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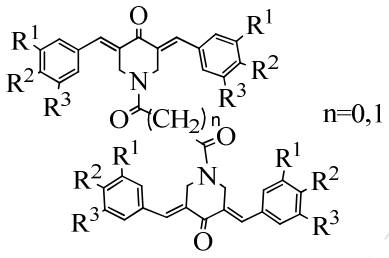
GRAPHICAL ABSTRACT



R^1, R^2, R^3=H, CH_3, F, Cl, OCH_3, $N(CH_3)_2$

A number of bis(arylidene)-4-piperidone dimers have submicromolar IC_{50} values towards HCT116 and HT29 colon cancer cells and are far more potent than 5-fluorouracil and curcumin to these neoplasms.

Novel 3,5-bis(arylidene)-4-piperidone dimers: Potent cytotoxins against colon cancer cells

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Abstract

Two novel series of dimeric 3,5-bis(arylidene)-4-piperidones **7** and **8** were prepared as cytotoxic agents. A specific objective of this study was the discovery of novel compounds displaying potent anti-proliferative activities against colon cancers. Most of the compounds demonstrate potent cytotoxicity against HCT116 and HT29 colon cancer cell lines in which the IC_{50} values range from low micromolar to nanomolar values. In general, the majority of the compounds showed greater cytotoxicity and some degree of selectivity towards HCT116 cells compared to HT29 cells. Compound **9** in which the amidic carbonyl groups were excised was substantially less potent than **8a** in both cell lines suggested that the amide groups are important components of the molecules for exhibiting cytotoxicity. Virtually all the compounds were more potent than a reference drug 5-fluorouracil which is used in treating colon cancers as well as a related enone curcumin. QSAR studies were undertaken and some guidelines for amplification of the project have been formulated. Flow cytometry analysis of a representative potent compound **7f** revealed that it induces apoptosis in HCT116 cells.

Keywords: piperidone, α,β-unsaturated ketone, cytotoxicity, selective toxicity

1. Introduction

Colorectal cancer is the second leading cause of death in men and women among all cancers [1]. The low incidence of colorectal cancer in Asian countries has been suggested to be due *inter alia* to the regular use of curcumin in the diet [2, 3]. Curcumin **1** produces a profound cytotoxic effect on colon carcinogenesis in rats and mice, reduces a number of colon tumours in a mouse model with a mutation in the APC gene [4] and decreases the multiplicity of colon adenomas [5]. Curcumin was found to protect against the development of colon cancer induced by azoxymethane during both the initiation and the promotion stages in both rats and mice [6,7] and also demonstrates potent anticancer effects on intestinal cancer, stomach cancer, and hepatocellular carcinoma [8]. The α,β-unsaturated keto motif is considered to contribute significantly to the bioactivity observed. Subsequently a large number of curcuminoids have been described in the literature, which display potent cytotoxic potencies against various cancers including colon cancers [9-11]. One of the reasons for the interest in these compounds is that α,β-unsaturated ketones display a selective affinity for thiols compared to hydroxyl and amino groups [12] which are present in nucleic acids. Hence these molecules should not elicit genotoxic properties associated with a number of alkylating agents used in cancer chemotherapy [13]. Cytotoxic α,β-unsaturated ketones are thiol alkylators [12] and the importance of multitargeted ligands has been documented very recently [14-16].

[PLEASE INSERT FIGURE 1]

Our major interest is the development of conjugated unsaturated ketones as candidate cytotoxins with special emphasis on those compounds which inhibit the growth of colon cancers [17,18]. In view of these considerations, a bifunctional 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore was incorporated into a variety of cyclic and acyclic analogs that led to the discovery of a number of potent cytotoxins possessing the general structure **2** [14]. In particular, some 3,5-bis(arylidene)-4-piperidones demonstrate IC_{50} values in the low micromolar to submicromolar range towards a number of malignant cell lines [19,20]. These compounds were designed based on the hypothesis of sequential cytotoxicity which states that successive chemical attacks may lead to greater damage in cancer cells compared to normal cells [19]. This theory was formulated on the observations that an initial lowering of the concentrations of cellular thiols followed by a second chemical attack would cause a greater deleterious effect to neoplasms than normal cells [21,22]. A N-acyl-3,5-bis(benzylidene)-4-piperidone **4a** displayed more than 8-fold

greater cytotoxic potency than its precursor **3** towards P388/MRI cells [23]. An investigation of the effect of these two compounds **3** and **4a** on hepatic glutathione levels in mice revealed reductions in the glutathione concentrations of 19 and 29%, respectively, suggesting that an increase in the number of thiol alkylating sites in the molecule contributed to an improvement in cytotoxic potency [23]. Based on this observation, the N-acryloyl-3,5-bis(arylidene)-4-piperidone **4b** and a number of aryl substituted analogs were developed which possess greater cytotoxic potencies over their 3,5-bis(arylidene)-4-piperidone precursors [19]. These observations unequivocally demonstrate the value of incorporating additional thiol alkylating groups in the molecule and led to a decision to prepare series **5.** In these compounds two molecules of **3** are attached by a linker with varying carbon chain lengths keeping in view that the relative location of one of the 1,5-diaryl-3-oxo-1,4-pentadienyl groups in series **5** with respect to the other one may be important for binding to the active sites of the proteins and may influence cytotoxicity substantially. The compounds in series **5** displayed potent cytotoxicity against human Molt4/C8 and CEM T-lymphocytes as well as murine L1210 lymphoid leukemic cells [24] while further investigations revealed the greater toxicity of these compounds to neoplasms than non-malignant cells [25]. From these studies two prototypic molecules **7a** and **8a** emerged which displayed remarkable cytotoxic potencies having average IC_{50} values of 0.17 and 0.11 μM, respectively, against a panel of seven different colon cancer cell lines [25]. These two compounds are clearly lead molecules for further analog development.

The aims of the present study are fourfold namely (i) examining the compounds in series **7** and **8** as candidate cytotoxins which are particularly effective against colon cancers, (ii) to evaluate the influence of the electronic, hydrophobic and steric properties of the aryl ring substituents in **7a-i** and **8a-j** on cytotoxic potencies and if possible to develop structure-activity relationships which will be helpful for designing further potent cytotoxic molecules, (iii) to establish the role of the amidic groups in governing cytotoxic properties of the molecules, and (iv) to investigate the mode action of a representative molecule whereby the cytotoxicity of this class of compounds is mediated.

[PLEASE INSERT SCHEME 1]

2. Chemistry

The synthesis of compounds in series **6-9** is outlined in **Scheme 1**. Briefly, the 3,5-bis(benzylidene)-4-piperidones **6a-j** were synthesized by acid-catalyzed condensation of 4-

piperidone hydrochloride with aryl aldehydes [19]. The dimers in series **7** and **8** were obtained from their corresponding 3,5-bis(benzylidene)-4-piperidones **6** by reacting with oxaloyl chloride or malonyl chloride, respectively. The synthesis of **9** was accomplished by a base catalyzed condensation of two mole equivalents of **6a** with one mole equivalent of 1,3-dibromopropane. The structures of all the compounds were established by ^{1}H NMR and elemental analysis.

The ^{1}H NMR spectra of **7a-i**, **8a-j** and **9** revealed that the compounds are isomerically pure and all four olefinic double bonds exhibit the *E*-configuration. The *E*-stereochemistry of these compounds was indicated based on the following observations. First, the olefinic protons of the dimers **7-9** appear at 7.39-7.79 ppm in the ^{1}H NMR spectra, which is an indicative of the *E* geometry [19]. Second, a number of 3,5-bis(benzylidene)-4-piperidones **6** adopt the *E*-stereochemistry as established by X-ray crystallography [19, 23]. In addition, recently an X-ray crystal structure of the 2-fluoro analog of **7** was shown to possess the *E*- geometry [26]. In order to evaluate the hypothesis that the amidic groups in the series **7-8** are essential for cytotoxicity, a representative molecule **9** was prepared which is bereft of both amidic carbonyl groups. Various physicochemical properties of the aryl substituents and the topography of the molecules were examined for correlations with the IC_{50} values.

