WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 2, 859-869 Research Article ISSN 2278 – 4357

SYNTHESIS OF 4-[5-(4-NITROPHENYL)-4, 5-DIHYDRO-1H-PYRAZOL-3-YL]-3-(2, 4-DICHLOROPHENYL) SYDNONE AND ANTICANCER SCREENING AGAINST 60 TUMOR CELL LINES

Sachin K. Bhosale[1]*, Shreenivas R. Deshpande[2], Rajendra D. Wagh[3] and Nirmala V. Shinde[1]

[1]Department of Pharmaceutical Chemistry, S. M. B. T. College of Pharmacy, Nandihills, Dhamangaon, Tah: Igatpuri, Dist: Nashik. Maharashtra, India.

[2]Department of Medicinal and Pharmaceutical Chemistry, HSK College of Pharmacy, BVVS Campus, Bagalkote, Karnataka, India.

[3]Department of Pharmaceutical Chemistry, A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra, India.

Article Received on
30 Nov. 2018,

Revised on 21 Dec. 2018,
Accepted on 11 Jan. 2019

DOI: 10.20959/wjpps20192-13084

***Corresponding Author**
Dr. Sachin K. Bhosale
Department of Pharmaceutical Chemistry, S. M. B. T. College of Pharmacy, Nandihills, Dhamangaon, Tah: Igatpuri, Dist: Nashik. Maharashtra, India.

ABSTRACT

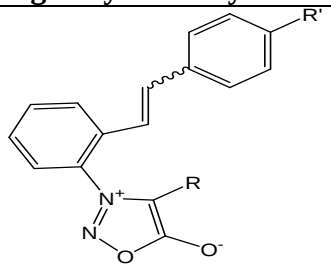
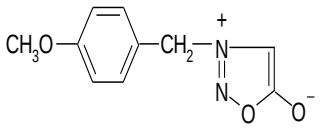
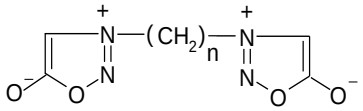
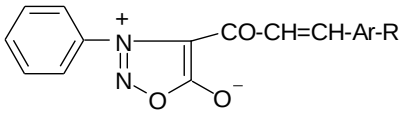
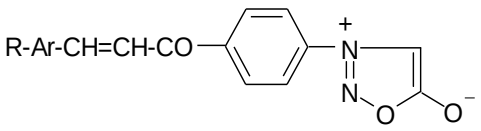
This research work seeks to synthesize of novel mesoionic compound 4-[5-(4-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-3-(2, 4-dichlorophenyl) sydnone, Ia containing sydnone moiety to transform antitumor molecule. Compound synthesized by cyclization of 4-[1-oxo-3-(4-nitrophenyl)-2-propenyl]-3-(2,4-dichlorophenyl)sydnone, I (sydnonyl-substituted α, β-unsaturated ketone) with hydrazine hydrate. Compound Ia was characterized by spectral study and evaluated against 60 human cancer cell lines for *in vitro* anticancer activity and was found to have greater anticancer activity than standard vincristine sulphate against some specific cell lines. Further structural modification of the molecule might lead to development of potent antitumor molecules.

