Synthesis and Cytotoxicity of Novel 3-Aryl-1-(3′-dibenzylaminomethyl-4′-hydroxyphenyl)-propenones and Related Compounds

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The reaction of various 4′-hydroxychalcones (1a—e) with paraformaldehyde and dibenzylamine led to the formation of a novel series of 4′-hydroxy-3′-dibenzylaminomethyl chalcones (7a—e) instead of 4′-hydroxy-3′,5′-bis-(dibenzylaminomethyl)chalcones 4. In order to rationalise the formation of monoadduct 7, energy minimized model structures of 4a and 7a were compared. The *in vitro* cytotoxic activities of 7a—e were tested against PC-3 cell lines for the first time in this study and compared with the precursor 4′-hydroxychalcones (1a—e). Except for compound 7a (IC_{50}: 19.85 μM), insertion of dibenzylaminomethyl function into 4′-hydroxychalcones resulted in complete loss of cytotoxic activity. The results suggested that it is not only the pK_a but also the shape and size of the amine that is critical in governing the cytotoxic activity.

Key words chalcone; Mannich base; dibenzylamine; cytotoxicity; androgen independent prostate cancer cell line

Mannich ketones are well documented as potent cytotoxic agents.[1—6] The bioactivity of Mannich ketones may be due to the alkylating ability of α,β-unsaturated ketones that are liberated *in situ* following deamination (Fig. 1).[2,4,7—11] Various studies on Mannich ketones have been reported from our laboratory[1—6] but toxicities[7,8] associated with this class of compounds have discouraged us to pursue them further. The unwanted toxicities associated with the Mannich ketones may be due to the rapid decomposition to form a reactive enone which is sequestered indiscriminately with cellular nucleophiles. This has prompted our interest in chalcones which carry an α,β-unsaturated keto motif and are associated with diverse biological activities[9—14] and are often devoid of undesirable toxicity.[15,16] We were very interested in studying 4′-hydroxychalcones due to their effectiveness as cytotoxic,[11,17,18] antitumour,[9,13] antioxidative,[9] antibacterial,[14,17] and antileishmanial agents.[14] An extensive study on the cytotoxic properties of various 4′-hydroxychalcones and the corresponding 3′,5′-bis(aminomethyl)-4′-hydroxychalcones was undertaken by Dimmock *et al.*[11] This study suggested that insertion of aminomethyl moieties at the *ortho* positions of the 4′-hydroxyl group leads to an increase in cytotoxic potency against human CEM T-lymphocytes and the cytotoxicity is influenced by the nature of the amino group *e.g.* replacement of dimethylamine (**2**, pK_a: 10.78[19]) by morpholine (**3**, pK_a: 8.49[19]) increased potency by 3 fold. Therefore, a negative correlation between cytotoxicity and the pK_a of the amine had emerged. In other words, cytotoxicity increases as the pK_a of the amine decreases. As both the amines differ by their shape and size, it is improper to conclude that the pK_a of the amine is the only factor that influences cytotoxicity. On occasions, the shape and size of the amines dramatically alter the bioactivity.[20] In order to explore this possibility, the energy minimized structures of **2** and **3** were modelled and superimposed at our end, but these exercises did not reveal any statistically significant topographical variations. No noticeable changes in the torsion angles of both structures were observed (**2**, θ_1 −175.28, θ_2 −35.30 and **3**, θ_1 −178.75, θ_2 −37.93). Therefore, in this case, it is conceivable that the magnitude of the pK_a, not the shape or size of the amine, is responsible for the greater cytotoxic potency associated with **3**. However, this study remained inconclusive to gain some insights of the influence of shape and size of the amines on cytotoxicity as both the amines, dimethylamine (V= 58.16 Å^3) and morpholine (V=90.38 Å^3) do not differ greatly in terms of their shape and size. In order to gain some meaningful conclusions in this regard, we were interested in developing further analogues of **2** and **3** *i.e.* series **4** carrying an amine function with a similar pK_a value that of morpholine but differing by its shape and size to a greater extent *e.g.* dibenzylamine (pK_a: 8.52,[19] V=201.46 Å^3).

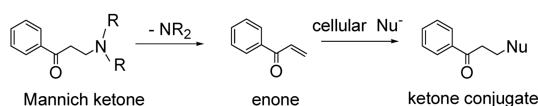

Fig. 1. Liberation of an Enone Following Deamination and Subsequent Reactions with Cellular Nucleophiles

The rationale to prepare **4** included the following considerations. First, conversion of various acyclic conjugated styryl ketones into the corresponding Mannich bases is often accompanied by increased bioactivity both *in vitro* and *in vivo*.[21] Second, the rate of deamination is inversely proportional to the pK_a of amine. An amino function with lower pK_a value is expected to undergo deamination easier and may generate an additional enone alkylating site *e.g.* cyclohexadienones (Fig. 2) for cellular thiols much more rapidly than an amine function with a higher pK_a value. So, higher potency of Mannich chalcones is expected compared to 4′-hydroxychalcones. A proposed mechanism of sequential cytotoxicity[11,22] by which this class of compounds exhibit cytotoxicity *in situ* is presented in Fig. 2. Third, selective affinity of α,β-unsaturated ketones towards thiols compared to hydroxyl and amino groups present in the nucleic acids may

