Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(5):1333-1343

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and *in vitro* anticancer evaluation of 2-methylphenyl sydnone derivatives against Human breast cancer cell line MDA-MB-231 and Human prostate cancer cell line PC3

Sachin K. Bhosale[a*], Shreenivas R. Deshpande[b] and Rajendra D. Wagh[c]

[a]*Department of Pharmaceutical Chemistry, S. M. B. T. College of Pharmacy, Nandi hills, Dhamangaon, Tah: Igatpuri, Dist: Nashik. Maharashtra (India)*
**Research and Development Cell, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, A. P. (India)*
[b]*Department of Medicinal and Pharmaceutical Chemistry, HSK College of Pharmacy, BVVS Campus, Bagalkote, Karnataka (India)*
[c]*Department of Pharmaceutical Chemistry, A. R. A. College of Pharmacy, Nagaon, Dhule, Maharashtra (India)*

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-(2-methyphenyl)-1,2,3-oxadiazolium-5-olates has been synthesized and evaluated against human prostate cancer cell line PC3 and human breast cancer cell line MDA-MB-231. Compound **2C** was found to have moderate cytotoxic activity (GI50=56.9µg/ml). 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

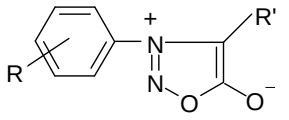
---

INTRODUCTION

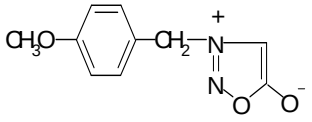
Substituted sydnone **1** are reported to explore highly potential activity against cancer cell lines [1-8]. Greco *et al* has screened a series of sydnone for anticancer activity, and it was found that, 3-(p-methoxybenzyl) sydnone **2** was effective for carcinoma-755 in mice. The same compound was 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) [4, 5]. 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.

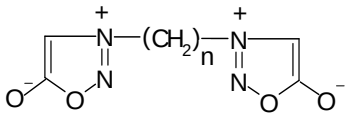
Potent anticancer 1, 2, 3-oxadiazol-5-olate molecules



1

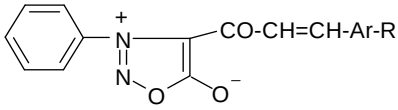


2

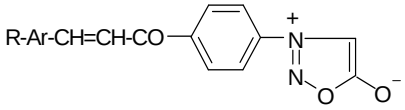


3

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

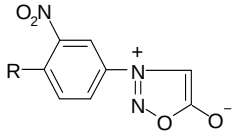


4

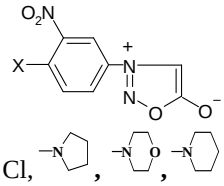


5

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



6



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

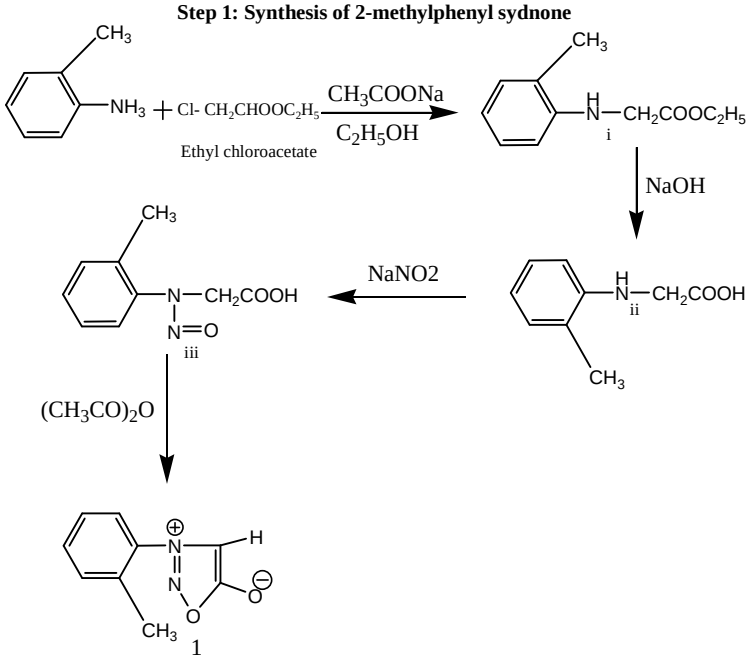
EXPERIMENTAL SECTION

Chemistry: All reagents were purchased from Sigma-Aldrich, Mumbai (India) Melting points of the intermediates, and the final products were recorded using a Systolic melting point apparatus and are uncorrected. 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 ^1H 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.

