Anticancer Activity of 4-[1-Oxo-(Substituted Aryl)-2-Propenyl]-3-Phenylsydnones

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Novel structural feature containing 4-[1-oxo-(substituted aryl)-2-propenyl]-3-phenylsydnones were synthesized and screened for anticancer activity. These compounds contain two pharmacophores,a,b unsaturated ketone moiety and sydnone nucleus. Three compounds were synthesized, and all of the three exhibited promising *in vitro* cytotoxicity in 56 cell lines representing cancers of non-small cell lung, colon, CNS, melanoma, ovarian, prostrate, breast and leukemia. Average growth inhibition of 50% was in the range of 1.7-3.5 μM. Methyl derivative was highly selective against SNB-75 tumor cell line of CNS. It was active at less than one nano mole. However, *in vivo* the activity was moderate by hollow fiber assay model.

Sydnones (1) belongs to mesoionic class of compounds and exhibit interesting physiological properties. These are aromatic, small in size, internally polar but behaves as nonpolar compounds. These are reported to posses wide variety of biological actions[1,2]. We have extensively studied their antiinflammatory, analgesic, anticancer and nitric oxide donor activities[3-7].

Curcumin (2), [(E,E)-1,7-bis(4-hydroxy-3-methoxy)-1,6-heptadiene-3,5-dione] an active ingredient of *curcima longa*; chalcones (3) the naturally occurring coloring pigments in plants are reported to posses anticancer activities in addition to host of other properties[8-11]. These two molecules contain, α,β unsaturated ketone pharmacophore. It is a reactive pharmacophore, prone to nucleophilic and electrophilic reactions. As part of structural activities we felt it interesting to add α,β unsaturated ketone pharmacophore to sydnones. This effort resulted in sydnones-substituted chalcones[6]; these compounds did exhibit both *in vitro* and *in vivo* anticancer activities. We felt it worthwhile to optimize this potential lead and study more detailed anticancer activity. As a part of this effort we describe here *in vitro* and cytotoxicity and *in vivo* anticancer activities of 4-[1-oxo-(substituted aryl)-2-propenyl]-3-phenylsydnones (4) and their plausible mode of action.

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All chemicals used were of LR grade for synthesis and AR grade for analysis. 4-[1-Oxo-(substituted aryl)-2-propenyl]-3-phenylsydnones (4) were synthesized according to a previously reported method from our laboratory[3]. The scheme involved condensation of aniline with ethyl chloroacetate to yield ethyl ester of N-phenylglycine. Nitrosation followed by cyclization gave 3-phenylsydnone. Which was then acetylated at 4-position to 4-acetyl-3-phenylsydnone. This was subjected to Claisen-Schmidt condensation to yield the title compounds. Double beam UV Spectrophotometer (UV-240 Shimadzu, Japan) was used to measure absorbance. Griess reagent was prepared by mixing equal volumes of 0.1% naphthyl ethylene diamine dihydrochloride in water and 1% sulfanilamide in phosphoric acid. Reagent was used within 12 h after preparation.

Compounds were screened for *in vitro* cytotoxicity at the National Cancer Institute (NCI), National Institute of Health (NIH), USA. Details of the test system have been previously published[12-14]. In this protocol, the compounds in five logarithmic dilutions were exposed to 56 cancer cell lines representing leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostrate and breast. The cell viability or growth after 48 h exposure is estimated by sulforhodamine (SRB) protein assay as optical density.

