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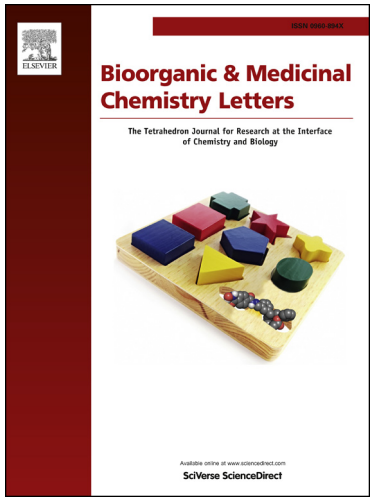

1-[3-(2-Hydroxyethylsulfanyl)propanoyl]-3,5-bis(benzylidene)-4-piperidones: A novel cluster of P-glycoprotein dependent multidrug resistance modulators

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1-[3-(2-Hydroxyethylsulfanyl)propanoyl]-3,5-bis(benzylidene)-4-piperidones: A novel cluster of P-glycoprotein dependent multidrug resistance modulators

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ABSTRACT

A series of 1-[3-(2-hydroxyethylsulfanyl)propanoyl]-3,5-bis(benzylidene)-4-piperidones **4a-e** display promising P-glycoprotein dependent multidrug resistance (MDR) revertant properties and are significantly more potent than a reference drug verapamil when evaluated against L-5178Y MDR lymphoma cells. These dienones may be referred to as dual agents having both MDR revertant properties and tumour-selective cytotoxicity. In particular, 3,5-bis(4-chlorobenzylidene)-1-[3-(2-hydroxyethylsulfanyl]propanoyl-4-piperidone **4d** emerged as a lead molecule for further development based on its MDR revertant properties, cytotoxic potencies and tumour-selective toxicity. The structure-activity relationships reveal important structural requirements for further designing of potent MDR revertants

The principal focus in these laboratories is the design and development of various conjugated styryl ketones as candidate antineoplastic agents.[1-3] One of the reasons for this interest is the observation that in general these compounds react preferentially or exclusively with thiols but not amino or hydroxy groups.[4,5] Hence these thiol alkylators will likely avoid interactions with nucleic acids and thus be bereft of the mutagenic and carcinogenic properties of some anticancer drugs.[6]

In recent years, compounds containing the 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore (ARCH=CHC(O)CH=CHAR) (Figure 1) have been a major focus of our research studies.[7] In these molecules, the potential for sequential interactions with thiol macromolecules exists, i.e., an initial interaction with cellular mercaptans can take place followed by a second thiol alkylation. On a number of occasions, an initial chemical insult followed by a second interaction with cellular constituents is more detrimental to neoplasms than normal cells.[8,9] Various studies from our laboratory have shown that the insertion of a 1,5-diaryl-3-oxo-1,4-pentadienyl group onto a piperidinyl scaffold led to a number of novel compounds that display potent cytotoxicity towards various tumour cells[7,10] and have substantially greater toxicity to neoplasms than normal cells.[11,12]

A major problem in cancer chemotherapy is the ability of tumours to develop multidrug resistance (MDR). Resistance to numerous anticancer agents is mainly associated with expression of the 170-kDa P-glycoprotein (P-gp), and/or the 190-kDa multidrug resistance protein (MRP) and/or breast cancer resistance protein (BCRP) which belong to the ABC transporter family of efflux proteins. Among these transporters, P-gp is one of the major causes of intrinsic and acquired resistance to anticancer drugs in tumor cells. Therefore P-gp is considered a promising target for cancer therapy, and enormous efforts have been expended on the development of effective modulators of the P-gp mediated MDR.[13] In humans, P-gp is encoded by a gene, mdr1. Recently efforts have been made in our laboratory to discover compounds having dual functions, namely displaying both tumour-selective toxicity and MDR-reversal properties. The assay utilized for P-gp mediated MDR-reversal uses murine L-5178Y lymphoma cells transfected with the human mdr1 gene.[14] The concentration of the dye rhodamine 123 in treated and untreated transfected and parental cells is measured and the relative intensities of fluorescence are referred to as fluorescence activity ratio (FAR). A FAR value of greater than one indicates reversal of MDR. Compounds **1a-e** are virtually bereft of MDR-revertant properties,