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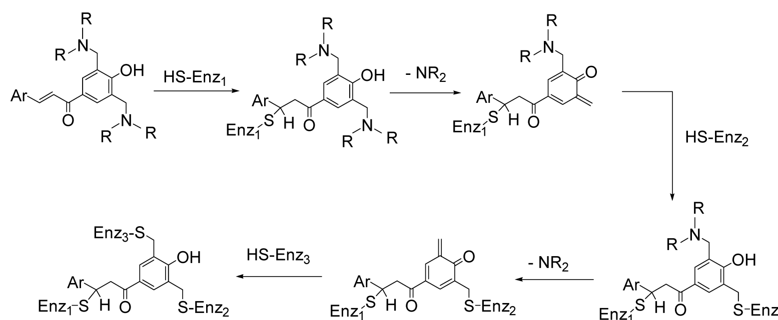
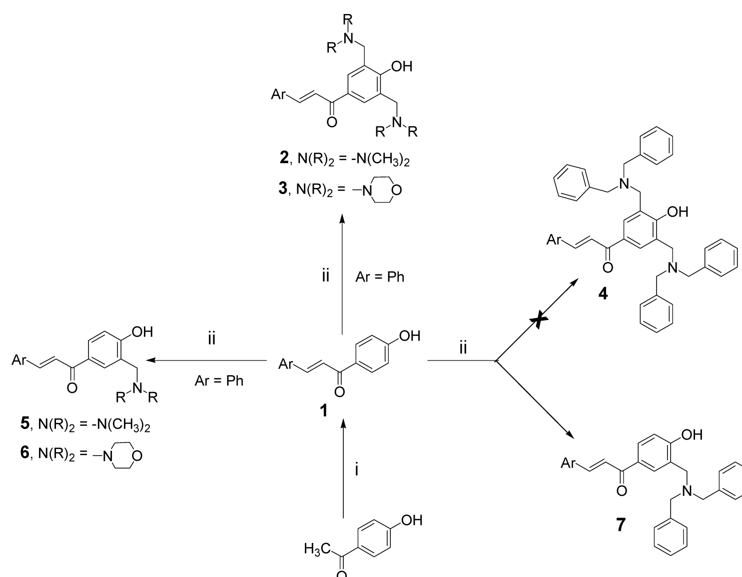


Fig. 2. Hypothesis of Sequential Cytotoxicity Proposed by Dimmock *et al.*[11,21]



Reaction condition: i, Ar-CHO/NaOH/ ethanol; ii, paraformaldeyde/amine/ethanol. For the compounds **1** and **7**: a: Ar=C_6H_5; b: Ar=4-ClC_6H_4; c: Ar=4-$CH_3OC_6H_4$; d: Ar=4-$CH_3C_6H_4$; e: 2-Thienyl.

Chart 1

eliminate the problems of mutagenicity and carcinogenicity, which has been associated with certain alkylating agents used in cancer chemotherapy.[23] Fourth, introduction of a bulkier amine would alter the conformation of the molecule dramatically which consequently influences radically the mode of interaction of the molecule at the binding site to exhibit any pharmacological action. Thereby, a correlation between topography of the molecules and cytotoxicity may emerge.

Results and Discussion

We intended to utilise the conventional Mannich reaction condition to prepare 3′,5′-bis(dibenzylaminomethyl)-4′-hydroxychalcone derivatives **4** (Chart 1) from 4′-hydroxychalcones **1**. The intermediate 4′-hydroxychalcones (**1a**—**e**) were prepared by the condensation of 4′-hydroxyacetophenone and an aryl aldehyde under alkaline conditions in 85—90% yield as described previously.[11,24] The chemical structures of the compounds **1a**—**e** were confirmed by ^{1}H-NMR. Melting points of **1a**—**e** were in accordance with the literature reports.[11,24] To our surprise, the reaction of a suitable 4′-hydroxychalcone **1** with paraformaldehyde and dibenzylamine in ethanol under refluxing condition resulted in the isolation of a novel series of 3′-dibenzylaminomethyl-4′-hydroxychalcone derivatives **7a**—**e** instead of expected 3′,5′-bis(dibenzylaminomethyl)-4′-hydroxychalcone derivative **4** (Chart 1). Various attempts to prepare **4** by varying the reactant ratio, reaction time and temperature proved to be unsuccessful. Bis(dimethylaminomethyl)-4′-hydroxychalcone **2** and bis(morpholinomethyl)-4′-hydroxychalcone **3** (Chart 1) were readily prepared from 4′-hydroxychalcone **1a** by Dimmock *et al.*,[11] whereas our efforts to prepare the corresponding bis(dibenzylaminomethyl)-4′-hydroxychalcones **4** led to the formation of only mono adducts **7**. This disparity in the product formation may be due to the pronounced steric effect of dibenzylamine compared to dimethylamine or morpholine that led to the formation of energetically more favourable mono adduct **7a** ($E=-1355.91$ au) than the bis-adduct **4a** ($E=-1987.12$ au) (Table 1). The bis(dibenzylaminomethyl) adduct **4a** possesses 1.46 fold higher energy than the corresponding mono-adduct **7a** whereas the bis(dimethylaminomethyl) adduct **2** ($E=-1068.95$ au) and bis(morpholinomethyl) adduct **3** (-1372.92 au) possess relatively lower energy than the bis(dibenzylaminomethyl) adduct **4a**. This relative energy of the molecules generated by HF/6-31* (*ab initio* calculation) partly rationalizes the disparity in the formation of mono-aminomethyl adduct when using dibenzylamine in place of dimethylamine and morpholine.

