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Research Article

# COMBINATION OF HETEROCYCLIC COMPOUNDS: AS ANTICANCER AGENTS

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**ABSTRACT**

Benzimidazole and Pyrazole both are heterocyclic aromatic organic compounds. These two heterocyclic compounds are occupied prominent place among various classes of aromatic organic compounds. Heterocyclic compounds having a variety of therapeutic uses, including antitumor, antifungal, antiparasitic, analgesics, antiviral, antihistamine, as well as use in cardiovascular disease, neurology, endocrinology, and ophthalmology. Both Benzimidazole and Pyrazole are also effective in anticancer. All the newly synthesized heterocyclic compounds were screened for the in-vivo cytotoxic activity by XTT assay methods using various cell lines like VERO, NCI, MDA-MB-453 and MDA-MB-468 using doxorubicin as standard drugs for comparison of results. Aims: To design and identify new heterocyclic compounds to potent anticancer activity. Methods: XTT assay method. Results: By using different cell lines for 10 compounds we find positive results for one or two compounds. From the discussion, we conclude that compound which contain o-nitrobenzene as attachment gives better anticancer activity in all cell lines, whereas compound contain p-flourobenzene as attachment gives better anticancer activity in cell line like NCI, MDA-MB-453 and MDA-MB-468. The compound contains m-nitrobenzene gives positive results in VERO cell line.

**KEY WORDS:** XTT, VERO, NCI, MDA-MB-453, MDA-MB-468

## INTRODUCTION

Benzimidazole and Pyrazole both are heterocyclic aromatic organic compounds. These two heterocyclic compounds are occupied prominent place among various classes of aromatic organic compounds. Heterocyclic compounds having a variety of therapeutic uses, including antitumor, antifungal, antiparasitic, analgesics, antiviral, antihistamine, as well as use in cardiovascular disease, neurology, endocrinology, and ophthalmology[1]. Benzimidazole and Pyrazole are also effective in anticancer. All the newly synthesized heterocyclic compounds were screened for the in-vivo cytotoxic activity by XTT assay methods using various cell lines like VERO, NCI, MDA-MB-453 and MDA-MB-468[2].

**AIMS AND OBJECTIVE:** To design and identify new heterocyclic compounds to potent anticancer activity.

## PHARMACOLOGICAL SCREENING METHODS

XTT assay Method[3]
Characterization of cell lines and culture media
Subculturing of cell lines (DMEM, FBS)
Drugs (Doxorubicin)
Initial cell viability, density and population doubling time
Preparation of plate for cytotoxic activity (Drug Addition)
After 4hrs, 12hrs, 24hrs cell viability calculation (XTT Assay)

### Cell lines and Culture Medium [4, 5]

VERO, NCI, MDA-MA-453 and MDA-MB-468 cell cultures used in the experiments were derived from National Centre for Cell Science, Pune. Stock cells of these cell lines were cultured in DMEM, supplemented with 10% FBS (fetal bovine serum). Along with media cells were also supplemented with penicillin, streptomycin and amphotericin-B, in a humidified atmosphere with 5% $CO_2$ at 37°C until confluence reached. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock cultures were grown initially in 25 $cm^2$ tissue culture flasks, then in 75 $cm^2$ and finally in 150 $cm^2$ tissue culture flasks and all cytotoxicity experiments were carried out in 96 well microtitre plates.

### Experiment Design

Cell lines in exponential growth phase were washed, trypsinized and re- suspended in complete culture media. Cells were plated in 96 well micro titer plate and incubated for 24 hours during which a partial monolayer form. The cells were then exposed to various concentrations of the test compounds and standard doxorubicin. Control wells were received only maintenance medium. The plates were incubated at 37°C in a humidified incubator with 5% $CO_2$ for a period of 24 hours. Morphological changes of drug treated cells were examined using an inverted microscope and compared with the cells serving as control. At the end of, cellular viability was determined by use of XTT assay.

### Screening of Test Compounds by XTT Assay

### Preparation of solutions [3-15]

XTT labeling reagent and activation reagent was put at room temperature. Each vial was mixed thoroughly to obtain a clear solution.