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except for the modest MDR-modulating properties of **1b**. However nonbasic analogs of **1**, namely series **2** and **3** (Figure 1) are potent MDR modulators and in general possess substantially greater MDR-reversal potencies that a reference drug verapamil.[10,15]

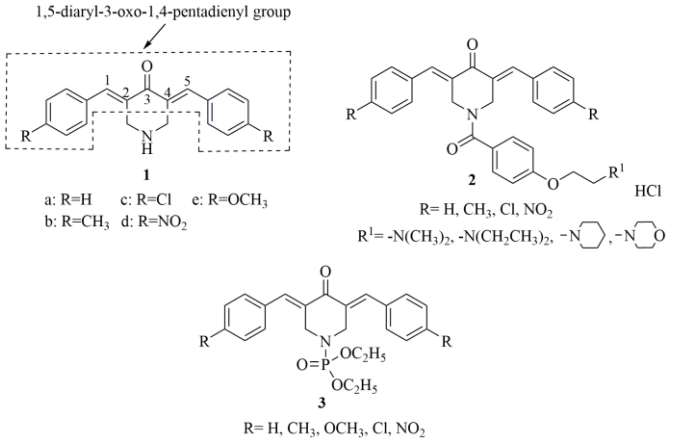
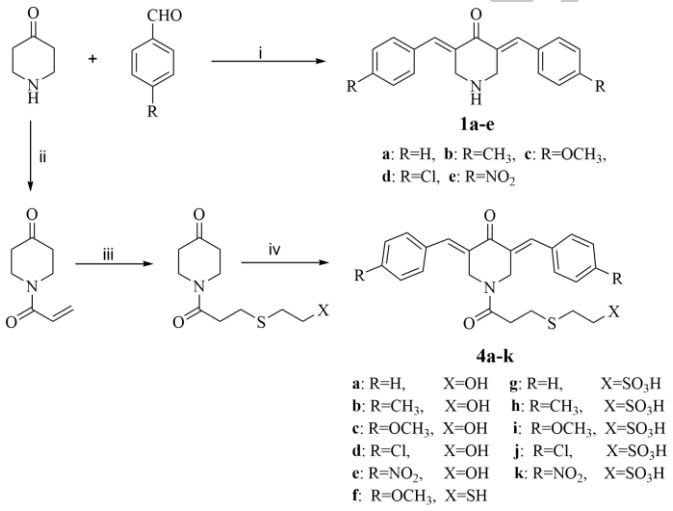


Figure 1: The structures of the compounds in series **1**-**3**

The present study aimed at evaluating series **4** as candidate P-gp mediated MDR-modulators for the following reasons. First, compounds **4** in this series contain some of the molecular features present in series **2** and **3**, namely the 1,5-diaryl-3-oxo-1,4-pentadienyl group and nonbasic substituents having atoms and groups capable of forming van der Waals and hydrogen bonding with cellular constituents. Second, a recent study revealed the excellent tumour-specificity and potent cytotoxicity of **4a-e**.[16] Hence, if these compounds also possess MDR-reversal properties, the impetus for their rapid development as anticancer drug candidates that can be used against P-gp mediated drug resistant tumours is axiomatic.



Scheme 1: Synthesis of series **1** and **4.** The reagents used are as follows: i: HCl(gas)/acetic acid; ii: CH_2=COCl/K_2CO_3/cat. tetrabutyl ammonium bromide; iii: $HSCH_2CH_2X/N(C_2H_5)_3$; iv: RC_6H_4CHO/HCl(gas)/acetic acid.

