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SYNTHESIS, CHARACTERIZATION AND ANTIMITOTIC ACTIVITY OF CYANOACETYL HYDRAZONE DERIVATIVES

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ABSTRACT

The present investigations describe about the synthesis, characterization and biological studies of novel cyanoacetyl hydrazone derivatives. However their derivatives have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities. The structures of all the synthesized compounds were elucidated by using spectral data. The synthesized compound was subjected to preliminary antimitotic studies by Allium cepa root MERISTAMATIC CELLS. The result from the study showed that the cyanoacetyl hydrazones had excellent anti-mitotic activity that was comparable to the activity of methotrexate. Mito inhibitory effect of the compounds were observed in higher concentrations. Our findings support the reported therapeutic use of these compounds as a antimitotic or anticancer agent in the Indian system of medicine.

KEYWORDS: cyanoacetyl hydrazone, antimitotic activity, meristamatic cells, methotrexate, mitotic Index.

Abbreviation

S1- 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetylhydrazone.
S2- 3-methyl-2,6 di(bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazine.
S3- 3-methyl-2,6 di(bis-*p*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazine.

INTRODUCTION

Organic chemistry and medicinal chemistry are becoming very vital chemistry. The primary objective of an organic chemist is to work towards isolation, characterization and synthesis of new compounds that are suitable for use as drugs. Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. However their derivatives having N-C linkage have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities.[1] Hydrazones and their derivatives constitute an important class of compounds that has found wide utility in organic synthesis. The chemistry of carbon-nitrogen double bond of hydrazone is becoming the backbone of condensation reaction in benzo-fused N-heterocyclics also it constitutes an important class of compounds for new drug development.

Anti-mitotic activity of cyano acetyl hydrazones were dose dependent. The increases in the concentrations are directly proportional to the activity.

MATERIALS AND METHODS

Chemicals were procured from E. Merck (India), S. D. Fine Chemicals (India) and reagent/solvents were used without distillation procedure. Melting points were taken in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (ν in cm–1) and NMR spectra were recorded on a Bruker spectrometer DPX-300MHz (Bruker, Germany) by using $CDCl_3$ as solvent with TMS as an internal standard. All the spectral data are consistent with the assigned structures of the desired product and the progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent.

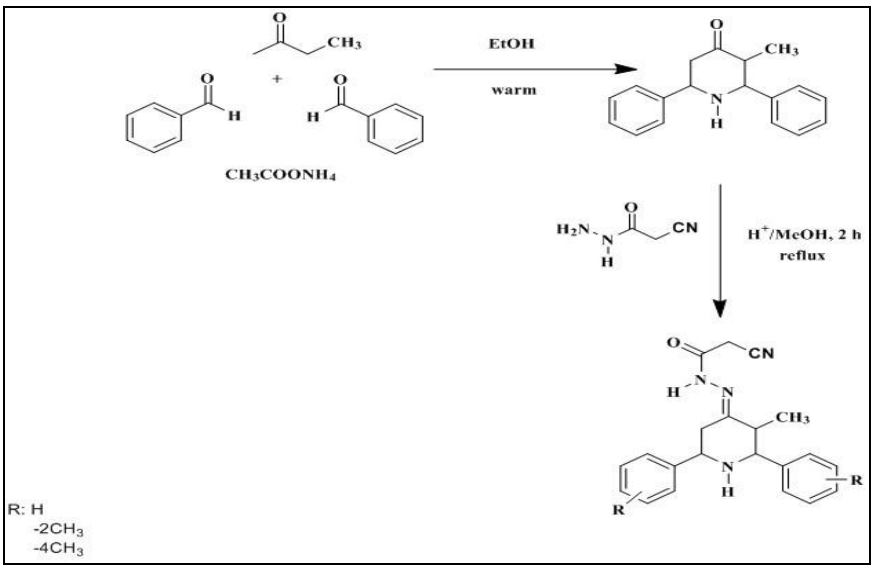
Preparation of S1, S2 and S3

3-methyl-2,6-diphenylpiperidin-4-one was prepared by adopting the literature method.[2] Condensation of 2-butanones, benzaldehyde and ammonium acetate in warm ethanol in the ratio of 1:2:1 respectively afforded the formation of 3-methyl-2,6-diphenylpiperidin-4-ones.