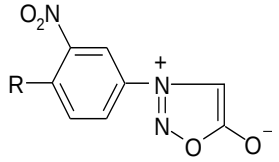
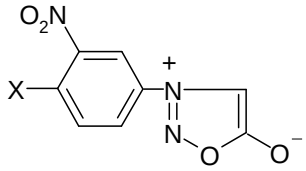
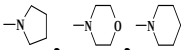
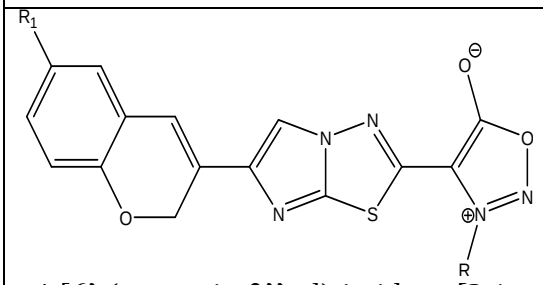
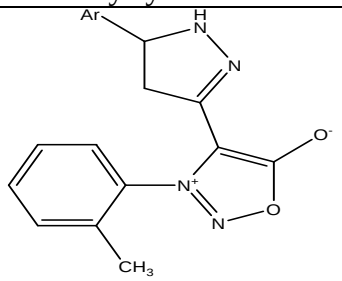
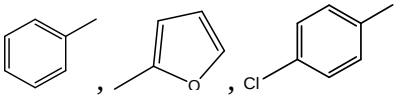
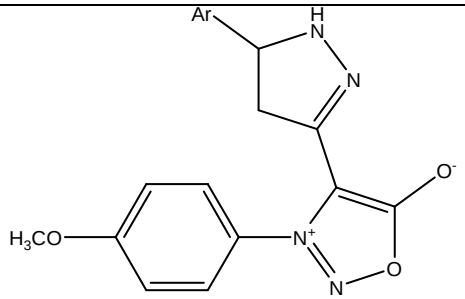
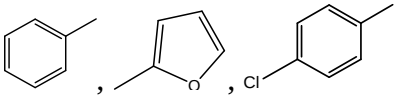
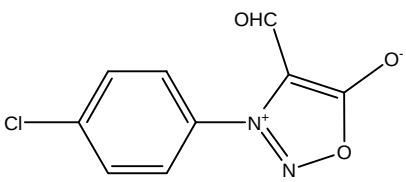
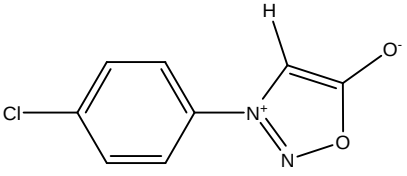
INTRODUCTION

Mesoionic 1, 2, 3-oxadiazol-3-ium-5-olate (sydnone) derivatives have been described for a variety of antitumor activities.(Butkovic et al, 2011; Grynberg et al**,** 1992; Greco et al, 1962; Satyanarayana et al, 2004; Satyanarayana et al, 1995; Dunkley et al, 2003; Dunkley et al, 2003; Tegginamath et al, 2013; Bhosale et al, 2015; Bhosale et al, 2015; Bhosale et al, 2017)

It has been observed that the ionic resonance structures of 1, 2, 3-oxadiazol-3-ium-5-olate ring enhances interactions with cancer cells. A series of 4-substituted-3-nitrophenyl-1, 2, 3-oxadiazol-3-ium-5-olates has shown antitumor activity.(Dunkley et al, 2003; Dunkley et al, 2003) Based on literature survey and reported antitumor molecules we have designed and synthesized molecule **Ia.**

Table 1: Reported sydnones and their derivatives having antitumor activity (Butkovic et al, 2011; Grynberg et al, 1992; Greco et al, 1962; Satyanarayana et al, 2004; Satyanarayana et al, 1995; Dunkley et al, 2003; Dunkley et al, 2003; Tegginamath et al, 2013; Bhosale et al, 2015; Bhosale et al, 2015; Bhosale et al, 2017).