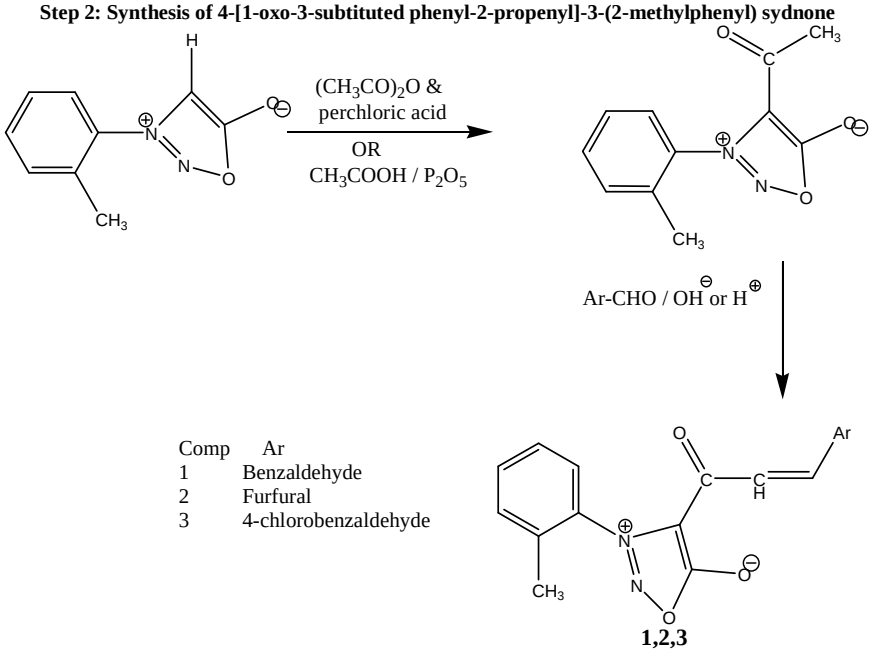
SYNTHESIS

Scheme1: Synthesis of 1A, 2A, 3A, 1B, 2B, 3C, 1D, 2D, 3D

Step 1: Synthesis of 2-methylphenyl sydnone

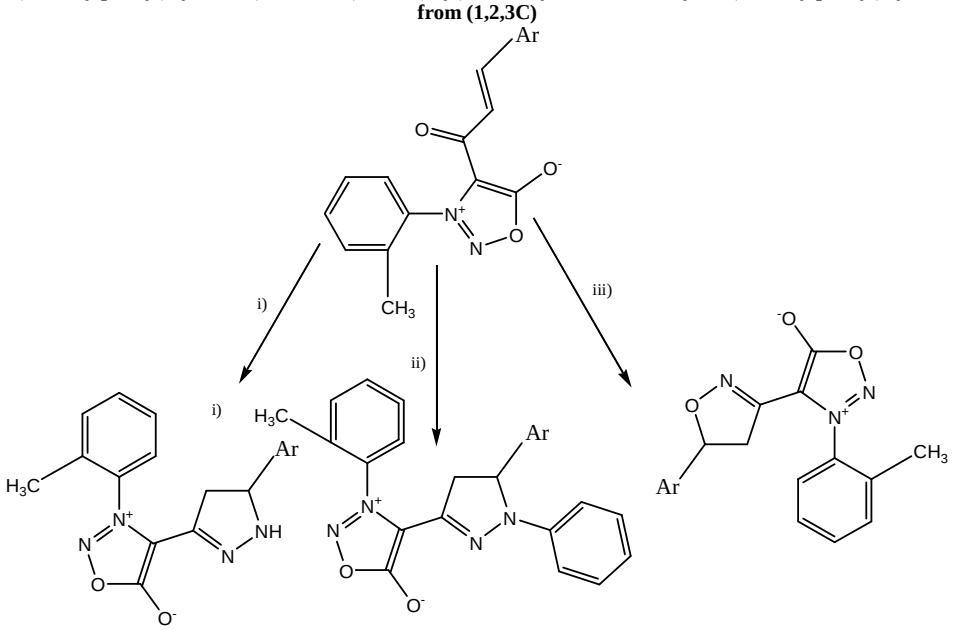


Step 2: Synthesis of 4-[1-oxo-3-subtituted phenyl-2-propenyl]-3-(2-methylphenyl) sydnone



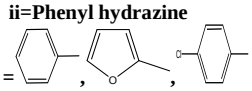
Comp	Ar
1	Benzaldehyde
2	Furfural
3	4-chlorobenzaldehyde