**XTT labeling mixture**

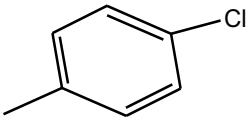
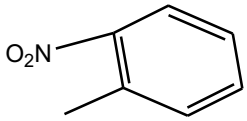
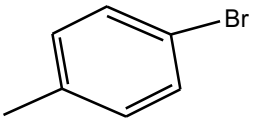
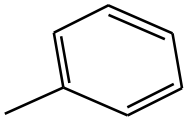
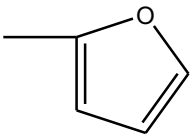
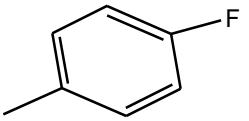
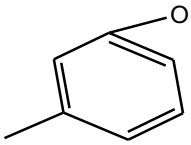
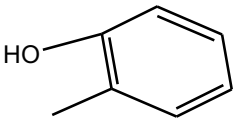
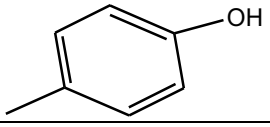
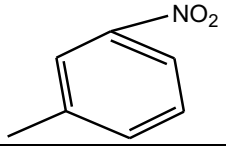
A cell proliferation assay (XTT) with one micro-plate (96 wells) was performed by mixing 5 ml XTT labeling reagent with 0.1 ml activation reagent.

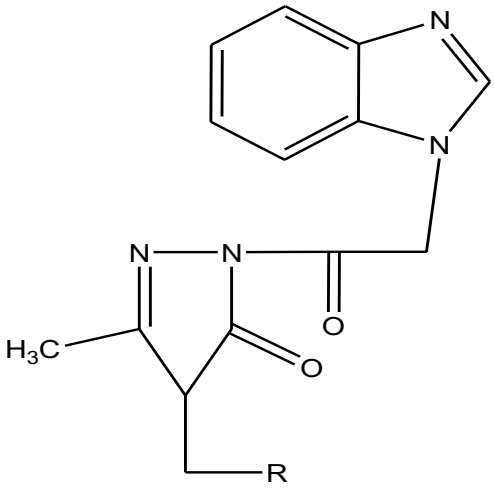
**Protocol**

Cells were preincubated at a concentration of $1\times10^6$ cells/ml in culture medium for 3 hours at 37°C and 5% $CO_2$. Cells were seeded at a concentration of $5\times10^4$ cells/well in 100 µl culture medium and various amounts of compound (final concentration, *e.g.* 100µM - 0.005µM) into microplates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated into microplates for 24 hours at 37°C and 5% $CO_2$.50 µl XTT labeling mixture was added and incubated for 18 hours at 37°C and 6.5% $CO_2$. The spectrophotometrical absorbance of the samples was measured using a microplates (ELISA) reader. The wavelength to measure absorbance of the formazan product was 450 nm. (The reference wavelength was not more than 650 nm.) Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula,

$$\% \text{ viability} = (A_T\text{-}A_B) / (A_C\text{-}A_B) \text{ X } 100$$

**Compounds Structure** [16, 17]

| Comp. No. | -R |
|---|---|
| 1 |  |
| 2 |  |
| 3 |  |
| 5 |  |
| 6 |  |
| 7 |  |
| 8 |  |
| 9 |  |
| 10 |  |
| 11 |  |