In vivo anticancer activity by Hollow fiber assay was performed according to NCI protocol, at NCI, Bethesda, USA. In this assay the human tumor cells are cultivated in polyvinylidene fluoride (PVDF) hollow fibers, and a sample

TABLE 1: CYTOTOXICITY OF 4-[1-OXO-(SUBSTITUTED ARYL)-2-PROPENYL]-3-PHENYLSYDNONES (4)

Cell line	Growth Inhibition 50 %(μM)[a]		
	4a	4b	4c
Leukemia			
CCRF-CEM	0.89	0.54	0.06
HL-60(TB)	1.13	2.52	0.18
K-562	>100	2.52	0.18
MOLT-4	2.30	2.01	2.06
SR	3.68	0.21	2.14
Non-Small Cell Lung Cancer			
A549/ATCC	4.06	5.98	6.05
EKVX	3.06	5.21	2.43
HOP-92	3.97	2.85	1.70
NCI-H226	3.66	1.72	12.90
NCI-H322M	4.97	4.85	9.10
NCI-H460	2.59	2.33	2.85
NCI-H522	1.59	1.62	2.85
Colon Cancer			
COLO 205	1.96	1.32	1.46
HCC-2998	1.92	3.14	2.06
HCT-116	1.79	3.77	1.98
HCT-15	1.95	3.85	1.25
HT 29	1.61	3.85	1.46
KM 12	2.49	2.61	2.37
SW-620	1.68	2.03	1.13
CNS Cancer			
SF-268	3.13	2.69	2.11
SF-295	6.14	1.05	3.38
SF-539	2.26	2.34	1.89
SNB-19	3.04	5.85	3.31
SNB-75	<0.001	—	1.58
U251	1.84	3.02	1.48
Melanoma			
LOXIMVI	2.09	1.91	0.32
M14	2.38	5.36	2.50

of each cell line is implanted into each of physiological compartments (intraperitoneally and subcutaneously) in mice representing 3 distinct cancer cell lines. Each compound is tested in 12 cell lines. Three mice are treated with the compound at each of 2 test doses by intraperitonea route using QDx4 treatment schedule. Compound 4a at 84 mg/kg and 125 mg/kg and compound 4c at 100 mg/kg and 150 mg/kg. Vehicle controls consist of 6 mice receiving the compound diluents only. The fiber cultures are collected on the day following the last day of treatment. To asses the anticancer effects, viable cell mass is determined for each of cell lines using a formazan dye (MTT) conversion assay. The results are expressed as percent growth (%T/C), which is calculated by dividing the average optical density of the compound treated samples by optical density of the vehicle treated controls.

Nitric oxide (NO) activity was measured according to our earlier reported method[7]. Test compounds at 1000 μM suspension in 2 ml phosphate buffer pH 7.4 (50 mM) and 5 mM of L-cysteine hydrochloride was incubated at 37±1°. After various time intervals, the reaction mixture added with 500 μl of Greiss reagent. After the reaction mixture stood for 20 min at room temperature, the absorbances were measured at 550 nm. Content of nitrite was determined by standard plot prepared using sodium nitrite.

For iron chelation activity, a 100 μM test compound solution was scanned in the range of 200-450 nm. To this, a solution of ferric chloride (5 mM) was added in increments of 20 μM and corresponding scan was recorded. This was continued until the concentration of Test compound:ferric chloride reached a ratio of 1:2.

Antimicrobial activity was tested against a gram positive (*Bacillus subtilis*) and a gram negative bacteria (*Escherichia coli*) by agar cup plate method. A loopful of bacteria was inoculated into 15 ml molten and cooled nutrient agar 50°. This was immediately poured into a petridish asceptically. Three cups were prepared in the solidified medium and filled with 40 μl DMSO, and test compound at 600 μg. The plate was allowed to stand for 2 h at room temperature for diffusion and finally incubated at 37±1° for 24 h.