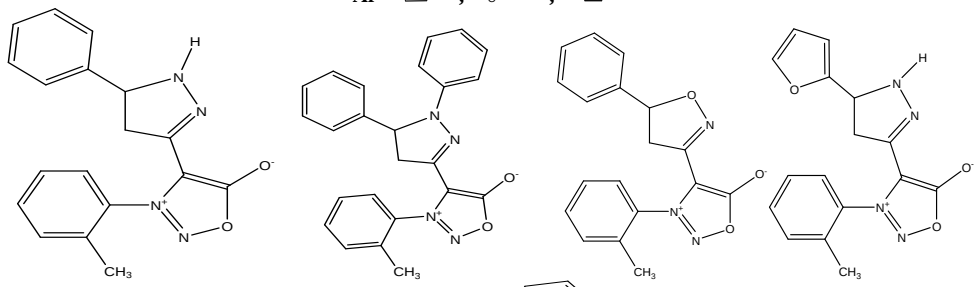
Step 3 synthesis of 4-[5-(aryl)-4, 5-dihydro-1h-pyrazol-3-yl]-3-(2-methylphenyl) sydnone (1A,2A,3A), 4-[5-(aryl)-4, 5-dihydro-1-phenyl-pyrazol-3-yl]-3-(2-methylphenyl) sydnone (1B, 2B, 2C), 4-[5-(aryl)-4, 5-dihydro-isoxazol-3-yl]-3-(2-methylphenyl) sydnone (1D, 2D, 3D) from (1,2,3C)



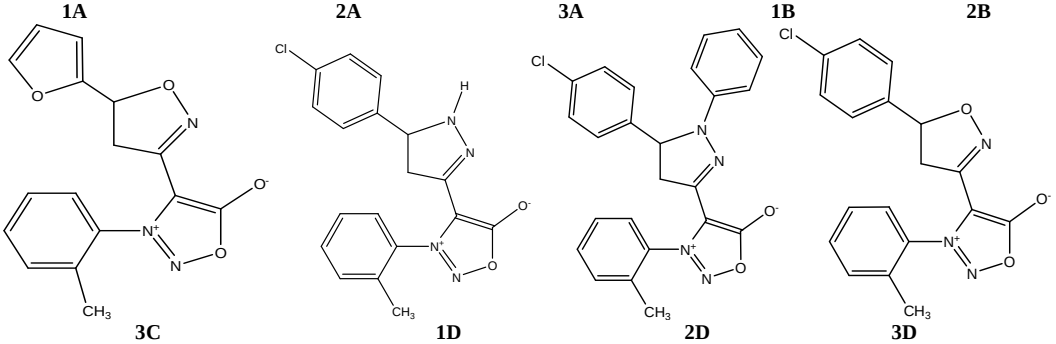
(1A, 2A, 3A) **(1B, 2B, 2C)** **(1D, 2D, 3D)**