In order to evaluate the relative stability of the mono-adduct **7a** and bis-adduct **4a**, the net change in energy ΔE (sum of the energies of products, **4a**+H_2O−sum of the energies of reactants, **7a**+HCHO+dibenzylamine) was calculated. The ΔE found to be −25 kcal/mol. This result suggests that the formation of the bis-adduct **4a** is associated with higher state of energy leading to lower stability than the mono-adduct **7a**. The calculated ΔE for **4a** (−31.37 kcal/mol) was found to be 5 fold higher than **7a** (−6.27 kcal/mol) when compared against **1a**, suggesting the higher stability of mono-adduct over the bis-adduct. The relative thermodynamic stabilities of mono and bis-adducts corresponding to various amines *e.g.*, dimethylamine, morpholine and dibenzylamines were also accounted for by calculating the enthalpy change (ΔH), defined as $H_{products}-H_{reactants}$ during the chemical transformation. The ΔH for the formation of mono-adducts from **1a** (dimethylamine, −176.57 kcal/mol; morpholine, −326.25 kcal/mol; dibenzylamine, −15.03 kcal/mol) and bis-adducts from mono-adducts (dimethylamine, −177.01; morpholine, −323.86 kcal/mol; dibenzylamine, −12.10 kcal/mol) suggests that the bis-(dibenzylaminomethyl) adduct **4a** is the least stable product when compared with bis(dimethylaminomethyl) adduct **2** and bis(morpholinomethyl) adduct **3**. The stabilities of bis-adducts **2** and **3** are higher by 15 and 27 times respectively than **4a**. Thermodynamically, the mono-adduct **7a** is more favoured over bis-adduct **4a**.

Table 1. Energy Values of the Mono-Mannich Adducts and Bis-Mannich Adducts of 4′-Hydroxychalcone **1a**

Amine	*E* (au)	
	Mono-Mannich adduct	Bis-Mannich adduct
Dimethylamine	**5**, −896.82	**2**, −1068.95
Morpholine	**6**, −1048.59	**3**, −1372.42
Dibenzylamine	**7a**, −1355.91	**4a**, −1987.12

We were successful in preparing the mono-aminomethyl adducts, 3′-dimethylaminomethyl-4′-hydroxychalcone **5** and 3′-morpholinomethyl-4′-hydroxychalcone **6** from 4′-hydroxychalcone **1a** in 21% and 40% yield respectively, by using the reactants (paraformaldehyde, amine, 4′-hydroxychalcone) in molar ratios (1 : 1 : 1). The structure of the products was confirmed by ^{1}H-NMR, CHN analyses and IR. The conclusion drawn from the above results was that using the appropriate molar ratios of the reactants and reaction condition, bis and mono-Mannich adducts were obtained for dimethylamine and morpholine whereas in the case of dibenzylamine, irrespective of the molar ratios of the reactants and reaction condition used, only mono-adducts **7** were obtained.

The optimum yields of **7** were obtained using paraformaldehyde, dibenzylamine and chalcone in molar ratios (2 : 2 : 1) while the use of molar ratios (1 : 1 : 1) extended the reaction time substantially accompanied by low yields. To obtain the best yield, 4′-hydroxychalcone was added to a previously heated reaction mixture of dibenzylamine and paraformaldehyde in ethanol. Refluxing a mixture of dibenzylamine, paraformaldehyde and chalcone even in 2 : 2 : 1 molar ratio

Table 2. ^{1}H- and ^{13}C-NMR Data of Mannich Chalcones (**7a**—**e**)