Biologically active sydnones	Substituents	Biological activity	Ref.
 **1a, 1b**	**1a** R=CH_3, R'=CH_3 **1b** R=Ph, R'=Cl cis-4-methyl-3-[2-[2-(4-methylphenyl) ethenyl] phenyl] sydnone (**1a)** cis-4-phenyl-3-[2-[2-(4-chlorophenyl)ethenyl]-phenyl] sydnone(**1b)**	Anticancer	01
 **2**	3-(p-methoxybenzyl) sydnone	Anticancer against carcinoma-755	02
 **3**	polymethylene-bis-sydnones	Potent antitumor	03
 **4**	**4a**Ar= Ph, R=4-CH_3 **4b**Ar= Ph, R=3-OCH_3, 4-OH, **4c**Ar= Ph, R=4-CF_3	Highly selective against SNB-75 tumour cell line of CNS	04
 **5**	**5a** Ar=Ph, R= H, **5b**Ar=Ph, R=4CH_3,**5c** Ar=Ph, R=4-OCH_3, **5d** Ar=PH, R=2,4-$(OCH_3)_2$, **5g** Ar=Ph, R=3- Cl, **5h** Ar=Ph, R=2-Cl **5e** Ar=Ph, R=4-$NHCOCH_3$, **5f** Ar=Ph, R=4-Cl, **5g** Ar=Ph, R=3-Cl,**5h** Ar=Ph, R=2-Cl	Anticancer	04,05
	6 R=F 4-substituted-3-nitrophenyl sydnone	Anticancer against MCF7 (Breast), NCI-H460 (Lung) and	06, 07

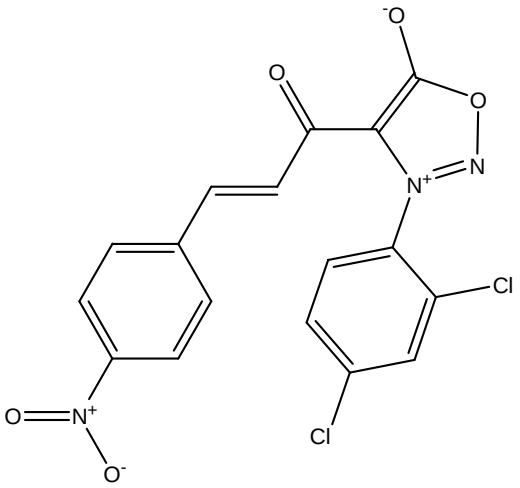
 **6**		SF-268 (CNS) cell lines	
 **7**	**7a, 7b, 7c, 7d** X= Cl,  3-[4-X-3-nitrophenyl]-1,2,3-oxadiazolium-5-olates	Anticancer against Sarcoma 180, Ehrlich carcinoma, B10MCII (Fibrous histiocytoma) and L1210 leukemia ascitictumours	06, 07
 4-[6'-(caumarin-3''-yl)-imidazo-[2,1-b][1,3,4]thiadiazol-2'-yl]-3-arylsydnone**8**	R_1=H ,Br, Cl, H, Br, Cl, H, Br, Cl, H, Br, Cl R= C_6H_5, C_6H_5, C_6H_5, P-CH_3-C_6H_4, P-CH_3-C_6H_4, p-CH_3-C_6H_4, P-OCH_3-C_6H_4, p-OCH_3-C_6H_4, p-OCH_3-C_6H_4, p-Cl-C_6H_4, p-Cl-C_6H_4, p-Cl-C_6H_4	Anticancer	08
 **9**	Ar= 	Antitumor against human breast cancer cell line MDA-MB-231 and human prostate cancer cell line PC3	09
 **10**	Ar= 	Antitumor against non-small cell lung cancer cell line (HOP-92), melanoma (M-14) and human prostate cancer cell line (PC3)	10
 3-(4-Chlorophenyl)-4-sydnonecarboxaldehyde**11**	 4-chlorophenyl sydnone **12**	Antitumor against non-small cell lung cancer cell line (NCI-H23), CNS cancer cell line (SNB-75)	11

MATERIALS AND METHODS

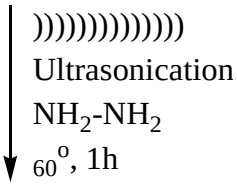
All chemicals and reagents were purchased from Sigma-Aldrich, Mumbai, India. Melting points of the intermediates and the final products were recorded on Systolic melting point apparatus and are uncorrected. TLC was carried out to monitor the completion of reaction by using E-Merck precoated 60 F254 plates. IR spectra were recorded by using KBr pellets on Jasco FTIR 1460 Plus spectrometer. NMR spectra were obtained on a BRUKER AVANCE II 400 NMR spectrometer at 400 MHz for ^{1}H and 40 MHz for ^{13}C (chemical shifts are expressed in δ, ppm). Mass spectra were recorded on WATERS, Q-TOF MICROMASS (LC-MS) instrument. The ultrasonic irradiation was performed by using a Biotechnics India (model-1510, frequency, 40 KHz).