Preparation of 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone

A mixture of 3-methyl-2,6-diphenylpiperidin-4-one (0.1 mol), cyanoacetic hydrazide (0.1 mol) in the presence of few drops of concentrated acetic acid in methanol was

refluxed for 2 hours. After the completion of reaction, the reaction mixture was cooled to room temperature. The solid product was separated by filtration and washed with warm water and recrystallized by methanol to afford 3-methyl-2,6-diphenylpiperidin-4-one cyanoacetyl hydrazone.



Scheme 1: Determination of antimitotic activity.

Evaluation of antimitotic activity using Allium cepa roots

Antimitotic activity study was conducted as per the methods reported by previous workers with modifications (Grant, 1982; Fiskesjo, 1988; Shweta*et al*. 2014).[6-7]

Allium cepa bulbs

Approximately equal size bulbs (40±10 g) of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Thanjavur, Tamil Nadu, India. Any onions that were dry, moldy or have started shooting green leaves were discarded.

Growing Allium cepa meristems:

The outer scales were removed from the healthy onion bulbs leaving the root primordia intact. These bulbs were grown in dark for 48 h over 100 ml of tap water at ambient temperature until the roots have grown to approximately 3 cm. The water was changed daily during this period. The viable bulbs were then selected and used for subsequent studies.

Exposure to text samples

The bulbs with root tips grown up to 2-3 cm were removed from the water and placed on a layer of tissue paper to remove excess of water. Various concentrations of the S1,S2 and S3 were prepared i.e; 10 µg/mL, 20 µg/mL, 30 µg/mL. The bulbs were divided into four groups. The first group served as control (tap water). Second group is Allium cepa roots were dipped in the compound *S1*. Third group is Allium cepa roots were dipped in the compound *S2*. Fourth group is Allium cepa roots were dipped in the compound *S3*. Fifth group is Allium cepa roots were dipped in the Methotrexate (0.10 mg/mL) was used as a standard control. All the groups were incubated at 25±2°C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), root number and the mitotic index were recorded after 96 h. The % of root growth inhibition was calculated by.

$$\% \text{ of root growth inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

The effective concentration for 50% root length inhibition (EC_{50} value) was determined by plotting the treatment concentrations against mean root lengths as percentage of water control group.

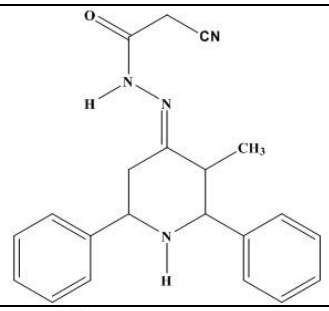
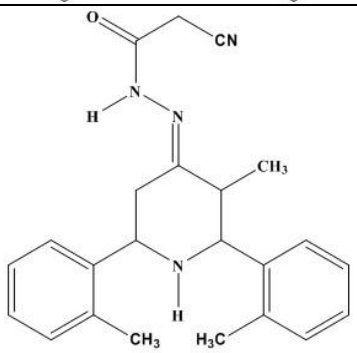
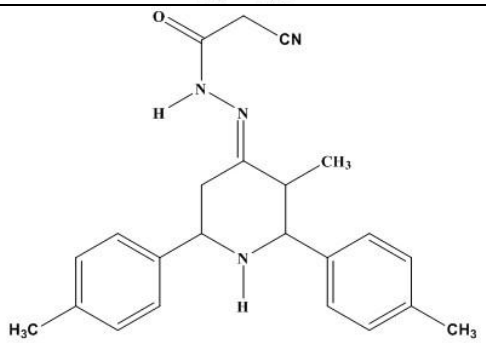
Microscopic studies and determination of mitotic index

After 96 h, the root tips were fixed with fixing solution of acetic acid and alcohol (1:3). Squash preparations were made by staining the treated roots with acetocarmine stain (Badria et al., 2001).[8] For each root tip, the numbers of mitotic cells and total meristematic cells were counted manually in 5-8 fields of view using high resolution (100x) bright field light microscope. The mitotic index was calculated by.

$$\text{Mitotic Index} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

RESULTS AND DISCUSSION

Table 1: The physical data of Synthesized Cyanoacetyl hydrazone derivatives.

Compound	Structure	Yield(%)	M.Formula	M.Weight	M.Point
S1		79.65	$C_{21}H_{20}N_4O$	344	137-140^oC
S2		82.6	$C_{23}H_{26}N_4O$	374	141-144 ^oC.
S3		78.69	$C_{23}H_{26}N_4O$	374	140-142^oC.