## RESULTS AND DISCUSSION

### Anticancer activity on NCI Cell line

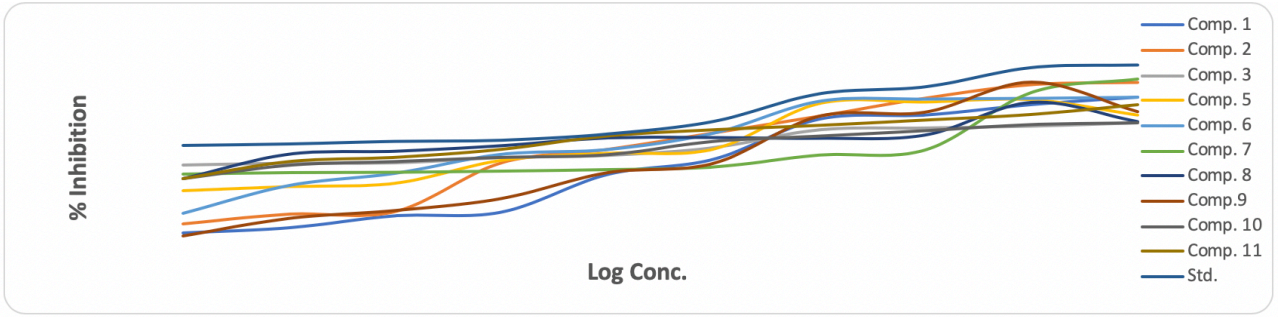



**Figure 1: anticancer activity on NCI cell line (log conc. Vs %inhibition)**

**Table 1: % inhibition on NCI cell line**

| LOG CONC. | 1 | 2 | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Std.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 73.113 | **78.349** | 63.207 | 66.037 | 73.113 | **79.905** | 63.632 | 67.169 | 63.207 | 70.012 | 85.283 |
| 1.5228 | 70.283 | **77.500** | 61.933 | 71.839 | 72.688 | **74.811** | 70.990 | 78.349 | 62.532 | 66.352 | 84.292 |
| 1.0457 | 66.320 | **72.405** | 61.226 | 70.990 | 72.405 | **53.018** | 58.537 | 67.020 | 60.254 | 64.252 | 76.933 |
| 0.5686 | 64.764 | **65.613** | 60.518 | 70.424 | 71.550 | **51.037** | 57.264 | 65.470 | 58.215 | 62.254 | 74.245 |
| 0.0915 | 49.481 | **59.245** | 53.726 | 53.160 | 59.386 | **46.509** | 57.547 | 47.641 | 56.21 | 60.592 | 63.632 |
| -0.385 | 43.679 | **53.018** | 50.754 | 51.603 | 53.160 | **45.518** | 57.405 | 44.103 | 51.215 | 58.265 | 58.820 |
| -0.862 | 29.386 | **47.924** | 49.905 | 48.915 | 51.320 | **44.952** | 54.433 | 34.339 | 50.214 | 53.256 | 56.415 |
| -1.339 | 27.971 | **29.528** | 47.924 | 40.283 | 44.103 | **44.520** | 52.311 | 29.811 | 48.654 | 50.245 | 55.990 |
| -1.816 | 23.443 | **28.537** | 47.783 | 39.009 | 39.433 | **44.386** | 51.179 | 26.839 | 47.256 | 48.654 | 55.000 |
| -2.294 | 21.462 | **24.858** | 47.075 | 37.452 | 28.820 | **43.820** | 41.839 | 20.188 | 42.235 | 42.251 | 54.433 |

*Doxorubicin

### Anticancer activity on MDA-MB-453 Cell line

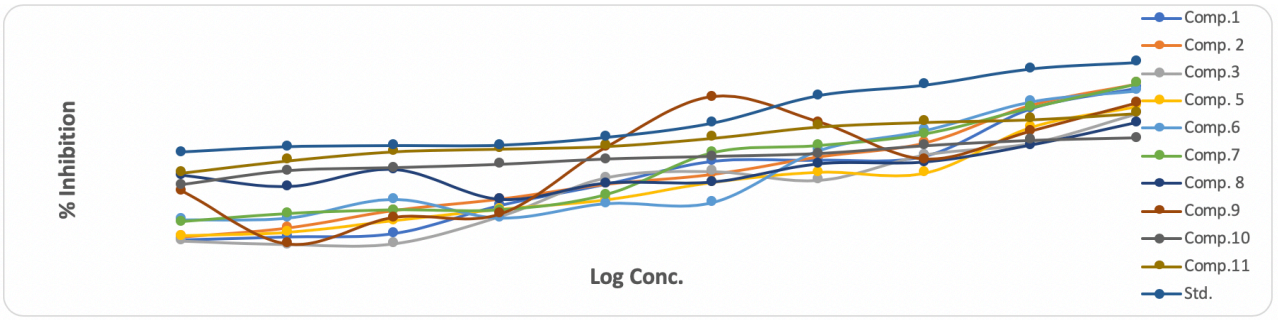



**Figure 2: anticancer activity on MDA-MB-453 cell line (log conc. Vs inhibition)**

**Table 2: % inhibition on MDA-MB-453 cell line**

| LOG CONC. | 1 | 2 | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Std.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 76.918 | **78.481** | 67.398 | 70.097 | 75.923 | **78.481** | 63.988 | 71.234 | 58.258 | 67.582 | 86.580 |
| 1.5228 | 69.245 | **70.524** | 56.315 | 62.282 | 71.802 | **69.529** | 55.462 | 60.719 | 57.265 | 65.254 | 84.164 |
| 1.0457 | 51.484 | **56.457** | 51.910 | 45.089 | 61.140 | **59.725** | 49.068 | 50.205 | 55.254 | 64.258 | 78.054 |
| 0.5686 | 49.921 | **51.199** | 42.390 | 45.374 | 53.615 | **55.320** | 48.358 | 63.990 | 52.325 | 62.548 | 74.076 |
| 0.0915 | 49.481 | **44.663** | 45.658 | 41.537 | 34.291 | **52.620** | 41.537 | 73.508 | 51.235 | 58.325 | 63.703 |
| -0.385 | 40.969 | **40.969** | 43.384 | 35.001 | 33.864 | **36.990** | 41.111 | 54.752 | 50.254 | 55.258 | 58.304 |
| -0.862 | 33.012 | **35.427** | 28.890 | 31.449 | 28.465 | **31.165** | 35.001 | 29.744 | 48.254 | 54.268 | 55.320 |
| -1.339 | 22.497 | **31.165** | 18.661 | 27.186 | 35.427 | **31.165** | 46.226 | 28.465 | 46.985 | 53.258 | 55.170 |
| -1.816 | 21.218 | **24.628** | 18.377 | 22.781 | 28.465 | **29.744** | 39.832 | 18.519 | 45.845 | 49.845 | 54.752 |
| -2.294 | 20.080 | **21.218** | 19.655 | 21.500 | 27.754 | **26.760** | 44.095 | 38.411 | 40.587 | 45.258 | 52.762 |