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

Ar = 



1A **2A** **3A** **1B** **2B**



3C **1D** **2D** **3D**

Synthesis of 3-(2-methylphenyl) sydnone (1)

Synthesis of N-nitroso (2-methylphenyl) glycine: Ethyl chloroacetate (73 g, 49 ml, 0.6 mol), *o*-toluidine (53 g, 0.5 mol) and anhydrous sodium acetate (50 g, 0.6 mol) in 120 ml of alcohol were refluxed for 6 h. The reaction mixture was left overnight at room temperature and poured into ice-cold water; a precipitate of N- (2-methylphenyl) glycine ethyl ester **1** was obtained (84 g, 0.43 mol., 87%, m.p. 48-49 ^{o}C). To **1** (84 g, 0.43 mol) was added sodium hydroxide (20 g, 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-methylphenyl) glycine **2** was filtered and washed in cold water (14.5 g, 0.09 mol 21%, m.p. 115-117^{o}C). A solution of sodium nitrite (6.3 g, 0.09 mol) in water (20 ml) was added to **2** (14.5 g, 0.09 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 methanol (12.5 g, 0.06 mol., 67%, m.p. 96-98^{o}C).

Synthesis of 3-(2-methylphenyl) sydnone: 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 (9 g, 0.05 mol, 83%) and recrystallized using alcohol. 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-(substitutedaryl)-2-propenyl]-3-(2-methylphenyl) sydnones (1, 2, 3c)

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

Typical procedure: A mixture of 4-acetyl-3-(2-methylphenyl) sydnone (2.6 g, 0.01 mol), sodium hydroxide aqueous solution and ethanol (95%, 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 **1** (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-(2-methy)-phenyl) sydnone (1A, 2A, 3A)

Synthesis of 4-[5-(phenyl)-4, 5-dihydro-1H-pyrazol-3-yl]-3-(2-methylphenyl) sydnone (1A): To an ice cooled solution of hydrazine hydrate (100 mg, 2.00 mmol) in ethanol (3 mL) was added **1** (0.50 mmol). The mixed solution was heated at 60 °C for 5–6 h until the reaction was complete and then cooled. The precipitate solid was collected by filtration and washed with ice-cold water, cold ethanol to afford 129 mg (80%) of **1A** 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* = 320. Element Calcd for $C_{18}H_{16}N_4O_2$: C, 67.45; H, 5.01; N, 17.46. Found: C, 67.47; H, 5.02; N, 17.49. In similar way compounds **2A and 3A** were synthesized from respective **2** and **3**

4-[5-(furyl)-4, 5-Dihydro-1H-Pyrazol-3-Yl]-3-(2-Methylphenyl) Sydnone (2A): Yield: 51%; mp 135–137 °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*= 310. Element calculated for ($C_{16}H_{14}N_4O_3$) C, 58.88; H, 4.32; N, 17.17. Found: C, 58.69; H, 4.35; N, 17.08.

4-[5-(4-chlorophenyl)-4,5-Dihydro-1H-Pyrazol-3-Yl]-3-(2-Methylphenyl)Sydnone(3A): Yield: 65%; mp 148–151 °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). 13C NMR (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*= 356. Element Calcd for $C_{18}H_{15}ClN_4O_2$: C, 60.94; H, 4.26; N, 15.79; O, 9.02. Found: C, 60.97; H, 4.31; N, 15.72.

4-[5-(phenyl)-4,5-dihydro-1-phenyl-pyrazol-3-yl]-3-(2-methylphenyl)sydnone(1B): $C_{24}H_{20}N_4O_2$

Typical Procedure: To an ice cooled solution of phenyl hydrate (2.00 mmol) in glacial acetic acid (3 mL) was added to **1** (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 135–137°C. Exact Mass: 396.16, Mol. Wt.: 396.441, C, 72.58 H, 5.02, N, 14.42, O, 8.09. Yellow orange colour crystals (98mg, 52%) IR (KBr):1757 (C=O) cm^{-1}. ^1H 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}C NMR ($CDCl_3$, 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: 396.173.