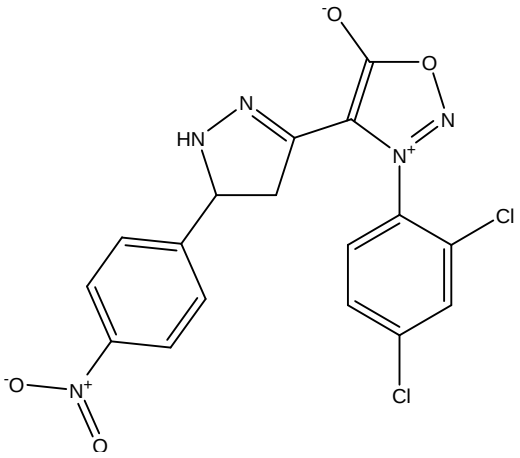
Synthesis of 4-[5-(4-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-3-(2, 4-dichlorophenyl) sydnone (Ia): To an ice cold solution of hydrazine hydrate (20 mM) in ethanol (30 ml), 4-[1-oxo-3-(4-nitrophenyl)-2-propenyl]-3-(2,4-dichlorophenyl)sydnone, **I** (5mM) (synthesized as per reported procedure by Bhosale et al, 2015)was added. The resultant solution was heated at 60 °C for 1h under ultra-sonication and then allowed to cool. The precipitate was collected through filtration and washed with ice-cold water and ethanol to afford yellow-orange crystals of **Ia**(187mg, Rf =0.488, Ethyl acetate: Benzene, 2:8).[05],[13],[14]

Characterization for compound Ia: IR (KBr) (cm^{-1}):1738.73 (C=O), 3118.78 (NH pyrazoline), ^{1}H NMR (δ ppm):8.03 ($C_6H_4NO_2$, 2H) 7.38 ($C_6H_4NO_2$, 2H) 7.1- 7.3 ($C_6H_3Cl_2$, 3H) 7.01 ($C_6H_4OCH_3$, 2H) 6.72 ($C_6H_4NO_2$, 2H) 7.0 (Pyrazol, N-H, 1H) 3.9 (Pyrazol, 5CH, 1H) 1.9 (Pyrazol, $4CH_2$, 2H), ^{13}C NMR (δ ppm): 40 (pyrazole, $4CH_2$), 53.4 (pyrazole, 5C), 105.7 (sydnone, 5C), 121.67 (sydnone, 4C), 127-135 ($C_6H_3Cl_2$), 155.6 (pyrazole, 3C), 123.4-148.5 ($C_6H_4NO_2$). Physicochemical data: $C_{17}H_{11}Cl_2N_5O_4$ Mol. wt. 420.206, m/z= 419.019, C, 48.49; H, 2.64; N, 16.67 % Yield=73, Rf=0.541 mp=156-158°C



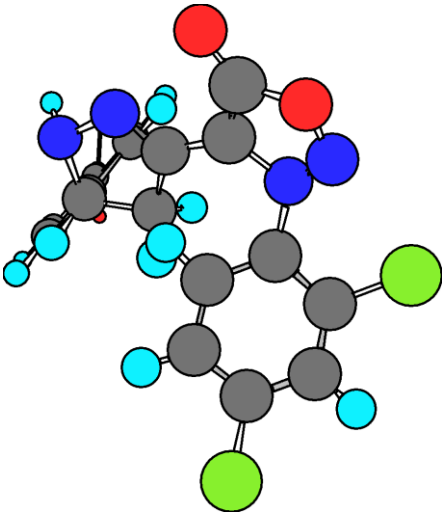
I





Ia

Scheme 1: Synthesis of 4-[5-(4-nitrophenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-3-(2,4-dichlorophenyl)sydnone, Ia from 4-[1-oxo-3-(4-nitrophenyl)-2-propenyl]-3-(2,4-dichlorophenyl)sydnone, I



3D model for compound Ia

Table 1: Anticancer screening data for compound Ia.