3-methyl-2,6-diphenylpiperidin-4-one cyanoacetylhydrazone (S1): Yield. 79.65%. mp.137-140^oC. **FT-IR (KBr) ν_{max} (cm^{-1}):** 3036-2834 (C-H Aliphatic & Aromatic stretching), 1702 (C=O), 1604 (C=N), 2263 (C≡N), 3212-3115 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm**:142.66(C-2 ipso carbon), 142.18 (C-6 ipso carbon), 126.56-128.79 (Aromatic carbons), 164.74 (C=O), 157.54 (C=N), 114.05 (C≡N), 24.59 (CH_2 carbon of cyanoacetohydrazone moiety), 69.20 (C-2), 60.80 (C-6), 45.36 (C-3), 36.04 (C-5), 12.06 (3-CH_3). **^1H NMR(300 MHz, $CDCl_3$), δ ppm:** 7.50-7.26(m, 10H, Aromatic Protons), 9.24 (b s, 1H, N-H Hydrazone Moiety), 2.08 (b s, 1H, N-H Piperidin moiety), 3.78 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.89 (d, J = 6.3Hz, 3H, 3-CH_3), 3.90 (dd, $J^3_{a,e}$ = 2.4Hz, $J^3_{a,a}$ = 11.4Hz, 1H, H-6a), 3.53 (d, $J^3_{a,a}$ = 9.9Hz, 1H, H-2a), 2.23 (dd, $J^3_{a,e}$ = 13.8Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 2.90 (dd, $J^3_{a,e}$ = 2.7 Hz, $J^2_{a,e}$ = 13.8Hz, 1H, H-5e), 2.58 (m, 1H, H-3a Proton).

3-methyl-2,6 di(bis-*o*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S2): Yield. 82.6%. mp. 141-144 ^oC. **FT-IR(KBr) ν_{max} (cm^{-1}):** 3025-2852 (C-H Aliphatic & Aromatic stretching), 1674 (C=O), 1568 (C=N), 2267 (C≡N), 3440-3184 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm:** 139.98 (C-2 ipso carbon), 140.49(C-6 ipso carbon), 126.49-129.77 (Aromatic carbons), 164.48 (C=O), 158.05 (C=N), 114.35 (C≡N), 24.16 (CH_2 carbon of cyanoacetohydrazone moiety), 76.57 (C-2), 56.12 (C-6), 44.89 (C-3), 34.56 (C-5), 11.15 (3-CH_3) 19.20 (*o*-CH_3). **^1H NMR(300 MHz, $CDCl_3$), δ ppm:** δ ppm 7.32-7.13 (m, 8H, Aromatic Protons),10.09(b s, 1H, N-H, Hydrazone Moiety), 2.09 (b s, 1H, N-H Piperidin moiety), 3.50 (q, 2H, CH_2 – Protons in hydrazone moiety), 0.92 (d, J = 6Hz, 3H, 3-CH_3), 3.89 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 10.2Hz, 1H, H-6a), 3.11(d, $J^3_{a,a}$ = 10.2Hz, 1H, H-2a), 2.39 (dd, $J^3_{a,e}$ = 11.4Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 3.07(dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ =

12Hz, 1H, H-5e), 2.57 (m, 1H, H-3a Proton), 2.33 (s, 3H, *o*-CH_3 protons).

3-methyl-2,6 di(bis-*p*-methyl phenyl) piperidin-4-one cyanoacetyl hydrazone (S3): Yield. 78.69%. mp. 140-142 ^oC. **FT-IR(KBr) ν_{max} (cm^{-1})**: 3026-2963(C-H Aliphatic & Aromatic stretching), 1701 (C=O), 1638 (C=N), 2266 (C≡N), 3195-3097 (N-H). **^{13}C NMR(300 MHz, $CDCl_3$) δ ppm**: 139.33 (C-2 ipso carbon), 139.83 (C-6 ipso carbon), 126.42-129.38 (Aromatic carbons), 164.87 (C=O), 158.07 (C=N), 114.13 (C≡N), 24.56 (CH_2 carbon of cyanoacetohydrazone moiety), 68.92 (C-2), 60.46 (C-6), 45.34 (C-5), 36.16 (C-3), 12.10 (3-CH_3), 21.13(*p*-CH_3). **^1H NMR(300 MHz, $CDCl_3$), δ ppm:** 7.14-7.36 (m, 8H, Aromatic Protons), 9.01(bs, 1H, N-H, Hydrazone Moiety), 2.06 (b s, 1H, N-H Piperidin moiety), 3.73 (q, 2H, CH_2 –Protons in hydrazone moiety), 0.89 (d, J = 6Hz, 3H, 3-CH_3), 3.87 (dd, $J^3_{a,e}$ = 3Hz, $J^3_{a,a}$ = 11.4Hz, 1H, H-6a), 3.49 (d, $J^3_{a,a}$=9Hz, 1H, H-2a), 2.24 (dd, $J^3_{a,e}$=12Hz, $J^3_{a,a}$ = 11.7 Hz, 1H, H-5a), 2.83 (dd, $J^3_{a,e}$ = 2.1 Hz, $J^2_{a,e}$ = 13.5Hz, 1H, H-5e), 2.57 ((m, 1H, H-3a Proton), 2.06 (s, 3H, p-CH_3 protons).