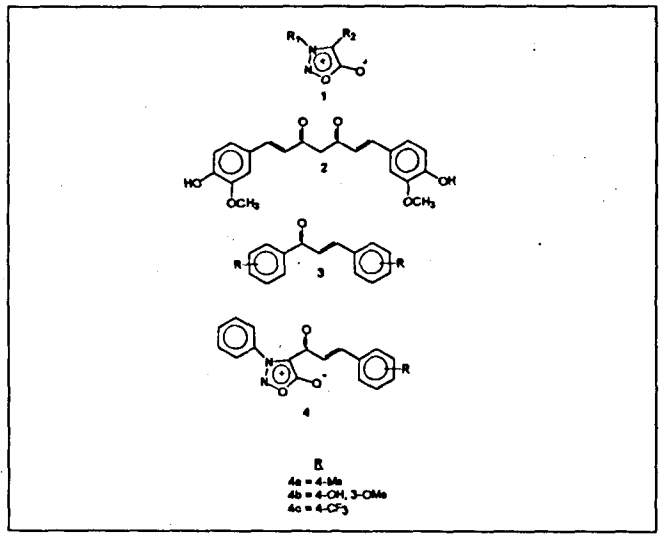
The title compounds were synthesized according to our previously reported procedure[3]. Each of the compounds was screened for *in vitro* cytotoxic activity in 56 cell lines representing the commonly found cancers like leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal,

Contd :

Cell line	Growth Inhibition 50 %(mM)[a]		
	4a	4b	4c
SK-MEL-2	2.30	3.37	2.59
SK-MEL-28	1.72	3.20	1.55
SK-MEL-5	2.75	3.68	2.74
UACC-257	3.73	7.08	2.79
UACC-62	1.72	3.44	1.47
Ovarian Cancer			
IGROVI	2.76	3.92	1.89
OVCAR-4	1.36	1.19	3.90
OVCAR-5	4.99	1.09	2.46
OVCAR-8	1.52	3.28	2.47
SK-OV-3	6.87	5.18	14.0
Renal Cancer			
786-0	2.00	2.49	0.80
A498	11.60	1.78	15.10
ACHN	1.69	3.19	1.44
CAK1-1	2.05	3.45	0.59
RXF393	1.94	2.53	1.51
SN12C	3.23	1.93	1.57
TK-10	3.13	4.06	1.68
UO-31	2.02	4.59	1.89
Prostate Cancer			
PC-3	6.00	11.30	5.02
DU-145	3.65	3.37	1.67
Breast Cancer			
MCF7	3.54	3.60	0.70
MCF7/ADR-RES	2.39	2.82	2.03
MDA-MB-231/ATCC	3.77	3.21	3.71
HS578T	1.46	1.57	3.03
MDA-MB-435	1.70	2.88	1.69
MDA-N	2.40	2.80	1.41
BT-549	2.72	2.99	1.59
T-47D	3.46	3.52	2.02
Average	2.34	3.47	1.74

a; Concentration giving 50% growth inhibition in various cell lines.

prostrate and breast in five different dilutions according to National Cancer Institute, Bethesda, USA protocol[12-14]. A good cytotoxic activity was observed in the primary screening, hence the primary screening was repeated, and Table 1 gives the molar concentrations of the compounds at 50% growth inhibition in the repeat test. All of the three compounds exhibited good activity with average 50% growth



Scheme1: Structures of Sydnones (1), Curcumin (2), Chalcones (3), 4-[1-oxo-(substituted aryl)-2-propenyl]-3-phenylsydnones (4)

inhibition in the range of 1.7-3.5 μM. Trifluoromethyl derivative (4c) was most active (1.74 μM).

Regarding the selectivity, methyl derivative (4a) was most selective towards SNB-75 cell line of CNS cancer. It was 100 times more specific to this cell line in comparison to its average (2.3 μM) cytotoxicity. 4-Hydroxy-3-methoxy derivative (4b) was not selective for any particular cell line; trifluoromethyl derivative (4c) exhibited selectivity towards CCRF-CEM cell line of leukemia (0.06 μM).