3. Bioevaluations

The compounds in series **7-9** as well as two reference compounds 5-fluorouracil (5-FU) and curcumin were evaluated against two human colon cancer cell lines HCT116 and HT29. These biodata are reported in Table 1. Solubility considerations mandated that the highest concentration of **8i** that could be used is 25 μM, which was insufficient to generate an IC_{50} value. A representative lead cytotoxin **7f** was examined for its ability to cause apoptosis in HCT116 cell lines. This result is presented in Figure 2.

4. Results and Discussion

The compounds **7a-i** and **8a-j** were evaluated against human HCT116 and HT29 colon cancer cells. The biodata presented in Table 1 show remarkable cytotoxic potencies of all the compounds except **7d,i** and **8d,i.** The majority of the IC_{50} values were in the submicromolar to nanomolar range. The compounds displaying IC_{50} values < 1μM are considered to be highly

cytotoxic. In series **7**, 78% of the IC_{50} values were <1 μM against both the cell lines and in series **8**, IC_{50} values of <1μM were observed in 80% of the compounds against HCT116 and HT29 cells.

[PLEASE INSERT TABLE 1]

In particular, **7a,e** and **8a,c,h** have IC_{50} values in double digit nanomolar range except in the case of **8a**, the IC_{50} value is 3 nM in the HT29 bioassay. The importance of the marked cytotoxic potencies of most of these compounds was confirmed when the IC_{50} values of **7**-**9** are compared with that of 5-FU which is a drug used in treating colon cancers. Relative potency (RP) figures were generated by comparing the IC_{50} figures of the compounds in series **7**-**9** with that of 5-FU in these two screens. These data are presented in Table 1 which reveals that in general the compounds are far more toxic to these two neoplasms than the clinically useful drug. In addition, the RP figures indicate that in general the HCT116 cells are more sensitive to these compounds than the HT29 neoplasms. This difference between the RP values for each compound may reflect a capacity to display greater toxic effects to malignant cells than normal tissues. This possibility is enhanced by the variation in efficacy of the compounds to inhibit the growth of the HCT116 and HT29 cells, e.g. the 304-fold difference between **7b** and **7i** in the HCT116 screen. In other words, the compounds in series **7** and **8** are not general biocidal agents and possibly are tumour-selective cytotoxins. Virtually all of the compounds in series **7**-**9** are substantially more potent than curcumin.

The following correlations were observed between the nature of the aryl substituents in series **7** and **8** and their cytotoxic potencies in both screens. First, the placement of a methyl (**7b**,**8b**), chloro (**7c**,**8c**), fluoro (**7e**,**8e**), or methoxy (**7f**,**8f**) group into the 4 position of the aryl rings led to compounds having similar potencies in the range of 0.02-0.78 μM. However, the introduction of a 4-dimethylamino substituent (**7i**,**8i**) into the aryl rings caused potency to plummet considerably. Second, the addition of a 3-chloro group to the 4-chloro analogs leading to the **7d** and **8d** causes a dramatic reduction in potency, e.g., there is a 668-fold difference between the IC_{50} values of **7c** and **7d** in the HCT116 screen.

[PLEASE INSERT TABLE 2]

The next part of the analysis of the biodata involved a comparison of the potencies of some of the compounds in series **7** and **8**. These comparisons are presented as $\Delta_{8/7}$ values which were obtained by dividing the IC_{50} value of a compound in **8a-i** by that of the analog in series **7**

which possesses the same aryl substituent. In this way one may be able to ascertain whether an oxalyl [-C(O)-C(O)-] or malonyl [-C(O)-CH_2-C(O)-] linker between the piperidinyl nitrogen atoms is optimal. Taking standard deviations into account, in the HCT116 bioassay **7b-g** are more potent than **8b-g** while **8h** has a lower IC_{50} value than **7h**. The unsubstituted analogs **7a** and **8a** are equipotent. In the case of the HT29 screen, **7b,e,f** are more potent than **8b,e,f** while **8a,c,d,h** have lower IC_{50} figures than **7a,c,d,h** and **7g** and **8g** have equal potency. Thus the oxalyl and malonyl linkers are favoured in 56% and 31%, respectively, of the enones **7a-h** and **8a-h** while equipotency was observed in 13% of the comparisons made. A quantitative estimate of the differences in potencies between **7a-h** and **8a-h**, which are dependent on the nature of the spacer group, is presented as $\Delta_{8/7}$ values in Table 2. Thus the oxalyl group is marginally preferable to the malonyl linker although excellent cytotoxic potencies were observed in each series of compounds.

A question to be addressed is whether the substituted dimers **7b-i** and **8b-i** display greater cytotoxic potencies than the unsubtituted analogs **7a** and **8a**, respectively. For series **7**, the enone **7a** has greater potency than **7d,h,i** and was equipotent with **7b,c,e-g** in the HCT116 assay. For the HT29 screen, **7a** was more potent than **7b-d,f-i** and was equipotent with **7e**. In the case of series **8**, the unsubstituted analog **8a** has lower IC_{50} values than **8b-g,i** and displays equal potency as **8h** in the HCT116 screen while against HT29 cells, **8a** is more potent than **8b-i**. Thus overall **7a** and **8a** are more potent than the substituted dimers **7b-i** and **8b-i** in 78% of the comparisons made while the same potency was noted in 22% of the cases. These comparisons are presented in quantitative form in Table 2 where, in general, the $\Delta_{7b\text{-}i/7a}$ figures are smaller than the $\Delta_{8b\text{-}i/8a}$ values. Hence substitution in the aryl rings in series **7** rather than **8** may lead to potency increases over the substituted components. Further, the importance of the amidic groups and their contributions towards the cytotoxicity of the dimers were suggested by comparing the IC_{50} values of **8a** and **9** against HCT116 and HT29 cells. Compound **9** was found to be a potent cytotoxin compared to 5-FU and curcumin towards both cell lines. However, it displayed more than 30-fold and 700-fold lower cytotoxic potencies in comparison to **8a** against HCT116 and HT29 cells, respectively. These observations suggest that the amidic carbonyl groups contribute significantly to cytotoxic potencies. Therefore, further analog development of these classes of compounds should retain the amidic groups.

In order to evaluate whether the magnitude of some of the physicochemical properties of

the aryl substituents such as electronic (σ), hydrophobic(π), and steric properties (MR) influence the cytotoxic potencies in series **7** and **8**, linear and semilogarithmic plots were made between these constants and the IC_{50} values of **7a-i** and **8a-j** in the HCT116 and HT29 screens. Statistically significant negative correlations ($p<0.05$) were noted between σ and the IC_{50} values of **7a-i** and **8a-j** in the HCT116 screen and a trend to a negative correlation ($p=0.06$) was noted between the σ constants and the IC_{50} values of **7a-i** in the HT29 bioassay. These results suggest that in general further development of this class of compounds should include placing stronger electron-withdrawing groups in the aryl rings. Such an approach will reduce the electron density on the olefinic carbon atoms which will enhance electrophilic attack with cellular thiols. However there are exceptions to this generalization. For example, analogs containing the 4-fluoro (an electron-withdrawing atom) and 4-methoxy (an electron-repelling group) substituents are at variance with this generalization in the HCT116 screen. Thus **7e** and **7f** are equipotent while **8f** is 10 times more potent than **8e** revealing the need for further exploration in this area. No correlations were noted between the π and MR constants of the aryl substituents and the IC_{50} values.

[PLEASE INSERT FIGURE 2]

A final issue to be investigated is the way in which these classes of compounds exert their cytotoxicity. A number of anticancer agents exert their activity by inducing apoptotic cell death in cancer cells. Compound **7a** was shown to trigger apoptosis, not necrosis, in a number of T-cell and B-cell lymphomas [24]. In order to examine whether the substituted dimers also induce apoptotic cell death in colon cancer cells, HCT116 cells was treated with a potent representative compound **7f** using a concentration of 1μM. The results are presented in Figure 2. After 24 h, 34.9% apoptotic cell death was observed in the treated cells compared to 2.82% in untreated cells. After 48 h, the percentage of apoptotic cells was increased slightly to 38.5%. This observation suggests that apoptosis is one of the ways by which **7f** displays cytotoxicity in HCT116 colon cells. Due to its structural similarity to other analogs in series **7** and **8**, it is possible that these compounds also follow the same mode of action.