The synthetic chemical route to the compounds in series **1** and **4** is outlined in Scheme 1. The synthesis of **1a**,[17] **b**,[18] **c-e**[17] has been reported previously. For the synthesis of compounds in series **4**, N-acryloyl-4-piperidone was condensed with 2-mercaptoethanol, 1,2-ethanethiol and 2-mercaptoethane sulphonate sodium (mesna) compounds to provide the N-acylated-4-piperidone intermediates which was further reacted with appropriate aryl aldehydes to give the desired compounds **4a-e**,[16] **4f** [19] and **4g-k**.[19]

Table 1. The fluorescence activity ratios (FAR) and summaries of the cytotoxic data of **4a-k**

Compound	FAR values[a]		FAR ratio			
	4 µg/mL (A)	40 µg/mL (B)	B/A	Ave CC_{50}[b] (µM)	SI[c]	PSE[d]
1a[e]	1.58	2.00	1.27	-	-	-
1b[e]	3.26	4.03	1.24	-	-	-
1c[e]	1.25	0.91	0.73	-	-	-
1d[e]	1.04	0.80	0.77	-	-	-
1e	2.19	0.92	0.42	-	-	-
4a	4.71	42.4	9.00	1.53	9.61	6.28
4b	55.4	44.4	0.80	1.56	5.34	3.42
4c	37.1	85.1	2.29	2.67	5.88	2.20
4d	49.2	45.0	0.92	0.66	12.2	18.5
4e	8.86	20.0	2.26	0.39	15.1	38.7
4f	18.9	126	6.67	3.37	3.41	1.01
4g	1.14	1.20	1.05	154	1.88	0.01
4h	1.20	1.60	1.33	126	1.93	0.02
4i	1.27	3.23	2.54	91.7	2.54	0.03
4j	1.14	1.71	1.50	29.7	5.49	0.19
4k	1.14	1.71	1.50	4.73	7.67	1.62
Verapamil[e]	4.2	-	-	-	-	-
Melphalan	-	-	-	14.0	15.0	1.07

[a]The letters FAR refer to the fluorescence activity ratio of each compound. These data are generated by measuring the relative fluorescence in treated and untreated parental and MDR murine L5178-Y cells.

[b]The designation CC_{50} indicates the concentration of the compounds required to kill 50% of the cells. The ave CC_{50} values are the average CC_{50} figures of the compounds towards HSC-2, HSC-4 and HL-60 neoplasms.

[c]The selectivity index (SI) is the quotient of the average CC_{50} values towards human HGF, HPC and HPLF normal cells and the average CC50 values towards HSC-2, HSC-4 and HL-60 neoplasms.

[d]The potency-selectivity expression (PSE) is the product of the reciprocal of the average CC_{50} value and the SI figures.

[e]The FAR value has been reported previously.[15]

All of the compounds in series **4** were evaluated for P-gp mediated MDR-revertant properties and the results are presented in Table 1. Two different concentrations of the compounds namely, 4 µg/mL and 40 µg/mL were used for screening. The MDR revertant property shown at a concentration of 4 µg/mL is more relevant since compounds with clinical potential likely display bioactivity using concentrations in the low micromolar range or less. Using a concentration of 4 µg/mL, the biodata reveals that the analogs **4a-e** demonstrate greater MDR-reversal properties than the precursors **1a-**

e. Thus the attachment of the 3-(2-hydroxyethylsulfanyl)-propanoyl group onto the 3,5-bis(benzylidene)-4-piperidone scaffold affords a cluster of novel lead molecules **4a-e**. In series **4**, compounds **4a-f** are potent MDR-revertants while **4g-k** are inactive. The replacement of a hydroxy substituent in the side chain of **4c** by a mercapto group retained MDR-reversal properties while a sulfonic acid group (SO_3H) (**4i**) led to almost a complete loss of this property. The three most potent compounds (**4b-d**) have 9-13 times the potency of verapamil hydrochloride and one may note that even the least potent compound among **4a-f**, namely **4a**, has approximately the same FAR value as the reference drug. In regard to **4a-e**, the two most potent compounds are **4b** and **4d** which have the highest Hansch π values of the aryl substituents of 0.56 and 0.71, respectively.[20] It is conceivable therefore that analogs with increased lipophilicity may have greater MDR-revertant potencies. The FAR value of **4f** is approximately half that of the oxo isostere **4c**. The molecular refractivity values of aliphatic hydroxy and mercapto groups are 2.55 and 8.76, respectively[21] and thus the smaller size of the hydroxy substituent in **4c** may contribute to its greater potency than **4f**. Clearly the replacement of the hydroxy and mercapto group of the *N*-acyl side chain by a sulfonic acid group leading to **4g-k** virtually abolishes MDR-revertant properties. This effect could be due to the sulfonic group causing a misalignment of **4g-k** with P-gp and/or the highly polar nature of the sulfonic group substituent which inhibits penetration of the molecule via cell membranes.

