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Design, synthesis and cytotoxic activity evaluation of mesoionic 4-methoxyphenyl sydnone analogs against 60 human tumors cell lines

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ABSTRACT

Heterocyclic analogues of 1, 2, 3-oxadiazolium-5-olate along with pyrazole ring and isoxazole ring have been designed for antineoplastic evaluation. A series of novel 4-[5-(aryl)-4, 5-dihydro-(1H-pyrazole/1-phenyl-pyrazol/isoxazole)-3-yl]-3-(4-methoxyphenyl)-1, 2, 3-oxadiazolium-5-olates has been synthesized and compounds ***(31d, 32d, 33d)*** *evaluated antitumor activity against 60 human tumor cell lines. Each compound is exposed to 60 human tumor cell lines, including non small cell lung, colon, melanoma, prostate, ovarian, breast, and kidney cancers. Data reported as a mean graph of the percent growth inhibition of treated cells. Out of 60 human tumor cell lines, compound* ***33d*** *is highly effective for non small cell lung cancer cell lines and melanoma (M14). Further designing with modifications and in vivo study of synthesized 1, 2, 3-oxadiazolium-5-olates may give a ray of light to search for a potent antitumor molecule.*

Keywords: 1, 2, 3-oxadiazolium-5-olate, sydnone, anticancer, pyrazole, isoxazole

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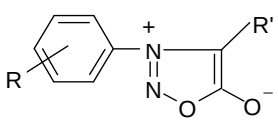
INTRODUCTION

Substituted sydnone **1** are reported to explore highly potential activity against cancer cell lines [1-8]. Greco *et al* has screened a series of sydnone for anticancer activity, and it was found that, 3-(p-methoxybenzyl) sydnone **2** was effective for carcinoma-755 in mice. The same compound was found inactive against sarcoma-180 and leukemia-1210[1]. A number of polymethylene-bis-sydnones **3** have been synthesized and shown strong antitumor activity [2]. The compounds of **4** and **5** series were cytotoxic to tumor cells *in vitro*, while only methyl substituted derivative showed powerful *in vivo* tumor reducing activity [3]. Satyanarayana *et al*., screened three derivatives (**4a, 4b, 4c**) for *in vitro* cytotoxicity in 56 cell lines representing cancers of non-small cell lung, colon, CNS, melanoma, ovarian, prostate, breast and leukemia and all these compounds exhibited promising activity. Average growth inhibition of 50% was in the range of 1.7-3.5 μM. **4a** was highly selective against the SNB-75 tumor cell line of CNS. A series of N- (4'-substituted-3'-nitrophenyl) sydnone **6** with potential antitumor activity was designed based

on potent analogues. 4'-fluoro derivative (**6**, R=F) has an improved activity against all three cell lines MCF7 (Breast), NCI-H460 (Lung) and SF-268 (CNS) (Anto et al., 1994; Satyanarayana et al., 2004). The effects of new aryl-sydnones, 3-[4-X-3-nitrophenyl]-1,2,3-oxadiazolium-5-olates **(7a, 7b, 7c, 7d)** on the survival of mice bearing Sarcoma 180, Ehrlich's carcinoma, B10MCII (Fibrous histiocytoma) and L1210 leukemia ascitic tumors, on proliferation of cultured tumor cells and on synthesis of DNA in L1210 leukemia were determined [3]. **7a** and **7b** *in-vivo* significantly enhanced the survival of S180, Ehrlich and B10MCII tumor-bearing mice. Furthermore, **7b** showed significant activity against L1210. **7c** and **7d** did not show antitumor activity. **7a** *in vitro* was the most cytotoxic and **7d** being the least active in all the above tumor cells. All screened derivatives inhibited thymidine uptake by L1210 cells [6]. Literature demonstrates that sydnones are associated with a wide range of physiological activities, including antimicrobial, anti-inflammatory, analgesic and antipyretic activities [1-13]. Consequently, chemists still enthusiastically pursue the syntheses of sydnones to screen as potential anticancer compounds. Moreover, pyrazole [14, 15] and isoxazole [16, 17] have been found to strong anticancer activity. In particular, they are reported to be powerful antibiotic, anticancer, antioxidating agents. Hence designing and synthesis of novel heterocyclic molecules of 1, 2, 3-oxadiazolium-5-olate along with pyrazole and isoxazole ring are very interesting.

Potent anticancer 1, 2, 3-oxadiazol-5-olate molecules



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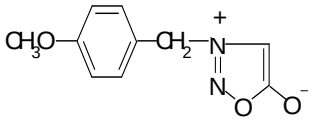

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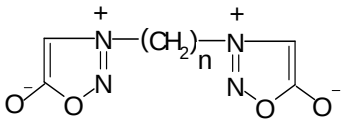
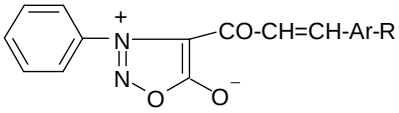


3

(Basic structure for 1, 2, 3-oxadiazolium-5-olate)



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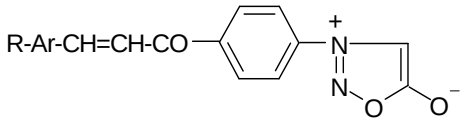
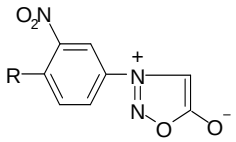


5

4a Ar= Ph, R=4-CH_3, **4b** Ar= Ph, R=3-OCH_3, 4-OH, **4c** Ar= Ph, R=4-CF_3
5a Ar=Ph, R= H, **5b** Ar=Ph, R=4-CH_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



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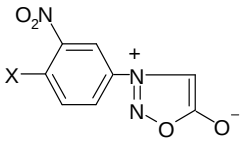
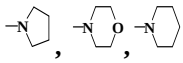


7a, 7b, 7c, 7d X= Cl, 

MATERIALS AND METHODS

Chemistry

All reagents were purchased from Sigma-Aldrich, Mumbai (India). Melting points of the intermediates and the products were recorded using a Systolic melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed on E-Merck precoated 60 F254 plates, and the spots were rendered visible by exposing to UV light and/or iodine vapours. Infra red spectra was recorded in KBr discs using Jasco FTIR 1460 Plus spectrometer. NMR spectra were obtained on a BRUKER AVANCE II 400 NMR spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts (δ) reported are with respect to internal reference tetramethyl silane. An electron impact mass spectrum was recorded on WATERS, Q-TOF MICROMASS (LC-MS) instrument. Elemental analyses (CHN) were in full agreement with the proposed structures within ±0.30% of the theoretical values. The ultrasonic irradiation was performed by using a Biotechnics (India) supersonic cleaner bath, model 1510, AC input 115 V, output 50 W, 1.9 liters with a mechanical timer (60 min with continuous hold) and heater switch, 47 KHz.

