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3-Arylidene-1-(4-nitrophenylmethylene)-3,4-dihydro-1*H*-naphthalen-2-ones and related compounds displaying selective toxicity and reversal of multidrug resistance in neoplastic cells

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Abstract—The incorporation of the 1-aryl-5-(4-nitrophenyl)-3-oxo-1,4-pentadienyl group into 3,4-dihydro-1*H*-naphthalenyl, cyclohexyl, 2,3-dihydro-1*H*-indenyl and cyclopentyl scaffolds led to the discovery of various compounds with selective toxicity for malignant cells and ability to reverse multidrug resistance.

The major emphases in these laboratories are the design, syntheses and antineoplastic evaluations of various conjugated arylidene ketones. These compounds react preferentially with thiols rather than hydroxy and amino groups.[1] Thus these enones may exert their bioactivities without interactions with the amino and hydroxy portions of nucleic acids thereby avoiding the genotoxic problems of a number of anticancer drugs.[2] In addition, the theory of sequential cytotoxicity states that successive chemical attacks by candidate antineoplastic agents may be more harmful to malignant cells rather than the corresponding normal tissues.[3,4] On the basis of this hypothesis, a number of 1,3-diarylidene-2-tetralones were prepared which had different electron densities on the olefinic carbon atoms which may permit sequential thiol addition by cellular constituents.[5] In general, these compounds were more than twice as cytotoxic as the corresponding 1-arylidene-2-tetralones suggesting that the theory of sequential cytotoxicity was worthy of further consideration. In particular, the aryl substituents of **1a** were chosen for the following reasons. First, the Hammett σ values of the 4-nitro and 3,4,5-trimethoxy substituents are 0.78 and −0.03, respectively,[6] indicating that nucleophilic attack on the olefinic carbon atom adjacent to ring A would be predicted to occur initially followed by thiolation at the second olefinic group. Second, under the hypoxic conditions of certain tumours, the nitro group could be reduced to one or more toxic metabolites;[7] this process would occur more readily in such tumours rather than normal cells. Third, the 3,4,5-trimethoxyphenyl group is an integral part of a number of anticancer agents such as podophyllotoxin[8] and various combretastatins.[9] These considerations were validated when **1a** displayed excellent cytotoxicity when evaluated against 56 human tumour cell lines having an average IC_{50} value of 0.59 μM.[5] Furthermore, mice tolerated doses up to and including 300 mg/kg of this compound[5] (see Fig. 1).

Thus **1a** was considered a lead molecule with demonstrated cytotoxicity without short-term marked toxicity. The objectives of the present study were to prepare a small group of prototypic molecules related to **1a** with a view to determining whether selective toxicity to malignant cells and reversal of multidrug resistance (MDR) would be demonstrated. If such properties were noted, an indication of those structural features which

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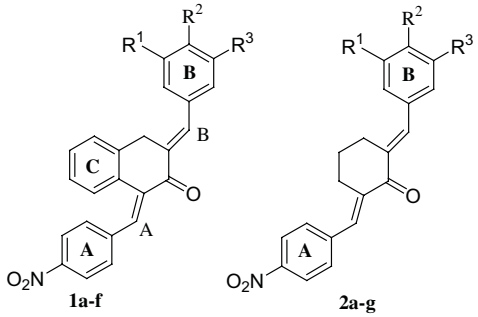

Figure 1. The general structures of compounds in series **1**–**4**. The nature of the aryl substituents are indicated in Table 1.

contributed to these favourable bioactivities would be sought.

The design of analogues of **1a** was based on the following reasoning. First, in order to ensure a differential between the fractional positive charges on C^A and C^B, the 4-nitrophenylmethylene group was retained while, in general, electron-releasing substituents were placed in ring B. The disparity between the combined Hammett σ values of the aryl substituents in rings A and B in series **1**–**4** is presented in Table 1. These data reveal that with the exception of **2g**, the $\Delta\sigma$ figures were equal or greater than **1a**. Second, further molecular modifications of series **1** were undertaken in order to glean some idea of the contributions to bioactivity of ring C as well as the size of the alicyclic ring; such considerations led to the decision to prepare series **2**–**4**.

The synthesis of the compounds in series **1**–**4** was accomplished as follows. 1-(4-Nitrophenylmethylene)-3,4-dihydro-1*H*-naphthalen-2-one was prepared by a literature method[10] and reacted with various aryl aldehydes in acidic media to yield **1a**–**f**. The enones **2a**–**g** were synthesized in a similar manner from 2-(4-nitrophenylmethylene)cyclohexanone.[11] Morpholine was reacted with either 2,3-dihydro-1*H*-inden-2-one or cyclopentanone to produce the corresponding enamines to which 4-nitrobenzaldehyde was added. After treating the products with acid, the resultant enones were condensed with the appropriate aryl aldehyde under acidic conditions giving rise to **3a** and **4a**,**b**. The purity of compounds was established by elemental analyses and ^{1}H NMR spectroscopy. This latter determination revealed the stereohomogeneity of the compounds and the olefinic double bonds were assigned the *E* configuration since X-ray crystallography had proved that various 1-arylidene-2-tetralones,[5] 2-arylidenecyclohexanones[10,12] and a 2,6-bis(arylidene)cyclohexanone[13] possessed the *E* stereochemistry.