Antimitotic activity of compounds (S1, S2 and S3) using Allium cepa root meristamatic cells

The antimitotic activity was screened using Allium cepa root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity. The inhibitory effect of compounds(S1,S2 and S3) were evaluated on the growth and mitotic activity of Allium cepa root meristems and the effect was compared with standard anticancer drug methotrexate. A progressive increase in average mean root length (8.10mm), average mean root numbers (7) and mitotic index (87.50%) observed in control group after 96 hrs of experimental period.

The compound (S1) and methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control. The average mean root length at 10, 20,and 30µg/mL of compound (S1) was 6.70mm, 3.80mm and 2.20mm at 96 hr respectively while standard shows 2.60mm. The average mean root numbers at 10, 20 and 30µg/mL of compound (S1) was 5,4 and 3 at 96 hr respectively while standard shows 2 numbers. The mitotic index at 10, 20, and 30 µg/mL of compound (S1) was 81.55, 66.66 and 35.89% at 96 hr respectively while standard shows 36.76%.

The compound (S2) and methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control. The average mean root length at 10, 20,and 30µg/mL of compound (S2) was 6.20mm, 4.00mm and 2.40mm at 96 hr respectively while standard shows 2.60mm. The average mean root numbers at 10, 20, and 30µg/mL of compound (S2) was 6, 5 and 4 at 96 hr respectively while standard shows 2 numbers. The mitotic index at 10, 20, and 30 µg/mL of compound (S2) was 76.92, 67.70 and 51.72% at 96 hr respectively while standard shows 36.76%.

The compound (S3) and methotrexate produced root decay and decreased the root length and root number significantly at 96 h as compared to control. The average mean root length at 10, 20,and 30µg/mL of compound (S3) was 6.10mm, 5.10mm and 3.20mm at 96 hr respectively while standard shows 2.60mm. The average mean root numbers at 10, 20,and 30µg/mL of compound (S3) was 5,5 and 4 at 96 hr respectively while standard shows 2 numbers. The mitotic index at 10, 20, and 30 µg/mL of compound (S3) was 81.25, 69.09 and 57.69% at 96 hr respectively while standard shows 36.76%.

The water control shows normal growth with greater root length and numbers. Treatment with different concentrations (10,20, and 30 µg/mL) of compounds (S1,S2 and S3) show decreased the growth gradually in dose dependent manner. The highest dose as 30µg/mL of compound (S1) has significant activity in root length, number and mitotic index and near to the standard.

The antimitotic activity was screened using Allium cepa root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity. The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and extends a few mm after that. Cell of this region undergo repeated divisions. The fate of cell division is higher in this region compare to that of the other tissues. This region is called the meristamatic region. This division is similar to the above mentioned cancer division in humans. Hence, these meristamatic cells can be used for preliminary screening of drugs with anticancer activity.

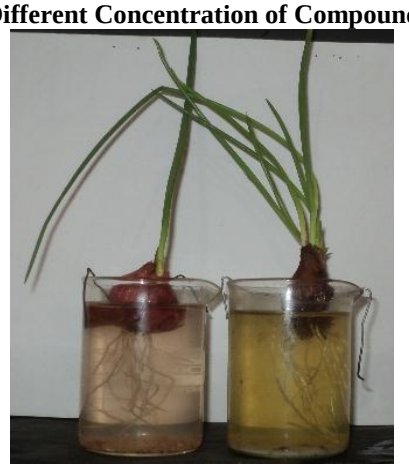
The antimitotic activity was screened using Allium cepa root meristamatic cells which have been used extensively in screening of drugs with anticancer compounds. Cytotoxicity at all concentrations test extract were evidenced by evaluating macroscopic parameters, i.e., reduction in root number and root length both of which were indicative of root growth inhibition. In the present study mitotic index of different concentrations of extract clearly indicates the efficiency in the inhibition of growth of cancer cells either by affecting microtubules or encouraging microtubule formation, and thus stopping the microtubules from being broken down. This makes the cells become so clogged with microtubules that they cannot continue to grow and divide. The rate of tumor growth is dependent upon a balance between the rates of proliferation and apoptosis.