Comp.	^{1}H-NMR (δ)	^{13}C-NMR (δ)
7a	δ: 3.66 (s, 4H, C17-H, C18-H, 2×NCH_2), 3.86 (s, 2H, C16-H, NCH_2), 6.93—6.91 (d, 1H, C14-H), 7.33—7.27 (m, 6H, C21-H, C22-H, C23-H, C27-H, C28-H, C29-H), 7.43—7.37 (m, 7H, C3-H, C4-H, C5-H, C20-H, C24-H, C26-H, C30-H), 7.56—7.53 (d, 1H, C8-H, *J*=15 Hz), 7.65—7.64 (dd, 2H, C2-H, C6-H), 7.83—7.79 (d, 1H, C7-H, *J*=15 Hz), 7.83 (d, 1H, C15-H), 7.92 (dd, 1H, C11-H).	δ: 188.93 (C9), 162.88 (C13), 144.10 (C7), 136.77 (C19, C25), 135.58 (C1), 130.69 (C11), 130.64 (C15), 130.45 (C10), 130.30 (C3, C5), 129.90 (C20, C24, C26, C30), 129.30 (C21, C23, C27, C29), 129.16 (C4), 128.72 (C22, C28), 128.30 (C2, C6), 122.58 (C12), 122.31 (C8), 116.42 (C14), 58.34 (C16), 57.06 (C17, C18).
7b	δ: 3.66 (s, 4H, C17-H, C18-H, 2×NCH_2), 3.85 (s, 2H, C16-H, NCH_2), 6.92—6.88 (d, 1H, C14-H), 7.33—7.31 (m, 6H, C21-H, C22-H, C23-H, C27-H, C28-H, C29-H), 7.41—7.37 (m, 6H, C3-H, C5-H, C20-H, C24-H, C26-H, C30-H), 7.52—7.49 (d, 1H, C8-H, *J*=15.63 Hz), 7.59—7.57 (d, 2H, C2-H, C6-H), 7.76—7.72 (d, 1H, C7-H, *J*=15.64 Hz), 7.82 (d, 1H, C15-H), 7.92—7.90 (dd, 1H, C11-H).	δ: 188.58 (C9), 163.01 (C13), 142.59 (C7), 136.71 (C19, C25), 136.51(C1), 134.07 (C4), 130.70 (C11), 130.45 (C15), 130.12 (C10), 129.98 (C3, C5), 129.86 (C20, C24, C26, C30), 129.58 (C21, C23, C27, C29), 129.17 (C22, 28), 128.33 (C2, C6), 122.70 (C12), 122.64 (C8), 116.46 (C14), 58.34 (C16), 57.04 (C17, C18).
7c	δ: 3.66 (s, 4H, C17-H, C18-H, 2×NCH_2), 3.85 (s, 2H, C16-H, NCH_2), 3.87 (s, 3H, C-31, OCH_3), 6.92—6.90 (d, 1H, C14-H), 6.95—6.94 (d, 2H, C3-H, C5-H), 7.37—7.27 (m, 6H, C21-H, C22-H, C23-H, C27-H, C28-H, C29-H), 7.40—7.38 (m, 4H, C20-H, C24-H, C26-H, C30-H), 7.44—7.41 (d, 1H, C8-H, *J*=15.56 Hz), 7.62—7.60 (d, 2H, C2-H, C6-H), 7.79—7.76 (d, 1H, C7-H, *J*=15.54 Hz), 7.82 (d, 1H, C15-H), 7.92—7.90 (dd, 1H, C11-H).	δ: 188.99 (C9), 162.65 (C13), 161.86 (C4), 143.95 (C7), 136.80 (C19, C25), 131.69 (C11), 130.45 (C15), 130.36 (C10), 129.99 (C20, C24, C26, C30), 129.16 (C21, C23, C27, C29), 128.28 (C22, C28, C1, C2, C6), 122.50 (C12), 119.97 (C8), 116.34 (C14), 114.77 (C3, C5), 58.33 (C16), 57.08 (C17, C18), 55.80 (C31).
7d	δ: 2.41 (s, 3H, C31-H), 3.66 (s, 4H, C17-H, C18-H, 2×NCH_2), 3.85 (s, 2H, C16-H, NCH_2), 6.93—6.91 (d, 1H, C14-H), 7.24—7.22 (d, 2H, C3-H, C5-H), 7.34—7.32 (m, 6H, C21-H, C22-H, C23-H, C27-H, C28-H, C29-H), 7.40—7.37 (m, 4H, C20-H, C24-H, C26-H, C30-H), 7.52—7.49 (d, 1H, C8-H, *J*=15.59 Hz), 7.56—7.54 (d, 2H, C2-H, C6-H), 7.83—7.77 (d, 1H, C7-H, *J*=15.59 Hz), 7.83 (d, 1H, C15-H), 7.93—7.91 (dd, 1H, C11-H).	δ: 189.02 (C9), 162.76 (C13), 144.20 (C7), 141.13 (C4), 136.79 (C19, C25), 132.83 (C1), 130.64 (C11), 130.41 (C15), 130.05 (C10), 129.99 (C3, C5), 129.17 (C20, C24, C26, C30), 128.76 (C21, C23, C27, C29), 128.29 (C22, C28, C2, C6), 122.53 (C12), 121.28 (C8), 116.38 (C14), 58.33 (C16), 57.07 (C17, C18), 21.91 (C31).
7e	δ: 3.66 (s, 4H, C15-H, C16-H, 2×NCH_2), 3.85 (s, 2H, C14-H, NCH_2), 6.93—6.91 (d, 1H, C13-H), 7.10—7.08 (q, 1H, C4-H), 7.41—7.32 (m, 13H, C3-H, C5-H, C7-H, C19-H, C20-H, C21-H, C22-H, C23-H, C25-H, C26-H, C27-H, C28-H, C29-H), 7.81 (d, 1H, C14-H), 7.92—7.81 (dd, 1H, C10-H), 7.95—7.92 (d, 1H, C6-H, *J*=15.33).	δ: 188.28 (C8), 162.87 (C12), 141.06 (C6), 136.77 (C5), 136.57 (C18, C24), 132.01 (C10), 130.59 (C2), 130.35 (C14), 129.99 (C9), 129.16 (C19, C23, C25, C29), 128.71 (C20, C22, C26, C28), 128.66 (C4), 128.30 (C21, C27, C3), 122.55 (C11), 121.06 (C7), 116.44 (C13), 58.33 (C15), 57.05 (C16, C17).

Table 3. UV and IR Data of Mannich Chalcones (**7a**—**e**)

Comp.	UV $\lambda_{max}^{CHCl_3}$ (log ε) nm	IR (stretching frequency, cm^{-1})		
		OH	C=O	C=C
7a	242 (4.05), 323 (4.46)	3029	1653	1586
7b	242 (4.08), 326 (4.51)	3000	1655	1589
7c	244 (4.16), 344 (4.49)	3023	1653	1589
7d	243 (4.12), 330 (4.49)	3026	1654	1589
7e	246 (3.88), 347 (4.45)	3028	1645	1575

prolongs the reaction time and results in significant yield loss.