Synthesis

Synthesis of 3-(4-methoxyphenyl) sydnone (4)

Synthesis of N-nitroso (4-methoxylphenyl) glycine (3)

Ethyl chloroacetate (0.6mol), *p*-methoxyaniline (0.5mol) and anhydrous sodium acetate (50 g, 0.6mol) in 120 ml of alcohol were refluxed for 5h. The reaction mixture was left overnight at room temperature and poured into ice-cold water; a precipitate of N- (4-methoxylphenyl) glycine ethyl ester **1** was obtained (0.45 mol., 87%, m.p. 53-56 ^{o}C). To **1** (0.45 mol) was added sodium hydroxide (0.5 mol) in 225 ml of water and the mixture was refluxed for 0.5 h. After cooling, the reaction mixture was acidified to pH 2 using hydrochloric acid under cooling. The precipitated N-(4-methoxylphenyl) glycine **2** was filtered and washed in cold water (0.1mol, m.p. 119-121^{o}C). A solution of sodium nitrite (6.3 g, 0.09 mol) in water (20 ml) was added to **2** (0.10 mol) in water (60 ml) at 0 ^{o}C during 0.5 h. Further stirring for an additional 2 h resulted in a clear solution which was acidified with hydrochloric acid. The precipitated **3** was washed in cold water, dried and recrystallized with ethanol (0.06 mol., 67%, m.p. 103-105^{o}C).

Synthesis of 3-(4-methoxyphenyl) sydnone (4): Acetic anhydride (25 ml) was added to **3** (12.5 g, 0.06 mol). The reaction mixture was left overnight at room temperature and poured into cold water. The separated **4** was filtered, dried (0.05 mol, 83%) and recrystallized using ethanol. mp 139-141 ^{o}C; IR, cm^{-1} 1753 (C=O, sydnone), 3139 (C-4 of sydnone C-H stretch);

Synthesis of 4-[1-oxo-3-(substituted aryl)-2-propenyl]-3(4-methoxyphenyl) sydnones (31-33)

***Synthesis of 4-[1-oxo-3-(phenyl)-2-propenyl]-3-(4-methoxylphenyl) sydnone (31)*:**

Typical procedure: A mixture of 4-acetyl-3-(4-methoxyphenyl) sydnone (0.01 mol), sodium hydroxide aqueous solution and ethanol (20 mL) was cooled at (5–10°C) and to this was added benzaldehyde (2 g, 0.012 mol) while being stirred. The reaction mixture was stirred further for 1 h. The precipitate obtained was filtered, washed in cold water and re-crystallized from ethanol and ethyl acetate (1:1) to give **31** (1.67 g, 0.0043 mol. 43%). Remaining compounds were prepared similarly using respective aryl aldehydes.

Typical procedure for preparation of 4-[5-(aryl)-4, 5-dihydro-(1H-pyrazole)-3-yl]-3-(4-methoxy)-phenyl) sydnone (31a, 32a, 33a)

Synthesis of 4-[5-(phenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-3-(4-methoxyphenyl) sydnone **(31a):** To an ice cooled solution of hydrazine hydrate (100 mg, 2.00 mmol) in ethanol (3 mL) was added **31** (153 mg, 0.50 mmol). The mixed solution was heated at 60 °C for 5–6 h until the reaction was complete and then cooled. The precipited solid was collected by filtration and washed with ice-cold water, cold ethanol to afford 129 mg (80%) of **31a** as yellow-orange crystals; m.p. 133–135°C. IR (KBr): 3286 (N–H), 1719 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.40 (s, 3H), 2.63 (dd, *J* = 16.5, 10.6 Hz, 1 H), 3.21 (dd, *J* = 16.5, 10.6 Hz, 1 H), 4.63 (td, *J* = 10.6, 2.5 Hz, 1 H), 7.22–7.35 (m, 5 H), 7.41 (d, *J* = 8.5 Hz, 2 H), 7.63 (d, *J* = 8.5 Hz, 2 H), 7.81 (d, *J* = 2.5 Hz, 1 H). ^{13}C NMR (DMSO-*d*6): δ = 21.04, 41.07, 62.91, 104.92, 125.78, 126.61, 127.43, 128.57, 129.95, 132.58, 135.28, 142.12, 142.36, 165.98. EIMS (30 eV): *m*/*z* (%) = 336.13. Element Calcd for $C_{18}H_{16}N_4O_3$: C, 64.28; H, 4.79; N, 14.27. Found: C, 64.31; H, 4.81; N, 16.64. In similar way compounds **32a, 33a,** were synthesized from respective **32**, **33**.

4-[5-(furyl)-4, 5-Dihydro-1H-Pyrazol-3-Yl]-3-(4-Methoxyphenyl) Sydnone **(32a):** Yield: 51%; mp 140–141 °C. IR (KBr): 3279 (N–H), 1739 (C=O) cm–1. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.40 (s, 3 H), 2.67 (dd, *J* = 16.5, 10.5 Hz, 1 H), 3.21 (dd, *J* = 16.5, 10.5 Hz, 1 H), 4.94 (td, *J* = 10.5, 2.9 Hz, 1 H), 6.91–6.97 (m, 2 H), 7.37–7.44 (m, 3 H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.89 (d, *J* = 2.9 Hz, 1 H). ^{13}C NMR (DMSO-*d*6): δ = 21.06, 41.72, 58.67, 104.68, 124.97, 125.02, 125.80, 126.98, 129.98, 132.53, 136.12, 142.22, 145.68, 165.96. FABMS: *m*/*z* (%) = 412.39 (M+ + H, 100), Element Calcd ($C_{24}H_{20}N_4O_3$) for C, 69.88; H, 4.84; N, 13.56. Found: C, 69.90; H, 4.85; N, 13.53.

4-[5-(4-chlorophenyl)-4, 5-Dihydro-1H-Pyrazol-3-Yl]-3-(4-Methoxylphenyl) Sydnone **(33a):** Yield: 65%; mp 159–161 °C. IR (KBr): 3331 (N–H), 1751 (C=O) cm–1. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.40 (s, 3 H), 2.60 (dd, *J* = 16.5, 10.5 Hz, 1 H), 3.23 (dd, *J* = 16.5, 10.5 Hz, 1 H), 4.70 (td, *J* = 10.6, 2.6 Hz, 1H), 7.24–7.49 (m, 6 H), 7.62 (d, *J* = 8.4 Hz, 2 H), 7.82 (d, *J* = 2.6Hz, 1 H). ^{13}CNMR (DMSO-*d*6): δ = 21.04, 41.04, 62.14, 104.79, 123.08, 125.76, 128.53, 129.95, 131.95, 132.56, 135.42, 141.41, 142.14, 165.98. EIMS (30 eV): *m*/*z* (%) = 337.11 (100%). Element Calcd for $C_{18}H_{15}ClN_3O_4$: C, 64.09; H, 4.48; N, 12.46; O, 9.02. Found: C, 64.11; H, 4.50; N, 12.44.