4-[5-(furyl)-4,5-dihydro-1-phenyl-pyrazol-3-yl]-3-(2-methylphenyl) sydnone(2B): Yield 73%. $C_{22}H_{18}N_4O_3$ Exact Mass: 386.138, Mol. Wt: 386.403, m/e: 386.138 (100.0%), C, 68.38; H, 4.70; N, 14.50; O, 12.42. IR (KBr):1756 (C=O) cm^{-1}. ^1H 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-(2-methylphenyl)sydnone(2C): $C_{24}H_{19}ClN_4O_2$, Exact Mass: 430.120, Mol. Wt.: 430.886, m/e: 430.120 (100.0%), C, 66.90; H, 4.44; Cl, 8.23; N, 13.00; O, 7.43. IR (KBr):1753 (C=O) cm^{-1}. ^1H 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-(2-methylphenyl) sydnone (1D): $C_{18}H_{15}N_3O_3$

Typical Procedure: To an ice cooled solution of hydroxylamine hydrochloride (0.1 mole) in pyridine (3 mL) was added to **1** (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 **1D** as yellow orange crystals; mp 106–108°C. Exact Mass: 321.111, Mol. Wt.: 321.330, m/e: 321.10. Elements C, 67.28; H, 4.71; N, 13.08; $C_{18}H_{15}N_3O_3$ mol wt. 321.14, mp m/z: 321.121, C, 67.29 H, 4.83, N, 13.47, O, 14.95. IR (KBr):1754 (C=O) cm^{-1}. ^1H 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-(2-methylphenyl)sydnone(2D): $C_{16}H_{13}N_3O_4$ Exact Mass: 311.091, Mol. Wt.:311.292, m/e: 311.10. Elements C, 61.73; H, 4.21; N, 13.50, IR (KBr):1759 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$, 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-(2-methylphenyl)sydnone(3D): $C_{18}H_{14}ClN_3O_3$, Exact Mass: 355.072, Mol. Wt. 355.775, m/e: 355.11, Elements C, 60.77; H, 3.97; Cl, 9.97; N, 11.81; O, 13.49. IR (KBr):1753 (C=O) cm^{-1}. ^1H 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.

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.

Table 1: Brine Shrimp lethality assay

Compounds	LC_{50}(µg/ml)
1A	15.31
2A	13.44
3A	16.82
1B	17.67
2B	14.41
2C	07.42
3C	10.64
1D	13.56
2D	14.21
3D	15.33
Vincristine sulphate	0.39

Values are mean to three tubes

***In vitro* anticancer evaluation**

Sulforhodamine B (SRB) assay: The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well micro titer plates in 90 µL. After cell inoculation, the micro titer plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental compounds. After 24 h, one plate of each cell line was fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental compound were solubilized in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of compound addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test article at a concentration of 10^{-3}. Additional three, 10-fold serial dilutions were made to provide a total of four drug concentrations plus control. Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations. After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells [20]. 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 compound 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)

The experiment data were estimated using linear regression method of plots of the cell viability against the molar drug concentration of tested compounds.

Observations: Refer Table **1**, Fig.**1-4**.

Graphical representation of anticancer effect of compounds: Fig. 1 and 2 shows cytotoxic effect of test compounds and Adriamycin (Doxorubicin)on human breast cancer cell line MDA-MB-231(1A, 1B, 1D, 3C, 3D) and human prostate cancer cell line PC3 (2A,2B,2C,2D,3D).

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 human breast cancer cell line MDA-MB-231(1A, 1B, 1D, 3C, 3D) and human prostate cancer cell line PC3 (2A,2B,2C,2D,3D). 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 **2C** showed moderate cytotoxic activity having an LC_{50} of 07.42µg/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]. 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.