The ^{1}H-NMR, ^{13}C-NMR, UV and IR spectra of the compounds **7a**—**e** are in accordance with their chemical structures. (Tables 2, 3). Elemental analyses (C, H, and N) results were within 0.4% of the calculated values. The presence of a mono dibenzylaminomethyl group was very much evident from the spectral data. For example in the case of **7a**, the dibenzylamine protons were observed at δ 7.43—7.37 and 7.33—7.27 ppm respectively in the ^{1}H-NMR spectra, where the methylene protons appeared at δ 3.86—3.66 ppm and the olefinic protons of Mannich chalcone **7a** were observed as doublets (J=15 Hz) at δ 7.56—7.53 and δ 7.83—7.79 ppm. On the basis of the J values, the structures were characterized as E isomers. The chemical shifts of the aromatic carbons of the dibenzylamino group were observed at δ 136.8—128.7 ppm. The carbon peaks of olefinic group in **7a** were observed at δ 144.1 and δ 122.3 ppm. In addition, a strong absorption was observed at 1653 cm^{-1} in the IR spectra of **7a** supports the α,β-unsaturated ketone structure[25] and the C=C stretching frequency shifts from 1586 to 1575 cm^{-1}. For the compounds **7a**—**e**, strong K bands were observed in the UV spectra at 347—323 nm, which results from the electronic transition of the styryl ketone chromophore $\pi \rightarrow \pi^*$. These bands are as important as olefinic proton signals in NMR spectra to follow adduct reactions carried out to determine the alkylating antineoplastic activity of the α,β-unsaturated ketones, and also to determine adduct–elimination ratios. The maximum absorption at 323 nm wave length in the UV spectrum of **7a** confirms the structure of the conjugated α,β-unsaturated ketone. The low intensity O–H stretching of the phenolic group of the Mannich chalcones was observed at 3029—3000 cm^{-1}. This lowering of intensity may be due to the intermolecular hydrogen bonding of hydroxyl groups. A positive ferric chloride test confirmed the presence of phenolic group in the compounds of series **7**.

The cytotoxic potencies of 4′-hydroxychalcones (**1a**—**e**) and their corresponding 3′-dibenzylaminomethyl derivatives (**7a**—**e**) were obtained against an androgen independent prostate cancer cell line (PC-3) (Table 4). All the compounds in series **1** displayed lower cytotoxicity than the reference drug 5-fluorouracil (5-FU) (Table 4). In series **1**, cytotoxicity was in the order of **1a**≥**1b**>**1c**>**1d**>**1e**. **1a** and **1b** were the most potent compounds and displayed moderate cytotoxicity (IC_{50}: 6.19 and 6.22 μM respectively). The potencies of **1a**—**d** may be influenced by the partition coefficient (log P) and the magnitude of the physicochemical properties *i.e.* electronic (σ), hydrophobic (π), molar refractivity (MR) of the aryl substituents. However, no correlations were noted ($p>0.05$). Replacement of the phenyl ring by thiophene

Table 4. Cytotoxic Potencies, log P and E_{LUMO} Values of **1a**—**e** and **7a**—**e**

Compound	IC_{50} (μM) PC-3 cells	log P	E_{LUMO} (kcal/mol)
1a	6.19	3.49	−54.25
1b	6.22	4.21	−72.17
1c	7.10	3.41	−49.70
1d	14.83	4.00	−52.29
1e	31.21	3.14	−83.93
7a	19.85	6.71	−46.77
7b	>25	7.43	−63.40
7c	>100	6.63	−42.84
7d	>100	7.21	−45.29
7e	>100	6.36	−75.33
5-FU	1.5	—	—

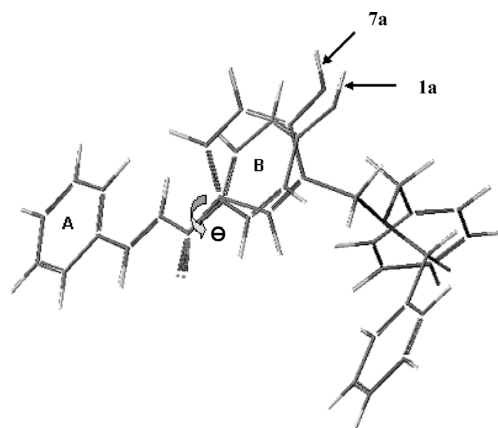


Fig. 3. Superimposition of **1a** and **7a** in Which Aromatic Ring A and C=C Atoms Are Overlapped

bioisostere led to a decrease in potency in **1e**. The unsubstituted compound **1a** was equipotent with **1b** which suggested that future modifications of series **1** should include various compounds bearing the aryl substituents such as 3-Cl, 3-CF_3, 3-$N(CH_3)_2$, 3-NO_2 as per the Topliss decision tree approach[26] that may lead to enhanced potency.