A number of cytotoxic agents are known to interfere at different phases of cell cycle progression [27-30]. To evaluate this phenomenon, HCT116 and HT29 cells were treated with 1 μM concentration of **7f** for 48 h and the effect of **7f** on cell cycle was investigated using flow cytotometry. Curcumin, a potent cytotoxic agent [31] that possesses some structural similarities

with **7f** and is also known to induce G2/M phase arrest in HCT116 [29] and HT29 cells [30] was used as a positive control for this study. When compared to untreated cells, the treatment of **7f** did not display any significant changes in the cell population in the S or G2/M phases of the cell cycle, either in HCT116 or in HT29 cells (data not given). These results suggest that **7f** causes cytotoxicity and apoptosis in HCT116 and HT29 cells via mechanisms other than cell cycle arrest. In future, further modes of studies of the lead cytotoxic agent **7f** are warranted to gain some insight of the ways this class of compounds display cytotoxicity.

5. Conclusions

This study reveals that in general the dimers in series **7**-**9** are potent cytotoxic agents towards HCT116 and HT29 colon cancer cells. In general, these molecules are substantially more potent than either 5-FU or curcumin. Evidence was obtained which suggested that the amidic groups in series **7** and **8** contributed to the cytotoxic potencies. In particular, **7a**, **e** and **8a, c, h** emerged as lead molecules for further development. QSAR revealed that the placement of strong electron withdrawing groups into the aryl rings may enhance cytotoxic potencies still further. A lead representative compound **7f** induced apoptosis in HCT116 cells. In conclusion, this investigation has provided adequate evidence for initiating further studies of these novel cytotoxins with a view to obtaining a clinical candidate. However structural modifications to improve a variety of physicochemical properties will be required. For example, the clogP values of **7a** and **8a** are 5.40 and 5.34, respectively [25]. Hence aryl substitution with hydrophilic moieties, such as a glucosidic group on one or more of the aryl rings, should be undertaken.

6. Experimental protocols

6.1. Chemistry

Melting points were determined on a Gallenkamp instrument and are uncorrected. ^{1}H and ^{13}C NMR were recorded using a Bruker Avance 500 MHz spectrometer equipped with a 5mm BBO probe. Chemical shifts (δ) are reported in ppm. Elemental analyses were undertaken using an Elementer CHNS analyzer. The ^{1}H and ^{13}C NMR spectra of three representative compounds namely **7b**, **8b** and **9** are presented as supplementary material.

*6.1.1. Synthesis of 3,5-bis(arylidene)-4-piperidones (**6a-j**)*

The synthesis of **6a**-**j** was reported previously [18,19].

*6.1.2. General procedure for the synthesis of 3,5-bis(arylidene)-4-piperidone dimers (**7**)*

Oxaloyl chloride (0.003 mol, 0.39 gm) in DCE (5 ml) was added dropwise to a stirred suspension of a 3,5-bis(benzylidene)-4-piperidone **6** (0.006 mol) in DCE (20 ml) containing triethylamine (0.006 mol, 0.61 gm) at ~20 ^{o}C for a period of 30 min. The reaction was stirred at room temperature 8-12 h. The solvent was removed under reduced pressure at 45 ^{o}C. An aqueous solution of potassium carbonate (25 ml, 5 % w/v) was added to the crude mass and stirred for 2 h. The solid obtained was filtered, dried, and crystallized from a suitable solvent to yield pure products.

*6.1.2.1. 1,2-bis[3,5-bis(Benzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7a**)*

The synthesis of **7a** was reported previously [24].

*6.1.2.2. 1,2-bis[3,5-bis(4-Methylbenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7b**)*

Yield: 53%; mp (chloroform/methanol) 275 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.79(s, 2H, 2×=CH), 7.65 (s, 2H, 2×=CH), 7.29 (d, J=7.70 Hz, 8H, Ar-H), 7.18(q, 8H, Ar-H), 4.53 (d, J=15.61 Hz, 8H, 4×NCH_2), 2.44 (s, 6H, 2×CH_3), 2.28 (s, 6H, 2×CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 185.04(CO), 162.73(CON), 140.31(C=C-Ph), 140.22(C=C-Ph), 139.15(C=C-Ph), 138.29(C=C-Ph), 131.57(Ar-C),131.07(Ar-C), 130.67(Ar-C), 130.33(Ar-C), 129.72(Ar-C), 129.62(Ar-C), 129.46(Ar-C), 129.00(Ar-C), 46.07(CH_2NCO), 42.05(CH_2NCO), 21.53(CH_3), 21.43(CH_3). Anal.calcd for $C_{44}H_{40}N_2O_4$.1.5 H_2O: C 76.76; H 6.30; N 4.07 %, found: C 76.46; H 6.39; N 3.93%.

*6.1.2.3. 1,2-bis[3,5-bis(4-Chlorobenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7c**)*

Yield: 61%; mp (methanol) 289 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.72(s, 2H, 2×=CH), 7.54 (s, 2H, 2×=CH), 7.46 (d, J=8.40 Hz, 2H, Ar-H), 7.37(d, J=8.37 Hz, 2H, Ar-H), 7.31(d, J=8.42 Hz, 4H, Ar-H), 7.20(d, J=8.38 Hz, 4H, Ar-H), 4.62 (s, 4H, 2×NCH_2), 4.52 (s, 4H, 2×NCH_2). Anal.calcd for $C_{40}H_{28}Cl_4N_2O_4$.3 H_2O: C 60.26; H 3.54; N 3.51 %, found: C 60.35; H 3.70; N 3.15%.

*6.1.2.4. 1,2-bis[3,5-bis(3,4-Dichlorobenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7d**)*

Yield: 64%; mp (chloroform/methanol) 271 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.68 (s, 2H, 2×=CH), 7.55 (s, 1H, =CH), 7.53 (s, 1H, =CH), 7.46 (d, J=8.15 Hz, 6H, Ar-H), 7.41(d, J=1.71 Hz, 2H, Ar-H), 7.20 (dd, J=1.67Hz, J=1.71Hz, 2H, Ar-H), 7.05 (dd, J=1.71 Hz, J=1.77 Hz, 2H, Ar-H), 4.67 (s, 4H, 2×NCH_2), 4.56 (s, 4H, 2×NCH_2). Anal.calcd for $C_{40}H_{24}Cl_8N_2O_4$: C 54.58; H 2.75; N 3.18 %, found: C 54.52; H 2.90; N 2.95 %.

*6.1.2.5. 1,2-bis[3,5-bis(4-Fluorobenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7e**)*

Yield: 67%; mp (chloroform/methanol) 253 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.73(s, 2H, 2×=CH), 7.57 (s, 2H, 2×=CH), 7.37(q, 4H, Ar-H), 7.25 (q, 4H, Ar-H), 7.18 (t, 4H, Ar-H), 7.098(t, 4H, Ar-H), 4.63 (s, 4H, 2×NCH_2), 4.51 (s, 4H, 2×NCH_2). Anal.calcd for $C_{41}H_{26}Cl_8N_2O_4.H_2O$: C 53.93; H 3.09; N 3.07 %, found: C 53.84; H 2.98; N 2.74%.

*6.1.2.6. 1,2-bis[3,5-bis(4-Methoxybenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7f**)*

Yield: 71%; mp (chloroform/methanol) >300 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.74(s, 2H, 2×=CH), 7.58 (s, 2H, 2×=CH), 7.35 (d, J=8.65 Hz, 4H, Ar-H), 7.22 (d, J=8.63 Hz, 4H, Ar-H), 6.98 (d, J=8.71 Hz, 4H, Ar-H), 6.91 (d, J=8.67 Hz, 4H, Ar-H), 4.60 (s, 4H, 2×NCH_2), 4.53 (s, 4H, 2×NCH_2), 3.90 (s, 6H, 2×OCH_3), 3.76 (s, 6H, 2×OCH_3). Anal.calcd for $C_{44}H_{40}N_2O_8$: C 72.91; H 5.56; N 3.86 %, found: C 72.57; H 5.94; N 3.75%.