A ten-fold increase in concentration, i.e., 40 μg/mL was also employed with a view to identifying MDR revertants which are either inactive or display weak potencies at the lower concentration. The quotients of the FAR values at 40 μg /mL and 4 μg /mL are presented in Table 1. A large increase in the FAR figures for **4a**,**f** was observed at the higher concentration. However in most cases there are little differences in the FAR values at 4 μg /mL and 40 μg /mL while for some compounds the FAR values diminished at the higher concentration, e.g., **4b**.This phenomenon suggests that in these instances, viz similar or lower FAR values at 40 μg /mL than 4 μg /mL, other biochemical processes are activated which diminish the MDR-revertant potencies. This phenomenon has been noted previously.[15,22] With the exception of the low potency of **4i**, raising the concentration of **4g-k** to 40 μg/mL did not reveal any compounds with noteworthy MDR-revertant properties.

The next phase of the investigation was to identify the optimal dual agents, i.e., those compounds which display both MDR-revertant properties and greater toxicity to neoplasms than non-malignant cells. The cytotoxicity of the compounds in series **4** have been assessed against human HSC-2 and HSC-4 squamous cell carcinomas as well as human HL-60 promyelocytic leukemia cells[16,19] using a reported methodology[23] and the average CC_{50} values are given in Table 1. In addition, **4a-k** were evaluated against human HGF gingival fibroblasts, HPC pulp cells and HPLF peridontal ligament fibroblasts which are non-malignant cells.[16,19] From these data, selectivity index (SI) figures were generated (average CC_{50} values towards normal cells/average CC_{50} figures versus neoplasms) which are displayed in Table 1. Of particular interest is the identification of those compounds achieving PL10 status, i.e., a *Promising Lead* compound having an average CC_{50} value of < 10 μM towards cancer cells and a SI value of >10.[11,24]

A review of the biodata in Table 1 reveals that **4a-f** and **4k** have average CC_{50} values below 10 μM. All of these compounds are substantially more potent than melphalan, e.g., **4e** has 36 times the potency of this reference drug. The SI values of **4d** and **4e** are greater than 10 and in particular **4e** has a similar selectivity as melphalan. Thus both **4d** and **4e** achieve PL10 status. In order to quantify potency and selectivity, the product of the average CC_{50} figures and the SI values were obtained and the potency-selectivity expression (PSE) data are given in Table 1. The PSE values reveal that the N-[3-(2-hydroxyethylsulfanyl)propanoyl] analogs **4a-e** are important lead molecules, especially **4d** and **4e**. Both of these compounds contain strongly electron-withdrawing substituents in the aryl ring (the Hammett σ_p values of the chloro and nitro group are 0.23 and 0.78, respectively[20]), and thus the creation of analogs with such aryl substituents as 4-cyano (Hammett σ_p=0.66) and 4-trifluoromethyl (Hammett σ_p=0.54)[20] should be considered. The retention of one or more nitro groups in the aryl rings requires careful consideration. On the one hand, under the hypoxic conditions of certain solid tumours, the rate of reduction of the nitro group to a variety of cytotoxins is likely to be faster than in the corresponding normal cells. Thus selective toxicity for tumours may result. On the other hand, reduction products such as nitroxyl and nitro radicals as well as nitroso groups can lead to a variety of pathological conditions[25] and unacceptable side effects. Thus to avoid these issues associated with a nitro group, an isosteric replacement of 4-nitrophenyl group in **4e** by a 4-pyridyl group should be carried out in future. Among the sulfonic acid derivatives **4g-k**, only **4k** had a greater PSE figure than melphalan.