Insertion of the dibenzylaminomethyl group at the *ortho* position of 4′-hydroxychalcones (**1a**—**e**) led to lowering of bioactivity unexpectedly except **7a** which showed very weak cytotoxicity (IC_{50}: 19.85 μM). The energy minimized structures of **1a** and **7a** were modelled and superimposed in order to gain an insight of the structural and conformational changes in series **7** that led to the loss of bioactivity. Superimposition of **1a** and **7a** in which aromatic ring A and the C=C atoms are overlapped is shown in Fig. 3. This study revealed that coplanarity of ring **B** with the adjacent carbonyl function was lost in **7a** due to the insertion of the dibenzylaminomethyl group at the *ortho* position to the 4′-hydroxy group of **1a** and exerted a significant change in the shape of **7a** (torsion angle θ changed from 0 degree to 38.21 degree). One may conclude that the change in topography of the molecules in series **7** may be a possible contributing factor that leads to an improper alignment of the substrate at the receptor site which results in the loss of potency observed. The loss of coplanarity of ring B with the adjacent carbonyl function is expected to affect the LUMO energy of the molecules and deamination reaction. Therefore, the LUMO energies of all the compounds in the series **1** and **7** were obtained (Table

4) and in all cases the insertion of a dibenzylaminomethyl group into the ortho position of 4′-hydroxychalcones **1a**—**e** resulted in the lowering of LUMO energy. The lowering of LUMO energy leads to the activation of the carbonyl function, thus, facilitating the nucleophilic attack at the β-carbon. Therefore, the compounds in series **7** are expected to display improved activity over the compounds in series **1**. However, the compounds in series **7** displayed lower cytotoxicity compared to the compounds in series **1**. A possible explanation for this observation could be as follows, namely that lowering in the LUMO energy of the molecules in series **7** results in its greater solvation. Thus, on one hand results its weak interaction at the active site leading to decrease in cytotoxic effect. On the other hand, solvation of hydroxyl and amine part would retard the deamination process to release the activated enone species, which is believed to be responsible for exerting the cytotoxic effect as well as the electropositive character of the carbonyl function, which results in diminished nuclophilicity at the β-carbon. In order to investigate the reactivity of the Mannich chalcones **7** towards nucleophilic attack and their ability to generate the corresponding enone, a representative compound **7a** was reacted with a biomimetic thiol, 2-mercaptoethanol in phosphate buffer under physiological conditions (pH 7.4/37 °C) for 48 h following the reported methodology[29] and the isolated product was analysed by ^{1}H-NMR. After 48 h of incubation, there was no indication of any product (Mannich ketone-thiol conjugate) formation was observed, only unreacted compound **7a** was isolated from the reaction mixture. But under similar conditions, incubation of **1a** with ethanethiol formed minute quantities of thiol conjugate. The following experiment was designed with the rationale that acyclic/cyclic Mannich bases,[27,28] styryl ketones[29] and Mannich bases of styryl ketones[28] react with ethanethiol in phosphate buffer (pH 7.4) at 37 °C form the corresponding thiol conjugates. The results obtained from the stability study suggest the following. First, there is no formation of activated enone species following deamination of Mannich chalcones **7**. Second, insertion of dibenzylaminomethyl function onto 4′-hydroxychalcones deactivated the carbonyl function which in turn reduced the nuclophilicity at the β-carbon. These conclusions support the solvation theory discussed before and provide an explanation for lowering in cytotoxic potencies of Mannich chalcones **7** compared to the 4′-hydroxychalcones **1**.

In conclusion, this study rationalized the formation of a novel series of mono Mannich chalcones **7a**—**e** instead of the bis Mannich adducts **4**. Possible correlations between cytotoxicity and the log P values of the compounds or Hammett values of the aryl substituents were investigated and future development of the cluster of the compounds is noted. The loss of bioactivity of the **7a**—**e** was accounted as due to the steric effect of the dibenzylamine group that led to a significant change in the shapes and E_{LUMO} energies of the molecules and provided guidelines for the future development of this class of compounds.

Experimental

Synthesis of Compounds. Materials All chemicals were purchased from Aldrich Chemical Co. (Munich, Germany). Melting points were determined on a Thomas Hoover Instrument (Philadelphia, PA, U.S.) and are uncorrected. UV spectra were recorded in $CHCl_3$ by a Thermo Electron Heλios (α) (UVA 114903, England). ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a Bruker DPX-500FT-NMR spectrometer (U.S.A.) (chemical shift in δ, ppm). Elemental analyses were performed on LECO C, H, N, S-932 (U.S.A.) machine. The reactions were monitored using silicagel HF254-366 TLC plates (E. Merck, Darmstadt, Germany).

Table 5. Physical Data of Compounds **1a**—**e**

Comp. No.	Mol. formula	Mol. wt.	Yield (%)	mp (°C)	mp (°C) reported
1a	$C_{15}H_{12}O_2$	224.26	85	174	172—173[11]
1b	$C_{15}H_{11}ClO_2$	258.71	90	190	187—189[11]
1c	$C_{16}H_{14}O_3$	254.29	86	182	180—182[11]
1d	$C_{16}H_{14}O_2$	238.29	86	193	190—192[11]
1e	$C_{13}H_{10}O_2S$	230.29	90	172	171[23]

1-(4-Hydroxyphenyl)-3-phenyl-propenone Derivatives (1a—e) These compounds were prepared by the following procedure.[11] An aqueous solution of sodium hydroxide (10% w/v, 10 ml) was added to a solution of the appropriate aryl aldehyde (0.02 mol) and 4-hydroxyacetophenone (0.02 mol) in ethanol (6 ml). The reaction mixture was stirred at room temperature for 12 h, poured onto water (100 ml) and neutralized with hydrochloric acid (10% w/v) to provide a yellow solid or a yellow oil, which solidified on cooling. The compounds were crystallized from a suitable solvent[11,23] to obtain analytically pure samples. The structure and purity of the compounds were confirmed by ^{1}H-NMR and the melting points reported in the literature. Physical data of the compounds are presented in Table 5.