*6.1.2.7. 1,2-bis[3,5-bis(3,4-Dimethoxybenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7g**)*

Yield: 60%; mp (chloroform/methanol) 282 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71(s, 2H, 2×=CH), 7.42 (s, 2H, 2×=CH), 6.99 (d, J=8.47 Hz, 4H, Ar-H), 6.94(d, J=8.08 Hz, 2H, Ar-H), 6.80(m, 6H, Ar-H), 4.70 (s, 4H, 2×NCH_2), 4.64 (s, 4H, 2×NCH_2), 4.00 (s, 6H, 2×OCH_3), 3.96 (d, 12H, 4×OCH_3), 3.74 (s, 6H, 2×OCH_3). Anal.calcd for $C_{48}H_{48}N_2O_{12}.H_2O$: C 66.75; H 5.84; N 3.24 %, found: C 66.31; H 5.62; N 3.06%.

*6.1.2.8.1,2-bis[3,5-bis(3,4,5-Trimethoxybenzylidene)-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7h**)*

Yield: 57%; mp (ethanol) 273 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.63(s, 2H, 2×=CH), 7.44 (s, 2H, 2×=CH), 6.66 (s, 4H, Ar-H), 6.49 (s, 4H, Ar-H), 4.75 (s, 4H, 4×NCH_2), 4.68 (s, 4H, 4×NCH_2), 3.96 (s, 12H, 4×OCH_3), 3.94 (s, 9H, 3×OCH_3), 3.88 (s, 9H, 3×OCH_3), 3.84 (s, 6H, 2×OCH_3). Anal.calcd for $C_{52}H_{56}N_2O_{16}$: C 64.72; H 5.85; N 2.90 %, found: C 64.95; H 6.16; N

2.78%.

*6.1.2.9.1,2-bis[3,5-bis{4-(N,N-Dimethylamino)benzylidene}-4-oxo-piperidin-1-yl]ethane-1,2-dione (**7i**)*

Yield: 46%; mp (chloroform/methanol) >300 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.75(s, 2H, 2×=CH), 7.65 (s, 2H, 2×=CH), 7.33 (d, J=8.75 Hz, 4H, Ar-H), 7.21(d, J=8.71 Hz, 4H, Ar-H), 6.73 (d, J=16.93 Hz, 8H, Ar-H), 4.58 (s, 4H, 2×NCH_2), 4.54 (s, 4H, 2×NCH_2), 3.08 (s, 12H, 4×NCH_3), 2.95 (s, 12H, 4×NCH_3). Anal.calcd for $C_{44}H_{40}N_2O_4$.1.5 H_2O: C 76.76; H 6.30; N 4.07 %, found: C 76.46; H 6.39; N 3.93%.

*6.1.3. General procedure for the synthesis of 3,5-bis(arylidene)-4-piperidone dimers (**8a-j**)*

Malonyl chloride (0.01 mol, 1.4 gm) in DCE (5 ml) was added dropwise to a stirred suspension of a 3,5-bis(arylidene)-4-piperidone **6** (0.02 mol) in DCE (20 ml) containing triethylamine (0.02 mol, 2.02 gm) at ~20 ^{o}C for a period of 30 min. The reaction was stirred at room temperature 8-12 h. The solvent was removed under reduced pressure at 45 ^{o}C. An aqueous solution of potassium carbonate (25 ml, 5% w/v) was added to the crude mass and stirred for 2 h. The solid obtained was filtered, dried, and crystallized from a suitable solvent to yield pure products.

*6.1.3.1. 1,3-bis-[3,5-bis(Benzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8a**)*

The synthesis of **8a** was reported previously[24].

*6.1.3.2. 1,3-bis-[3,5-bis(4-Methylbenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8b**)*

Yield: 43%; mp (chloroform/methanol) 251 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (s, 2H, 2×=CH), 7.74 (s, 2H, 2×=CH), 7.40 (d, J=7.90 Hz, 4H, Ar-H), 7.27 (d, J=7.60 Hz, 4H, Ar-H), 7.21(q, 8H, Ar-H), 4.89 (s, 4H, 2×NCH_2), 4.65 (s, 4H, 2×NCH_2), 3.15 (s, 2H, CH_2), 2.43 (s, 6H, 2×CH_3), 2.41(s, 6H, 2×CH_3). ^{13}C NMR (125MHz, $CDCl_3$): δ 186.39(C=O), 165.30(C=ON), 140.31(C=C-Ph), 140.17(C=C-Ph), 138.62(C=C-Ph), 137.34(C=C-Ph), 131.86(Ar-C), 131.39(Ar-C), 130.88(Ar-C), 130.53(Ar-C), 130.45(Ar-C), 130.39(Ar-C), 130.33(Ar-C), 129.68(Ar-C), 129.61(Ar-C), 128.77(Ar-C), 46.79(CH_2NCO), 43.85(CH_2NCO), 40.54 ($COCH_2CO$), 21.53(CH_3). Anal.calcd for $C_{45}H_{42}N_2O_4$.H_2O: C 77.94; H 6.40; N 4.04 %, found: C

77.94; H 6.63; N 3.98%.

*6.1.3.3. 1,3-bis-[3,5-bis(4-Chlorobenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8c**)*

Yield: 58%; mp (chloroform/methanol) 260 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.64 (s, 2H, 2×=CH), 7.60 (s, 2H, 2×=CH), 7.57 (d, J=11.46 Hz, 4H, Ar-H), 7.54 (d, J=7.60 Hz, 4H, Ar-H), 7.49 (q, 8H, Ar-H), 4.62 (d, 8H, J=10.15 Hz, 4×NCH_2), 3.51 (s, 2H, CH_2). Anal.calcd for $C_{41}H_{30}Cl_4N_2O_4$.2.5 H_2O: C 61.38; H 3.77; N 3.49 %, found: C 61.10; H 3.69; N 3.30%.

*6.1.3.4. 1,3-bis-[3,5-bis(3,4-Dichlorobenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8d**)*

Yield: 65%; mp (chloroform/methanol) 236 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.71 (s, 2H, 2×=CH), 7.63 (s, 2H, 2×=CH), 7.56 (q, 8H, Ar-H), 7.51 (d, J=8.29 Hz, 2H, Ar-H), 7.30 (d, J=1.45 Hz, 2H, Ar-H), 7.31 (dd, J=1.45 Hz, J=1.53 Hz, Ar-H), 7.15 (dd, J=1.48 Hz, 2H, Ar-H), 4.78 (d, 8H, J=6.72 Hz, 4×NCH_2), 3.30 (s, 2H, CH_2). Anal.calcd for $C_{41}H_{26}Cl_8N_2O_4$. H_2O: C 53.93; H 3.09; N 3.07 %, found: C 53.84; H 2.98; N 2.74%.

*6.1.3.5. 1,3-bis-[3,5-bis(4-Fluorobenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8e**)*

Yield: 56%; mp (chloroform/methanol) >300 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81 (s, 2H, 2×=CH), 7.75 (s, 2H, 2×=CH), 7.49(q, 4H, Ar-H), 7.33 (q, 4H, Ar-H), 7.18 (t, 4H, Ar-H), 7.12 (t, 4H, Ar-H), 4.84 (s, 4H, 2×NCH_2), 4.79 (s, 4H, 2×NCH_2), 3.22 (s, 2H, CH_2). Anal.calcd for $C_{41}H_{30}F_4N_2O_4$.H_2O: C 69.42; H 4.27; N 3.95 %, found: C 69.58; H 4.30; N 3.71%.

*6.1.3.6. 1,3-bis-[3,5-bis(4-Methoxybenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8f**)*

Yield: 61%; mp (chloroform/methanol) 245 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.80 (s, 2H, 2×=CH), 7.73 (s, 2H, 2×=CH), 7.48 (d, J=8.6 Hz, 4H, Ar-H), 7.29 (d, J=10.17 Hz, 2H, Ar-H), 6.98 (d, J=8.49 Hz, 4H, Ar-H), 4.85(s, 4H, 2×NCH_2), 4.72 (s, 4H, 2×NCH_2), 3.88 (d, J=7.72 Hz, 12H, 4×OCH_3), 3.22 (s, 2H, CH_2). Anal.calcd for $C_{45}H_{42}N_2O_8$.4 H_2O: C 66.59; H 5.22; N 3.45 %, found: C 66.68; H 5.38; N 3.27%.