All of the compounds in series **1**–**4** were evaluated against four neoplastic cell lines, namely HSC-2, HSC-3, HSG and HL-60 cells as well as the nonmalignant

Table 1. The aryl substituents and cytotoxic evaluation of the compounds in series **1**–**4**

Compound	Aryl substituents[a]				CC_{50}[b] (μM)							
	R^1	R^2	R^3	$\Delta\sigma$[c]	HSC-2	HSC-3	HSG	HL-60	HGF	HPC	HPLF	SI[d]
1a	OCH_3	OCH_3	OCH_3	0.81	0.55	2.9	3.0	0.55	>400	>400	>400	>229
1b	OCH_3	OCH_3		0.93	0.92	4.2	4.4	0.56	>400	>400	>400	>159
1c	OCH_3	OH		1.03	1.45	3.8	5.8	0.95	33	83	21	15
1d		OCH_3		1.05	11	32	47	4.4	>400	>400	>400	>17
1e	OCH_2O			1.10	2.6	7.6	5.0	2.2	>400	>400	>400	>92
1f		OH		1.15	5.2	14	11	2.5	85	113	75	12
2a	OCH_3	OCH_3	OCH_3	0.81	0.76	4.7	8.3	1.1	336	347	378	95
2b	OCH_3	OCH_3		0.93	2.3	4.4	13	4.3	53	82	95	13
2c	OCH_3	OH		1.03	2.8	5.2	11	3.4	165	>400	171	>44
2d		OCH_3		1.05	>400	>400	>400	>400	>400	>400	>400	~1.0
2e	OCH_2O			1.10	308	189	>400	>400	>400	391	330	~1.2
2f		OH		1.15	8.1	13	>400	4.4	>400	>400	>400	~3.8
2g		Cl		0.55	1.5	4.6	15	4.1	191	345	332	46
3a	OCH_3	OCH_3	OCH_3	0.81	221	>400	>400	318	89	153	>400	~0.6
4a	OCH_3	OCH_3	OCH_3	0.81	>400	>400	>400	>400	>400	>400	>400	~1.0
4b		OCH_3		1.05	63	>400	>400	>400	>400	>400	>400	~1.3
Doxorubicin	—	—	—	—	0.68	2.9	1.9	0.47	233	414	>400	>235

[a] For clarity, hydrogen atoms are omitted.

[b] Concentrations of compound to kill 50% of the cells.

[c] $\Delta\sigma$ refers to the differences in the σ values between the substituents in rings A and B in series **1**–**4**. The sigma values used in these calculations were taken from Ref. 16.

[d] The letters SI indicate the selectivity index which was calculated as follows: $SI = [CC_{50}(HGF)+CC_{50}(HPC)+CC_{50}(HPLF)]/[CC_{50}(HSC\text{-}2)+CC_{50}(HSC\text{-}3)+CC_{50}(HSG)+CC_{50}\ (HL\text{-}60] \times 4/3$.

HGF, HPC and HPLF cells.[14] The potency of the lead compound **1a** towards four cancer cell lines is revealed in Table 1. The average CC_{50} figure of **1a** to HSC-2, HSC-3, HSG and HL-60 cells was 1.75 μM or 85% of the average potency of doxorubicin towards these four cell lines. The average potencies of the other compounds prepared in this study were less than **1a** revealing that 3,4,5-trimethoxy substitution in aryl ring B was optimal (**1a** > **1b**–**f**), the presence of ring C increases potency (**1a** > **2a**) and a six-membered alicyclic ring was preferred to a five-membered one (**1a** > **3a**). In order to obtain an answer to the first query as to whether preferential toxicity for malignant cells was displayed, a selectivity index (SI) value was calculated for each of the compounds in series **1**–**4**. These figures are presented in Table 1 which reveal that within series **1** and **2**, many of the compounds displayed excellent selectivity. In particular, **1a** rivals doxorubicin in this regard. A review of the SI data revealed the following structure–activity relationships (SAR). First, marked selective potencies for malignant cells was noted in series **1** and **2** but not **3** and **4** indicating the importance of a six-membered rather than a five-membered alicyclic ring. Second, in general, the presence of ring C in series **1** enhanced selectivity since the SI figures of **1a**,**b**,**d**–**f** were greater than **2a**,**b**,**d**–**f**, respectively. Third, **1a** and **2a** possessed the highest SI figures in series **1** and **2**, respectively, indicating that the 3,4,5-trimethoxy substitution pattern was optimal. However no correlation exists between the SI values and the $\Delta\sigma$ figures revealing that other factors impinge on the selective toxicity for malignant cells such as the specific locality of the nuclear methoxy groups which enable good ligand–receptor binding to occur. In the future, chemically divergent substituents should be placed in both rings A and B and comparisons made between the $\Delta\sigma$ and SI figures in a further evaluation of the theory of sequential cytotoxicity.