4-[5-(phenyl)-4,5-dihydro-1-phenyl-pyrazol-3-yl]-3-(4-methoxylphenyl)sydnone**(31b):** $C_{16}H_{14}N_4O_4$ *Typical Procedure:* To an ice cooled solution of phenyl hydrate (2.00 mmol) in glacial acetic acid (3 mL) was added to **31** (0.50 mmol) under ultrasonication conditions (frequency 25 KHz) and allowed to react at room temperature for 2h.

The reaction mixture was poured in to crushed ice. The precipited solid was collected by filtration and washed with ice-cold water, cold ethanol, mp 149–151°C. Exact Mass: 326.102, Mol. Wt.: 326.307, C, 58.89 H, 4.32, N, 17.17, O, 19.61. Yellow orange colour crystals (98mg, 52%) IR (KBr):1757 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.98-1.90 (m, 1H), 2.26-2.29 (m, 1H), 4.12 (1H). δ = 2.36 (t, 3H, *J* = 6.9, 7.0 Hz), 2.43 (dd, *J* = 16.5, 10.6 Hz, 1 H), 3.21 (dd, *J* = 16.5, 10.6 Hz, 1 H), 4.63 (td, *J* = 10.6, 2.5 Hz, 1 H), 7.07-6.45 (5H), 7.23-7.10 (5H), 7.41 (d, *J* = 8.5 Hz, 2 H), 7.63 (d, *J* = 8.5 Hz, 2 H), 7.81 (d, *J* = 2.5 Hz, 1 H). ^{13}CNMR ($CDCl_3$, 125 MHz): 143.3, 42.3, 138.34, 129.4, 129.32, 129.12, 129.33, 128.54, 128.28, 128.4, 128.3, 127.3, 127.42, 126-09, 126.23, 116.12, 112.12, 112.78, 20.79; ESI-MS: 326.11.

4-[5-(furyl)-4,5-dihydro-1-phenyl-pyrazol-3-yl]-3-(4-methoxylphenyl) sydnone (32b): Yield 73%. $C_{22}H_{18}N_4O_4$ Exact Mass: 402.133, Mol. Wt: 402.403, m/e: 402.131 (100.0%), C, 65.66; H, 4.50; N, 13.90; O, 15.92. IR (KBr):1756 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.17 (s, 3 H), 2.29 (dd, *J* = 16.5, 10.5 Hz, 1 H), 3.18 (dd, *J* = 16.5, 10.5 Hz, 1 H), 4.94 (td, *J* = 10.5, 2.9 Hz, 1 H), 6.91–6.97 (m, 2 H), 7.19–7.32(m, 5 H), 7.37–7.44 (m, 3 H), 7.59 (d, *J* = 8.4 Hz, 2H), 7.97 (d, *J* = 2.9 Hz, 1 H). ^{13}C NMR: 21.26, 43.83, 56.8, 109.6, 110.52, 121.83, 121.83, 124.57, 124.57, 124.72, 129.15, 129.15, 130.52, 130.52, 136.19, 140.39, 142.42, 143.25, 146, 149.39, 153.83, 171.3

4-[5-(4-chlorophenyl)-4,5-dihydro-1-phenyl-pyrazol-3-yl]-3-(4-methoxylphenyl sydnone (33b): $C_{16}H_{13}N_3O_5$, Exact Mass: 327.086, Mol. Wt.: 327.292, m/e: 327.09 (100.0%), C, 58.73; H, 4.01; N, 12.83; O, 24.43. IR (KBr):1753 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.17 (s, 3 H), 2.58 (dd, *J* = 16.5, 10.5 Hz, 1 H), 3.24 (dd, *J* = 16.5, 10.5 Hz, 1 H), 4.70 (td, *J* = 10.6, 2.6 Hz, 1H), 7.10-6.39 (m, 5H), 7.22–7.43 (m, 2 H), 7.83 (d, *J* = 8.4 Hz, 2 H), 7.99 (d, *J* = 2.6Hz, 1 H). ^{13}C NMR: 21.26, 43.83, 59.35, 121.83, 121.83, 124.57, 124.72, 124.57, 129.01, 129.01, 129.10, 129.10, 129.15, 129.15, 130.52, 130.52, 135.68, 136.19, 137.84, 140.39, 143.25, 146, 153.83, 171.3.

4-[5-(phenyl)-4, 5-dihydro-isoxazol-3-yl]-3-(4-methoxyphenyl) sydnone **(31d):** $C_{18}H_{15}ClN_4O_3$

Typical Procedure: To an ice cooled solution of hydroxylamine hydrochloride (0.1 mole) in pyridine (3 mL) was added to **31** (0.50 mmol) under ultrasonication conditions (frequency 47 KHz) and allowed to react at room temperature for 1.5 hr. The reaction mixture was poured in to crushed ice. The precipited solid was collected by filtration and washed with ice-cold water, cold EtOH to afford 98 mg (52%) of **31d** as yellow orange crystals; mp 121–123 °C. Exact Mass: 370.083, Mol. Wt.: 370.790, m/e: 370.09 (100.0%), Elements C, 58.31; H, 4.08; N, 17.17; IR (KBr):1754 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.19 (s, 3H), 2.35 (dd, *J* = 16.5, 10.6 Hz, 1 H), 3.45 (dd, *J* = 16.5, 10.6 Hz, 1 H), 7.42–7.55 (m, 5 H), 7.42 (d, *J* = 8.5 Hz, 2 H), 7.89 (d, *J* = 8.5 Hz, 2 H), 8.05 (d, *J* = 2.5 Hz, 1 H). ^{13}C NMR: 21.26, 42.03, 82.31, 124.57, 124.57, 125.78, 125.78, 128.49, 128.49, 128.92, 130.52, 130.52, 136.19, 140.73, 143.25, 146, 153.83, 171.3.

4-[5-(furyl)-4,5-dihydro-isoxazol-3-Yl]-3-(4-methoxyphenyl) sydnone**(32d):** $C_{24}H_{19}ClN_4O_3$ Exact Mass: 446.115, Mol. Wt: 446.886, m/e: 446.12 (100.0%), Elements C, 64.50; H, 4.29; N, 12.54. IR (KBr):1759 (C=O) cm^{-1}. ^{1}H NMR (CDCl3, 500 MHz): δ = 2.22 (s, 3 H), 2.71 (dd, *J* = 16.5, 10.5 Hz, 1 H), 3.13(dd, *J* = 16.5, 10.5 Hz, 1 H), 6.38–6.69 (m, 2 H), 7.35–7.48 (m, 3 H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.95 (d, *J* = 2.9 Hz, 1 H). ^{13}C NMR: 21.26, 42.03, 74.63, 108.83, 109.09, 124.57, 124.57, 130.52, 130.52, 136.19, 142.59, 143.25, 146, 152.22, 153.83, 171.3.