*6.1.3.7. 1,3-bis-[3,5-bis(3,4-Dimethoxybenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8g**)*

Yield: 65 %; mp (chloroform/methanol) >300 ^{o}C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.86 (s, 4H, 4×=CH), 7.17 (s, 4H, Ar-H), 7.13 (s, 8H, Ar-H), 4.54 (s, 8H, 4×NCH_2), 3.84 (d, J=7.27 Hz,

24H, 8×OCH_3), 3.48 (s, 2H, CH_2). Anal.calcd for $C_{49}H_{50}N_2O_{12}$.7 H_2O: C 59.69; H 6.54; N 2.84 %, found: C 59.82; H 6.18; N 2.71%.

*6.1.3.8. 1,3-bis-[3,5-bis(3,4,5-Trimethoxybenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8h**)*

Yield: 53%; mp (chloroform/methanol) 115 ^oC; ^1H NMR (500 MHz, $CDCl_3$): δ 7.78 (s, 2H, 2×=CH), 7.74 (s, 2H, 2×=CH), 6.72 (s, 4H, Ar-H), 6.58 (s, 4H, Ar-H), 4.96 (s, 4H, 2×NCH_2), 4.90 (s, 4H, 2×NCH_2), 3.92 (d, J=15.37 Hz, 30H, 10×OCH_3), 3.86 (s, 6H, 2×OCH_3), 3.37 (s, 2H, CH_2). Anal.calcd for $C_{53}H_{58}N_2O_{16}$.H_2O: C 63.83; H 6.07; N 2.81 %, found: C 63.84; H 6.27; N 2.51%.

*6.1.3.9. 1, 3-bis-[3, 5-bis{4-(N,N-Dimethylamino)benzylidene}-4-oxo-piperidin-1yl]propane-1,3-dione (**8i**)*

Yield: 42%; mp (chloroform/methanol) >300 ^oC; ^1H NMR (500 MHz, $CDCl_3$): δ 7.78 (s, 4H, 4×=CH), 7.36 (d, J=8.64 Hz, 8H, Ar-H), 6.73 (d, J=8.70 Hz, 8H, Ar-H), 4.21(s, 8H, 4×NCH_2), 3.05 (s, 26H, 8×NCH_3 and CH_2). Anal.calcd for $C_{49}H_{54}N_6O_4$.1.5 H_2O: C 71.88; H 7.02; N 10.61 %, found: C 71.59; H 7.28; N 10.78 %.

*6.1.3.10. 1,3-bis-[3,5-bis(4-Hydroxybenzylidene)-4-oxo-piperidin-1yl]propane-1,3-dione (**8j**)*

Yield: 56%; mp (chloroform/ethanol) >300 ^oC; ^1H NMR (500 MHz, DMSO-d_6): δ 7.64 (s, 2H, 2×=CH), 7.60 (s, 2H, 2×=CH), 7.57 (d, J=11.46 Hz, 4H, Ar-H), 7.54 (d, J=7.60 Hz, 4H, Ar-H), 7.49 (q, 8H, Ar-H), 4.62 (d, 8H, J=10.15 Hz, 4×NCH_2), 3.51 (s, 2H, CH_2). Anal.calcd for $C_{41}H_{34}N_2O_8$.4.5 H_2O: C 64.46; H 5.68; N 3.67 %, found: C 64.59; H 5.31; N 3.59%.

*6.1.4. Synthesis of 1,3-bis-[3,5-bis(benzylidene)-4-oxo-piperidin-1yl]propane (**9**)*

A mixture of 3,5-bis(benzylidene)-4-piperidone **6a** (0.004 mol, 1 gm), 1,3-dibromopropane (0.002 mol, 0.41 gm), potassium carbonate (0.002, 0.28 gm) and a catalytic amount of potassium iodide (10 mg) in acetonitrile (30 ml) was heated at reflux temperature for 6-7 h. Further quantities of 1,3-dibromopropane (0.001 mol, 0.2 gm) were added and the reaction continued for another 3-4 h. The completion of the reaction was monitored by TLC (solvent: chloroform-methanol 98:2). After the reaction was complete, the solvent was removed under reduced pressure at 45 ^oC. An aqueous solution of potassium carbonate (25 ml, 10% w/v) was

added to the crude mass and stirred for 2 h. The solid obtained was filtered, dried, and crystallized from acetone to yield pure product. Yield: 63 %; mp (acetone) 135 ^{o}C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.81(s, 4H, 4×=CH), 7.39 (m, 20H, Ar-H), 3.78 (s, 8H, 4×NCH_2), 2.54 (t, 4H, 2×CH_2), 1.60 (p, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 187.34 (CO), 136.57 (C=C-Ph), 135.20 (C=C-Ph), 133.11 (C=C-Ph), 130.40 (Ar-C), 129.05 (Ar-C), 128.60(Ar-C), 55.38 (NCH_2CH_2), 54.97 (NCH_2-piperidone), 25.91 (CH_2). Anal.calcd for $C_{41}H_{38}N_2O_2$: C 83.36; H 6.48; N 4.74 %, found: C 83.74; H 6.79; N 5.05%.

6.2. Biology

6.2.1. Cell culture

Phosphate buffered saline (PBS), pH 7.8, versene, and trypsin (2.5%) was obtained from Invitrogen Inc. (Burlington, ON, Canada). Fetal bovine serum (FBS) was purchased from Fisher Scientific (Toronto, ON, Canada). McCoy's 5A medium (ATCC cat No: 30-2007) was purchased from the ATCC (American Type Culture Collection, Rockville, USA). The penicillin-streptomycin antibiotic solution and all other chemicals unless otherwise indicated were purchased from Sigma (Oakville, ON, Canada). MilliQ water was obtained from a MilliQ water purification system (Millipore, MA, USA). Dr. Keith Bonham, Saskatoon Cancer Center, Saskatoon kindly gifted the HCT116 and HT29 colon cancer cell lines. The HCT116 and HT29 cell lines were subcultured in McCoy's 5A medium (ATCC cat No: 30-2007) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin antibiotic solution. The cells were grown in an atmosphere of 95% O_2 and 5% CO_2 with 95% humidity.

6.2.2. Cytotoxicity assay

The cytotoxicity of the compounds **7-9,** 5-fluorouracil and curcumin were determined using the sulforhodamine B assay as reported previously [32,33]. The cells were grown in McCoy's 5A medium supplemented with 10% FBS. The cells were harvested using 0.25 % trypsin in versene and the cell count was determined using the trypan blue exclusion method. In a 96-well plate, about 5×10^3 cells in 100 µl of complete media were plated per well and incubated for 24 h. The test compounds were serially diluted in DMSO and added to media for a final concentration of 1% DMSO. From this solution an aliquot of 100 µl was added to each well and incubated for 48 h. The cells were fixed by adding 50 µl of 50% w/v aqueous trichloroacetic acid to each well and

incubated at 4°C for 1 h. To obtain the cell numbers at zero time point (Tz), a plate was fixed at the time of treatment of the cells. The plates were rinsed with tap water four times and air dried. The fixed cells were stained with 0.4% w/v sulforhodamine in 1% v/v acetic acid for 10 min and washed four times with 1% v/v acetic acid and air dried. The dye was re-dissolved in 200 µl of 10 mM Trizma base and the absorbance read at 515 nm. The percent growth was calculated as per the equation below. The IC_{50} values were derived by fitting a four-parameter curve into the percent cell growth versus log concentration data using GraphPad Prism 5.0 for Windows (GraphPad Software, San Diego, California, USA).

$$\% \text{ Growth inhibition} = \frac{OD_{sample} - ODT_z}{OD_{control} - ODT_z}$$

6.2.3. Apoptosis assay

Apoptosis assay was conducted using Annexin V-FITC apoptosis detection kit (Biovision; Catalogue No: ALX-850-250-KI02) as per the suggested protocol. HCT116 cells were grown in McCoys'5A Modified media supplemented with 10% FBS and 1% antibiotic-antimycotic solution in a humidified incubator at 37°C with 5% CO_2. About 1.5×10^3 cells per well were seeded in 6 well plates and incubated for 24 h at 37 ^oC following which the cells were treated with 1µM conc. of **7f** in media with a final conc. of 1% DMSO. Apoptotic cell death was examined at different time points 1, 5, 11, 24 and 48 h and compared against untreated cells. The cells were collected by trypsinization (0.2% Trypsin-EDTA) followed by centrifugation for 5 min at 1000g at 4 °C. The pellet was washed with 1ml of 1X PBS, resuspended in 500 microlitres of 1X binding buffer and treated with 5 µl of Annexin V-FITC (fluorescein isothiocyanate labeled annexin V) and 5 µl of propidium iodide. After incubating the samples in the dark for 5 min at room temperature, apoptotic cell death was detected by a fluorescence-activated cell sorter (FACS) and analyzed by CellQuest (Becton-Dickinson) software.