*Doxorubicin

**Anticancer activity on MDA-MB-468 Cell line**

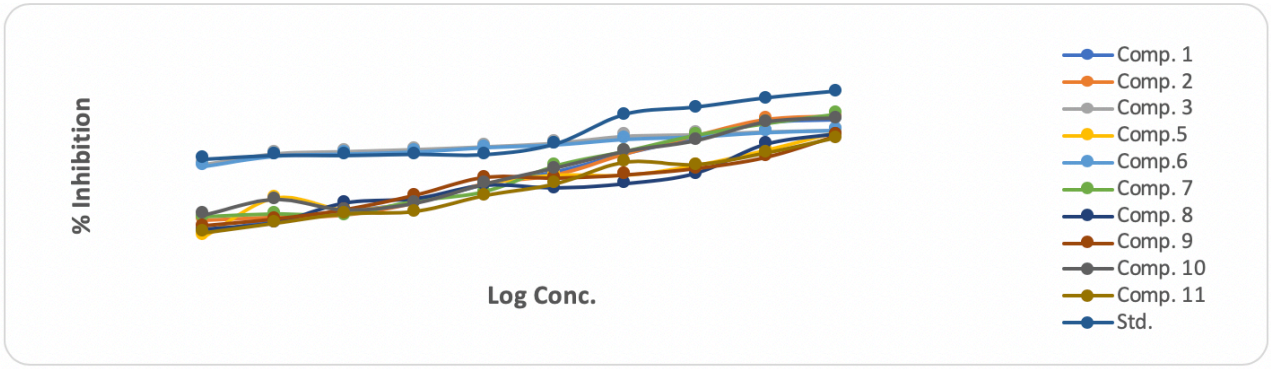



**Figure 3: anticancer activity on MDA-MB-468 cell line (log conc. Vs %inhibition)**

**Table 3: % inhibition on MDA-MB-468 cell line**

| LOG CONC. | 1 | 2 | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Std.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 71.029 | **72.575** | 66.358 | 65.266 | 66.352 | **73.840** | 64.001 | 63.579 | 71.450 | 62.450 | 83.960 |
| 1.5228 | 69.623 | **71.029** | 65.685 | 56.973 | 65.35 | **69.483** | 59.360 | 53.599 | 69.623 | 55.427 | 80.868 |
| 1.0457 | 62.595 | **63.579** | 64.358 | 49.945 | 63.352 | **64.001** | 46.009 | 48.399 | 61.049 | 50.080 | 76.651 |
| 0.5686 | 55.427 | **55.286** | 63.589 | 45.447 | 62.21 | **56.551** | 41.230 | 45.447 | 56.129 | 51.210 | 73.418 |
| 0.0915 | 46.990 | **45.588** | 60.358 | 45.447 | 59.645 | **49.664** | 39.262 | 43.900 | 48.539 | 41.230 | 59.784 |
| -0.385 | 41.230 | **41.652** | 58.648 | 41.371 | 58.358 | **37.710** | 40.527 | 44.322 | 41.230 | 35.889 | 54.864 |
| -0.862 | 32.797 | **32.797** | 57.354 | 32.937 | 56.358 | **33.210** | 34.343 | 36.029 | 32.375 | 28.580 | 55.005 |
| -1.339 | 28.861 | **27.174** | 56.358 | 29.564 | 55.351 | **26.893** | 32.375 | 29.283 | 29.283 | 27.455 | 54.443 |
| -1.816 | 24.640 | **26.190** | 55.325 | 34.905 | 54.365 | **27.174** | 23.379 | 24.925 | 34.062 | 23.098 | 54.443 |
| -2.294 | 18.881 | **24.785** | 50.255 | 17.195 | 49.652 | **25.909** | 20.287 | 21.833 | 27.030 | 18.741 | 52.756 |