	Human Breast Cancer Cell Line MDA-MB-231															
	% Control Growth															
	Drug concentrations (µg/ml) calculated from graph															
	MDA-MB-231				Drug Concentrations (µg/ml) TGI				GI50							
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
1A	93.6	90.4	82.3	78.3	91.0	90.9	90.0	88.3	89.5	87.3	82.6	71.0	92.4	89.5	85.2	79.2
1B	100.0	90.9	86.7	86.1	100.0	96.3	94.0	93.0	100.0	100.0	100.0	98.5	100.0	95.7	93.7	92.6
1D	96.5	93.6	89.4	78.7	100.0	97.2	93.0	87.0	100.0	100.0	100.0	100.0	98.8	96.9	94.2	88.6
3C	92.8	90.4	80.8	64.9	97.8	89.4	84.7	81.2	100.0	100.0	100.0	88.2	96.9	93.3	87.5	78.1
3D	86.1	81.6	79.7	59.7	84.1	83.7	75.5	64.5	100.0	100.0	99.4	74.6	90.1	88.4	84.8	66.3
ADR	-46.5	-55.4	-64.6	-64.6	-46.6	-54.6	-60.3	-61.9	-58.1	-62.3	-70.0	-70.1	-50.4	-57.5	-64.9	-65.5

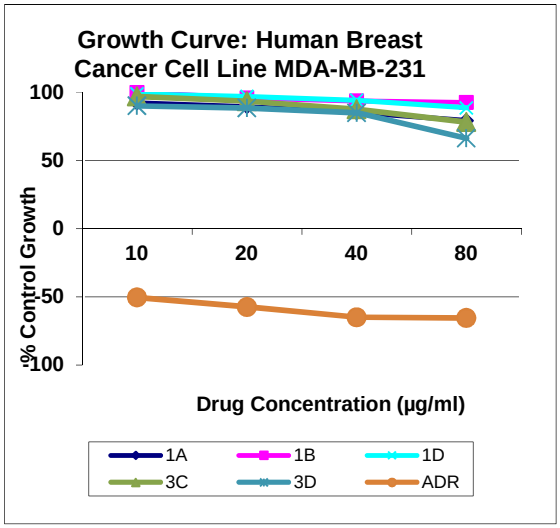
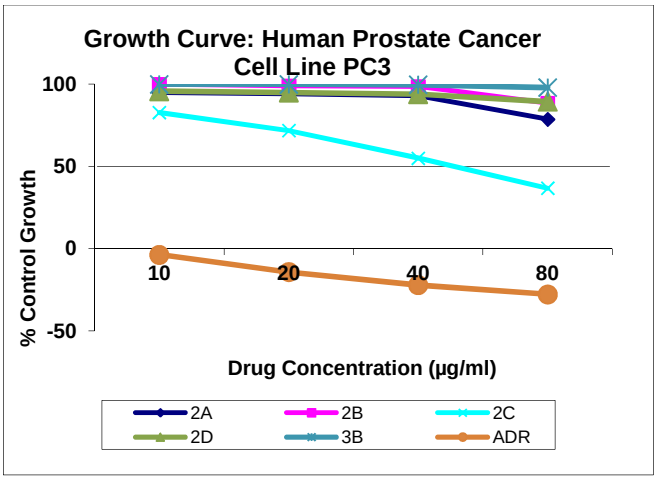


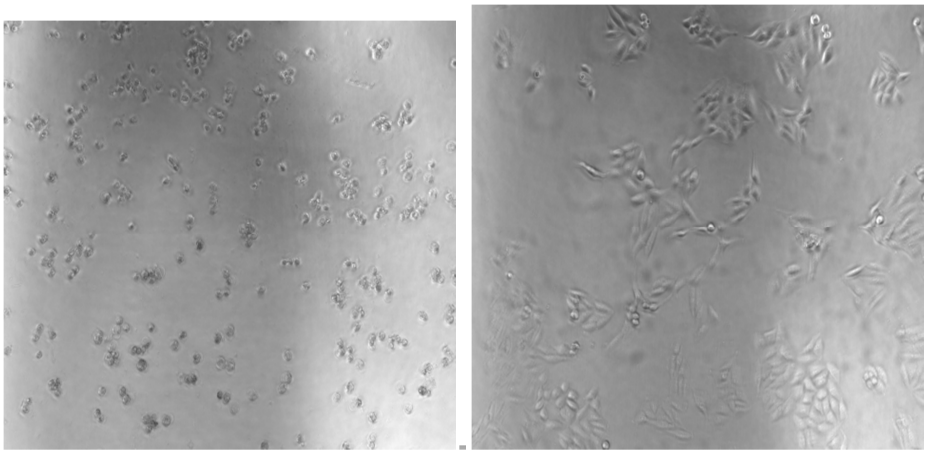
Fig.1. Cytotoxicity activity evaluation of compound 1A, 1B, 1D, 3C, 3D against human breast cancer cell line MDA-MB-231