In order to address the second question as to the efficacy or otherwise of these novel cytotoxins to MDR, murine lymphoma L-5178 cells transfected with the human MDR1 gene as well as human colon cancer Colo320 neoplasms were treated with two concentrations of the compounds in series **1**, **2** and **4**.[15] The accumulation of rhodamine 123 was measured in the treated and untreated cells. The data are summarized in Table 2. In those cases where the fluorescence activity ratio (FAR) values are greater than 1, reversal of MDR has taken place. MDR is due, inter alia, to an increase in the efflux of a compound from the cell. Consequently a reversal of MDR means that the exodus of rhodamine 123 from the cells will be either the same or lower than in the parental cells. The data in Table 2 reveal that **1a**–**c**,**f**, **2a**,**b** displayed a remarkable inhibition of the MDR of human MDR1 gene-transmitted mouse lymphoma cells. These cells overexpress the P-gp 170 protein responsible for drug efflux. The same compounds were also the most effective on the elevated drug accumulation of human colon cancer Colo320 cells but in each case the FAR values were lower than for the murine lymphoma cells. In general, the SAR of the compounds inhibiting MDR were the same as for the SI values generated, namely the presence of ring C was beneficial (**1a**–**f** > **2a**–**f**), a six-membered alicyclic ring was preferable to the 2,5-disubstituted cyclopentanones (**2a**,**d** > **4a**,**b**) and in series **1** and **2**, maximum inhibition of MDR was found in **1a** and **2a** having the 3,4,5-trimethoxy groups in ring B.

Table 2. Effect of **1a**–**f**, **2a**–**g** and **4a**,**b** on multidrug resistance in murine L-5178 cells and human colon cancer Colo320 neoplasms

Compound	Fluorescence activity ratios at different concentrations[a]			
	L-5178		Colo320	
	4 μg/mL	40 μg/mL	4 μg/mL	40 μg/mL
1a	134	120	10.9	13.0
1b	44.1	55.9	7.19	11.4
1c	51.8	123	3.38	6.20
1d	4.36	7.68	2.22	4.30
1e	3.27	3.87	1.95	2.27
1f	135	39.3	1.99	11.7
2a	15.6	75.1	7.43	4.38
2b	20.4	47.3	6.12	4.62
2c	1.50	0.83	1.98	1.13
2d	0.76	0.82	0.50	0.58
2e	0.91	1.23	0.67	0.59
2f	0.94	0.97	1.15	0.60
2g	1.16	0.94	0.60	0.53
4a	3.06	6.62	1.96	1.28
4b	0.59	0.69	0.63	0.37

[a] The fluorescence activity ratio (FAR) values are the ratios of the fluorescence intensities of the treated cells/untreated cells. The FAR value of a reference compound verapamil was 38.2 and 8.05 using L-5178 and Colo320 cells, respectively, when a concentration of 10 μg/mL of verapamil was used (limitations of solubility precluded a higher concentration of verapamil being utilized).

In summary, this study of several series of compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore has led to novel, potent cytotoxins, some of which demonstrated remarkable selective toxicity to malignant cells and the ability to reverse MDR. The reasons for these important findings may have included the fact that the theory of sequential cytotoxicity was verified and/or preferential reduction of the nitro group took place in those tumours in which greater hypoxia exists.

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14. Human tumour cells (squamous cell carcinoma HSC-2, HSC-3, submandibular gland carcinoma HSG) and human normal oral cells (gingival fibroblast HGF, pulp cell HPC, periodontal ligament fibroblast HPLF) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS. Human promyelocytic leukaemia HL-60 cells were cultured in RPMI1640 + 10% FBS. Normal cells were prepared from the periodontal tissues, according to the guideline of Meikai University Ethic Committee (No. 206) after obtaining the informed consent from the patients. Since normal cells have a limited life span, they were used at the population doubling level of 5–7. Near confluent cells were incubated for 24 h with or without various concentrations of each compound and the relative viable cell number, that is, the absorbance of 540 mm of the MTT-stained cell lysate, was determined by the MTT method. The viable cell number of HL-60 cells was obtained by trypan blue exclusion. The CC_{50} values were determined from the dose–response curves. Each value in Table 1 represents the mean from duplicate determinations.
15. The compounds were added to mouse lymphoma L-5178 cells or human colon cancer Colo320/MDR and incubated for 10 min. Rhodamine 123 was added to the media and the accumulation of this dye in the cells was measured using an argon laser beam. The FAR value for the non-treated MDR cells divided by the non-treated parental cells is approximately 1.
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