6.2.4. Cell cycle analysis

Cell cycle analysis was carried out by modifying a literature procedure [34]. HCT116 and HT29 cells were maintained in McCoys'5A Modified media supplemented with 10 % FBS and 1% antibiotic-antimycotic solution. About 1×10^6 cells were seeded in 75 cm^2 flasks and incubated for 24 h in a humidified incubator at 37 °C with 5% CO_2. The cells were treated with 1µM of **7f** in media having 1% DMSO. The treated cells and untreated control were incubated in a

humidified incubator for 48 h at 37° C with 5 % CO_2. Floating cells were collected and adherent cells were harvested with trypsin-EDTA (0.2%) and pooled. The samples were washed with cold PBS, fixed in 70 % ethanol and left on ice for 2 h. Further samples were washed after 2 h with PBS and resuspended in PBS containing RNase (300 μg/ml). The samples were incubated for 20 min in the dark with propidium iodide (20 μg/ml) and RNase (300 μg/ml). The samples were analyzed by a FACScalibur (BD) flow cytotmeter and data were analyzed using Modfit LT free trial version 3.3 available from Variety software house. Cells were gated to include G_0/G_1, S-phase, and G_2/M populations.

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Captions for the figures and schemes

Figure 1. Structures of the compounds in series **1**-**5**.

Figure 2. Analysis of the effect of **7f** on HCT116 cells after incubation for 24 h and 48 h by flow cytometry. The experiments were carried out in duplicate and the % of live, dead and apoptotic cells are the average values of two independent experiments.

Scheme 1. Syntheses of the compounds in series **7**-**9**. i = acetic acid/dry HCl; ii = oxalyl chloride (for **7a-i**) and malonyl chloride (for **8a-j**); iii = 1,3-dibromopropane/K_2CO_3/KI. The aryl substituents are presented in Table 1.

Table 1: Evaluation of **7a-i**, **8a-j** and **9** against HCT116 and HT29 colon cancer cell lines.

Compound	Aryl substituents			IC_{50} (µM)		RP[a]	
	R^1	R^2	R^3	HCT116	HT29	HCT116	HT29
7a	H	H	H	0.04±0.03	0.014±0.002	113	454
7b	H	CH_3	H	0.09±0.02	0.129±0.035	50	49
7c	H	Cl	H	0.03±0.01	0.78±0.13	151	8
7d	H	Cl	Cl	20.05±1.01	49.46±4.50	0.23	0.13
7e	H	F	H	0.02±0.01	0.03±0.001	226	212
7f	H	OCH_3	H	0.02±0.01	0.17±0.04	226	37
7g	H	OCH_3	OCH_3	0.03±0.01	0.568±0.265	151	11
7h	OCH_3	OCH_3	OCH_3	0.27±0.06	0.27±0.15	17	24
7i	H	$N(CH_3)_2$	H	27.37±3.68	18.56±2.30	0.17	0.34
8a	H	H	H	0.02±0.01	0.003±0.002	226	2117
8b	H	CH_3	H	0.41±0.25	0.20±0.02	11	32
8c	H	Cl	H	0.05±0.001	0.09±0.05	90	71
8d	H	Cl	Cl	26.73±2.35	6.05±0.10	0.17	1.1
8e	H	F	H	0.60±0.01	0.18±0.08	8	35
8f	H	OCH_3	H	0.06±0.01	0.77±0.05	75	8
8g	H	OCH_3	OCH_3	0.18±0.07	0.31±0.12	25	20
8h	OCH_3	OCH_3	OCH_3	0.02±0.003	0.04±0.02	226	159
8i	H	$N(CH_3)_2$	H	>25	>25	<1	<1
8j	H	OH	H	0.72±0.10	0.46±0.21	6	14
9	-	-	-	0.68±0.19	2.15±0.65	7	3
5-FU	-	-	-	4.52±0.54	6.35±1.12	1.00	1.00
Curcumin	-	-	-	11.54±4.45	13.20±2.08	0.39	0.48

[a]The relative potency (RP) of the compounds against a reference drug, 5-fluorouracil was calculated by dividing the IC_{50} value of 5-fluorouracil by the IC_{50} value of the compound against a particular cell line. Only the mean IC_{50} values were considered for calculating RP values.

Table 2. Comparisons between some of the potencies of the compounds in series **7** and **8**.

Series **7**, **8**	Aryl substituent	$\Delta_{8/7}$[a]		$\Delta_{7b\text{-}i/7a}$[b]		$\Delta_{8b\text{-}i/8a}$[c]	
		HCT116	HT29	HCT116	HT29	HCT116	HT29
a	H	0.50	0.21	---	---	---	---
b	4-CH_3	4.56	1.55	2.25	9.21	20.5	66.7
c	4-Cl	1.67	0.12	0.75	55.7	2.50	30.0
d	3,4-Cl_2	1.33	0.12	501	353	1337	2017
e	4-F	30.0	6.00	0.50	2.14	30.0	60.0
f	4-OCH_3	3.00	4.53	0.50	12.1	3.00	257
g	3,4-$(OCH_3)_2$	6.00	0.54	0.75	40.7	9.00	103
h	3,4,5-$(OCH_3)_3$	0.07	0.15	6.75	19.3	1.00	13.3
i	4-$N(CH_3)_2$	>0.19	>1.35	684	1326	>1250	>8333

[a]The $\Delta_{8/7}$ values are the quotients of the IC_{50} values in series **8** divided by the analog in series **7** which has the same aryl substituent.

[b]The $\Delta_{7b\text{-}i/7a}$ values are the quotients of the IC_{50} values of **7b-i** divided by the figure for the unsubstituted compound **7a**.

[c]The $\Delta_{8b\text{-}i/8a}$ values are the quotients of the IC_{50} values of **8b-i** divided by the figure of the unsubstituted compound **8a**.