4-[5-(4-chlorophenyl)-4,5-dihydro-isoxazol-3-yl]-3-(4-methylphenyl)sydnone**(33d):** $C_{18}H_{14}ClN_3O_4$, Exact Mass: 371.067, Mol. Wt. 371.774, m/e: 371.066 (100.0%), Elements C, 58.15; H, 3.80; Cl, 9.54; N, 11.31. IR (KBr):1753 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.32 (s, 3 H), 2.48 (dd, *J* = 16.5, 10.5 Hz, 1 H), 3.24 (dd, *J* = 16.5, 10.5 Hz, 1 H), 7.22–7.43 (m, 2 H), 7.83 (d, *J* = 8.4 Hz, 2 H), 8.01 (d, *J* = 2.6Hz, 1 H). ^{13}C NMR: 21.26, 42.035, 82.31, 124.57, 124.57, 127.68, 127.68, 128.66, 128.66, 130.52, 130.52, 135.68, 136.19, 140.73, 143.25, 146, 155.78, 172.9.

BIOLOGICAL ACTIVITY

Anticancer screening

Preliminary Cytotoxicity Study (Brine shrimp lethality bioassay): Brine shrimp lethality bioassay is widely used for the bioassay for the bioactive compounds. The brine shrimp, *Artemia salina*, was used as a convenient monitor for the screening. The eggs of the brine shrimps were collected from an aquarium shop (Nashik, Maharashtra) and hatched in artificial seawater (3.8% NaCl solution) for 48 hr to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using Meyer method [18, 19]. The test compounds were prepared by dissolving in DMSO (not more than 50 µl in 5 ml solution) and sea water (3.8% NaCl in water). A vial containing 50µl DMSO diluted to 5ml was used as a control. Standard Vincristine sulfate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were

inspected using a magnifying glass and the number of surviving nauplii in each vial were counted. The lethal concentrations of compounds resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 7); the LC_{50} was derived from the best-fit line obtained.

Table1: Brine Shrimp lethality assay

Compounds	LC_{50}(µg/ml)
31a	15.36
32a	14.41
33a	13.49
31b	11.21
32b	12.98
33b	13.68
31d	10.33
32d	09.51
33d	03.55
Vincristine sulphate	0.39

Values are mean to three tubes

In vitro anticancer evaluation against 60 human tumor cell lines

Synthesized compounds were evaluated for preliminary anticancer assay at National Cancer Institute (NCI), Bethesda, USA in an *in-vitro* 60 human tumor cell panel derived from nine neoplastic cancers (leukemia, Non small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancers) at a single dose of 10^{-5}M. [20-23].

Methodology of the in-vitro Cancer Screen

All compounds were screened for anticancer activity as per the protocol of NCI. Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as: [(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz [(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz. Each compound is exposed to 60 human tumor cell lines, including lung, colon, melanoma, prostate, ovarian, breast, and kidney cancers at five different doses for 48 hours. Compounds tested initially at a single high dose (10^{-5} M) in the full NCI 60 cell panel. The data reported as a mean graph of the percent growth inhibition of treated cells [20-23].

Observations: Refer Table **1** and **2**.

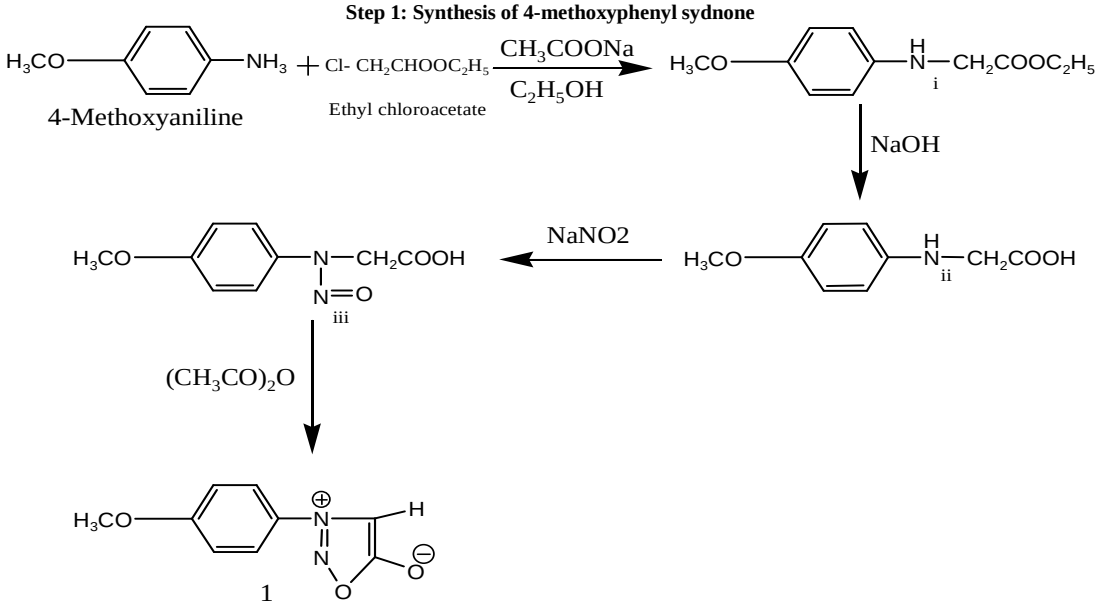
Graphical representation of anticancer effect of compounds: Fig. 1, 2, 3, and 4 shows anticancer activity of **31d, 32d, 33d** and Vincristine sulfate against 60 human cancer cell lines.