Human tumor cell line	% GI for Ia	% GI for Std.
Leukemia		
CCRF-CEM	-	4.49
HL-80	-	35.39
K-562	-	10.89
MOLT-4	-	5.30
RPMI-8226	24.17	16.2
SR	-	0.5
Non-Small Cell Lung Cancer		
HOP-92	-	-62.00
A549/ATCC	15.36	-6.3
NCI-H226	12.08	-.17.9
NCI-H23	-	-280.1
NCI-H322M	-	-33.4
NCI-H460	-	13.00
NCI-H522	-	23.4
Colon Cancer		
HCT-116	-	47.00
HCT-15	-	-0.6
SW-620	-3.47	-5.2
CNS Cancer		
SNB-75	-	-5.4
SF-295	-	-3.3
SF-268	4.96	-13.6
Melanoma		
M-14	-	ND
MALME-3M	9.06	-35.1
UACC-257	-	4.6
Ovarian Cancer		
OVCAR-04	8.27	-38.9

OVCAR-8	-	ND
SK-OV-3	25.50	18.2
IGROV1	8.37	6.3
Renal Cancer		
786-0	-	-2.1
A498	-	ND
CAKI-1	-	-16.2
UO-31	14.12	-18.3
SN-12C	17.84	-29.6
Prostate Cancer		
PC-3	7.91	-8.00
Breast Cancer		
MDA-MB-231/ATCC	-	37.4
MCF7	10.63	7.9
BT-549	8.57	34.5
T-47D	9.29	-48.5
Mean **Delta** **Range**	96.55 25.50 47.05	10.298 83.798 363.9

Range = highest growth percent- lowest growth percent.

Delta = mean growth percent - lowest growth percent.

% GI: % growth inhibition = mean growth percent- % growth,

Standard –Vincristine sulphate, ND-not determined, -- poor GI

Anti-cancer screening

'Brine shrimp lethality bioassay' was used to carry out preliminary cytotoxicity study and then compound **Ia** was evaluated against a panel of 60 different human tumor cell lines at National Cancer Institute, USA (NCI)as per reported standard procedure.

Preliminary anticancer activity by Brine shrimp lethality bioassay: (Meyer et al, 1982 Zhao et al 1992) The assay was performed on brine shrimp naupulli using Meyer's method. The *Artemia salina* was used as brine shrimp to monitor the screening and the eggs were procured from an aquarium shop (Nasik, Maharashtra). The eggs were allowed to hatch in artificial seawater (3.8% NaCl solution) for 48 h to obtain mature shrimp called naupulli. The various test compounds were set by using Dimethyl sulfoxide (DMSO) (not more than 50 µl in 5 ml solution) and sea water (3.8% NaCl in water). Test control was prepared in a vial by diluting 50µl DMSO to 5ml. Vincristine sulphate was used as positive control. The matured shrimps were placed in each of all experimental vials and control vial. After 24 h, the vials were examined with magnifying glass and the number of surviving naupulli for each vial was counted. The lethal concentrations resulting in 50% mortality of the brine shrimp (LC_{50}) was

determined from the 24 h counts. The dose-response data were transformed into a straight line through trade line fit linear regression analysis. It was observed 08.21LC_{50} (µg/ml)

***In-vitro* anticancer evaluation against 60 human tumor cell lines**: (Adams et al, 2005; Al-Suwaidan et al, 2013; Lorenzi et al, 2009; Mingyi et al, 2013; Roschke et al, 2003; Dudhe et al, 2014). Evaluation of compound **Ia,** for anticancer activity was done at NCI, Bethesda, USA as per standard procedure. The evaluation was carried out against 60 human tumor cell panels. The cells were obtained from various nine neoplastic cancers (leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers).The data reported as a mean graph for % growth inhibition of treated cells. Results of anticancer activity against 60 human tumor cell lines of compound was represented as one dose DTP curve and is shown in Fig. 1.