1-(3-Dimethylaminomethyl-4-hydroxyphenyl)-3-phenyl-propenone (5) The preparation of **5** was accomplished by using dimethylamine (40 wt% in water, 0.002 mol) following the same methodology as described for the compound **6** in 40% yield after purifying on basic alumina using chloroform/methanol (7 : 3) as eluent. Melting point: 118—121 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.4 (s, 6H, C17-H, C18-H), 3.74 (s, 2H, C16-H), 6.93—6.91 (d, 1H, C14-H), 7.46—7.41 (m, 3H, C3-H, C4-H, C5-H), 7.59—7.56 (d, 1H, C8-H, J=15.65 Hz), 7.68—7.67 (d, 2H, C2-H, C6-H), 7.81 (s, 1H, C11-H), 7.84—7.80 (d, 1H, C7-H, J=16 Hz), 7.97—7.94 (dd, 1H, C15-H). IR: 3056 (phenolic OH stretching), 1655 (C=O stretching), 1608 (C=C olefinic stretching). UV $\lambda_{\max}^{\mathrm{CHCl_3}}$ (log ε): 244 (3.94), 322 (4.49).

1-(3-4-Morpholinomethyl-4-hydroxyphenyl)-3-phenyl-propenone (6) A solution of chalcone **1a** (0.002 mol), paraformaldehyde (0.002 mol) and morpholine (0.002 mol) in ethanol was heated at 80 °C for 20 h. The crude product obtained after evaporating the solvent was purified on a silica gel column using chloroform/methanol (9 : 1) as eluent. Yield: 21%; melting point: 126—129 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.79—2.51 (t, 4H, C17-H, C20-H), 3.80—3.77 (t, 4H, C18-H, C19-H), 3.82 (s, 2H, C16-H), 6.91—6.89 (d, 1H, C14-H), 7.44—7.39 (m, 3H, C3-H, C4-H, C5-H), 7.57—7.51 (d, 1H, C8-H, J=15.7 Hz), 7.65—7.63 (d, 2H, C2-H, C6-H), 7.80—7.79 (d, 1H, C11-H), 7.82—7.78 (d, 1H, C7-H, J=15.7 Hz), 7.95—7.93 (dd, 1H, C15-H). IR: 3050 (phenolic OH stretching), 1657 (C=O stretching), 1608 (C=C olefinic stretching). UV $\lambda_{\max}^{\mathrm{CHCl_3}}$ (log ε): 244 (3.84), 321 (4.36).

1-(3-Dibenzylaminomethyl-4-hydroxyphenyl)-3-aryl-propenones (7a—e) A solution of the appropriate chalcone in ethanol (30 ml) was added to a previously heated mixture of paraformaldehyde and dibenzylamine in ethanol (20 ml) for 30 min. The reaction mixture was refluxed until the disappearance of starting material. The completion of the reaction was monitored by TLC and took 16—50 h. The solution was kept for 24 h cooling at −4 °C from which yellow solids were obtained. The products obtained were filtered and crystallized from suitable solvent. The molar ratios of reactants (paraformaldehyde, dibenzylamine and chalcone) along with the physical data of the compounds are presented in Table 2. ^{1}H-NMR and ^{13}C-NMR data of the compounds **7a**—**e** are presented in Table 2, while the UV and IR data are presented in Table 3. The carbon numbering of **7a**—**d** and **7e** are shown in Fig. 4.

Determination of the Cytotoxicity of the Compounds against Androgen Independent Prostate Cancer Cells (PC-3). Materials PC-3 cells were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.). RPMI 1640 medium, PBS, FBS, glutamine and gentamicin reagent solution were purchased from Gibco (Grand Island, N.Y., U.S.A.). Trypsin-EDTA solution (10X) and trypan blue solution were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Cell Titre 96 Aqueous Non-Radioactive Cell Proliferation kit was purchased from the Promega Corporation (Madison, WI, U.S.A.). Cell culture flasks, plates *etc.* were purchased from Corning Inc (U.S.A.). Marathon 3200 centrifuge was purchased from Fisher Sci-

Table 6. Physical Data of Compounds **7a**—**e**

Comp.	Mol. formula	Mol. wt.	Mol ratios (mmol) [paraformaldehyde : dibenzylamine : chalcone]	Reaction time (h)	Crystallisation solvent	Yield (%)	mp (°C)
7a	$C_{30}H_{27}NO_2$	433.55	1 : 2 : 2	34	Ethanol	70	165
7b	$C_{30}H_{26}ClNO_2$	468.01	1 : 3 : 3	29	Ethanol	16	199
7c	$C_{31}H_{29}NO_3$	463.59	1 : 2 : 2	50	Methanol–chloroform	16	167—168
7d	$C_{31}H_{29}NO_2$	447.59	1 : 3 : 3	22	Ethanol–chloroform	24	192
7e	$C_{28}H_{25}NO_2S$	439.59	1 : 2 : 2	16	Ethanol	67	169