RESULTS AND DISCUSSION

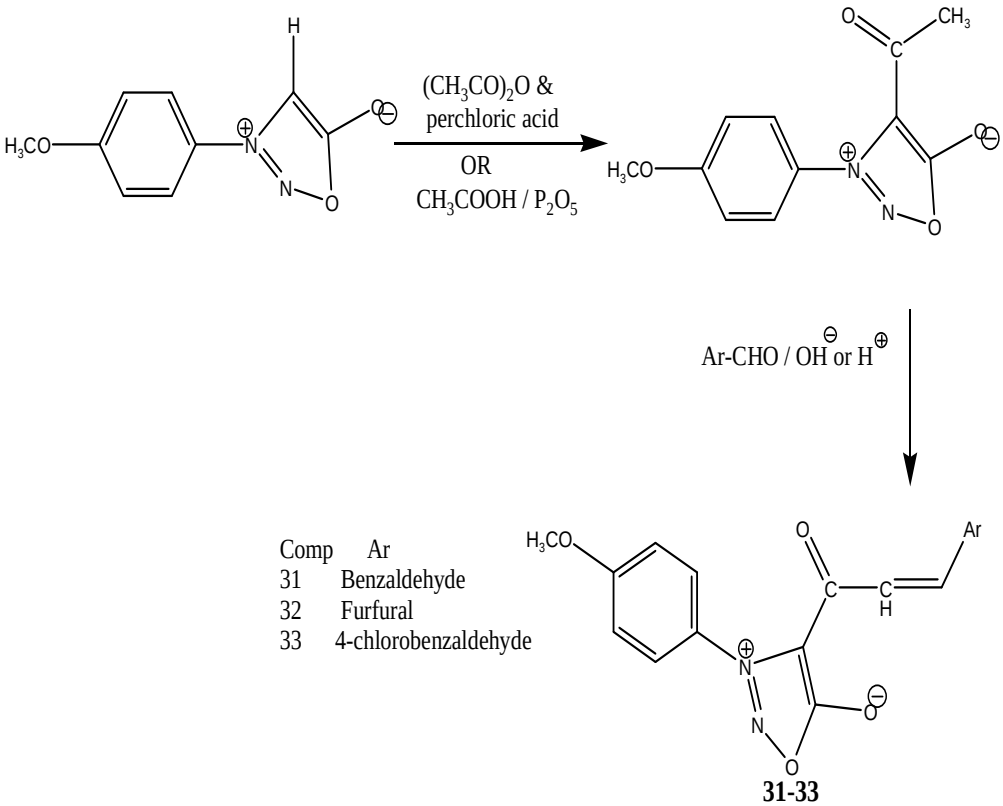
We synthesized and characterized new sydnone derivatives. Newly synthesized compounds were evaluated for preliminary brine shrimp lethality bioassay and *in vitro* anticancer activity against 60 human cancer cell lines. The lethality of the compounds to brine shrimp was determined after 24 hours of exposure to the test solutions and the positive control, Vincristine sulfate. The compound **33d** showed potential cytotoxic activity having an LC_{50} of 03.55µg/ml in contrast to the standard vincristine sulfate of 0.39µg/ml. The BSLT technique is easily mastered, of little cost, and utilizes small amount of test material. The aim of this method is to provide a front-line screen that can be backed up by more specific and more expensive bioassays on the active compounds. It appears that BSLT is predictive of cytotoxicity activity [19]. The result obtained used as a direction to carry *in vitro* anticancer activity against 60 human cancer cell lines. Compound **33d** is highly efficient for non small cell lung cancer HOP-92(118.43% GI), melanoma M-14(78.78% GI) and prostrate cancer (100.59% GI) human tumor cell lines comparatively Vincristine sulphate. In the future, modification may lead to safer and potential anticancer molecules. Further *in vivo* study of newly 1, 2, 3-oxadiazolium-5-olate derivative can give a ray of light over the field of antitumor molecule research.

Scheme1: Synthesis of **31a-33a, 31b-33b, 31d - 33d**

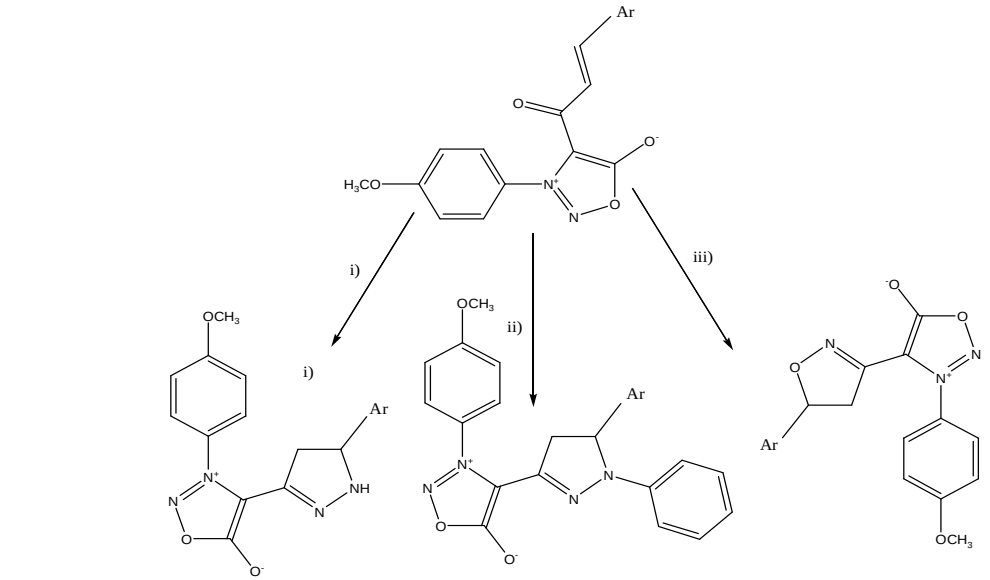
Step 1: Synthesis of 4-methoxyphenyl sydnone



Step 2: Synthesis of 4-[1-oxo-3-subtituted phenyl-2-propenyl]-3-(4-methoxyphenyl) sydnone

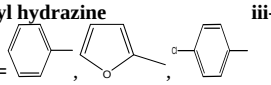


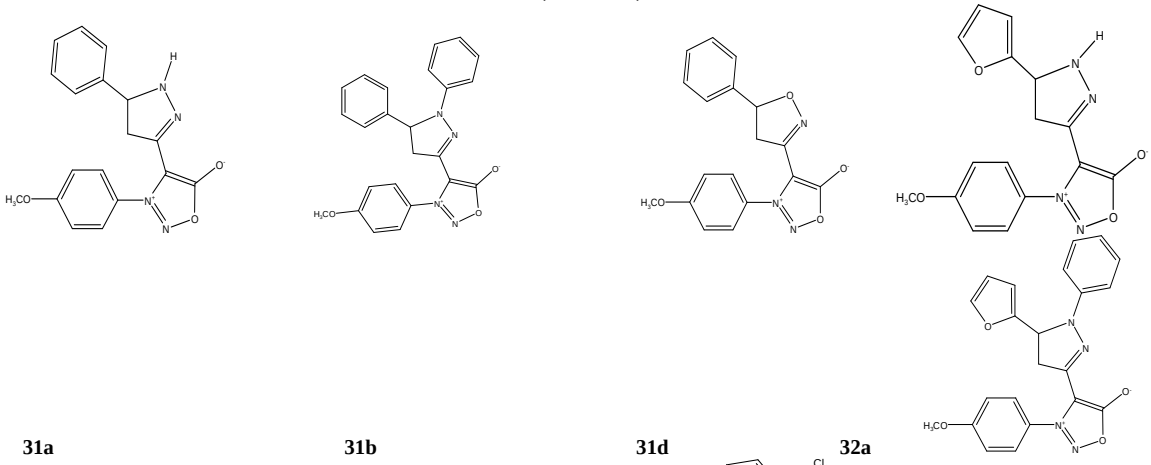
Step 3: Synthesis of 4-[5-(aryl)-4, 5-dihydro-1H-pyrazol-3-yl]-3-(4-methoxylphenyl) sydnone (31a-33a), 4-[5-(aryl)-4, 5-dihydro-1-phenyl-pyrazol-3-yl]-3-(4-methoxylphenyl) sydnone (31b-33b), 4-[5-(aryl)-4, 5-dihydro-isoxazol-3-yl]-3-(4-methoxylphenyl) sydnone (31d-33d) from (31,32,33)