*Doxorubicin

**Anticancer activity on VERO Cell line**

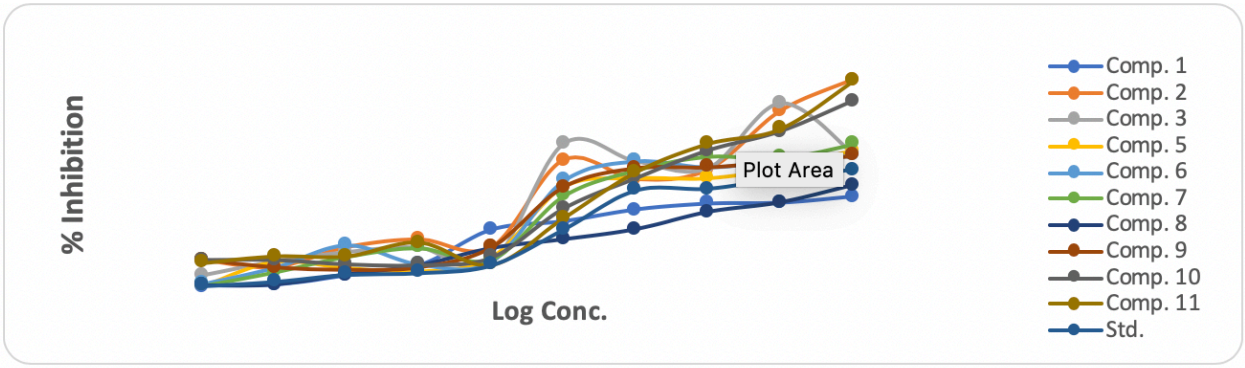



**Figure 4: anticancer activity on VERO cell line (log conc. Vs %inhibition)**

**Table 4: % inhibition on VERO cell line**

| LOG CONC. | 1 | 2 | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Std.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 10.022 | **22.922** | 14.800 | 15.090 | 14.517 | 15.854 | 11.235 | 14.517 | 20.534 | **22.731** | 12.989 |
| 1.5228 | 9.325 | **19.484** | 20.343 | 13.180 | 14.040 | 14.326 | 9.325 | 13.940 | 17.191 | **17.478** | 12.034 |
| 1.0457 | 9.235 | **12.989** | 13.180 | 12.034 | 13.276 | 14.517 | 8.268 | 13.276 | 15.090 | **15.854** | 10.888 |
| 0.5686 | 8.564 | **11.938** | 13.940 | 12.034 | 13.940 | 12.989 | 6.356 | 13.180 | 12.034 | **12.702** | 10.697 |
| 0.0915 | 7.265 | **14.040** | 15.854 | 11.079 | 11.747 | 10.124 | 5.235 | 11.079 | 8.695 | **7.640** | 6.399 |
| -0.385 | 6.3254 | **4.393** | 2.674 | 3.342 | 2.865 | 3.151 | 4.259 | 4.393 | 3.304 | **2.483** | 2.473 |
| -0.862 | 2.356 | **5.253** | 4.393 | 1.814 | 2.578 | 4.393 | 2.358 | 2.196 | 2.483 | **4.966** | 1.623 |
| -1.339 | 1.25 | **4.393** | 3.915 | 2.196 | 4.680 | 3.438 | 1.365 | 1.910 | 2.578 | **3.438** | 1.442 |
| -1.816 | 0.215 | **2.196** | 2.865 | 2.86 | 2.196 | 1.719 | 0.236 | 2.196 | 3.056 | **3.438** | 0.668 |
| -2.294 | 0.111 | **2.960** | 1.337 | 0.191 | 0.382 | 0.191 | 0.135 | 3.056 | 3.056 | **2.674** | 0.191 |

*Doxorubicin

From the results tables and graphs we can discuss that in the cell line NCI, MDA-MB-453 and MDA-MB-468 compound no. 2 and 7 gives prominent anticancer activity with comparison of standard drug doxorubicin, whereas in the cell line VERO compound no. 2 and 11 gives prominent anticancer activity with comparison of standard drug doxorubicin.

## CONCLUSION

From the discussion, we conclude that compound which contain o-nitrobenzene as attachment gives better anticancer activity in all cell lines, whereas compound contain p-flourobenzene as attachment gives better anticancer activity in cell line like NCI, MDA-MB-453 and MDA-MB-468. The compound contains m-nitrobenzene gives positive results in VERO cell line. Both the nitro and fluorine groups are highly electronegative so it may give better activity on different cell line, hence give prominent antitumor activity.

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