CONCLUSION

Based on *in-vitro* evaluation, Compound **Ia** showed higher and broader spectrum of anticancer activity against RPMI-8226 (%GI=24.17) of Leukemia Cancer A549/ATCC (%GI=15.36), NCI-H226 (%GI=12.08) of Non-Small Cell Lung Cancer SF-268 (%GI=4.96) of CNS Cancer OVCAR-04 (%GI=8.27), SK-OV-3 (%GI=25.50), IGROV1 (%GI=8.37) of Ovarian Cancer UO-31 (%GI=14.12), SN-12C (%GI=17.84) of Renal Cancer PC-3 (%GI=7.91) of Prostate Cancer MCF7 (%GI=10.63), T-47D (%GI=9.29) of Breast Cancer than standard drug vincristine sulphate. Compound **Ia** showed prominent anticancer activity due to active sydnone ring and substitution of 3^{rd} and 4^{th} position of sydnone with aryl ring having electron withdrawing functional groups like chloro (-Cl) and nitro (-NO_2) which make benzene ring more stable and may also increases lipophilicity to penetrate easily into cancer cells. Compound may showed their antitumor activities through multiple mechanisms including inhibiting protein kinase (CDK, MK-2, PLK1, kinase-like protein Eg5 and IKK), topoisomerase I and II, microtubule inhibition and many others. (Jawaid et al, 2000) Further research and development with designing necessary structural modifications of molecule **Ia** may lead to safer and effective potential anticancer drug candidates. The finding of the study inferred that the molecule **Ia** renders as a lead for further development of novel potent anticancer molecules against specific tumor cell line.

ACKNOWLEDGEMENT

Authors are thankful to NCI, USA for carrying out anticancer activity, SAIF Punjab University, Chandigarh, for spectral study, Dr. Dhake A. S., SMBT College of Pharmacy,

Dhamangaon, Nasik (MS) India for providing necessary facilities to carry out the research work.