This research has proceeded to determine the antimitotic effect of the S1, and S2 and S3. The antimitotic activity in the following order: S1> S2> S3.

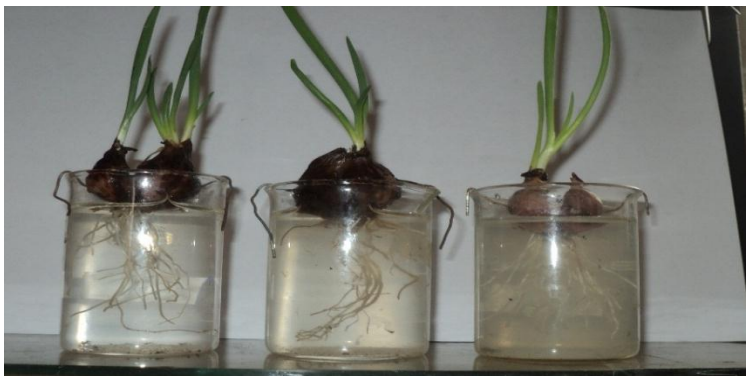
Table 2: Effect of Compounds (S1,S2 and S3) on Root length, Root number and Mitotic Index of Allium cepa roots.

Group	S1			S2			S3		
	Avg Root Growth (mm)	Avg Root Numbers	Mitotic Index (%)	Avg Root Growth (mm)	Avg Root Numbers	Mitotic Index (%)	Avg Root Growth (mm)	Avg Root Numbers	Mitotic Index (%)
Water (Control)	8.10	7	87.5	8.10	7	87.5	8.10	7	87.5
10 µg/ml	6.70	5	81.53	6.20	6	76.92	6.10	5	81.25
20 µg/ml	3.80	4	66.66	4.00	5	67.70	5.10	5	69.09
30 µg/ml	2.20	3	35.89	2.40	4	51.72	3.20	4	57.69
Std Methotrexate (0.1mg/ml	2.60	2	36.76	2.60	2	36.76	2.60	2	36.76

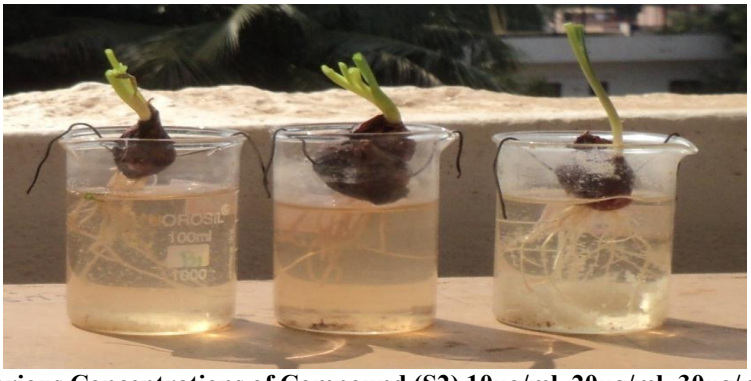
Treatment of Allium cepa roots with Different Concentration of Compounds(S1, S2, S3)



Control(Water) Std (Methotrexate) (0.1mg/ml).



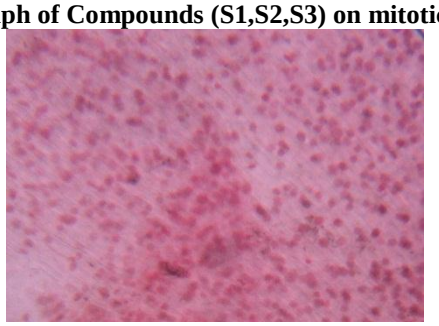
Various Concentrations of Compound (S1) 10µg/ml, 20µg/ml, 30µg/ml



Various Concentrations of Compound (S2) 10µg/ml, 20µg/ml, 30µg/ml.

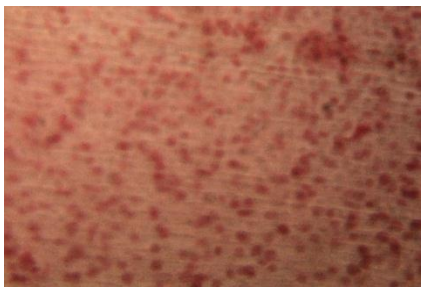
Various Concentrations of Compound (S3) 10µg/ml, 20µg/ml, 30µg/ml.

