greater selectivity index (SI) values than 5-FU, and the huge SI value of **4** is noteworthy.

The dienones **2**–**4** were designed as thiol alkylators whereby their olefinic carbon atoms could react with cellular thiols. If these interactions take place, the ratios of oxidised glutathione (GSSG) and the reduced species (GSH) will increase. The glutathione redox ratio is a major factor controlling apoptotic cell death.[16] The GSSG/GSH ratios in HCT-116 cells which were treated with **2**–**5** and 5-FU