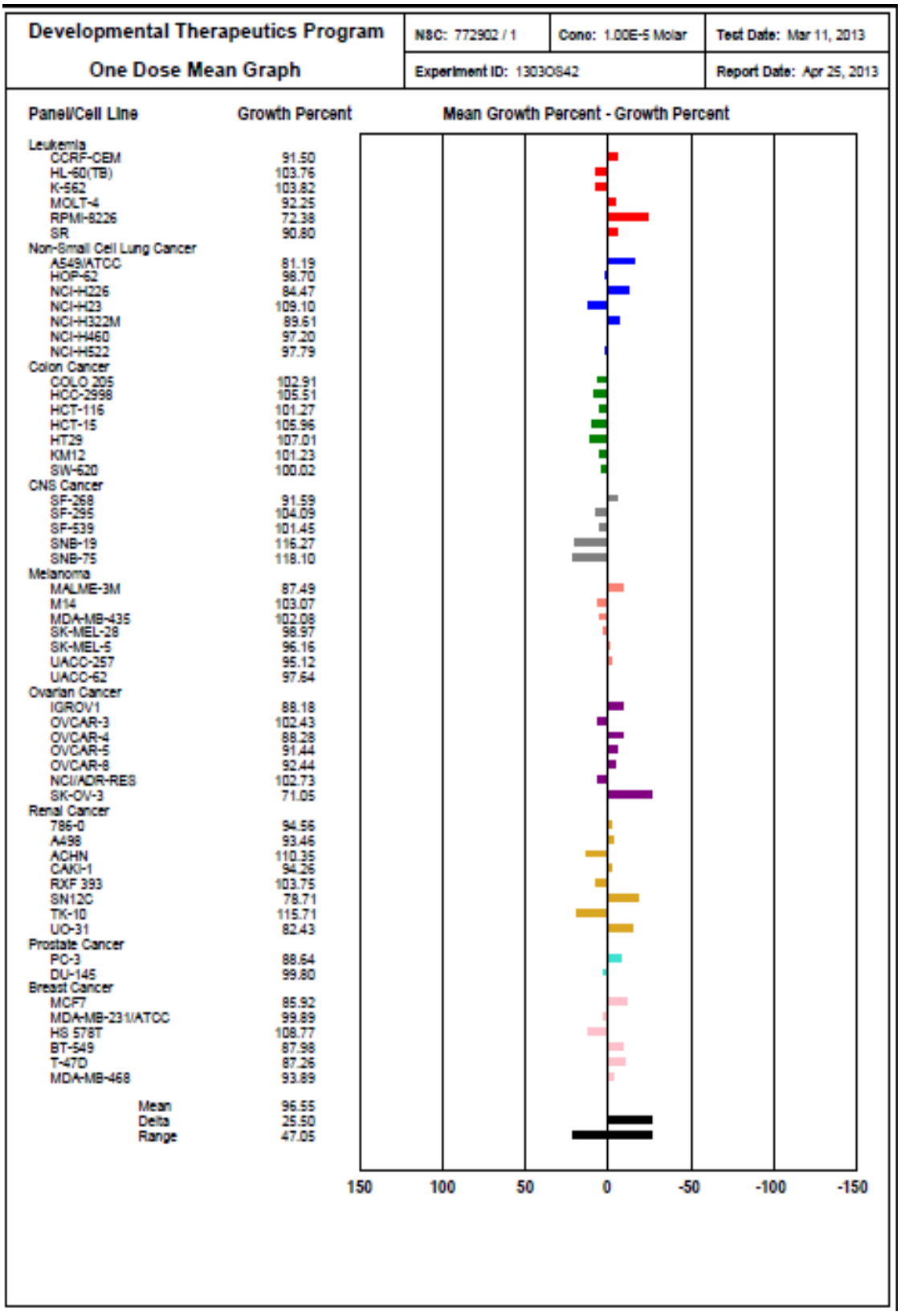


Figure 1: Anticancer activity for Ia against 60 human cancer cell lines.

REFERENCES

1. Butkovic K, Marinic Z, Sindler-Kulyka M. Synthesis of 3-(o-stilbenyl) sydnone and 3-(o-stilbenyl) -4-substituted sydnone derivatives and their antitumor evaluation. ARKIVOC, 2011; 10: 1-15.
2. Grynberg V, Gomes R, Shinzato T, Echevarria A,Miller J. Some new aryl-sydnones: effects on murine tumours. Anticancer Res., 1992; 12: 1025-8.
3. Greco CV, Nyberg, WH, Cheng CC. Synthesis of sydnones and sydnone imines. J Med Chem., 1962; 5: 861-5.
4. Satyanarayana K, Deshpande SR, Subbarao B, Rao MNA. Anticancer activity of 4-[1-oxo-(substitutedaryl)-2-propenyl]-3-phenylsydnones. Indian J Pharm Sci., 2004; 66: 679-83.
5. Satyanarayana K, Rao MNA. Synthesis, antiinflammatory, analgesic and antipyretic testing of 4-[1-oxo-(3-substituted aryl)-2-propenyl]-3-phenylsydnones and of 3-[4-[3-(substituted aryl)-1-oxo-2-propenyl] phenyl] sydnone. J Pharm Sci., 1995; 84: 263-6.
6. Dunkley CS, Thoman CJ. Synthesis and biological evaluation of a novel phenyl substituted sydnone series (VII) as potential antitumor agents. Chem Inform, 2003; 34: 129.
7. Dunkley CS, Thoman CJ. Synthesis and biological evaluation of a novel phenyl substituted sydnone series as potential antitumor agents. Bioorg Med ChemLett., 2003; 13: 2899-01.
8. Tegginamath G, Kamble RR, Taj T, Kattimani PP, Meti GY. Synthesis of novel imidazo [2, 1-b][1,3,4]thiadiazoles appended to sydnone as anticancer agents. Med Chem Res., 2013; 22: 4367–75.
9. Bhosale SK, Deshpande SR, Wagh RD. Synthesis and in vitro anticancer evaluation of 2-methylphenyl sydnone derivatives against human breast cancer cell line MDA-MB-231and human prostate cancer cell line PC3. Journal of Chemical and Pharmaceutical research, 2015; 7(5): 1333-43.
10. Bhosale SK, Deshpande SR, Wagh RD. Design, synthesis and cytotoxic activity evaluation of mesoionic 4-methoxyphenyl sydnone analogs against 60 human tumors cell lines. Der Pharma Chemica, 2015; 7(5): 306-18
11. Bhosale SK, Deshpande SR, Wagh RD. 3-(4-chlorophenyl)-[1, 2, 3] oxadiazol-3-ium-5-olate and its 4-formyl Analog: Ultrasound assisted synthesis and *in-vitro* anticancer evaluation against human tumor cell lines. Pakistan Journal of Pharmaceutical Sciences, 2017; 30(2): 513-20.