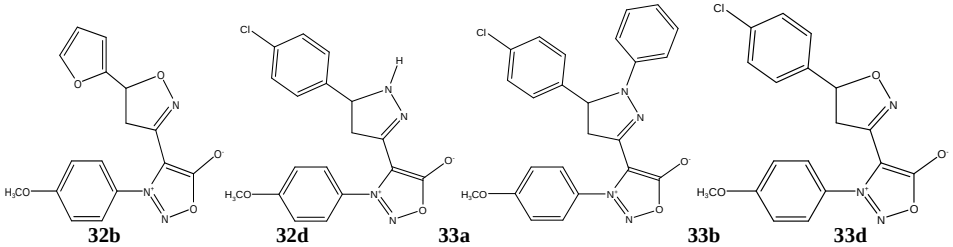
(31a, 32a, 33a) **(31b, 32b, 33b)** **(31d, 32d, 33d)**

i= Hydrazine **ii=Phenyl hydrazine** **iii-Hydroxylamine**

Ar = 



31a **31b** **31d** **32a**



32b **32d** **33a** **33b** **33d**

Table 2:- Anticancer screening data of compounds 31d, 32d, 33d

Cell Line	Percent Growth Inhibition (% GI)			
	31d	**32d**	**33d**	Standard Vincristine Sulphate
Leukemia				
CCRF-CEM	25.21	24.78	80.04	94.20
HL-60	0.13	32.44	94.81	125.10
K-562	9.99	48.62	79.09	106
MOLT-4	43.54	8.96	92.01	95
RPMI-8226	27.79	24.54	92.49	91.2
SR	21.24	71.32	79.95	83.40
Non Small Cell Lung Cancer				
HOP-92	5.32	-26.77	118.43	27.7
NCI-H23	18.15	0.68	17.49	-190.40
Colon Cancer				
HCT-116	34.22	21.84	81.24	136.7
CNS Cancer				
SNB-75	3.14	27.65	45.65	84.3
Melanoma				
M14	12.82	8.06	78.78	54.60
MDA-MB-435	7.17	36.21	25.96	143.70
Ovarian Cancer				
IGROV1	-13.38	32.51	36.36	96
OVCAR-3	20.89	3.11	42.42	141.4
OVCAR-4	18.35	1.86	40	50.8
OVCAR-5	-1.49	-9.07	10.09	145.6
OVCAR-8	17.85	8.38	31.69	50.82
NCI/ADR-RES	3.95	3.27	35.51	162.6
SK-OV-3	10.55	7.45	21.68	107.9
Renal Cancer				
786-O	11.50	7.7	60.07	87.60
A-498	-04.37	9.51	73.93	63.70
CAKI-1	32.98	4.67	41.31	73.50
UO-31	17.90	25.34	63.18	71.40
Prostate Cancer				
PC-3	54.39	8.64	100.59	81.7
Breast Cancer				
MCF	35.20	41.23	32.58	97.6
MDA-MB-231/ATCC	15.39	-4.54	73.58	127.1
BT-549	-3.02	8.04	00	124.2
Mean	88.84	90.55	57.10	-
Delta	43.23	61.87	75.53	-
Range	67.77	98.09	133.97	-

Range = highest growth percent- lowest growth percent. Delta = mean growth percent - lowest growth percent. % GI: % growth inhibition = mean growth percent- % growth.