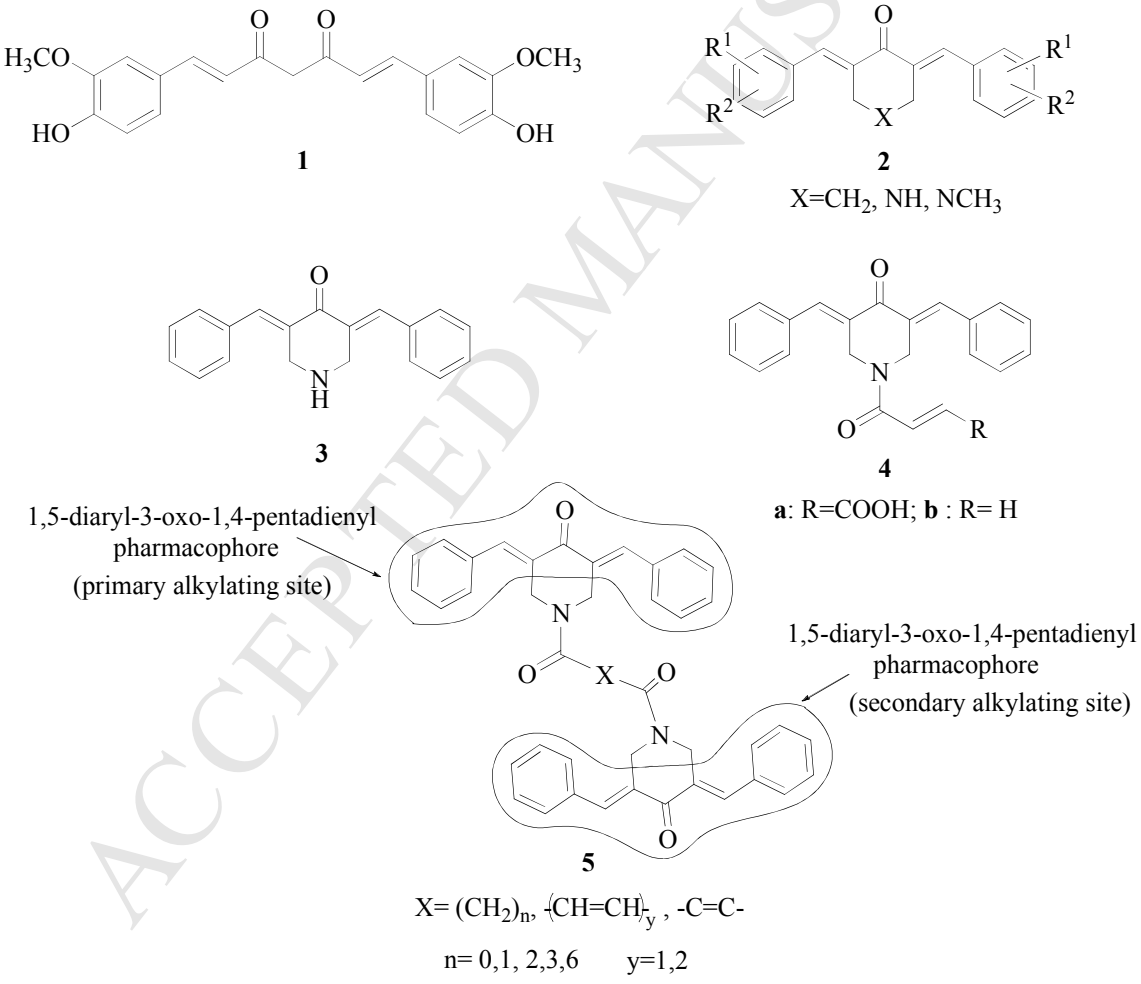

H3CO
HO
OCH3
OH
O
O
1
R1
R2
X
2
X=CH2, NH, NCH3
N
H
3
N
O
R
4
a: R=COOH; b : R= H
1,5-diaryl-3-oxo-1,4-pentadienyl
pharmacophore
(primary alkylating site)
1,5-diaryl-3-oxo-1,4-pentadienyl
pharmacophore
(secondary alkylating site)
5
X= (CH2)n, -(CH=CH)y , -C=C-
n= 0,1, 2,3,6 y=1,2

Figure 1

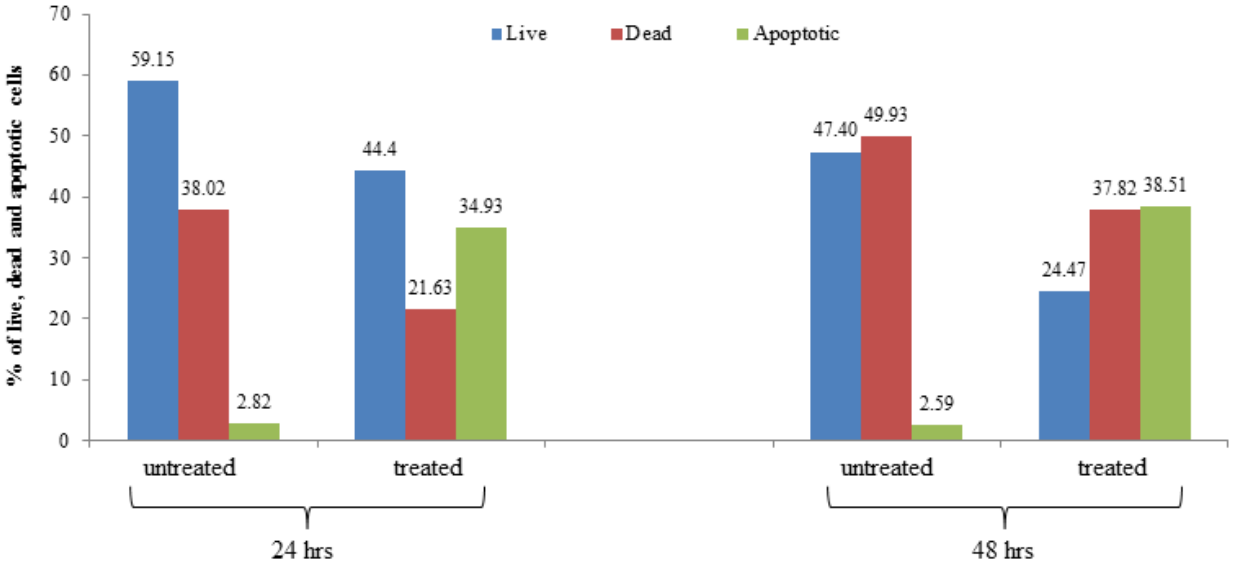
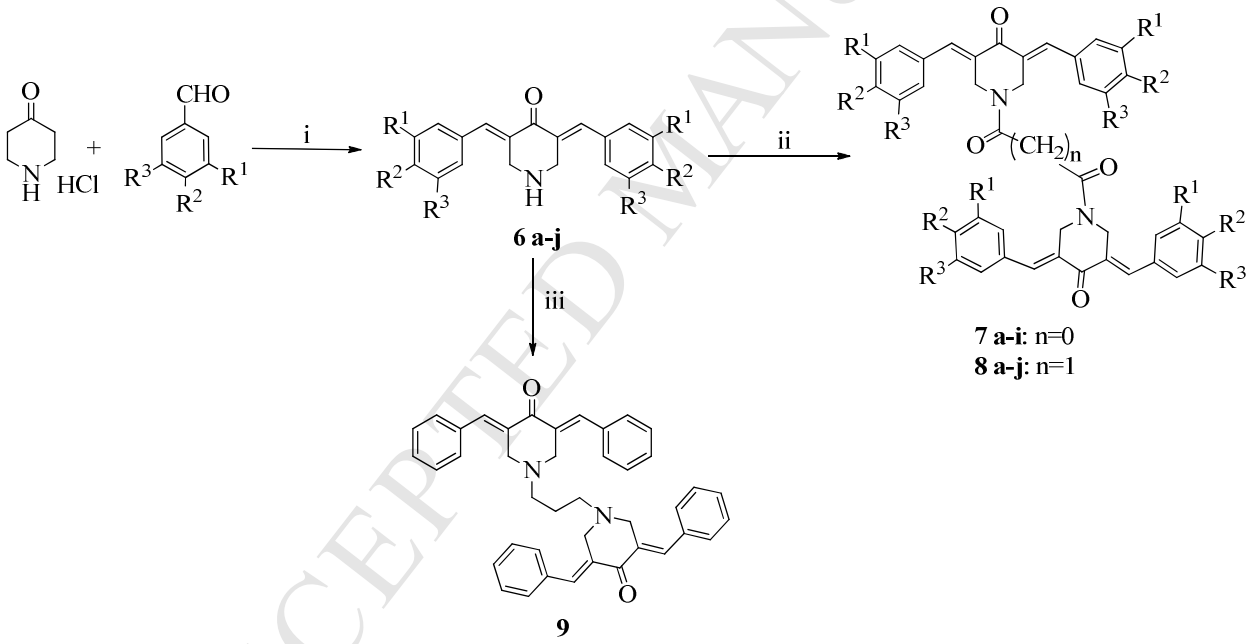

% of live, dead and apoptotic cells
70
60
50
40
30
20
10
0
Live
Dead
Apoptotic
59.15
38.02
2.82
44.4
21.63
34.93
47.40
49.93
2.59
24.47
37.82
38.51
untreated
treated
untreated
treated
24 hrs
48 hrs