In conclusion, the noteworthy MDR-revertants among series **4** are **4b-d**. The introduction of a 3-(2-hydroxyethylsulfanyl)- propanoyl scaffold onto 3,5-bis(benzylidene)-4-piperidones in series **1** led to a novel group of potent P-gp modulators. In terms of cytotoxicity, SI values and PSE figures, **4d** and **4e** emerge as the lead molecules. Hence the optimal dual agent having MDR reversal properties and selective-cytotoxicity is **4d** which should be pursued in a variety of directions. First, investigation of molecular mechanisms by which **4d** binds to P-gp which will give an insight for further designing of potent P-gp modulators. Second, evaluation of **4d** against other efflux transporters such as MRPs and BCRPs. Third, the isosteric replacement of 4-chloro or 4-chloroaryl groups with the groups possessing similar electronic, hydrophobic and steric properties should be undertaken and the compounds evaluated for MDR-revertant properties and selective toxicity for neoplasms.

Acknowledgments

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References and notes

1. Pati, H. N.; Das, U.; Sharma, R. K.; Dimmock, J. R. *Mini-Rev. Med. Chem.* **2007**, *7*, 131-139.
2. Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. *Curr. Med. Chem.* **1999**, *6*, 1125-1149.
3. Dimmock, J. R.; Wong, M. C. L. *Can. J. Pharm.Sci.* **1976**, *11*, 35-53.
4. Baluja, G.; Municio, A. M.; Vega, S. *Chem. Ind.* **1964**, 2053-2054.
5. Mutus, B.; Wagner, J. D.; Talpas, C. J.; Dimmock, J. R.; Phillips, O. A.; Reid, R. S. *Anal. Biochem.* **1989**, *177*, 237-243.
6. Okey, A. B.; Harper, P. A. In *Principles of Medical Pharmacology*; Kalant, H.; Grant, D. M.; Mitchell, J., Eds., 7th ed.; Elsevier: Toronto, Canada, 2007; p. 902.