	Drug concentrations (µg/ml) calculated from graph		
PC3	**LC50**	**TGI**	**GI50**
2A	>80	>80	>80
2B	>80	>80	>80
2C	>80	>80	56.9
2D	>80	>80	>80
3B	>80	>80	>80
ADR	>80	36.9	<10

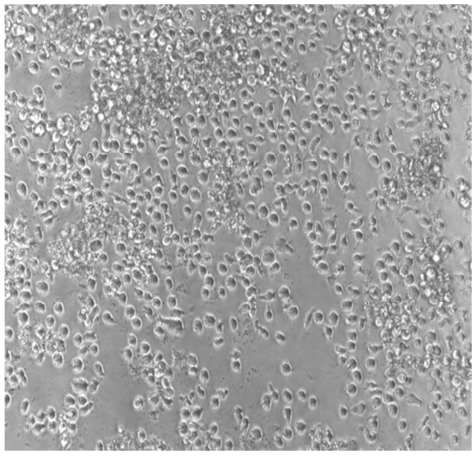
	Human Prostate Cancer Cell Line PC3															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				**Experiment 2**				**Experiment 3**				**Average Values**			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
2A	92.3	91.1	89.7	81.4	92.3	91.7	89.4	83.2	100.0	100.0	100.0	71.9	94.9	94.2	93.0	78.8
2B	100.0	100.0	100.0	97.6	100.0	97.0	96.0	94.7	100.0	100.0	100.0	72.9	100.0	99.0	98.7	88.4
2C	77.2	64.0	48.8	36.5	74.7	62.9	47.4	34.9	96.7	88.7	69.2	39.1	82.9	71.9	55.1	36.9
2D	94.2	93.3	91.2	86.4	93.2	91.7	90.8	81.9	100.0	100.0	100.0	100.0	95.8	95.0	94.0	89.4
3B	100.0	100.0	99.0	97.6	100.0	100.0	100.0	96.5	100.0	100.0	100.0	100.0	100.0	100.0	99.7	98.0
ADR	-3.7	-17.8	-33.6	-37.2	-9.7	-16.5	-19.8	-28.7	2.5	-8.1	-12.9	-17.0	-3.7	-14.1	-22.1	-27.6

Fig.2. Cytotoxicity activity evaluation of compound 2A, 2B, 2C, 2D, 3B against human prostate cancer cell line PC3



(a) **(b)**

Fig. 3: Cytotoxic activity of Adriamycin against a) Human Breast Cancer Cell Line MDA-MB-231 b) Human Prostate Cancer Cell Line PC3



(c)

Fig. 4: Cytotoxic activity of 2C against Human Prostate Cancer Cell Line PC3

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 human breast cancer cell line MDA-MB-231(1A, 1B, 1D, 3C, 3D) and human prostate cancer cell line PC3 (2A,2B,2C,2D,3D). The derivative **2C** was found to possess moderate anticancer activity (56.9µg/ml). *In vivo* anticancer evaluation studies can also be carried out for newly synthesized sydnone derivatives in future. Structural modification may lead to more potent anticancer molecules.

Acknowledgment

Authors are thankful to BCUD, University of Pune, INDIA (Project-13PHM000018) for financial assistance, Tata Memorial Centre, ACTREC, Kharghar, Navi Mumbai for carrying out *in vitro* anticancer activity, SAIF Punjab University for spectral study, JNTU Hyderabad, Dr. Dhake A. S., Ms. Nirmala S. Bhosale and SMBT College of Pharmacy, Dhamangaon, Nasik (MS) India, for providing necessary facilities to carry out the research work.