Figure 2



Scheme 1

Highlights

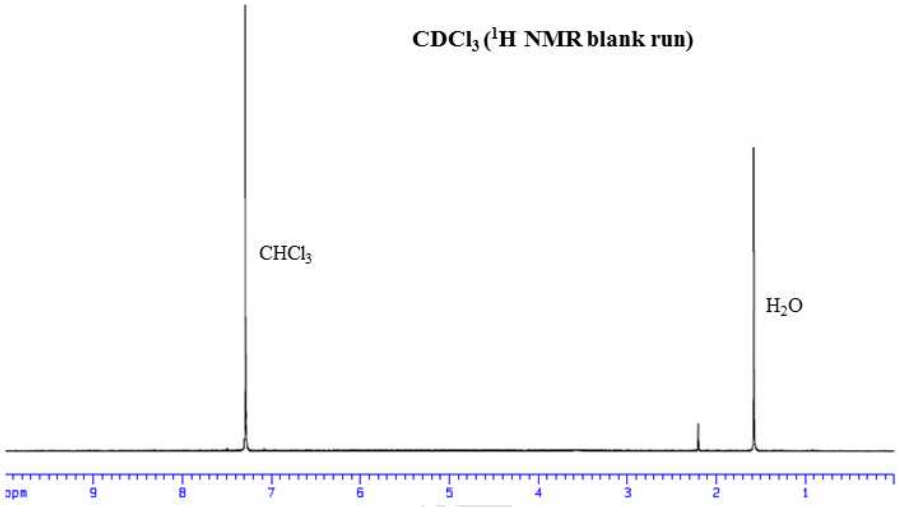
- Novel 3,5-bis(benzylidene)-4-piperidones were developed as cytotoxic agents
- The compounds were effective against HCT116 and HT29 colon cancer cells
- The IC_{50} values of most of the compounds are in the 10^{-7} to 10^{-9} M range
- QSAR studies were undertaken
- -A lead compound demonstrated apoptosis in HCT116 cells

SUPPLIMENTARY MATERIALS

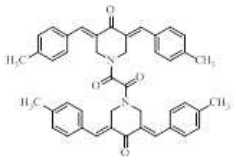
Novel 3,5-bis(arylidene)-4-piperidone dimers: Potent cytotoxins against colon cancer cells

Swagatika Das, Umashankar Das*, Deborah Michel, Dennis K. J. Gorecki, Jonathan R. Dimmock*

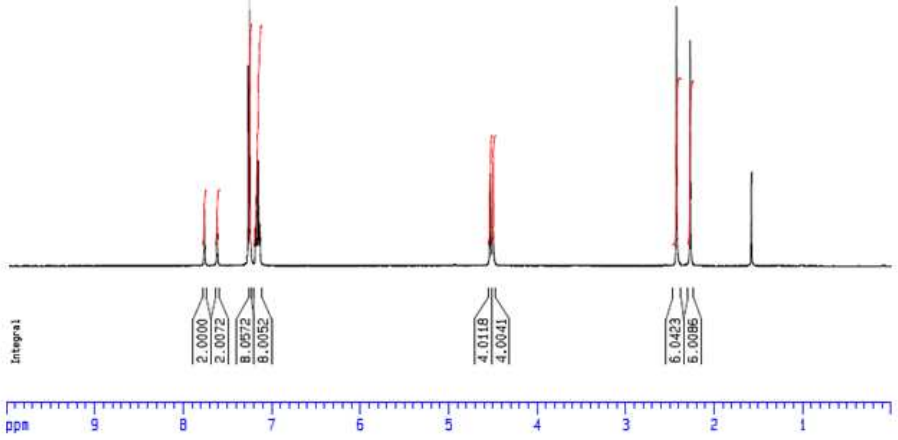
^{1}H and ^{13}C NMR spectra for representative compounds



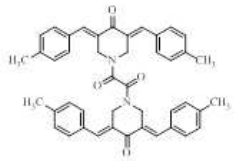
^{1}H NMR



7b

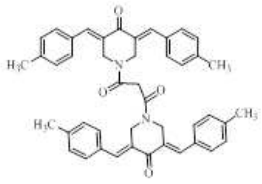


^{13}C NMR



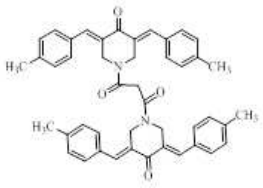
7b

^{1}H NMR

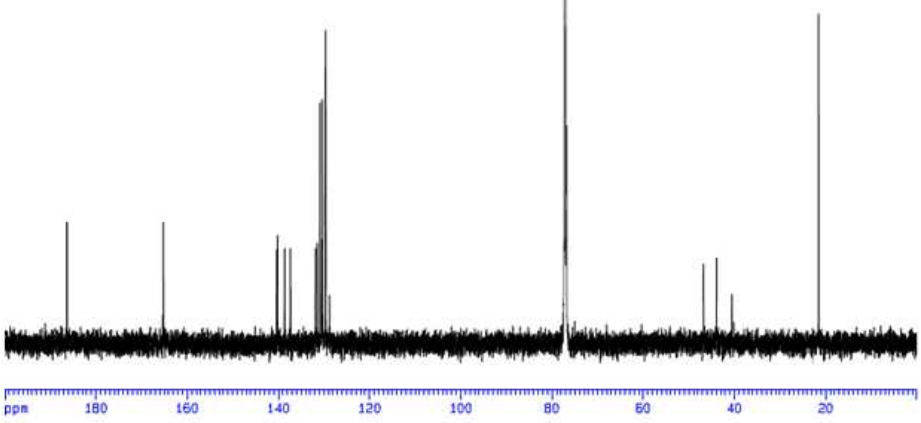


8b

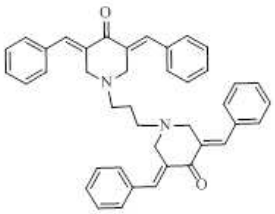
^{13}C NMR



8b

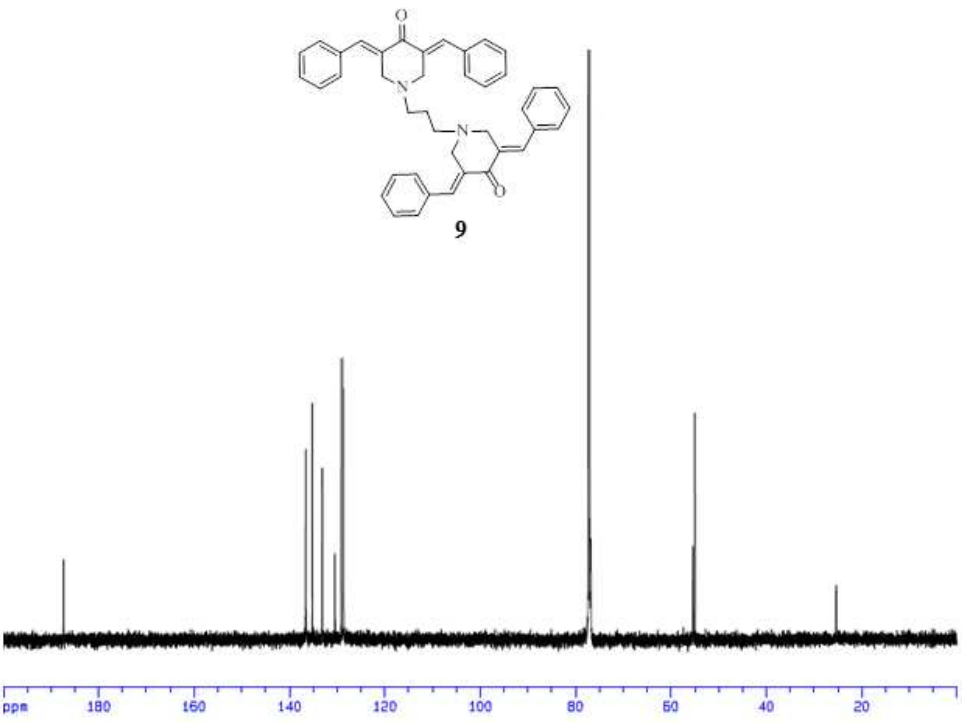


^{1}H NMR



9

^{13}C NMR



9