7. Das, U.; Sharma, R. K.; Dimmock, J. R. *Curr. Med. Chem.* **2009**, *16*, 2001-2020.
8. Chen, G.; Waxman, D. J. *Biochem. Pharmacol.* **1994**, *47*, 1079-1087.
9. Mitchell, J. B.; Russo, A. *Br. J. Cancer* **1987**, *55*, 96-104.
10. Das, S.; Das, U.; Selvakumar, P.; Sharma, R. K.; Balzarini, J.; De Clercq, E.; Molnár, J.; Serly, J.; Baráth, Z.; Schatte, G.; Bandy, B.; Gorecki, D. K. J.; Dimmock, J. R. *ChemMedChem* **2009**, *4*, 1831-1840.
11. Das, S.; Das, U.; Sakagami, H.; Hashimoto, K.; Kawase, M.; Gorecki, D. K. J.; Dimmock, J. R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 6464-6468.
12. Das, U.; Sakagami, H.; Chu, Q.; Wang, Q.; Kawase, M.; Selvakumar, P.; Sharma, R. K.; Dimmock, J. R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 912-917.
13. Kibria, G.; Hatakeyama, H.; Harashama, H. *Arch. Pharm. Res.* **2014**, 37, 4-15.
14. Kawase, M.; Sakagami, H.; Motohashi, N.; Hauer, H.; Chatterjee, S. S.; Spengler, G.; Vigyikanni, A. V.; Molnár, J. *In Vivo* **2005**, *19*, 705-712. In brief, both parental and MDR murine L-5178Y cells were grown in supplemented McCoy's 5A medium. The compounds were dissolved in dimethylsulfoxide, added to the cells and the mixture was incubated at room temperature for 10 minutes (max. DMSO conc. 1% v/v in the mixture). After the addition of a solution of rhodamine 123 dye in dimethylsulfoxide to the culture, the cells were incubated for 20 minutes at 37 ^{o}C. After washing, the cells were placed in PBS pH 7.4 and the fluorescence measured using a Becton Dickinson FACScan instrument. The fluorescence in treated and untreated MDR cells as well as treated and untreated parental cells was determined. From these data, the FAR values were calculated.
15. Das, U.; Molnár, J.; Baráth, Z.; Bata, Z.; Dimmock, J. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3484-3487.
16. Das, U.; Pati, H. N.; Sakagami, H.; Hashimoto, K.; Kawase, M.; Balzarini, J.; De Clercq, E.; Dimmock, J. R. *J. Med. Chem.* **2011**, *54*, 3445-3449.
17. Dimmock, J. R.; Padmanilayam, M.P.; Puthucode, R.N.; Nazarali, A.J.; Motaganahalli, N.L.; Zello, G.A.; Quail, J.W.; Oloo, E.O.; Kraatz, H.B.; Prisciak, J.S.; Allen, T.M. *J. Med. Chem.* **2001**, *44*, 586-593.
18. Das, U.; Alcorn, J.; Shrivastav, A.; Sharma, R.K.; De Clercq, E.; Balzarini, J.; Dimmock, J. R. *Eur. J. Med. Chem.* **2007**, *42*, 71-80.
19. Pati, H. N.; Das, U.; Quail, J. W.; Kawase, M.; Sakagami, H.; Dimmock, J. R. *Eur. J. Med. Chem.* **2008**, *43*, 1-7.
20. Hansch, C.; Leo, A. J. Substituent Constants of Correlation Analysis in Chemistry and Biology; John Wiley and Sons, New York, 1979, p. 49.
21. Hansch, C.; Leo, A. J. Substituent Constants of Correlation Analysis in Chemistry and Biology; John Wiley and Sons, New York, 1979, p. 52.
22. Dimmock, J. R.; Das, U.; Gul, H. I.; Kawase, M.; Sakagami, H.; Baráth, Z.; Ocsovsky, I.; Molnár, J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1633-1636.
23. Motohashi, N.; Wakabayashi, H.; Kurihara, T.; Fukushima, H.; Yamada, T.; Kawase, M.; Sohara, Y.; Tani, S.; Shirataki, Y.; Sakagami, H.; Satoh, K. *Phytother. Res.* **2004**, *18*, 212-223. In brief, compounds were added to the neoplastic and non-malignant cells and incubated at 37 ^{o}C for 48 hr in supplemented DMEM medium except HL-60 cells were cultured in supplemented RPMI1640 medium. Cell viability was determined by the MTT assay except a trypan blue exclusion procedure was used in the case of the HL-60 cells.
24. Pati, H. N.; Das, U.; Sakagami, H.; Kawase, M.; Chu, Q.; Wang, Q.; Stables, J. P.; Dimmock, J. R. *Arch. Pharm. Chem. Life Sci.* **2010**, *9*, 535-541.
25. Kerns, E. H.; Li, D. Drug-like Properties: Concepts, Structure Design and Methods: from ADME to Toxicity Optimization, Academic Press, Amsterdam, 2008, p. 220.

Graphical Abstract

1-[3-(2-Hydroxyethylsulfanyl)propanoyl]-3,5-bis(benzylidene)-4-piperidones: A novel cluster of P-glycoprotein dependent multidrug resistance modulators

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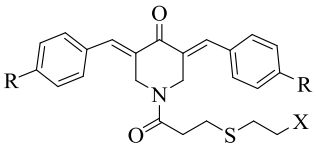

R= H, CH_3, OCH_3, Cl, NO_2

X = OH, SH Potent MDR revertants (FAR values 5-126)

X = SO_3H Virtual loss of MDR revertant properties