Photomicrograph of Compounds (S1,S2,S3) on mitotic Index of Allium cepa.

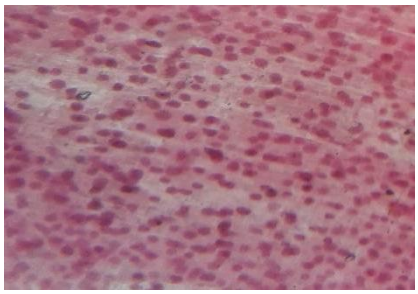


Water(Control)

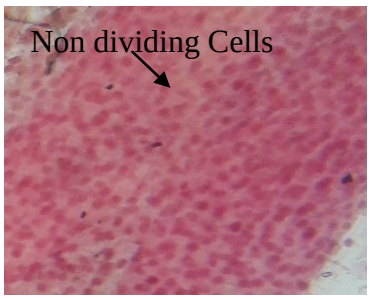
Std(Methotrexate) (0.1mg/ml



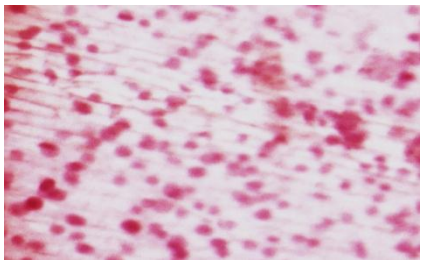
Compound (S1) 10µg/ml



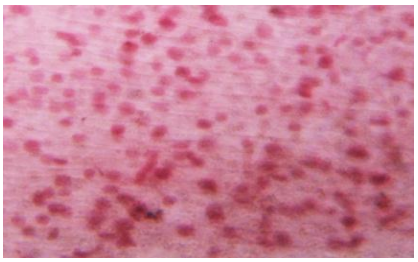
20µg/ml



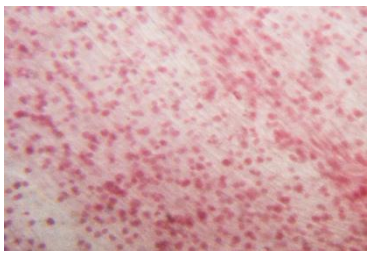
30µg/ml



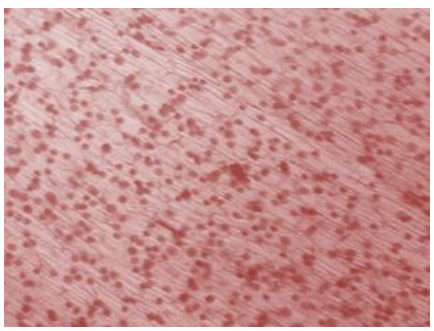
Compound (S2) 10µg/ml



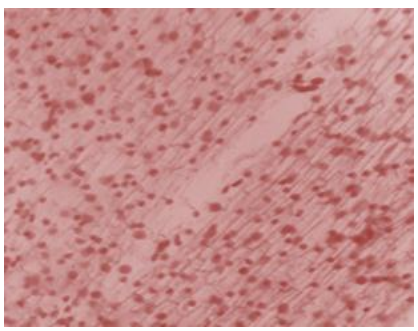
20µg/ml



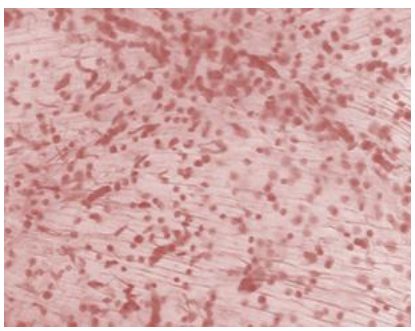
30µg/ml



Compound (S3) 10µg/lml



20/µg/ml



30µg/ml

CONCLUSION

Synthesized a series of new Cyanoacetyl hydrazone derivatives obtained with good yield. All the compounds were characterized by using IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. Among the three compounds, S1 has greated activity than S2 and S3. The antimitotic activity in the following order: S1> S2> S3. Maximum numbers of non dividing cells were observed. As a result of this cells arrest in mitosis and eventually die by apoptosis. Our findings support the reported therapeutic use of this compound as a antimitotic or anticancer agent in the Indian system of medicine.

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