Definitions and Notes:

GI50	Growth inhibition of 50 % (GI50) calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, drug concentration resulting in a 50% reduction in the net protein increase
TGI	Drug concentration resulting in total growth inhibition (TGI) will calculated from Ti = Tz
LC50	Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50.
ADR	Adriamycin (Doxorubicin). Known drug.
GI50 value of ≤ 10^-6 (i.e. 1 µmole) or ≤ 10µg/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value ≤ 20µg/ml is considered to demonstrate activity.	
Yellow highlighted test values under GI50 column indicate activity.	

REFERENCES

[1] CV Greco; WH Nyberg; CC Cheng, *J. Med. Pharm. Chem.*, **1962,** 91, 861.
[2] WH Nyberg; CC Cheng, *J. Med. Chem.*, **1965,** 8, 531.
[3] V Grynberg; R Gome; T Shinzato; A Echevarria; J Miller, *Anticancer Res.*, **1992**, 12, 1025.
[4] JR Anto; G Kuttan; R Kuttan; K Satyanarayana; MNA Rao, *J. Clin. Biochem Nutr.*, **1994**, 17, 73.
[5] K Satyanarayana; SR Deshpande; B Subbarao; MNA Rao, *Ind. J. Pharm. Sci.*, **2004**, 66 (5), 679-683.
[6] CS Dunkley; CJ Thoman, *Bioorg. Med. Chem. Lett*., **2003**, 13, 2899.
[7] K Butkovic; Z Marinic; M Sindler-Kulyka, *ARKIVOC*, **2011**, 1:15.
[8] A Senff-Ribeiro A Echevarria EF Silva CRC Franco, SS Veiga, MBM Oliveira, *Br. J. Cancer*, **2004**, 91(2), 297–304.
[9] SR Deshpande; KV Pai; RS Pai, *Arzneimittelforschung*, **2011**, 61(3), 180-185.
[10] K Satyanarayana; M NA Rao, *Eur. J. Med. Chem.*, **1995,** 30, 641-645.
[11] SR Deshpande; KV Pai; RS Pai, *J. Enz. Inhibition Med. Chem.*, **2011**, 27(2), 241-248.
[12] SK Bhosale; SR Deshpande; RD Wagh, *J. Chem. Pharm. Res.*, **2012**, 4(2), 1185-1199.
[13] SK Bhosale; SR Deshpande; RD Wagh; AS Dhake, *Der. Chemica Sinica*, **2015**, 6(4), 79-95.
[14] K Irfan;O Aykut; AC Kubra; T Yusuf, *Bioorg. Med. Chem.*, **2013**, 21(13), 3859–3865.
[15] B Alessandro; A Maria; M Chiara; A Cinzia; M Mauro; G Rosaria; C Patrizio; M Mariangela; R Camillo; V Maurizio, *Eur. J. Med. Chem.*, **2011**, 46(11), 5293–5309.
[16] JP Yong; CZ Lu; X Wu, *Anticancer Agents Med. Chem.* , **2014**, 15(1), 131-136.
[17]A Kamal; JS Reddy; MJ Ramaiah; D Dastagiri; EV Bharathi; MA Azhar; F. Sultana; SN Pushpavalli; M Pal-Bhadra; A Juvekar; S Sen; S Zingde, *Eur. J. Med. Chem.*, **2010**, 45(9), 3924-3937.
[18]BN Meyer; RN Ferrign; JE Putnam; LB Jacobson; DE Nicholas; JL McLaughlin, *Planta Medica*, **1982**, 45, 31-34.
[19]GX Zhao; YH Hui; JK Rupprecht; JL McLaughlin; KV Wood, *J. Nat. Prod.*, **1992**, 55, 347-356.
[20]P Houghton; R Fang; I Techatanawat; G Steventon; PJ Hylands; CC Lee, *Methods*, **2007**, 42 (4):377-87.