Because of the appreciable *in vitro* cytotoxicity and novel structural features, the compounds were tested for *in vivo* anticancer activity in hollow fiber assay. This is a preliminary *in vivo* test before screening the compounds in human xenograft model. In this assay, the compounds in two test doses (QDx4) are screened against 12-tumor cell lines cultivated in polyvinyl fluoride hollow fibers in mice. Methyl derivative (4a) showed modest activity against NCI-H23 (28%) OVCAR-3(27%), MDA-MB-435 (23%), UACC (28%) of non-small cell lung, ovarian, breast, melanoma, respectively. However, trifluoromethyl (4c) exhibited moderate activity against LOX IMVI (78%) and modest activity against NCI H23 (23%), MDA-MB-231 (23%) of melanoma, non-small cell lung and breast cancers respectively. In our earlier studies compound 4a exhibited promising (212%) *in vivo* tumor reducing activity in mice when injected in liposome-encapsulated formulation[6]. Lack of appreciable activity in hollow fiber assay, despite promising *in vitro* and

TABLE 2: *IN VIVO* ANTICANCER ACTIVITY BY HOLLOW FIBER ASSAY

Cell line in Fiber	% Growth (%T/C) [a]							
	Compound 4a				Compound 4c			
	84 mg/kg		125 mg/kg		100 mg/kg		150 mg/kg	
	i/p	s/c	i/p	s/c	i/p	s/c	i/p	s/c
Breast								
MDA-MB-435	71	92	71	89	87	84	100	100
MDA-MB-231	100	97	100	96	97	93	77	77
Ovarian								
OVCAR-5	87	84	100	94	100	98	100	86
OVCAR-3	100	98	84	73	100	95	95	85
CNS								
SF-295	86	90	98	85	100	92	99	97
U25193	97	96	100	96	100	100	100	
Non-Small Cell Lung								
NCI-H522	86	89	96	100	97	90	100	91
NCI-H23	100	91	72	92	97	93	77	77
Melanoma								
VACC-62	72	98	100	98	97	96	100	98
LOX IMVI	88	100	98	99	91	100	100	32
Colon								
COLO205	96	98	98	99	100	94	100	93
SW-620	90	96	100	97	100	95	100	96

a; Compounds were given by i/p in two doses to mice implanted (i/p and s/c) with human tumor cell in polyvinylidene (PVDF) fibers. Activity measured as % growth (%T/C), which is computed by dividing the average optical density of treated animals by control.

earlier tumor reducing activity indicates that formulation and pharmacokinetic parameters have a key role to play.

Nitric oxide is cytotoxic and the NO donor, mesoionic oxatriazole is reported to possess appreciable cytotoxic activity[15]. Since sydnones are also NO donors, we were interested to know whether above cytotoxic activity is mediated through NO. However, no NO donor activity was observed by classical Griess test in aqueous media. We feel lack of activity is due to poor water solubility of the compounds.

Many iron chelators are known to have cytotoxic acitivity[16,17]; however, in our iron chelation experiments no chelation property was observed. Cytotoxic activity is usually associated with antimicrobial activity. Hence these compounds were also tested for antimicrobial activity by agar cup plate method against gram negative (*E. coli*) and a gram positive (*B. subtilis*) no activity was observed.

Thus the present study indicate that 4-[1-oxo-(substituted aryl)-2-propenyl]-3-phenylsydnones are novel chemical class of anticancer compounds with appreciable *in vitro*

activity with moderate *in vivo* activity in hollow fiber assay. Further structural modifications of this lead, to improve pharmacokinetic parameters may yield potential anticancer compounds.

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Synthesis and Antimicrobial Evaluation of Some Novel Organometallic Compounds Against *Helicobacter pylori*

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Some novel organometallic compounds were prepared by complexing the antibiotics, tetracycline, azithromycin, cefotaxime, cephalexine, and antibacterials, ofloxacin, norfloxacin and gatifloxacin with bismuth citrate. They were characterized by UV, IR, NMR and elemental analysis. Their antibacterial activity against *Helicobacter pylori* and other microorganisms was investigated. Tetracycline-bismuth citrate was found to possess strong activity against *H. pylori* with a lowest inhibitory concentration of 125 mg/l. These complexes exhibited moderate activity against *Escherichia coli, Klebsiella pneumoniae, Bacillus pumilis, Staphylococcus aureus* and *Candida albicans.* The find-

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