12. Bhosale SK, Deshpande SR, Wagh RD. Ultrasound Assisted One Pot Synthesis, Spectral, Antimicrobial and Antioxidant studies of Novel 4-[1-Oxo-3-(substituted phenyl)-2-propenyl]-3-substituted Phenyl Sydnones. Asian Journal of Chemistry, 2015; 27(8): 3063-68.
13. Satyanarayana K, Rao MNA. Synthesis of 4-[5-(substitutedaryl) -4,5-dihydro-1H-pyrazol-3-yl] -3-phenyl sydnones as antiinflammatory, antiarthritic and analgesic agents. Eur J Med Chem., 1995; 30: 641-5.
14. Shih MH. A Concise Synthetic Method for Sydnonyl-Substituted Pyrazoline Derivatives, Synthesis, 2004; 1: 26-32.
15. Meyer BN, Putnam JE, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. J Med Plant Res., 1982; 45: 31-4.
16. Zhao GX, Hui YH, Rupprecht JK, McLaughlin JL, Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. J Nat Prod., 1992; 55: 347-56.
17. Adams S, Robbins FM, Chen D, Wagage D, Holbeck SL, Morse HC, Stroncek D and Marincola FM. HLA class I and II genotype of the NCI-60 cell lines. J Transl Med.2005; 3:11(b)Al-Suwaidan IA, AlanaziAM, Abdel-Aziz AA, Mohamed MA, El-Azab AS. Design, synthesis and biological evaluation of 2-mercapto-3-phenethylquinazoline bearing anilide fragments as potential antitumor agents: Molecular docking study. Bioorg Med Chem Lett., 2013; 23: 3935-41.
18. Lorenzi PL, Reinhold WC, Varma S, Hutchinson AA, Pommier Y, Chanock SJ, Weinstein JN. DNA Fingerprinting of the NCI 60 cell line panel. Mol Cancer Ther., 2009; 8: 713-24.
19. Mingyi M, Longru S, Ji LM. Synthesis and biological evaluation of Combretastatin A-4 derivatives containing a 3'-O-substituted carbonic ether moiety as potential antitumor agents. Chem Central J., 2013; 7: 179-81.
20. Roschke AV, Tonon G, Gehlhaus KS, McTyre N, Bussey KJ, Lababidi S, Scudiero DA, Weinstein JN, Kirsch IR. Karyotypic complexity of the NCI-60 drug screening panel. Cancer Res., 2003; 63: 8634-47.
21. Dudhe R, Sharma PK, Verma PK. Pyrimidine containing furanose derivative having antifungal, antioxidant, and anticancer activity, Org Med ChemLett., 2014; 4: 1-18.
22. Jawaid A, Ahsan AK, Zulphikar A, Rafi HM, Shahar Y. Structure-activity relationship (SAR) study and design strategies of nitrogen containing heterocyclic moieties for their anticancer activities. Eur J Med Chem., 2000; 125: 143-189.