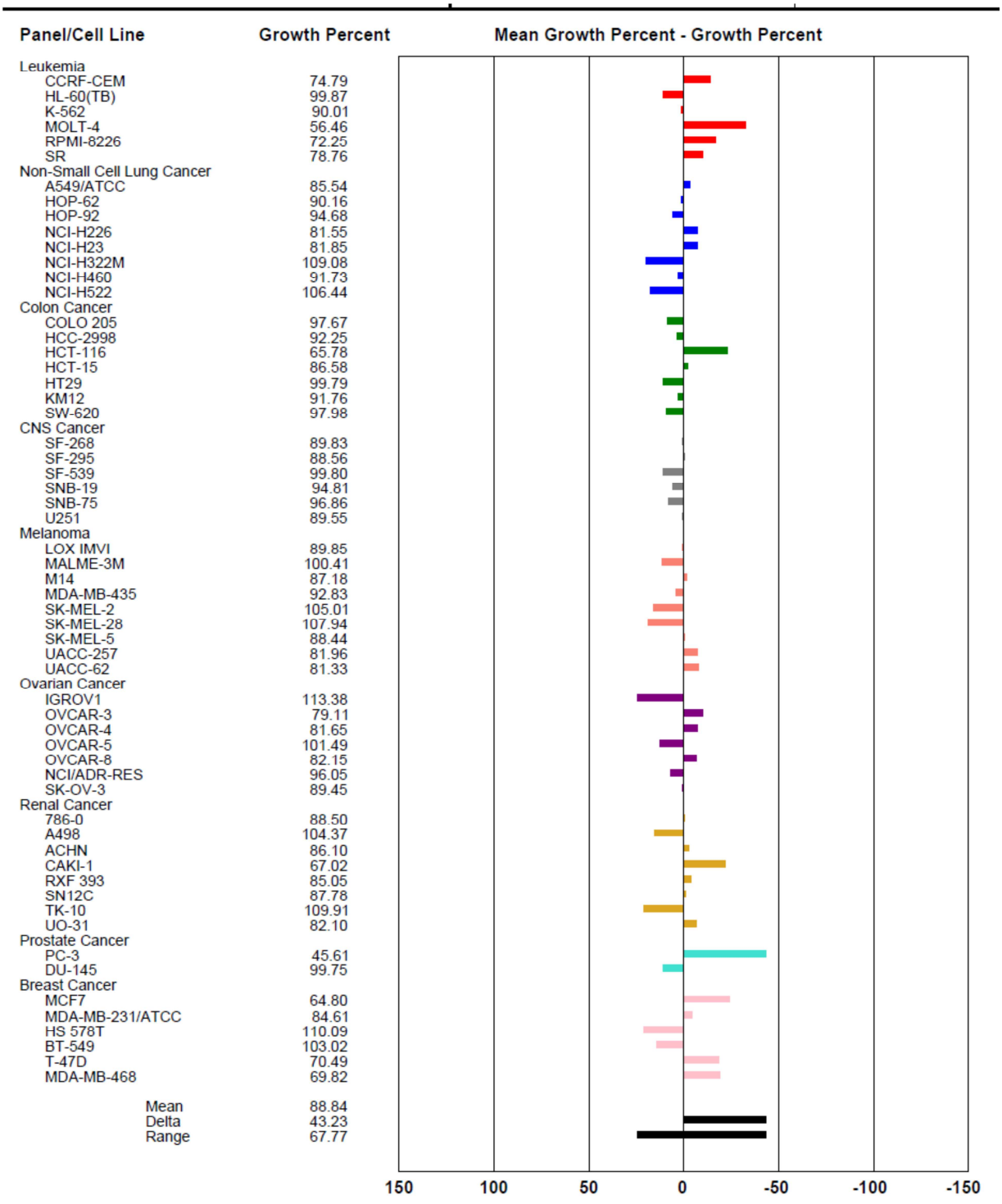


Fig 1:- Cytotoxicity activity of compound 31d against 60 human cancer cell lines

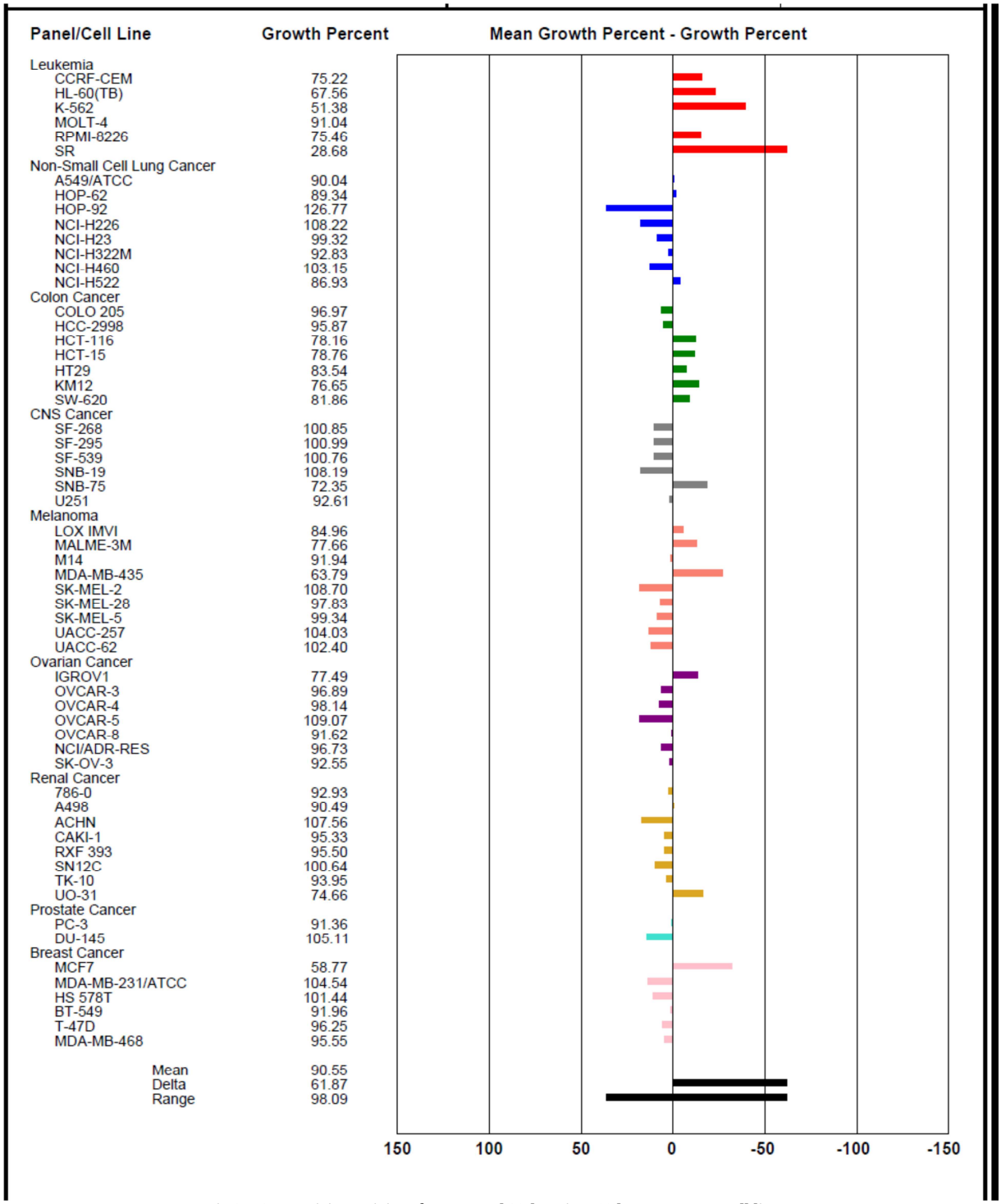


Fig 2:- Cytotoxicity activity of compound 32d against 60 human cancer cell lines

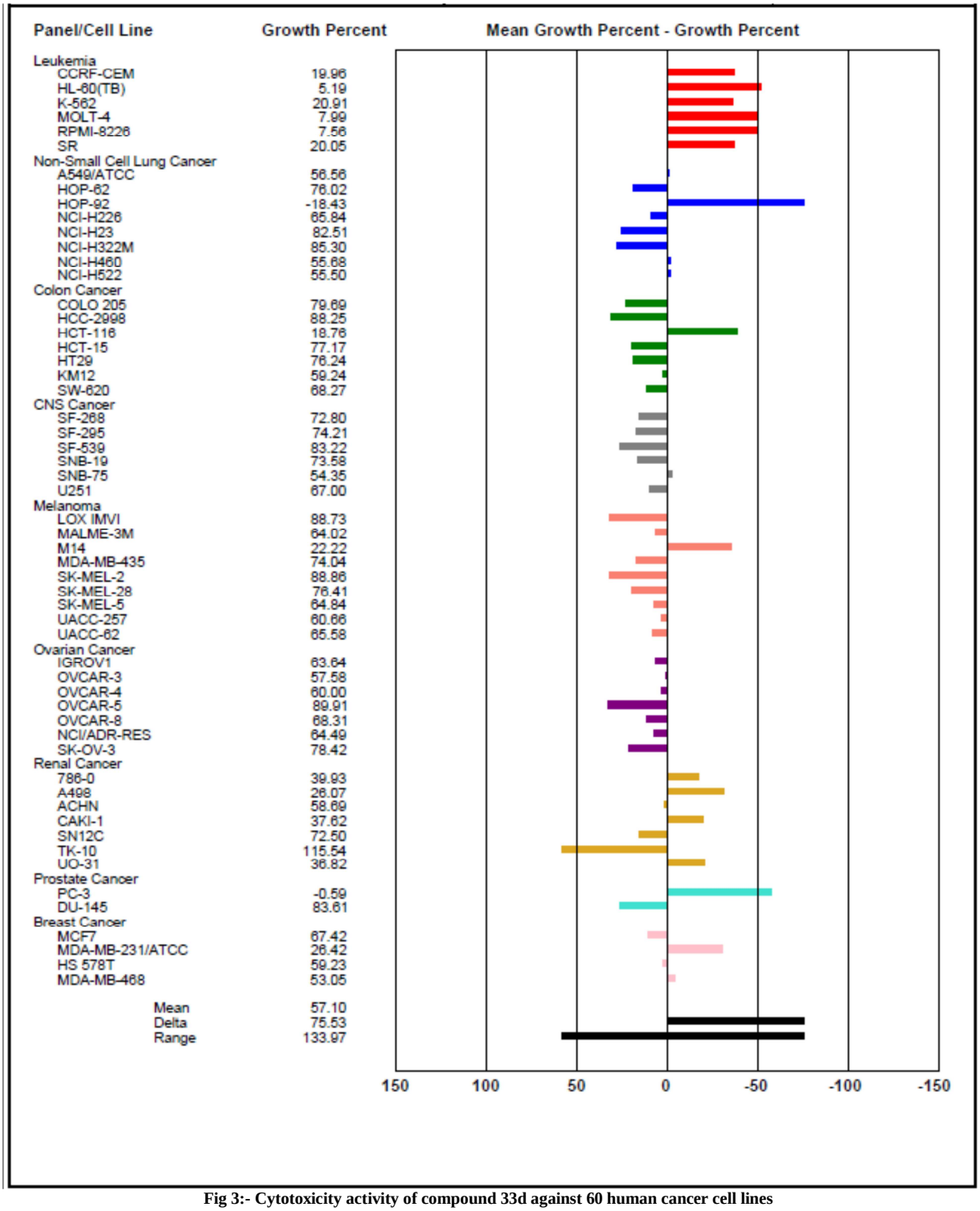


Fig 3:- Cytotoxicity activity of compound 33d against 60 human cancer cell lines

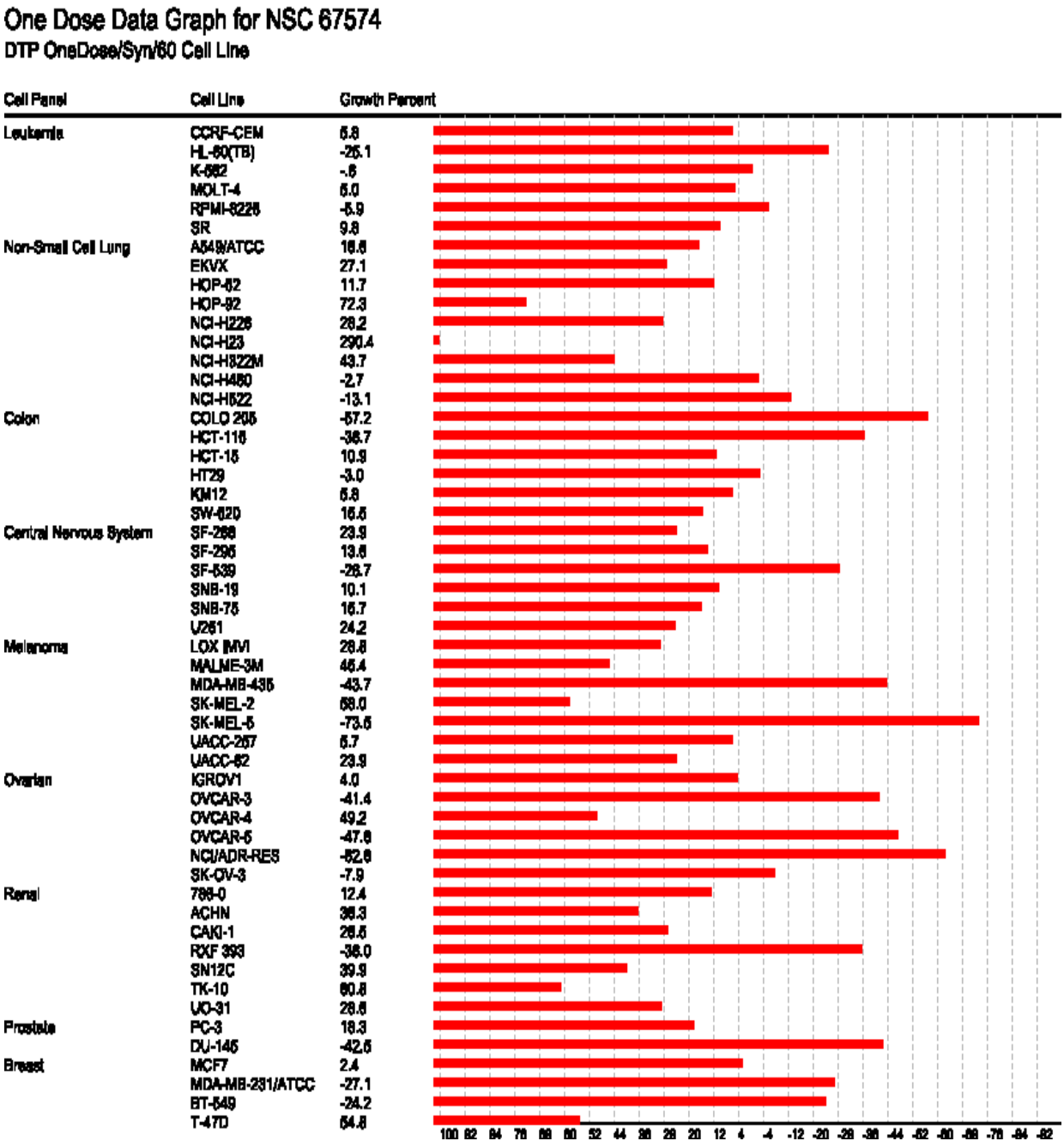


Fig 4:- Cytotoxicity activity of standard compound Vincristine sulphate against 60 human cancer cell lines

CONCLUSION

Novel 1, 2, 3-oxadiazolium-5-olate derivatives were synthesized and characterized by thin layer chromatography, ^{1}H NMR, ^{13}C NMR, mass and IR techniques. Newly synthesized compounds were evaluated for preliminary brine shrimp lethality bioassay and *in vitro* anticancer activity against 60 human cancer cell lines (Comp.**31d, 32d, 33d)**. Comp **33d** having cytotoxicity activity close to Vincristine against cell lines Leukemia, colon cancer (HCT-116), ovarian cancer (OVCAR-4) and highly cytotoxic than Vincristine against non small cell lung cancer (HOP-92), melanoma (M14) and prostrate cancer (PC-3) whereas comp **31d** and **32d** found to possess moderate anticancer activity. *In vivo* anticancer evaluation studies can also be carried out for newly synthesized sydnone derivatives in

future. Structural modification may lead to the synthesis of more sydnone derivatives and can be evaluated for their anticancer activities *in vitro* as well as *in vivo*.

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The financial support of the Council of scientific and Industrial Research, Delhi and Center Drug Research Institute, Lucknow, INDIA under OSDD (Project-OSDD/SMBT0003), is greatly appreciated, NCI, USA, SAIF Punjab University, Chandigarh (INDIA) for providing facilities for spectral study, Dr. A. S. Dhake, Ms. Nirmala S. Bhosale and SMBT College of Pharmacy, Dhamangaon (MS) for providing research facility.

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