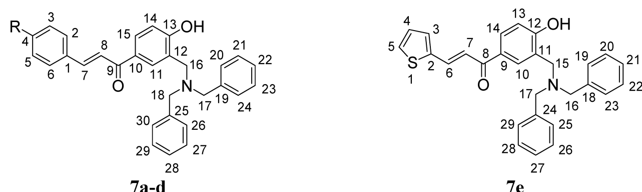


Fig. 4. Carbon Numbering of Compounds **7a**—**d** and **7e**

entific (Pittsburg, PA, U.S.A.). HERA cell incubator and HERA safe cell culture safety hood were purchased from Kendro Laboratory products (Newton, CT, U.S.A.). An IV900 series inverted microscope was purchased from Microscoptics, Inc. (Holy, MI, U.S.A.). SpectraMax 384 Plus spectrophotometer was purchased from Molecular devices (Sunnyvale, CA, U.S.A.).

Cell Maintenance PC-3 cells were maintained in T-75 flasks at 37 °C and 5% CO_2 with the RPMI-1640 medium supplemented with 10% FBS, 2 mM glutamine and 0.02% gentamicin solution (10 mg/ml).

Cytotoxicity Evaluation[30] Cells were plated into 96-well plates at a cell density of 1000 cells per well in 100 μl of medium. The cells were incubated at 37 °C and 5% CO_2 for 24 h to allow the cells to attach to the plate surface. The compounds were weighed out and dissolved in DMSO giving a 10 mM solution. Dilutions were made in the medium to give final concentrations of 50 and 10 μM and a final volume of 200 μl. With compounds that had solubility problems at the higher concentrations, a lower starting concentration of 25 μM was used. The concentrations of DMSO did not exceed 0.2% of the total volume in each well. Three replicates per compound per plate were performed and a no drug control received 100 μl of the medium with DMSO only. The compounds were incubated with the cells for 72 h at 37 °C and 5% CO_2. A Promega Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation assay kit was used to determine the viability of the cells. For 4 plates, 8 ml of MTS solution was combined with 400 μl of PMS solution. 20 μl of the MTS/PMS solution was added to each well and the 96-well plates were incubated for 3—5 h at 37 °C and 5% CO_2. Absorbance was determined at 490 nm using a Molecular devices Spectra Max 384 Plus spectrophotometer with SOFTmax Pro software. The absorbance data were copied into Microsoft Excel and the replicates of each compound were averaged, as were values for the control of each plate. A background value of 0.41 representing the absorbance of media and MTS/PMS solution alone was subtracted from each average. The percent inhibition of cell proliferation was calculated for each compound on each plate as follows:

$$\%I=\frac{[\{\text{control absorbance}-0.41\}-\{\text{sample absorbance}-0.41\}]}{[\{\text{control absorbance}-0.41\}]}\times 100$$

Compounds that inhibited 50% or higher of the cell viability at 50 μM concentration were then used for IC_{50} determinations with the starting concentration of the compounds being 150 μM. Three fold serial dilutions were performed and the concentration of the last well was 8 nM. Compounds with percent inhibition between 30—50% had a starting concentration of 200 μM. Following incubation with the drug for 72 h MTS assay was performed using the CellTiter 96® AQ_{ueous} kit. IC_{50} values were calculated using the SOFT max Pro plate reader programme. The reference used was 5-FU with an IC_{50} value of 1.5 μM. Each compound was tested at least three times.

Computational Methods and Molecular Modeling Preliminary geometry optimisations of the compounds 4′-hydroxychalcones (**1a**—**e**), and Mannich chalcones (**7a**—**e**) were performed using a Spartan '06 (Wavefunction Inc.) software package. Then the structures were reoptimised using semiempirical quantum mechanical calculations with the semiempirical AM1 method for E_{LUMO} energy calculations. To evaluate the relative stability of the mono-adducts (**5**, **6**, **7a**) and bis-adducts (**2**, **3**, **4a**), the structures were reoptimised using semiempirical quantum mechanical calculations (AM1 method), previous to their treatment by the *ab initio* methods. *Ab initio* calculations were performed using GAUSSIAN-94 with the 6-31* basis set. Geometry optimisations were performed at the Hartree–Fock (HF) levels of theory. For structural optimisation, models of **1a** and **7a** (Fig. 3) were built using BioMedCache 6.1 for Windows (Fujitsu Limited, Chiba, Japan) and energy minimized conformations were generated by performing an optimized geometry calculation in mechanics using augmented MM2 parameters. The structures of **1a** and **7a** were superimposed in which the aromatic ring A and C=C atoms are overlapped. BioMedCache 6.1 for windows was used to calculate the energies (heat of formation) of the reactants (**1a**, HCHO, amine) and products (**2**, **3**, **4a**, **5**, **6**, **7a** and H_2O). The structures were optimised by using semiempirical AM1 parameters. The enthalpy change was calculated as $\Delta H_{\text{mono-bis}}=H_{\text{bis-adduct}+H_2O}-H_{\text{mono-adduct}+\text{HCHO}+\text{amine}}$ and $\Delta H_{\text{1a-mono}}=H_{\text{mono-adduct}+H_2O}-H_{\text{1a}+\text{HCHO}+\text{amine}}$.

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